

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**202067Orig1s000**

**PHARMACOLOGY REVIEW(S)**

## Tertiary Pharmacology Review

**By:** Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

**NDA:** 202067

**Submission date:** December 23, 2010

**Drug:** clobazam

**Applicant:** Lundbeck, Inc.

**Indication:** Treatment of Lennox Gastaut Syndrome in patients  $\geq 2$  years old

**Reviewing Division:** Division of Neurology Products

### **Introductory Comments:**

The pharmacology/toxicology reviewer and supervisor have identified significant deficiencies in the nonclinical information necessary to characterize the toxicity of clobazam. They conclude that these deficiencies can be addressed postmarketing if the clinical data are deemed sufficient to support approval.

### **Discussion:**

Clobazam has not been previously approved in the United States but has been marketed in other countries since the 1970s. Many of the nonclinical studies of clobazam were conducted prior to 1975. The pharm/tox reviewer and supervisor identified a number of deficiencies with these studies. In particular, the developmental and reproductive toxicity studies were found to be inadequate because of a lack of justification of dose selection, lack of evidence of drug stability, inadequate dosing periods, and lack of complete individual line listings.

The sponsor and review division have recommended that clobazam be labeled with pregnancy category C. This differs from other benzodiazepines, some of which are labeled with pregnancy category X. The division's recommendation on pregnancy category seems appropriate based on review of the information currently available for clobazam and other benzodiazepines.

Carcinogenicity studies were also found to be inadequate because of replacement of high dose mice at 6 and 9 weeks (with younger animals), missing histopathology (inability to determine the number of tissues examined and the number of animals examined for histopathology), group housing during a feed study, and other reasons detailed in the pharm/tox review. The executive carcinogenicity assessment committee concurred that the mouse and rat studies were inadequate but also concluded that thyroid follicular cell adenomas in male rats at the high dose (100 mg/kg/day) were drug related.

### **Conclusions:**

The pharm/tox reviewer and supervisor have conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that clobazam may be approved for the above indication from a nonclinical

perspective with additional postmarketing assessments of carcinogenicity and developmental and reproductive toxicity. I reviewed the labeling changes proposed by the pharm/tox reviewer and supervisor and find them acceptable.

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/s/  
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PAUL C BROWN  
10/20/2011

# MEMORANDUM

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

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

Date: October 17, 2011

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

Subject: NDA 202-067 (ONFI, clobazam), dated 12/23/2010

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NDA 202-067 was submitted by Lundbeck, Inc. for Onfi (clobazam) Tablets for adjunctive treatment of seizures associated with Lennox-Gastaut syndrome (LGS) in patients 2 years of age and older. Clinical development of clobazam for this indication was conducted under IND 70125 (initial submission, 10/5/2005); orphan drug designation was granted on 12/18/2007.

The original nonclinical studies of clobazam (listed below) were conducted prior to 1975 (by or for (b)(4) clobazam was first approved in 1975, as an anxiolytic in France.

- Pharmacology
- PK/ADME: *in vitro* and *in vivo* (rat, dog, monkey)
- Toxicology
  - Acute dose: mouse and rat (p.o., iv, ip, sc), guinea pig (p.o.), rabbit (p.o.), dog (p.o.).
  - Repeat-dose (p.o.) rat (up to 24 and 26 weeks, and 18 months), dog (up to 26 and 52 weeks), and monkey (up to 52 weeks).
- Reproductive and developmental toxicity (p.o.)
  - Fertility and early embryonic development in mouse and rat.
  - Embryofetal development in mouse, rat, and rabbit.
  - Pre- and postnatal development (including maternal function) in mouse.

Therefore, those studies were not conducted according to current standards and not under GLP (GLP was instituted in 1978). In a PIND meeting held on 10/13/2004, the Division (then DNDP) informed the sponsor that additional nonclinical studies were needed to support clinical development of clobazam for treatment of LGS. These included: a complete battery of genotoxicity studies, a juvenile animal toxicology study in rat, safety pharmacology studies, and steady-state toxicokinetic data in the animal species used in

the pivotal toxicity (including general, reproductive, and carcinogenicity) studies. In response to the sponsor's specific question regarding the adequacy of the carcinogenicity studies, the Division noted that the adequacy of those studies would be a matter of review, but that deviations from GLP would need to be provided for each study. IND 70125 was originally submitted by Ovation Pharmaceuticals, Inc. (now Lundbeck, Inc.) on 5/25/2005.

The nonclinical studies submitted in support of NDA 202-067 include the original studies, and the following additional studies conducted by Lundbeck:

- *In vitro* receptor binding studies of clobazam (CBZ) and N-desmethyloclobazam (N-CBZ).
- Safety pharmacology studies: *in vitro* hERG and Purkinje fiber assays for CBZ and N-CBZ; cardiovascular safety in Beagle dog.
- Toxicokinetic studies: 28-day oral study in dog and monkey.
- Juvenile animal toxicology study in rat.
- A complete battery of genotoxicity studies: *in vitro* Ames assays for CBZ and N-CBZ, *in vitro* chromosomal aberration assays for CBZ and N-CBZ, and *in vivo* micronucleus assays in mouse for CBZ and N-CBZ.

These studies have been reviewed by Dr. Fisher (*Pharmacology/Toxicology Review and Evaluation NDA 202-067, October 9, 2011*). Based on his review, Dr. Fisher has concluded that the nonclinical data provided by the sponsor do not adequately assess the toxicity of clobazam, especially the reproductive and developmental toxicity and carcinogenic potential. This conclusion is based on the numerous deficiencies in the conduct and documentation of the pivotal nonclinical studies.

In addition to the lack of GLP compliance (noted above) for the majority of the pivotal studies, deficiencies include (but may not be limited to) the following:

- For the chronic (24-week, 6- and 18-month) oral toxicity studies in rat: incomplete microscopic examination, lack of individual animal line listings, and/or lack of summary histopathology tables. For none of the studies was toxicokinetic analysis conducted or signed pathology report provided.
- For the chronic (6-month, two 1-year) oral toxicity studies in dog: lack of individual animal line listings and evidence of toxicity due to presence of parasites (6-month), lack of signed pathology reports (one 1-year study report provided a signed Pathology Report but only for liver findings).
- For the chronic (52-week) oral toxicity study in monkey: histopathology conducted only on animals that died prematurely, evidence of compromised health unrelated to drug (i.e., malaria, tuberculosis, mites), lack of signed pathology report.
- For the dietary carcinogenicity studies in mouse and rat: early deaths (with replacement of high-dose animals with animal younger at initiation than the original animals) due to fighting-induced injury (mouse), group housing resulting in an inability to ensure accurate dosing, lack of data to document stability of

drug in diet, lack of toxicokinetic analysis, lack of documentation that a full battery of tissues was examined microscopically in any or all animals, lack of signed QA statement, study report or pathology report.

- For the reproductive and developmental studies: generally a lack of evidence of toxicity in the F<sub>0</sub> generation or justification for dose selection, use of dietary administration (fertility studies in mouse and rat) with no documentation of stability of drug in diet or plasma exposure, inadequate dosing period (i.e., dosing only during gestation days 9 to 14 in rat, or gestations days 7 to 12 in mouse in the embryo-fetal studies; dosing only from gestation day 17 to postnatal day 21 in the pre [peri] and postnatal study), and/or the lack of complete individual animal line listings for all parameters.

The sponsor did conduct additional studies (listed previously), as requested, except for toxicokinetic bridging studies for mouse, rat, or rabbit; therefore, no estimates of plasma exposure are available for these species. This is a serious deficiency, particularly affecting the pivotal studies conducted using dietary administration, and compounded by group housing in and the lack of drug stability data for the carcinogenicity studies. The sponsor provided no explanation for the lack of these data.

Taking these deficiencies into consideration, Dr. Fisher acknowledges the extensive previous human experience and the seriousness of the clinical indication, and recommends that the nonclinical deficiencies be addressed postmarketing if the clinical data are deemed to be sufficient to support approval. I concur with this recommendation. I also note that Dr. Fisher presumably has concluded that the nonclinical data provide some support, as evidenced by his recommendations for labeling. I would recommend that the sponsor be required to conduct a full battery of reproductive and developmental toxicity studies (as described in ICH guidance) and carcinogenicity studies in two species, all under GLP. In addition, the sponsor should be required to provide steady-state toxicokinetic data in the animal species used for these studies, at relevant doses. Although the chronic toxicity studies were inadequate, I don't believe repeat chronic toxicity studies are needed since some chronic toxicity data (with estimates of plasma exposure at relevant doses) are available for dog, chronic toxicity will be assessed in the carcinogenicity studies, and taking into consideration the extent of human experience.

Recommended labeling: labeling recommendations are based on the labeling changes made by Dr. Fisher to the sponsor's proposed wording. Safety margins are not included, due to the overall inadequacy of the nonclinical studies.

Regarding Section 8.1 (Pregnancy), the sponsor has proposed Pregnancy Category C, based on the available post-marketing data for clobazam and published literature on benzodiazepine use during pregnancy. This information has been reviewed by Dr. Boehm (*Clinical Safety Review, NDA 202-067, Gerard Boehm, M.D., M.P.H., 8/23/11*). (It should be noted that the literature provided by the sponsor did not include several more recent articles relevant to this issue.)

Dr. Fisher recommends Pregnancy Category C for clobazam, although other approved benzodiazepines are Pregnancy Category D or X, depending on the indication. According to approved class labeling for these products, the concern is based on data from “several studies” that suggest “An increased risk of congenital malformations associated with the use of minor tranquilizers (chlordiazepoxide, diazepam and meprobamate) during the first trimester of pregnancy...” (or similar language, depending on the drug). There are no human data for clobazam that suggest an increased risk of malformations. However, clobazam is approved in foreign countries, and product labeling available online ([http://www.hakimpharm.com/products/drugs/CNS\\_AGENTS/CLOZAM/CLOZAM.asp](http://www.hakimpharm.com/products/drugs/CNS_AGENTS/CLOZAM/CLOZAM.asp)) states under the Pregnancy section:

“FDA pregnancy category D. Clobazam crosses the placenta and has been reported to increase the risk of congenital malformations when used during the first trimester of pregnancy. Therefore clobazam should be avoided during pregnancy, especially during the first trimester.”

In previous reviews of data on the effects of *in utero* exposure to various benzodiazepines (*Memorandum, dated May 7, 1996, Gregory M. Dubitsky, M.D.; Pharmacology and Toxicology Review, March 5, 1996, J.E. Fisher, Ph.D.*), both Drs. Dubitsky and Fisher concluded that the human data suggesting adverse effects are inconsistent and, as Dr. Fisher noted, “...initial indications of an increased incidence of facial clefts in the offspring of exposed mothers have not been confirmed by more recent studies...” More recent published reviews have also concluded that there is no consistent or convincing evidence of a teratogenic potential for benzodiazepines, and that the effects of benzodiazepine use during pregnancy “remains unknown” (Gentile S. *Depress Anxiety* 27:675-686, 2010; also: Eros E. *et al. Eur J Obst Gyn Repro Biol* 101:147-154, 2002; Wikner BN *et al. Pharmacoeppi Drug Safe* 16:1203-1210, 2007; cf. Koren G, Nickel C *J Popul Ther Clin Pharmacol* 18(1):e28-e32, 2011 *comment on citation bias*). In particular, early reports of an association between *in utero* exposure to benzodiazepines and cleft lip/palate (e.g., Miller, Becker *TAP* 32:53-61, 1975; Walker, Patterson *Teratol* 10:159-164, 1074; cf. Dolovich LR *et al. Brit Med J* 317:839-843, 1998) have not been confirmed in subsequent evaluations (e.g., Wikner *et al.*, 2007). With that said, concerns regarding a potential relationship between benzodiazepine use during pregnancy and selected congenital malformations (e.g., anal atresia and/or pylorostenosis) have been raised (Bonnot O *et al. J Clin Psychopharm* 21(4):456-458, 2001; Wilner *et al.*, 2007); however, the authors of both reports state that these findings are preliminary and require additional investigation.

In his NDA review, Dr. Fisher points out that “...there is more evidence for long-term functional impairment associated with prenatal exposure to BDZs than for malformations...”, although “...evidence of neurobehavioral effects following prenatal BDZ exposure is largely anecdotal and is limited to effects...seen soon after birth.” The perinatal effects observed with clobazam (e.g., floppy infant syndrome) are similar to those reported for other benzodiazepines (cf. Wikner *et al.*, 2007).

It is my understanding that there is consensus within the division that Pregnancy Category C is appropriate for clobazam. However, there is concern that clobazam, if approved, will be the only benzodiazepine labeled in this way, which could be interpreted by physicians and patients as suggesting that clobazam is safer for use during pregnancy than other members of the class. How this may be addressed for clobazam is a clinical decision;

## HIGHLIGHTS OF PRESCRIBING INFORMATION

### -----INDICATIONS AND USAGE-----

ONFI is a benzodiazepine indicated for:

- Adjunctive treatment of seizures associated with Lennox-Gastaut syndrome (LGS) in patients 2 years of age or older (1.1)

### -----USE IN SPECIFIC POPULATIONS-----

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## FULL PRESCRIBING INFORMATION

### 8. USE IN SPECIFIC POPULATIONS

#### 8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies of clobazam in pregnant women and no adequate developmental toxicity studies of clobazam in animals.

Although limited, the available animal data suggest developmental toxicity, including an increased incidence of fetal abnormalities, following oral administration to pregnant animals at doses similar to those used clinically.

Data for other benzodiazepines suggest the possibility of adverse effects in animals and humans. Long-term effects on neurobehavioral and immunological function have been reported in rodents following prenatal exposure to benzodiazepines. Neonatal flaccidity, respiratory and feeding difficulties, hypothermia, and withdrawal symptoms have been reported in infants born to mothers who received benzodiazepines, including clobazam, late in pregnancy.

Therefore, ONFI should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

**Pregnancy Registry:** To provide information regarding the effects of *in utero* exposure to ONFI, physicians are advised to recommend that pregnant patients taking ONFI enroll in the North American Antiepileptic Drug (NAAED) Pregnancy Registry. This can be done by calling the toll free number 1-888-233-2334, and must be done by patients themselves or their caregiver. Information on the registry can also be found at the website <http://www.aedpregnancyregistry.org/>.

### 8.3 Nursing Mothers

ONFI is excreted in human milk.

### 8.4 Pediatric Use

The safety and effectiveness in patients less than 2 years of age have not been established.

In a study in which clobazam (4, 36, or 120 mg/kg/day) was orally administered to rats during the juvenile period of development (postnatal days 14-48), adverse effects on growth (decreased bone length and density) and behavior (altered motor activity and auditory startle response, learn deficit) were observed at the high dose. The no-effect dose for juvenile toxicity (36 mg/kg/day) was associated with plasma exposures (AUC) to clobazam and its major active metabolite, N-desmethyloclobazam, less than those expected at therapeutic doses in pediatric patients.

## 12 CLINICAL PHARMACOLOGY

### 12.1 Mechanism of Action

The mechanism of action of clobazam, a 1,5-benzodiazepine, is not fully understood but is thought to involve potentiation of GABAergic neurotransmission resulting from binding at the benzodiazepine site of the GABA<sub>A</sub> receptor.

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

#### *Carcinogenesis*

The carcinogenic potential of clobazam has not been adequately assessed. In a limited study in rats, oral administration of clobazam (4, 20, and 100 mg/kg/day) for 2 years resulted in an increased incidence of thyroid follicular cell adenomas in males at the high dose.

#### *Mutagenesis*

Clobazam and the major active metabolite, N-desmethyloclobazam, were negative for genotoxicity, based on data from a battery of *in vitro* (bacterial reverse mutation, mammalian clastogenicity) and *in vivo* (mouse micronucleus) assays.

#### *Impairment of Fertility*

There are no adequate studies of the effects of clobazam on fertility.

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/s/  
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LOIS M FREED  
10/17/2011



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 202-067  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: 12/23/10  
PRODUCT: Onfi (clobazam) Tablets  
INTENDED CLINICAL POPULATION: Treatment of Lennox-Gastaut Syndrome (LGS)  
SPONSOR: Lundbeck  
REVIEW DIVISION: Division of Neurology Products (HFD-120)  
PHARM/TOX REVIEWER: Ed Fisher  
PHARM/TOX SUPERVISOR: Lois Freed  
DIVISION DIRECTOR: Russell Katz  
PROJECT MANAGER: Sulin Sun

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Note: All figures and tables in this review were excerpted from the sponsor's submission or literature.

## I. INTRODUCTION AND DRUG HISTORY

NDA number: 202067

Date of submission: 12/23/10

Sponsor: Lundbeck

Drug:

Trade name: Onfi

Generic name: clobazam

Code names: H 4723, RU 4723, HR 376

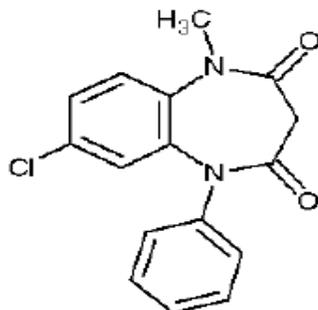
Chemical name: 7-chloro-1-methyl-5-phenyl-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)-dione

CAS registry number: CAS-22316-47-8

Molecular formula:  $C_{16}H_{13}ClN_2O_2$

Molecular weight: 300.7

Structure:



Relevant IND: 70,125

Drug class: benzodiazepine

Indication: adjunctive treatment of seizures associated with Lennox Gastaut syndrome (LGS) in patients  $\geq 2$  years of age

For comparison purposes, human AUC(0-24) values at the MRHD of 40 mg are 17649 and 57693 ng.h/ml for clobazam and N-desmethyclobazam (after repeated administration in Clinical Study OV-1022)

Route of administration: oral (tablets)

## II. PHARMACOLOGY

### A. BRIEF SUMMARY (partially taken from sponsor's summary; see initial IND review dated 9/11/97)

Clobazam (CLB) belongs to the class of short to intermediate acting benzodiazepines (BDZs), and has a long history of use in the treatment of epilepsy and anxiety. In *in vitro* receptor binding assays, significant binding of CLB was observed only at the central BDZ receptor ( $IC_{50} = 0.43 \mu M$ ;  $K_i = 0.36 \mu M$ ), the peripheral BDZ receptor ( $IC_{50} = 5.9 \mu M$ ;  $K_i = 5.3 \mu M$ ), and the  $\gamma$ -aminobutyric acid (GABA)-gated chloride channel ( $IC_{50} = 0.11 \mu M$ ;  $K_i = 0.095 \mu M$ ). These data indicate that CLB was selective among 78 receptors evaluated and binding was 10 times more potent at central BDZ receptors than at peripheral BDZ receptors.

CLB was effective in several animal models of chemically- or electroshock-induced convulsions, including those induced by pentylenetetrazole, strychnine, picrotoxin, and nicotine (**Table IIA.1**). In these models, CLB  $ED_{50}$  values (0.75 to 26 mg/kg) were similar to those of diazepam (0.75 to 21 mg/kg), and lower than those of chlordiazepoxide (2.5 to 86 mg/kg). CLB was also effective in preventing sound-induced seizures in DBA/2 mice and photic seizures in baboons. In the Ihara rat epileptic model, CLB (30 or 60 mg/kg BID po) suppressed abnormal circling and generalized tonic convulsions without adversely affecting behavior. Oral CLB induced dose-dependent inhibition of seizure stage and after-discharge duration of septal- and amygdala-kindled seizures. In the lateral geniculate nucleus-kindled rat seizure model, single oral doses of CLB (10 or 20 mg/kg) inhibited both seizure stage and after-discharge duration. CLB also had anticonvulsant activity in immature (12 to 25 days old) rats.

**Table IIA.1** Effects of CLB and Comparators in Various Nonclinical Seizure Models

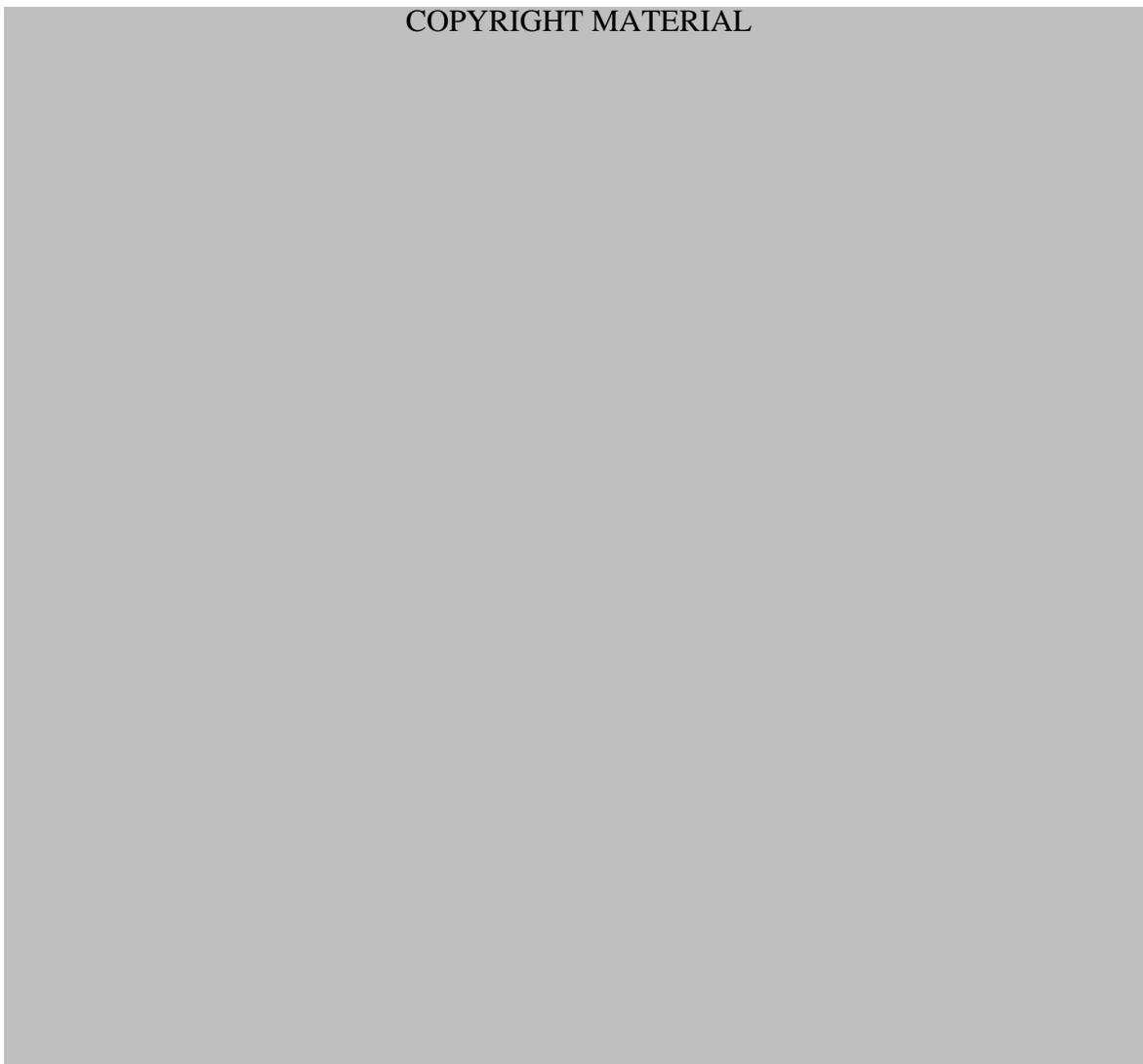
Species	Route	Seizure Stimulus	MED or $ED_{50}$ (mg/kg)		
			Clobazam	Diazepam	Chlordiazepoxide
Mouse	Oral	pentylenetetrazole	1.7	NT	NT
Mouse	Oral	pentylenetetrazole	10	5	25
Mouse	IP	pentylenetetrazole	4.3	NT	NT
Mouse	Oral	strychnine	16	21	86
Mouse	Oral	picrotoxin	26	ineffective	ineffective
Mouse	Oral	nicotine	14.0	4.5	19.0
Mouse	Oral	electroshock	23	16	31
Rat	Oral	electroshock	>400	NT	NT
Rat	IP	electroshock	6.4 - 10	NT	NT

From LNCP-001 or [1]

MED = Minimum effective dose;  $ED_{50}$  = Median effective dose; NT = Not tested; IP = Intraperitoneal

As with other BDZs, tolerance to the anticonvulsant effects of CLB has been observed in nonclinical seizure models. Loss of maximal activity following repeated administration of CLB was noted in several seizure models (electroshock, pentylenetetrazole, NMDA, amygdala-kindled, audiogenic, and spontaneous), although partial activity was still evident after repeated administration in some studies (**Figures IIA.1-2**; taken from Loscher and Schmidt, *Epilepsia* 47(8):1253-1284, 2006).

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**Figure IIA.2**

#### Effects of Human Metabolites

In *in vitro* receptor binding assays, significant binding of N-desmethyloclobazam (N-CLB), the major human metabolite of CLB, was observed only at the central BDZ receptor ( $IC_{50} = 0.39 \mu M$ ;  $K_i = 0.33 \mu M$ ), the peripheral BDZ receptor ( $IC_{50} = 2.8 \mu M$ ;  $K_i = 2.5 \mu M$ ), and the GABA-gated chloride channel ( $IC_{50} = 1.8 \mu M$ ;  $K_i = 1.5 \mu M$ ). These data indicate that N-CLB was selective among 78 receptors evaluated and binding was 7 times more potent at central BDZ receptors than at peripheral BDZ receptors. The  $IC_{50}$  of N-CLB at the central BDZ receptor was similar to that of CLB, while at the peripheral BDZ receptor, the  $IC_{50}$  was about half that of CLB. The  $IC_{50}$  of N-CLB at the GABA-gated chloride channel was 163 times greater than that of CLB. The primary pharmacodynamic activity of N-CLB was evaluated in selected studies indicative of anticonvulsant and anxiolytic action, and results indicate that N-CLB is active, but not as potent as CLB (**Table IIA.2**). The separation between efficacious and toxic doses for N-CLB was greater than for CLB (up to 90 times). This was thought to be due in part due to less potent binding of N-CLB at the GABA acid-gated chloride channel. However, it is difficult to distinguish the effects of CLB from N-CLB since mice and rats rapidly metabolize CLB to N-CLB.

**Table IIA.2** Effects of N-CLB in Selected Primary Pharmacodynamic Models

Test	Species	Route	ED <sub>50</sub> Values (mg/kg)		
			N-desmethyl-clobazam	Clobazam	Diazepam
Inclined Plane	Mice	Oral	~80	~20	~7.5
Horizontal Wire	Mice	Oral	>100	~40	~7.5
Metrazol Seizures	Mice	Oral	1.1	0.45	0.17
Rotorod	Mice	IP	>1000	40	~2.0
Rotorod	Rat	IP	>1000	30	~3.5
Footshock fighting	Rat	IP	15.5	2.2	0.49
Geller Conflict	Rat	IP	>100	20.0 (MED)	5.0 (MED)

From [1] IP = Intraperitoneal; MED = Minimal effective dose

## B. SAFETY PHARMACOLOGY

The secondary pharmacodynamic effects of CLB were evaluated in a series of traditional general pharmacology studies (LNCP-001). CLB had no effect on urine output or electrolyte excretion in rats given 50 mg/kg. Analgesic effects were observed in the acetic acid- and phenylquinone-induced writhing and foot-pad tests in mice and the dental-pulp pain model in rabbits. CLB prolonged hexobarbital-, thiopental-, secobarbital-, and alcohol-induced sleep time in mice. Analgesic effects and prolongation of sleep time were observed at doses comparable to or slightly higher than the ED<sub>50</sub> values in animal seizure models. CLB at oral doses up to 300 mg/kg did not affect body temperature in rats or rabbits, and had no effect on methamphetamine-induced hyperthermia in rats. No anti-inflammatory effects were observed in the rat carrageen-induced paw edema model at doses up to 160 mg/kg. Intestinal transit in mice given up to 100 mg/kg was unaffected, and only slight inhibition of histamine-induced contractions of isolated guinea pig ileum was observed at the maximum achievable concentration of 3000 ng/mL, suggesting that CLB has no significant effect on gastrointestinal motility. CLB at up to 100 mg/kg had no specific effects on apomorphine-induced emesis in dogs and did not affect pupil size in mice. There were no significant effects on uterine contractions *in vitro* or in the isolated rat phrenic nerve/diaphragm preparation at concentrations up to 6000 ng/mL. Along with the effects seen in the *in vivo* pharmacodynamic models of epilepsy, these data confirm a wide margin between desired and untoward effects of CLB.

In a hERG assay (OVNC-9008, conducted by (b) (4) report dated 7/31/09; GLP) CLB displayed a concentration dependent inhibition of hERG currents ranging from approximately 17% to 53% in a 100-fold concentration range from 2.5  $\mu$ M to 250  $\mu$ M. The estimated IC<sub>50</sub> was approximately 296  $\mu$ M with a Hill co-efficient of 0.42. N-CLB inhibited hERG currents by approximately 28.9% to 47.5% when tested at concentrations ranging from 1.27 to 110  $\mu$ M. Test concentrations greater than 110  $\mu$ M could not be achieved due to solubility limitations. An inhibition of nearly 48% of hERG currents observed with 110  $\mu$ M indicating this to be close to IC<sub>50</sub>. According to the sponsor, based on the hERG assay alone, the inhibitory effects of CLB and N-CLB on IKr suggest that if either compound alone or the two compounds in combination achieve free plasma concentrations in the range of 1 to 2.5  $\mu$ M ( $\geq$ 300 ng/mL), prolongation of the QT interval might be evident.

In a study of the *in vitro* effects on cardiac action potentials in rabbit Purkinje fibers (OVNC-9009, conducted by (b) (4) report dated 7/31/09; GLP) CLB produced statistically significant (SS) shortening (or less prolongation) of the action potential duration upon exposure to concentrations

ranging from 0.25 to 25  $\mu\text{M}$  at both stimulus frequencies when compared to the change in action potential duration upon exposure to vehicle alone. It did not induce SS changes in resting membrane potential, action potential amplitude and the maximum rate of depolarization ( $V_{\text{max}}$ ) at two stimulus intervals at concentrations up to 25  $\mu\text{M}$  except the resting membrane potential at 2.5  $\mu\text{M}$  at 0.5 BCL. N-CLB also produced SS of the action potential duration upon exposure to concentrations ranging from 12.9 to 30  $\mu\text{M}$  at both stimulus frequencies when compared to the change in action potential duration upon exposure to vehicle alone. It did not induce SS changes in resting membrane potential, action potential amplitude and the maximum rate of depolarization ( $V_{\text{max}}$ ) at two stimulus intervals at concentrations up to 30  $\mu\text{M}$  except the resting membrane potential at 2.5  $\mu\text{M}$  at 0.5 BCL. These changes were not in the expected direction based on the hERG results. According to the sponsor, the finding of APD shortening is most consistent with inhibition of other, non-IKr cardiac ion channels.

In a cardiovascular safety study in conscious freely moving telemeterized beagle dogs (OVNC-9034, (b) (4) 11/25/08, GLP), the same four male beagle dogs were administered oral doses of 0 (empty gelatin capsules), 1, 10, and 50 mg/kg CLB, with a 7 day washout period between each treatment until each animal received all treatments in ascending order.  $\leq 10$  mg/kg did not produce any T-R effects on mortality, body weight, serum cardiac troponin (cTpn I), hematology, coagulation, clinical chemistry, or the ECG (including QT and QTc intervals). Slight increases in heart rate were observed at all doses compared to C; however, these increases fell within historical control values and therefore not considered adverse. Administration of 50 mg/kg was associated with increased body temperature, decreased blood pressure, and various adverse, dose limiting clinical signs including, but not limited to seizures, convulsions, and tremors; however, there were no effects on QT or QTc intervals. Based on extrapolation of TK information from the 28-day dog study (**Table IIIB.1**), a dose of 50 mg/kg CLB is thought unlikely to exceed  $C_{\text{max}}$  values of 560 ng/mL (1.9  $\mu\text{M}$ ) while N-CLB free plasma  $C_{\text{max}}$  in the conscious dog cardiovascular study was estimated to be 23  $\mu\text{M}$  (6900 ng/mL) which is in the range at which alterations of cardiac ion channels might be expected (although *in vitro* to *in vivo* extrapolations are problematic).

### III. PHARMACOKINETICS

#### A. ADME SUMMARY (taken in part from sponsor's summary)

##### Absorption and Plasma Levels

Following administration of single or repeated oral doses of  $^{14}\text{C}$ -carbonyl-labeled CLB, absorption of total radioactivity (CLB and metabolites) was rapid and essentially complete in rats, dogs, and monkeys. In rats (0.57 mg/kg, single or multiple dose), the  $C_{\text{max}}$  of total radioactivity in blood was observed at 0.5 hours post-dose. Corresponding total concentrations were consistent after a single dose or after 10 days of dosing at this low dose.

In dogs (0.5 mg/kg, single oral dose), absorption was slightly lower than in the rat, with the  $C_{\text{max}}$  of total radioactivity observed between 2 and 4 hours postdose. Comparison of dose-normalized AUC values after oral and IV dosing suggested that at least 80% of the oral dose was absorbed, while recovery of radioactivity in urine indicated complete absorption of the oral dose. Monkeys were given a single oral dose of  $^{14}\text{C}$ -carbonyl-labeled CLB (2.5 or 20 mg/kg) or repeated doses of unlabeled CLB for 4 weeks followed by a single radiolabeled dose (above doses) on the last day. Concentrations of total radioactivity increased with increasing dose, and maximum observed concentrations were noted 4 to 8 hours postdose following single or repeated doses. Comparison of dose-normalized AUC values following a single dose of 2.5 and 20 mg/kg suggested a small decrease in absorption (<20%) at the high dose. Maximum concentrations of total radioactivity were 27% to 400% higher after repeated dosing than after a single dose at both dose levels. AUC values following repeated dosing were 70% higher at 2.5 mg/kg and 250% higher at 20 mg/kg than after a single dose. However, the half-lives were not significantly different

between the first and last <sup>14</sup>C-labeled CLB dose suggesting no delayed metabolism of CLB following repeated dosing.

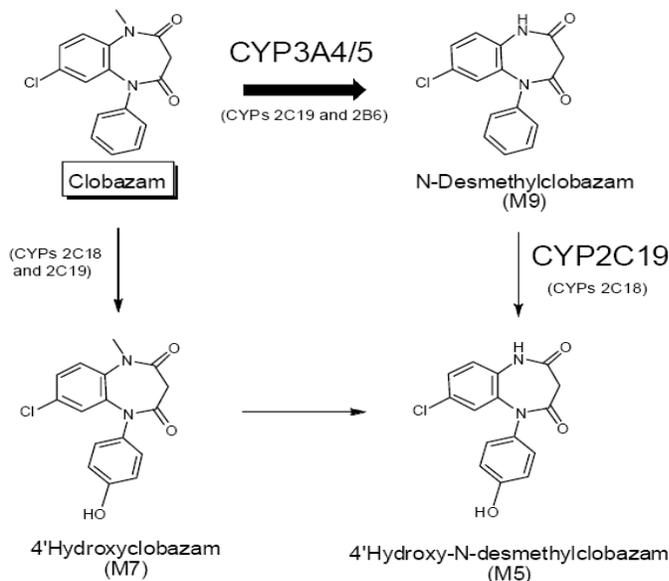
#### Distribution

The *in vitro* binding of <sup>14</sup>C-carbonyl-labeled CLB to serum proteins was moderate: 85, 83, 75, and 66% in human, dog, monkey, and rat serum, respectively. In these species the radioactivity was preferentially distributed to the plasma compartment. Results of a recent *in vitro* study (OVNC-9015) indicate that binding of CLB and N-CLB to human plasma proteins was 79 and 73%, respectively. The extent of CLB binding decreased in the presence of increasing concentrations of N-CLB, but the converse was not true. Following single and multiple dose administration of <sup>14</sup>C-carbonyl-labeled CLB to rats and dogs, total radioactivity was extensively distributed to the liver and kidney in both species. Liver-to-plasma ratios ranged from 9:1 to 16:1 in rats and from 3:1 to 5:1 in dogs; kidney-to-plasma ratios ranged from 2 to 3:1 in rats and were approximately 2:1 in dogs. Brain concentrations of total radioactivity were approximately 22% of plasma concentrations in rats and 60% to 75% of plasma concentrations in dogs. In pregnant rats, less than 1% of the administered dose was present in the fetus at the time of maximum levels in the dams.

#### Metabolism

The metabolism of CLB was examined in rats, dogs, monkeys, and humans (LNCPK-002; **Figure IIIB.1**). In all species, CLB was rapidly metabolized (only a small fraction of the dose was recovered in urine). N-dealkylation was the major metabolic route in all species. In human serum, N-CLB (M9), a biologically active metabolite, was the only major circulating metabolite. Additional metabolites identified were 4'OH-N-CLB (M5) and 4'OH-CLB (M7), each representing <3% of the parent AUC (**Figure IIIB.2**). These metabolites were present in rat and monkey urine and/or feces. M7 was not detected in the dog, however, it is possible that it was rapidly conjugated in this species. These *in vivo* metabolism studies indicated that there were no human unique metabolites. However, quantitative plasma metabolite data across species were limited to measurements of N-CLB in juvenile rat, dog, monkey, and human TK/PK studies (**Tables IIIC.1 and IVE.1.7**). These data indicate significant species differences in N-CLB/CLB ratios.





**Figure IIIB.1** Major Metabolic Pathway of CLB in Humans

#### Excretion

In rats, urinary and fecal excretion of CLB and metabolites (measured as total radioactivity) was similar with both routes of administration. Approximately 88% of the administered radioactivity was eliminated within 24 hours postdose, and almost 98% was eliminated within 48 hours postdose. With both routes of administration, urinary and fecal excretion accounted for approximately 28% and 72%, respectively, of total radioactivity administered. In dogs, urinary and fecal excretion of CLB and metabolites (measured as total radioactivity) was again similar with both routes of administration. Urinary and fecal excretion accounted for approximately 73% to 78% and 28%, respectively, of total radioactivity administered. Of the total radioactivity excreted in urine, >90% was excreted in 24 hours, and >98% was eliminated in 48 hours. In feces, 97% to 99% of total radioactivity excreted in feces was recovered within 3 days. Urinary elimination half-life values after IV and oral administration were 3.8 hours and 4.8 hours, respectively. In monkeys, overall recovery of 55.1% to 82.5% of administered radioactivity limited the conclusions that could be drawn from this study. Following single or repeated doses, urinary excretion was higher than fecal elimination. Half-life values of renal elimination, approximately 7 hours at 2.5 mg/kg and 11 hours at 20 mg/kg, were similar to half-life values of clearance from blood.

#### Toxicokinetics

Due to the legacy nature of toxicology studies conducted with CLB, systemic exposures of CLB and metabolites were not measured in the majority of toxicology studies with CLB. Therefore, TK studies were conducted recently to evaluate systemic exposure to CLB and N-CLB following oral administration of CLB to dogs (OVNC-9017) and monkeys (OVNC-9064); doses were the same as those used in chronic toxicity studies. In addition, TK parameters were assessed in the juvenile rat toxicology study. In these studies, CLB was rapidly absorbed following oral administration with  $C_{max}$  observed 0.5 to 7 hours postdose. Systemic exposure increased with increasing dose, and increases were more than dose-proportional in juvenile rats and male monkeys, and less than dose-proportional in dogs and female monkeys. In juvenile rats, exposures at the end of treatment (PND 48) were considerably lower than at the start of treatment (PND 14), which thought likely due to induction of clearance mechanisms but could also have reflected the development of metabolizing systems. In dogs, systemic exposure to CLB and N-CLB also decreased with repeated dosing, but less markedly than in juvenile rats, while in monkeys levels accumulated over

the course of the 28-day study. In a study of the potential inductive effects of CLB in the rat which was referred to in the discussion section of the 6-month rat study report, po administration of 400 mg/kg for 7 days reportedly resulted in approximately 2-fold increases in the activity of aminopyrine-N-demethylase and aniline-P-hydroxylase. It was demonstrated that both CLB and N-CLB are inducers of human CYP3A4/5 *in vitro* in human hepatocytes.

#### B. COMPARISON OF EXPOSURE IN ANIMALS AND HUMANS

Steady-state exposure (AUC<sub>0-24</sub>) to CLB and N-CLB in dogs, monkeys, and humans are summarized in **Table III C.1-2**. Similar data in adult rats is not available, and steady-state exposure in juvenile rats (**Table IV E.1.7**) was considerably lower than in dogs, monkeys, and humans, according to the sponsor “likely due to induction of clearance mechanisms.” Exposures to CLB at the highest doses in the chronic repeat-dose toxicity studies (40 mg/kg/day in dogs; 20 mg/kg/day in monkeys) were <0.1 in dogs and <0.5 in monkeys those at the maximum recommended human daily dose (MRHD) of 40 mg, while exposures to N-CLB were 2(F)-3(M)X in dogs and 7 (F)-9(M)X in monkeys those at the MRHD.

**Table III.C.1** Steady-State Exposure in Dogs, Monkeys, and Human

Species/Strain		Steady State AUC <sub>0-24</sub> (ng·hr/mL)					
		Dog/beagle		Monkey/rhesus		Human <sup>a</sup>	
Method of Administration		Oral (capsule)		Oral (gavage)		Oral	
Gender (M/F)		4M, 4F		4M, 4F			
Study Day		Day 14		Day 14		Day 29	
Analyte		Clobazam	N-CLB	Clobazam	N-CLB	Clobazam	N-CLB
Dose (mg/kg)	Sex						
2.5	M	188	37837	457	45462	NA	NA
	F	366	41935	744	52080	NA	NA
4	M	NA	NA	NA	NA	NA	NA
	F	NA	NA	NA	NA	NA	NA
20	M	277	64193	9023	527450	NA	NA
	F	1336	120833	3735	425223	NA	NA
36	M	NA	NA	NA	NA	NA	NA
	F	NA	NA	NA	NA	NA	NA
40	M	1445	175815	NA	NA	NA	NA
	F	749	104686	NA	NA	NA	NA
120	M	NA	NA	NA	NA	NA	NA
	F	NA	NA	NA	NA	NA	NA
40 mg <sup>a</sup>	NA	NA	NA	NA	NA	17649	57693
160 mg	NA	NA	NA	NA	NA	41389	206768

M = Male; F = Female; N-CLB = N-CLB; NA = Not applicable; BLQ = Below the limit of quantitation (1.00 ng/mL)

<sup>a</sup> Day 29 values from 66 (40 mg) or 62 (160 mg) healthy subjects given 20 mg for 3 days, followed by 40 mg for 15 days, followed by a single dose of 20 mg on Day 29, or escalating doses from 20 mg to 160 mg over 28 days followed by single dose of 80 mg on Day 29. Data from Clinical Study Report [OV-1022](#)

<sup>b</sup> Maximum recommended human dose

**Table III.B.2** Pharmacokinetic parameters in clinical study OV-1022

Parameter	Clobazam 40 mg TDD Pharmacokinetic Population Mean (%CV) N = 66		Clobazam 160 mg TDD Pharmacokinetic Population Mean (%CV) N = 62	
	Clobazam	N-CLB	Clobazam	N-CLB
AUC <sub>0-24h</sub> (ng·hr/mL)	10,350 (26%)	30,464 (66%)	25,445 (18%)	117,405(55%)
AUC <sub>0-24</sub> (ng·hr/mL)	17,649 (28%)	57,693 (65%)	41,389 (20%)	223,023(55%)
C <sub>max</sub> (ng/mL)	1,076 (24%)	2,783 (71%)	2,884 (18%)	11,020 (60%)
T <sub>max</sub> (hr) (a)	1.62 (0.62 – 6.12)	4.12 (0.00 – 12.12)	1.87 (0.62 – 6.12)	4.12 (0.00 – 12.12)

<sup>a</sup> Median (range) for T<sub>max</sub>.

#### IV. TOXICOLOGY

Study Type and Duration	Route of Administration	Species
<b>Single-dose toxicity</b>		
	Oral, IV, IP, SC	Mouse, Rat
	Oral	Guinea pigs, Rabbit, Dog
<b>Repeated-dose toxicity</b>		
2-4 days	Oral	Dog
4 weeks	Oral	Rat, Dog <sup>a</sup> , Monkey
10 weeks	Oral	Monkey
24 weeks	Oral	Rat
26 weeks	Oral	Rat, Dog
52 weeks	Oral	Dog, Monkey
16 months	Oral	Dog
18 months	Oral	Rat
<b>Genotoxicity</b>		
Reverse mutation assay <sup>a</sup>	<i>in vitro</i>	Bacteria
Chromosome aberration assay <sup>a</sup>	<i>in vitro</i>	CHO-K <sub>1</sub> cells
Micronucleus assay <sup>a</sup>	Oral	Mouse
<b>Carcinogenicity</b>		
80 weeks	Oral	Mouse
104 weeks	Oral	Rat
<b>Reproductive and developmental toxicity</b>		
Fertility and early embryonic development	Oral	Mouse, Rat
Embryo-fetal development	Oral	Mouse, Rat, Rabbit
Prenatal and postnatal development, including maternal function	Oral	Mouse
Toxicity in juvenile animals	Oral	Rat <sup>a</sup>
<b>Local Tolerance</b>	None	None
<b>Other Studies</b>		
Dependence	IV	Monkey
<b>Metabolite Studies (N-CLB)</b>		
52-week repeated-dose toxicity	Oral	Dog
Reverse mutation assay <sup>a</sup>	<i>in vitro</i>	Bacteria
Chromosome aberration assay <sup>a</sup>	<i>in vitro</i>	CHO-K <sub>1</sub> cells
Micronucleus assay <sup>a</sup>	Oral	Mouse

IV = Intravenous; IP = Intraperitoneal; SC = Subcutaneous; CHO-K<sub>1</sub> = Chinese hamster ovary

<sup>a</sup> Conducted in compliance with Good Laboratory Practice regulations

## A. REPEAT DOSE GENERAL TOXICITY

### 1. Rat

In the 6-month study (INCT-009; conducted by (b) (4), dated 4/30/74, non-GLP), HR 376 (lot # not provided) was administered (po) to rats (S-D, 18/sex/group) at doses of 0 (2% starch vehicle, 25, 100, or 400 mg/kg. There were 4 MD and 3 HD male deaths between days 60 – 153. In these animals, decreased spontaneous behavior, piloerection, and prone position were seen prior to death, but no cause of death was determined. In the surviving rats at the same doses, decreased spontaneous behavior was seen transiently after drug administration (up to 3 hrs) for the first 2-4 weeks of the study. Body weight gains were decreased somewhat in HD males starting at about 3 month (only graph shown). There were no clinical pathology changes due to treatment. In HD rats of both sexes, liver weights were slightly increased, and hepatocellular hypertrophy around the central area of the lobule was noted histopathologically, with males somewhat more affected than females. There was no clear evidence of T-R necrosis. Some recovery from these effects was observed, but changes were still present 1 month after cessation of dosing. Thyroid weights were slightly increased at the MD and HD and slight to moderate decrease in colloid within the follicles and cuboidal follicular cells suggestive of hypofunction of the thyroid was observed histopathologically. Recovery was seen for these effects as well. Adrenal and ovary weights were increased in a few MD and HD animals, but were not associated with histopathological abnormalities. Slight swelling and slight hypercellularity of the glomerulus were observed in all groups, but appeared to be increased in HD males, and, while some recovery was seen, the changes were still present in a few HD males after 1 month. The LD was considered a NOAEL.

In the chronic toxicity study (INCT-011; conducted by (b) (4), report dated 11/22/74, non-GLP), CLB (A 50 376, batch # Op 1 E0836.) was administered in the diet to rats (S-D, 30/sex/group) for 18 months at approximate doses of 0 (control diet), 12, 35, or 100 (increased to 200 after 2 weeks and 600 after 36 weeks) mg/kg. No effects were observed during the first two weeks when the HD was 100 mg/kg. When the HD was increased to 200 mg/kg, a decrease in spontaneous activity and mild sedation was observed in the males and hyperactivity was observed in the females; however, these pharmacologic effects had disappeared after the first week at this dose. The dose was further increased to 600 mg/kg after thirty-six weeks; however, no pharmacologic effects were observed after this increase. There were no T-R effects on mortality. BW was decreased (SS) in HD males (22% below C at 18 mo) and females (27% below C) starting at about the time of the last dose adjustment. There were no differences in food consumption, however. There were no apparent clinical pathology changes related to treatment. Increased (SS) liver and thyroid weights were seen in HD males and females. Histopathologic examination of the liver revealed a high incidence of spherical eosinophilic inclusions and increased incidences of bright yellow colored granules at the HD in both sexes (**Tables IVA.1.1-2**). Based on the light microscopic examination it was suspected that the inclusions were associated with hepatic enzyme induction, and ultrastructural examination of liver from 3 HD males showed that the inclusions were associated with proliferation of smooth endoplasmic reticulum thought to be indicative of enzyme induction. The hepatocytic granules in the liver of both sexes were considered to probably represent lipofuscin pigmentation, but according to the report, "the possibility exists that they might be some other material." There was also a T-R accumulation of yellow granules in the proximal convoluted tubular epithelial cells of treated females. According to the report, "while a portion of these renal granules may be lipofuscin or formalin pigmentation, the remainder might be some other material. However, neither the hepatic nor the renal granules elicited any tissue response and thus their exact toxicological importance, if any, is unknown." Other findings in the kidney included increased incidences of focal cortical tubular dilatation in HD males and hyaline casts in HD females. There were no increases in thyroid tumors as seen in the rat carcinogenicity study, but thyroid colloid cysts were increased in HD females.

Table IVA.1.1

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS IN TISSUES OBTAINED FROM MALE RATS WHICH RECEIVED A 50 376 BY CONTINUOUS, DIETARY INTAKE FOR EIGHTEEN MONTHS

TISSUE - RESPONSE	DOSE LEVEL (mg/kg): NO. RATS IN GROUP :	NUMBER OF RATS AFFECTED			
		CONTROL	12.0	35.0	600.0
		26	27	26	25
<b>HEART</b>					
- focal inflammatory cell infiltration		3	5	4	3
- focal fibrosis		1	1	5	2
<b>TRACHEA</b>					
- chronic tracheitis		6	3	3	5
- focal dilatation of tracheal glands		5	1	1	6 (24)
<b>LUNG</b>					
- chronic murine pneumonia		22	24	23	20
- focal accumulations of alveolar macrophages		5	8	3	9
- focal chronic granulomatous inflammation		2	1	0	0
- focal adenomatosis		0	1	1	1
<b>LIVER (Hematoxylin and eosin stain)</b>					
- focal bile duct proliferation		15	11	8	2
- hepatocytic vacuolization		6	12	3	16
- focal chronic inflammation		8	4	5	5
- focal cytoplasmic rarefaction		4	1	1	9
- focal hepatic necrosis		2	3	2	4
- focal telangiectasis (sinusoidal ectasia)		4	7	4	10
- focal cystic bile ducts		0	0	0	0
- cyst (tapeworm larvae, <i>Cysticercus fasciolaris</i> )		2	0	2	1
- presence of multiple, spherical, eosinophilic inclusions within hepatocyte cytoplasm		0	0	0	21
- presence of bright yellow granules within the hepatocytic cytoplasm		0	0	0	5
- anaplastic bile duct carcinoma		1	0	0	0
- hepatocellular carcinoma		1	0	0	0
- presence of hepatic glycogen (PAS stain)		4	13	13	10
- presence of hepatic iron (Mallory's stain)		12	17	17	19
- presence of periportal lipid (Oil Red-O stain)		26	26	26	25
<b>KIDNEY</b>					
- chronic nephritis		10	11	6	11
- focal inflammatory cell infiltration		22	23	23	25
- focal cortical tubular dilatation		14	13	7	21
- cortical and medullary tubules with proteinaceous content (hyaline casts)		20	18	16	24
- cortical tubular cell basophilia		22	25	20	18
- glomerular atrophy		21	19	20	19
- mineralization		0	0	0	0
- focal tubular hypoplasia		0	0	0	0
- microcalculi		0	4	0	0
- bright yellow cortical tubular granules		23	19	18	22
- renal adenocarcinoma		1	0	0	0
- transitional cell carcinoma		1	0	0	0
- cyst		1	0	0	0
- acute ascending pyelonephritis		0	2	1	0
<b>URINARY BLADDER</b>					
- chronic inflammatory cell infiltration		0 (23)	1 (25)	1 (22)	1 (19)
<b>ADIPOSE TISSUE</b>					
- lipoma		0	0	0	0
<b>TESTIS</b>					
- organ atrophy		3	0	1	3 (24)
- focal interstitial edema		8	17	18	12 (24)
- focal tubular atrophy		2	5	7	2 (24)
- hyperplasia		0	0	1	0
- interstitial cell testicular tumor		0	1	0	0

<u>MAMMARY GLAND</u>				
- fibroadenoma	-	-	-	1 (2)
- present and normal	-	-	-	1 (2)
- galactoceles	-	-	-	-
- adenocarcinoma	1 (1)	-	-	-
- fibroma	-	1 (1)	-	-
<u>PITUITARY GLAND</u>				
- cyst	6 (21)	1 (26)	1 (23)	3 (20)
- adenoma	2 (21)	3 (26)	4 (23)	2 (20)
<u>ADRENAL GLAND</u>				
- focal cortical cell vacuolization	6	10	15	12
- focal cortical capillary ectasia (hematocysts)	10	7	4	3
- focal cortical modular hyperplasia	3	2	4	3
- focal cortical cell rarefaction	2	9	7	5
- pheochromocytoma	1	0	1	0
- adrenal cortical tumor	0	0	0	0
<u>THYROID</u>				
- focal chronic thyroiditis	1	1 (26)	0	0
- ultimobranchial cyst	4	7 (26)	0	2
- colloid cyst	4	4 (26)	2	6
- adenocarcinoma	0	1 (26)	0	1
- adenoma	0	0	1	0
- papillary cystadenoma	0	0	0	1

Note: The number in parenthesis indicates the total number of a particular tissue examined when less than the number of rats in the group.

Table IVA.1.2

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS IN TISSUES OBTAINED FROM FEMALE RATS WHICH RECEIVED A 50 576 BY CONTINUOUS DIETARY INTAKE FOR EIGHTEEN MONTHS

TISSUE - RESPONSE	DOSE LEVEL (mg/kg):	NUMBER OF RATS AFFECTED			
		CONTROL	12.0	35.0	600.0
	NO. RATS IN GROUP :	26	26	28	26
<u>HEART</u>					
- focal inflammatory cell infiltration		1	4	3	0
- focal fibrosis		2	3	2	2
<u>TRACHEA</u>					
- chronic tracheitis		4 (23)	5 (25)	4 (27)	8 (25)
- focal dilatation of tracheal glands		10 (23)	6 (25)	3 (27)	7 (25)
<u>LUNG</u>					
- chronic murine pneumonia		25	22	26	25 (25)
- focal accumulations of alveolar macrophages		4	13	9	4 (25)
- focal chronic granulomatous inflammation		8	4	8	6 (25)
- focal adenomatosis		0	0	2	0
<u>LIVER (Hematoxylin &amp; eosin stain)</u>					
- focal bile duct proliferation		10	3	9	10
- hepatocytic vacuolization		14	13	9	13
- focal chronic inflammation		10	6	8	6
- focal cytoplasmic rarefaction		4	4	3	4
- focal hepatic necrosis		1	2	1	1
- focal telangiectasis (sinusoidal ectasia)		0	0	0	5
- focal cystic bile ducts		0	0	0	1
- cyst (tapeworm larvae, <i>Cysticercus fasciolaris</i> )		0	0	0	0
- presence of multiple, spherical, eosinophilic inclusions within hepatocyte cytoplasm		0	0	0	0
- presence of bright yellow granules within the hepatocytic cytoplasm		0	0	2	18
- anaplastic bile duct carcinoma		0	0	0	0
- hepatocellular carcinoma		0	0	0	0
- presence of hepatic glycogen (PAS stain)		8	8	8	10
- presence of hepatic iron (Mallory's stain)		26	25	26	25
- presence of periportal lipid (Oil Red-O stain)		26	25 (25)	27 (27)	25 (25)

<u>KIDNEY</u>				
- chronic nephritis	4	3	4	6
- focal inflammatory cell infiltration	18	16	14	24
- focal cortical tubular dilatation	8	4	6	10
- cortical and medullary tubules with proteinaceous content (hyaline casts)	9	12	13	17
- cortical tubular cell basophilia	13	14	11	14
- glomerular atrophy	13	7	6	8
- mineralization	3	2	1	1
- focal tubular hypoplasia	4	5	2	4
- microcalculi	0	3	3	4
- bright yellow cortical tubular granules	17	17	25	26
- renal adenocarcinoma	0	0	0	0
- transitional cell carcinoma	0	0	0	0
- cyst	0	0	0	0
- acute ascending pyelonephritis	0	0	0	0
<u>URINARY BLADDER</u>				
- chronic inflammatory cell infiltration	0 (19)	0 (19)	0 (25)	0 (25)
<u>ADIPOSE TISSUE</u>				
- lipoma	0	0	1	0
<u>TESTIS</u>				
- organ atrophy	-	-	-	-
- focal interstitial edema	-	-	-	-
- focal tubular atrophy	-	-	-	-
- hyperplasia	-	-	-	-
- interstitial cell testicular tumor	-	-	-	-
<u>MAMMARY GLAND</u>				
- fibroadenoma	2 (5)	2 (10)	1 (12)	4 (13)
- present and normal	2 (5)	6 (10)	8 (12)	6 (13)
- galactoceles	1 (5)	2 (10)	4 (12)	4 (13)
- adenocarcinoma	0	1 (10)	0	0
- fibroma	0	0	0	0
<u>PITUITARY GLAND</u>				
- cyst	1 (21)	1 (21)	1 (24)	0
- adenoma	12 (21)	9 (21)	10 (24)	4 (25)
<u>ADRENAL GLAND</u>				
- focal cortical cell vacuolization	22	11	13	10
- focal cortical capillary ectasia (hematocysts)	24	21	21	23
- focal cortical nodular hyperplasia	3	4	2	1
- focal cortical cell rarefaction	9	12	10	7
- pheochromocytoma	0	0	0	0
- adrenal cortical tumor	0	0	0	1
<u>THYROID</u>				
- focal chronic thyroiditis	0	0	1	0
- ultimobranchial cyst	4	4	4	2
- colloid cyst	0	0	0	4
- adenocarcinoma	0	0	0	0
- adenoma	1	0	0	0
- papillary cystadenoma	0	0	0	0

Note: The number in parenthesis indicates the total number of a particular tissue examined when less than the number of rats in the group.

## 2. Dog

In the 6-month study (INCT-012, conducted by (b) (4) report dated 7/18/72, non-GLP), CLB (A 50 376, batch #Op 1 E 0836) was given orally (capsules) at doses of 0, 5, 20, or 80 mg/kg to beagle dogs (3/sex/grp, 10 months of age). Clinical signs included D-R sedation, somnolence, emesis, ataxia and convulsive seizures (1 LD, 1MD, and 2 HD). Fluctuations in BW and food consumption were seen at the HD. A progressive rise in serum alk phos was observed in HD dogs. Two dogs (1 MD male and 1 HD female, during the 26th and 20th week, respectively), and another HD female was sacrificed moribund (9th week). No cause of death was determined in those that died, but the dog that was sacrificed showed pulmonary and rectal lesions compatible with parasitic migration and associated complications. Livers weights were significantly increased at the HD. A microscopic lesion of the liver, characterized by the presence of intracytoplasmic eosinophilic inclusions, was observed in 3 of 4 surviving HD dogs.

In the initial 1-year study (INCT-013, conducted by (b) (4) report dated 2/20/74, non-GLP), CLB (A 50 376, batch #Op 1 E 0836) was given orally (capsules) at doses of 0, 5, 10, or 40 mg/kg to beagle dogs (4/sex/grp, 12 months of age). Nine dogs died during the study: 1 LD, 2 MD, and 6 HD from the 77th to the 365th day. The cause of death was not established; however, from observations and special trials done during the study, it was thought that the probable cause was withdrawal seizures. No other adverse effects were observed except for a slight increase in serum alkaline phosphatase levels at the HD. There were no liver lesions associated with this change. The pharmacologic effects included marked sedation and somnolence which gradually subsided over the course of treatment. No other parameters were affected and no microscopic findings were associated with treatment except for an increased accumulation of pigments in hepatocytes and Kupffer cells (**Table IVA.2.1**).

**Table IVA.2.1**

Tissue/Response	Compound :	Incidence of Response						
		Control		A50376				
		0		10		5		
		M	F	M	F	M	F	
	Animals Per Group :	4	4	3	3	4	2	3
<u>Epididymis</u>	- chronic arteritis with subintimal hyaline change	0		0		1		
<u>Prostate</u>	- localized dilatation of acini	0		1		0		
<u>Ovary</u>	- hemorrhage within stroma of one ovary	0	1	0		0		
<u>Uterus</u>	- hypertrophy of endometrium (pseudocystosis)	1	0	0		0		
<u>Salivary gland</u>	- periductular inflammatory cell infiltration (neutrophils)	1/3	0	0				
<u>Lymph node</u>	- erythrophagocytosis (medulla)	1	2	1/2		0	0/1	0
<u>Spleen</u>	- congestion	0	0	1/2	1	0	1	2
<u>Liver</u>	- congestion	0	0	1	0	0	0	0
	- pigment (hemosiderin?) within Kupffer cells	0	0	1	0	0	0	0
	- localized granulomatous focus	0	0	1	0	0	0	0
<u>Heart</u>	- sub-endocardial hemorrhage	0	0	1	0	0	0	0

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A second 1-year study (INCT-014, conducted by (b) (4) report dated 12/23/74, non-GLP), CLB (A 50 376, batch #Op 1 E 0836) was conducted in beagle dogs (4/sex/grp, 12 months of age) with oral (capsules) doses of 0, 2.5, or 5 mg/kg. No significant toxic effects were observed. Serum alkaline phosphatase values were slightly elevated in the HD group. A slightly increased incidence of accumulation of a pigment was observed in hepatocytes and Kupffer cells (**Table IVA.2.2**). The report stated, "As was the case with the rat study, there was no evidence of hepatic necrosis or fibrosis associated with these granules in any dog in the present study. While the staining characteristics of the hepatic granules are indicative of endogenous pigmentation, the possibility exists that they might be some other material including (b) (4) or a metabolite therefrom." As in rats, yellow granules were observed in the epithelial cells of the proximal convoluted tubules. However, these changes were not associated with

other evidence of organ damage. According to the report, "while they appear to be lipofuscin pigmentation, the possibility cannot be excluded that a portion of them, especially in the high-dose dogs, are compound-related. Regardless of their exact classification and origin, these granules did not elicit any histological evidence of renal tubular, glomerular, or interstitial necrosis or fibrosis."

**Table IVA.2.2**

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS IN TISSUES OBTAINED FROM DOGS WHICH RECEIVED A DAILY ORAL DOSE OF A 50 376 FOR ONE YEAR

TISSUE -- RESPONSE	DOSE LEVEL (mg/kg): SEX OF ANIMALS : NO. DOGS IN GROUP :	NUMBER OF DOGS AFFECTED					
		CONTROL		2.5		5.0	
		M	F	M	F	M	F
<u>AORTA</u>							
-- focal mineralization		1	0	0	0	0	0
<u>HEART</u>							
-- valvular endocardiosis		1	0	0	0	0	0
<u>LUNG</u>							
-- septal cell proliferation		3	3	4	3	2	4
-- pulmonary atelectasis		2	2	2	2	2	2
-- pulmonary congestion and/or hemorrhage		2	2	1	1	1	2
-- parasitic (?) granulomatous inflammation		1	2	2	3	2	2
-- accumulation of alveolar macrophages		1	0	0	0	0	0
-- bronchopneumonia		0	0	1	0	0	0
<u>LIVER</u>							
-- hepatocytic vacuolization		3	3	3	4	4	4
-- finely granular yellow hepatocytic and Kupffer cell pigmentation		3	3	4	3	4	4
-- focal mononuclear cellular infiltration		0	1	1	0	0	0
-- presence of diffuse hepatocytic lipid		4	4	4	4	4	4
-- presence of periportal hepatocytic lipid (oil-red-O stain)		1	1	0	1	3	1
-- presence of hepatocytic glycogen (PAS stain)		4	4	4	4	4	4
-- presence of hepatocytic iron (Gomori's stain)		0	0	0	1	0	1
<u>GALL BLADDER</u>							
-- chronic inflammation		1	1	0	0	1(3)	1

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<u>PANCREAS</u>						
-- focal chronic inflammation	1	0	0	0	0	0
<u>SALIVARY GLAND</u>						
-- focal inflammatory cell infiltration	0	1	0	0	1	0
<u>STOMACH</u>						
-- submucosal lymphocytic infiltration	0	2	1	2	3 (3)	2 (3)
<u>DUODENUM</u>						
-- lymphoid hyperplasia	1	1	1	0	0	0
-- atypical mucosal cell hyperplasia	2	1	1	2	1	3
-- yellow-brown pigment in lamina propria	3	2	2	2	4	4
<u>ILEUM</u>						
-- lymphoid hyperplasia	0	1	2	2	3	0
-- atypical mucosal cell hyperplasia	0	1	2	2	1	1
<u>COLON</u>						
-- lymphoid hyperplasia	2	2	1	1	3	3
<u>KIDNEY</u>						
-- yellow cortical tubular granules	2	4	4	4	4	4
-- cortical tubular cell basophilia	1	1	2	1	1	1
-- glomerular atrophy	2	1	0	0	0	1
-- mineralization	1	0	1	1	0	0
<u>PROSTATE GLAND</u>						
-- chronic inflammation	1	-	0	-	1	-
<u>UTERUS</u>						
-- endometrial hyperplasia	-	1 (3)	-	1	-	0
<u>PITUITARY GLAND</u>						
-- cyst	0	2	1	0 (3)	2	0
<u>THYROID GLAND</u>						
-- interfollicular cell hyperplasia	0	1	0	1	1	0
-- chronic thyroiditis	0	0	0	1	0	0
<u>PARATHYROID GLAND</u>						
-- cyst	0	0	1	0	1	0 (2)
<u>SPLEEN</u>						
-- hemosiderin (?) pigment	4	4	4	4	4	4
-- congestion	2	3	4	3	3	0
-- lymphoid hyperplasia	0	0	0	0	1	1
<u>LYMPH NODES (MESENTERIC)</u>						
-- lymphoid hyperplasia	0	0	0 (3)	0	1	0
<u>THYMUS</u>						
-- lymphoid hyperplasia	0 (2)	0 (2)	0 (2)	1 (3)	0 (3)	0

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NOTE: The number in parenthesis indicates the total number of a particular tissue examined when less than the number of dogs in the group.

A chronic (12-month) oral (capsules) toxicity study of the major metabolite N-CLB (R 72 5818) was also conducted in beagle dogs (INCT-015, conducted by (b) (4), report dated 5/13/75, non-GLP) at a single dose of 40 mg/kg administered to only 1 male and 1 female for 363 days. After the 356th treatment the administration was discontinued for 2 days for the determination of withdrawal symptoms. These symptoms occurred about 48 hours after the last treatment and consisted of clonic-tonic convulsions in the male and persistent tremor in the male and female. Increased serum transaminases were seen in the female dog, but no other changes in hematological, clinical chemistry, or urinalysis parameters were reported. No T-R macroscopic and microscopic changes were seen.

At the Division's request, a 4-week bridging study (OVNC-9017, conducted by (b) (4), report dated 6/26/09; GLP) was conducted in beagle dogs (4/sex/group) at oral (capsule) doses of 0 (vehicle control), 2.5, 20, and 40 mg/kg CLB (Lot/Batch #: 317712). The HD was based on the findings in the first 12-month study. Clinical signs, body weights, and food consumption were monitored throughout the study. Blood samples were collected from fasted animals prior to dose administration on Days 1, 14, 28, and 35 for clinical pathology and for the determination of plasma CLB and N-CLB concentrations. On Day 35 of the study all animals were sacrificed, and subject to a comprehensive necropsy. The protocol specified tissues were collected, weighed, and preserved. There were no deaths in the study. Clinical signs, primarily at the MD and HD, consisted of lethargy, somnolence, ataxia, tremors, excessive saliva secretion, gasping, overly passive/submissive behavior, emesis, and minor abnormal gastrointestinal changes. A dose response trend was observed in the frequency, duration, and incidence of these observations, with some persisting throughout the dosing period. No convulsions were reported during treatment or during the recovery period (Days 29 - 35). There were no effects on body weight during the treatment period, but weight loss occurred during the recovery period in all dose groups. Clinical pathology investigations revealed no clear treatment related findings; any differences found were not considered biologically meaningful or a toxicological effect caused by the test substance. There were no treatment related findings in absolute or relative organ weights, with the exception of the lung weights, which were statistically significantly decreased. There were no macroscopic correlates of these findings; however, no histopathology was conducted "due to the lack of any dose related adverse findings reported during necropsy." TK data are shown below (**Tables IVA2.3-4**). Metabolite levels were higher than those of parent, and levels of both parent and metabolite declined over the course of treatment. There were no consistent sex differences, although metabolite levels tended to be higher in males at the HD.

**Table IVA.2.3** PK parameters for CLB in 28-day dog toxicology study

**Mean Pharmacokinetic Parameters for Clobazam on Day 1**

Sex	$C_{max}$	$T_{max}$	$AUC_{last}$	$t_{1/2}$	CL/F	V/F
	(ng/mL)	(hours)	(ng h/mL)	(hours)	mL/h/kg	mL/kg
<b>2.5 mg/kg</b>						
Males	74.70	0.63	101	0.8	26245	28337
Females	53.98	1.25	80.59	0.4	24454	24920
Combined	64.34	0.94	90.85	0.6	25349	26629
<b>20 mg/kg</b>						
Males	260.75	1.75	824	4.0	23885	130440
Females	422.00	1.50	1395	3.7	18107	98214
Combined	341.38	1.63	1110	3.8	20996	114327
<b>40 mg/kg</b>						
Males	463.33	1.33	1856	7.5	19189	197872
Females	519.00	1.75	2422	4.5	19968	148738
Combined	491.17	1.54	2139	6.0	19578	173305

**Mean Pharmacokinetic Parameters for Clobazam on Day 14**

Sex	$C_{max}$	$T_{max}$	$AUC_{last}$	$t_{1/2}$	CL/F	V/F
	(ng/mL)	(hours)	(ng*h/mL)	(hours)	mL/h/kg	mL/kg
<b>2.5 mg/kg</b>						
Males	37.70	6.63	188	3.1	22378	94938
Females	105.2	6.88	366	2.4	27540	99814
Combined	71.45	6.76	278	2.7	24959	97376
<b>20 mg/kg</b>						
Males	74.50	1.63	277	4.6	94293	554723
Females	224.33	4.00	1336	3.0	85028	247156
Combined	149.42	2.82	806	3.8	89661	400939
<b>40 mg/kg</b>						
Males	183.58	3.63	1445	3.6	106927	546961
Females	151.60	6.63	749	7.7	57757	590036
Combined	167.59	5.13	1097	5.7	82342	568499

**Mean Pharmacokinetic Parameters for Clobazam on Day 28**

Sex	$C_{max}$	$T_{max}$	$AUC_{last}$	$t_{1/2}$	CL/F	V/F
	(ng/mL)	(hours)	(ng h/mL)	(hours)	mL/kg	mL/kg
<b>2.5 mg/kg</b>						
Males	143.60	0.75	278	2.9	26654	60582
Females	39.35	0.88	45	0.6	73874	64595
Combined	91.48	0.82	162	1.8	50264	62589
<b>20 mg/kg</b>						
Males	91.73 *	1.17	269	4.8	73471	468806
Females	204.75*	0.88	449	3.5	47533	166739
Combined	148.24	1.03	359	4.2	60502	317772
<b>40 mg/kg</b>						
Males	126.30	1.63	502	6.6	115046	320779
Females	136.98	0.5	426	6.1	116866	864332
Combined	131.64	1.07	464	6.4	115956	592557

$AUC_{last}$  Area under the plasma concentration-time curve up to the last sampling time with measurable concentrations.  
 $C_{max}$  Maximum plasma concentration.  
 $T_{max}$  Time to maximum concentration.  
 $t_{1/2}$  Observed elimination half-life.  
V/F Volume of distribution  
CL/F Total clearance  
\* Indicates a statistically significant difference between sexes (p<0.05)

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**Table IVA.2.4** PK parameters for N-desmethylobazam in 28-day dog toxicology study

**Mean Pharmacokinetic Parameters for N-Desmethylobazam on Day 1**

Sex	$C_{max}$	$T_{max}$	$AUC_{last}$	$t_{1/2}$
	(ng/mL)	(hours)	(ng h/mL)	(hours)
<b>2.5 mg/kg</b>				
Males	1860.00	2.75	21819*	8.5
Females	1417.50	2.00	13887*	8.1
Combined	1638.75	2.38	17853	8.3
<b>20 mg/kg</b>				
Males	8085.00	3.75	133465	12.1
Females	8242.50	6.25	128476	14.24**
Combined	8163.75	5.00	130970	13.42
<b>40 mg/kg</b>				
Males	18300.00*	3.67	308037*	29.6
Females	9413.33*	6.67	169567*	20.9
Combined	13856.67	5.17	238799	25.3

**Mean Pharmacokinetic Parameters for N-Desmethylobazam on Day 14**

Sex	$C_{max}$	$T_{max}$	$AUC_{0-last}$	$t_{1/2}$
	(ng/mL)	(hours)	(ng h/mL)	(hours)
<b>2.5 mg/kg</b>				
Males	4100.00	4.00	37837	6.4
Females	3940.00	3.25	41935	9.0
Combined	4020.00	3.63	39886	7.7
<b>20 mg/kg</b>				
Males	6135.00	2.50	64193	8.4
Females	10370.00	3.00	120883	5.3
Combined	8252.50	2.75	92538	6.9
<b>40 mg/kg</b>				
Males	16450.00*	3.50	175815	5.6
Females	7025.00*	3.00	104686	9.8
Combined	11737.50	3.25	140251	7.7

**Mean Pharmacokinetic Parameters for N-Desmethylobazam on Day 28**

Sex	$C_{max}$	$T_{max}$	$AUC_{last}$	$t_{1/2}$
	(ng/mL)	(hours)	(ng•h/mL)	(hours)
<b>2.5 mg/kg</b>				
Males	4405.00	2.25	70247	5.1
Females	1458.75	2.75	14835	10.8
Combined	2931.88	2.50	42541	8.0
<b>20 mg/kg</b>				
Males	5395.00	2.25	73146	4.3
Females	8397.50	2.50	98025	11.2
Combined	6896.25	2.38	85586	7.7
<b>40 mg/kg</b>				
Males	12967.50	2.50	142134	4.8
Females	7240.00	2.50	108303	13.4
Combined	10103.75	2.50	125218	9.1

$AUC_{last}$  Area under the plasma concentration-time curve up to the last sampling time with measurable concentrations.  
 $C_{max}$  Maximum plasma concentration.  
 $T_{max}$  Time to maximum concentration.  
 $t_{1/2}$  Observed elimination half-life.

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3. Monkeys

In a 10-week study (INCT-017, conducted by (b) (4), report dated 10/73, non-GLP), CLB (A50376) doses of 0, 5, 10, or 20 mg/kg were administered to rhesus monkeys

(2/sex/grp) by stomach tube (1% MC suspension). There were no deaths, and the only clinical observation was ataxia in 1 HD male. There were no clinical chemistry findings clearly related to treatment and no T-R findings at necropsy (C and HD examined microscopically).

In a 52-week study (INCT-018, conducted by (b) (4), report dated 9/30/74, non-GLP), CLB (A 50 376) doses of 0, 2.5, 7.1, or 20 mg/kg were administered to rhesus monkeys (4/sex/grp) by stomach tube (1% MC suspension). One MD male died with signs "apathy," ataxia and coma. One HD male died after lateral position and coma. At the HD animals were described as "strongly sedated" and "dazed," and the EEG showed bradycardia. No other findings were reported for external appearance, body weight, or clinical pathology parameters. This study included a specific examination of withdrawal phenomena. At the end of dosing, LD animals showed some slight withdrawal symptoms consisting of aggressiveness and piloerection; MD withdrawal symptoms were as described above accompanied by restlessness, "periodic erection," and poor appetite; and at the HD, the same observations were made and in addition, the animals were said to be "found at times in unusual dorsal positions." At all three doses the reaction started on the second day after withdrawal and disappeared 2 hr after readministration. Immediately after this 'withdrawal test administration was resumed at the same dose as before. Then the dose was doubled from the third week. In the second withdrawal test two monkeys manifested severe withdrawal signs such as convulsions and delirium. 5 mg/kg diazepam given orally was sufficient for the suppression of withdrawal. It was noted that similar results have been reported for chlordiazepoxide with higher doses. No withdrawal symptoms were observed during the treatment period in the intervals between dosing. At necropsy, the two animals that died showed macroscopic changes in lungs, liver, kidney and lymph nodes, and gastric mucosa, but the cause of death was not determined and similar findings were observed in the same organs in surviving animals (Table IVA.3.1). Apparently, histopathology was only performed in the animals that died.

**Table IVA.3.1** Macroscopic findings in 1-year monkey study

Group Dose	Animal No	Macroscopic Findings
Gruppe Dosis	Tier No	Makroskopische Sektionsbefunde
Autopsy after 52 test weeks		
(I) 2,5 mg HR 376/kg	1 ♂	no pathological findings
	2 ♂	no pathological findings
	3 ♂	no pathological findings
	4 ♂	in the lungs, especially near hilus, multiple (about 15) whitish foci (diameter approx. 3 mm), otherwise no pathological findings
	5 ♀	in the mediastinum (left) one necrotic lymph node (diameter approx. 1 cm), otherwise no pathological findings
	6 ♀	in the lungs, especially near hilus, multiple (about 15) whitish foci (diameter approx. 3 mm), otherwise no pathological findings
	7 ♀	in the lungs, especially near hilus, multiple (about 15) whitish foci (diameter approx. 3 mm), otherwise no pathological findings
	8 ♀	no pathological findings
(II) 7,1 mg HR 376/kg	9 ♂	no pathological findings
	10 ♂	died during experiments (38th test week): both kidneys imbibed with multiple localized bleedings in renal medulla and cortex (diameter up to 2 mm); lungs enlarged and slightly indurated; in the heart muscle (left and right) multiple localized bleedings (diameter up to 2 mm); in the liver strong marking of lobules; all lymph nodes colored dark red
	11 ♂	in the right pleural cavity old adhesions with the thoracic cavity; here also one encapsulated focus, filled with necrotic masses

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(II) 7,1 mg HR 376/kg	12 ♂	in the liver one encapsulated focus, filled with necrotic masses (near hilus, diameter approx. 2 cms), otherwise no pathological findings
	13 ♀	in the liver sporadic foci (about 4), filled with necrotic masses (diameter approx. 3 mm); in the lungs one big encapsulated necrotic focus (diameter approx. 1 cm), furthermore about 10 scattered smaller foci (diameter approx. 1 - 2 mm)
	14 ♀	in the lungs 3 whitish foci (diameter approx. 2 mm), otherwise no pathological findings
	15 ♀	no pathological findings
	16 ♀	no pathological findings
	(III) 20,0 mg HR 376/kg	17 ♂
18 ♂		no pathological findings
19 ♂		died during experiments (17th test week): in the stomach near the cardia rubor and swelling of the mucosa; slight pulmonary edema.
20 ♂		no pathological findings
21 ♀		in the liver near hilus single encapsulated necrotic focus (diameter approx. 3 mm); in the mediastinum (left) one lymph node strongly enlarged and decayed necrotically (diameter approx. 2 cms)
22 ♀		no pathological findings
23 ♀		no pathological findings
24 ♀		in the right pleural cavity scarred adhesions with the thoracic cavity, at this side indurated areas near pleura; in the thymus one encapsulated necrotic focus (diameter approx. 1 cm); at the right ventricle (near the base) scar-like, indurated area (approx. 1 x 2 cms)
(IV) Control	25 ♂	no pathological findings
	26 ♂	no pathological findings
	27 ♂	in the left pleural cavity scarred adhesions with the thoracic cavity, otherwise no pathological findings
	28 ♂	no pathological findings
	29 ♀	no pathological findings
	30 ♀	left lung joined with the thorax by single scarred funicles; in the lungs multiple (about 20) whitish foci (diameter approx. 1 - 3 mm)
	31 ♀	in the right kidney (cortex) one cyst filled with transparent mucinous liquid; in the lungs one big indurated area (diameter approx. 5 cms), and sporadic whitish foci (diameter approx. 1 - 3 mm)
	32 ♀	in the lungs sporadic whitish foci (diameter approx. 1 - 3 mm)

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A study (OVNC-9064, conducted by (b)(4) report dated 2/12/10; non-GLP) was conducted in rhesus monkeys with the primary purpose of determining plasma PK of CLB and N-CLB following oral (gavage) doses of 2.5 or 20 mg/kg CLB (Lot No. N002) for 28 days. Clinical observations noted for the LD animals were mild, while the observations for the HD animals were much more significant. According to the report, HD females appeared to adjust to the dose formulation more readily than their male counterparts. On day 1, all animals experienced varying degrees of ataxia, lethargy, and disorientation (with the exception of 1 HD female which was not disoriented). While clinical observations from the HD females generally became less frequent beginning on day 3 postdose, observations increased in frequency for HD males (Animal Nos. RQ6990 and RQ7007). On day 21, 1 HD male was euthanized per veterinary directive due to clinical observations including recumbency, thin appearance, and unresponsiveness. On day 23, another HD male, was euthanized per veterinary directive due to clinical observations including recumbency, thin appearance, and distended abdomen. Clinical chemistry

and hematology and gross necropsy were performed on both animals but no cause of death was determined. There were no apparent T-R gross pathologic findings. Histopathologic examination of tissues was not conducted. These were considered likely T-R deaths.

PK parameters are shown below (Tables IVA.3.2-3). Exposure to CLB increased with the increase in dose and the increases in mean C<sub>max</sub> and AUC<sub>0-24</sub> were inconsistently dose proportional. Mean CLB C<sub>max</sub> and AUC<sub>0-24</sub> for females were generally greater than in males at the LD; however, males were greater than 2-fold higher than females at the HD on Days 1 and 14. Accumulation of CLB was observed after multiple dosing. Exposure to N-CLB increased with the increase in CLB dose and the increases in C<sub>max</sub> and AUC<sub>0-24</sub> were generally dose proportional. Sex differences were not observed in N-CLB C<sub>max</sub> and AUC<sub>0-24</sub> values. Accumulation of N-CLB was observed after multiple dosing.

Table IVA.3.2

Summary of the Mean Pharmacokinetic Parameters for Clobazam in Monkey Plasma

Interval	Group	Clobazam Dose Level (mg/kg)	Sex	C <sub>max</sub> (ng/mL)	DN C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	AUC <sub>0-24</sub> (ng·h/mL)	AUC <sub>0-24</sub> (ng·h/mL)	DN AUC <sub>0-24</sub> (ng·h/mL)	AUC <sub>0-24</sub> (ng·h/mL)	AUC <sub>0-24</sub> (ng·h/mL)	t <sub>1/2</sub> (h)	CL <sub>CR</sub> (mL/kg)	
Day 1	1	2.5	M	Mean N	105 2	42.1 2	2.00 2	305 2	345 2	138 2	467 1	NA NA	1.89 1	5349 1
			F	Mean N	175 2	49.8 2	4.790 2	435 2	463 2	185 2	308 1	NA NA	1.06 1	7616 1
	2	10	M	Mean N	421.4 2	211 2	2.00 2	27037 2	27037 2	1342 2	27176 2	NA NA	-4.25 2	2341 2
			F	Mean N	198.5 2	79.3 2	1.50 2	7249 2	7475 2	374 2	7562 2	NA NA	4.80 2	3613 2
Day 14	1	2.5	M	Mean N	127 2	58.8 2	1.25 2	403 2	457 2	183 2	NA NA	NA NA	1.47 2	4719 2
			F	Mean N	297 2	119 2	4.580 2	498 2	744 2	298 2	NA NA	NA NA	1.18 2	3929 2
	2	10	M	Mean N	3445 2	172 2	4.790 2	8877 2	9023 2	451 2	NA NA	NA NA	3.17 2	2334 2
			F	Mean N	884 2	44.2 2	1.50 2	3572 2	3735 2	187 2	NA NA	NA NA	1.96 2	5536 2
Day 28	1	2.5	M	Mean N	304 2	121 2	4.790 2	411 2	434 2	174 2	NA NA	NA 2	1.15 2	4179 2
			F	Mean N	435 2	174 2	4.580 2	884 2	620 2	248 2	NA NA	420 2	2.26 2	4294 2
	2	10	F	Mean N	2020 2	101 2	4.580 2	3822 2	3994 2	200 2	NA NA	3994 2	1.79 2	5661 2

Note: No concentration data was received for Group 2 males on Day 28.

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Table IVA.3.3

Summary of Mean Pharmacokinetic Parameters for N-desmethyloclobazam in Monkey Plasma

Interval	Group	Clobazam Dose Level (mg/kg)	Sex	C <sub>max</sub> (ng/mL)	DN C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	AUC <sub>0-24</sub> (ng·h/mL)	AUC <sub>0-24</sub> (ng·h/mL)	DN AUC <sub>0-24</sub> (ng·h/mL)	AUC <sub>0-24</sub> (ng·h/mL)	AUC <sub>0-24</sub> (ng·h/mL)	t <sub>1/2</sub> (h)	MIP AUC <sub>0-24</sub> (ng·h/mL)	
Day 1	1	2.5	M	Mean N	1399 2	560 2	6.00 2	23268 2	23268 2	9307 2	NA 0	NA NA	NA 0	65.9 2
			F	Mean N	1510 2	404 2	4.50 2	23259 2	23259 2	9344 2	36865 1	NA NA	18.8 1	53.7 2
	2	10	M	Mean N	11494 2	573 2	12.0 2	234807 2	234807 2	11700 2	NA 0	NA NA	NA 0	26.4 2
			F	Mean N	8095 2	455 2	6.00 2	185711 2	185711 2	9284 2	NA 0	NA NA	NA 0	35.1 2
Day 14	1	2.5	M	Mean N	2900 2	1089 2	4.00 2	45462 2	45462 2	18183 2	NA NA	NA NA	NA 0	96.1 2
			F	Mean N	2945 2	1178 2	4.00 2	52088 2	52088 2	28832 2	NA NA	NA NA	NA 0	76.2 2
	2	10	M	Mean N	27694 2	1383 2	5.00 2	527450 2	527450 2	24373 2	NA NA	NA NA	NA 0	42.2 2
			F	Mean N	22589 2	1129 2	4.00 2	425225 2	425223 2	21261 2	NA NA	NA NA	NA 0	118 2
Day 28	1	2.5	M	Mean N	2330 2	832 2	1.25 2	41737 2	37365 2	14946 2	NA NA	62888 2	13.0 2	81.4 2
			F	Mean N	2660 2	164 2	3.25 2	47521 2	48990 2	14756 2	NA NA	67886 2	13.8 2	47.9 2
	2	10	F	Mean N	24989 2	1245 2	1.25 2	667322 2	412475 2	28024 2	NA NA	667322 2	18.8 2	113 2

Note: No concentration data was received for Group 2 males on Day 28.

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B. GENOTOXICITY

CLB and N-CLB at doses up to 5000 µg/plate were not mutagenic in valid Ames tests (OVNC-7001 (CLB) and OVNC-9046 (N-CLB), conducted by (b) (4) reports dated 8/15/05 and 3/12/09). In the *in vitro* chromosomal aberration study in CHO cells (OVNC-7002, conducted by (b) (4), report dated 8/12/05), CLB significantly increased (8-fold solvent control) structural aberrations in the non-activated 4-hour exposure group at the highest concentration tested, 200 µg/ml (concentrations limited by solubility; **Table IVB.1**). Similar results were seen in the 4-hour exposure group with metabolic activation at the highest concentration of 400 µg/ml. However, based on the pre-determined criteria, the response was considered negative (values fell within the historical control reference range of 0-5%). Concentrations of 200 and 400 µg/ml were associated with 58 and 31% cell growth inhibition in the non-activated and activated assays, respectively. N-CLB did not increase numbers of numerical or structural aberrations at concentrations of up to 2500 µg/ml in the absence or presence of S9 (OVNC-9044, conducted by (b) (4) report dated 12/11/08). No increases in micronucleated polychromatic erythrocyte frequency in bone marrow were noted in mice given a single oral dose of CLB up to 150 mg/kg or N-CLB up to 2000 mg/kg (OVNC-7003 (CLB) and OVNC-9045 (N-CLB), conducted by (b) (4) reports dated 8/16/05 and 12/5/08).

**Table IVB.1**

SUMMARY									
Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
DMSO	-S9	4	8.5	200	200	0.005	±0.071	1.5	0.5
clobazam									
50	-S9	4	8.4	200	200	0.005	±0.071	0.0	0.5
100	-S9	4	8.2	200	200	0.005	±0.071	1.5	0.5
200	-S9	4	6.2	200	200	0.045	±0.231	2.5	4.0*
MMC, 0.2	-S9	4	5.9	200	100	0.280	±0.830	2.0	17.0**
DMSO	+S9	4	8.7	200	200	0.000	±0.000	0.5	0.0
clobazam									
50	+S9	4	7.3	200	200	0.000	±0.000	0.0	0.0
200	+S9	4	6.6	200	200	0.015	±0.158	2.0	1.0
400	+S9	4	3.4	200	200	0.045	±0.208	4.0*	4.5**
CP, 10	+S9	4	6.1	200	100	0.220	±0.543	0.5	17.0**
DMSO	-S9	20	7.3	200	200	0.000	±0.000	1.0	0.0
clobazam									
100	-S9	20	6.4	200	200	0.000	±0.000	1.0	0.0
200	-S9	20	5.7	200	200	0.005	±0.071	0.5	0.5
600	-S9	20	4.2	200	200	0.010	±0.100	1.5	1.0
MMC, 0.1	-S9	20	6.3	200	100	0.350	±1.140	1.0	20.0**

**Treatment:** Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.

**Aberrations per Cell:** Severely damaged cells were counted as 10 aberrations.

**Percent Aberrant Cells:** \*, p≤0.05; \*\*, p≤0.01; using Fisher's exact test.

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C. CARCINOGENICITY

1. RU 4723: ONCOGENIC RESPONSE IN MICE TO DIETARY ADMINISTRATION FOR 80 WEEKS (Study: LNCT-019 [79/RUC028/295]; conducted by (b) (4), (b) (4), (b) (4) report dated 10/24/79; non-GLP [GLP introduced 6/79])

a. Methods

CLB (RU 4723, Lot Nos.: 29 and 36) was administered in the diet to CD-1 mice ( (b) (4) (b) (4) d; 60 /sex/group) at doses of 0 (standard diet), 4, 20, or 100 mg/kg/day for 80 weeks. Dietary concentrations were adjusted at weekly intervals during the first 20 weeks and at four-weekly intervals thereafter. Based on analysis of drug concentration in the diet, quantitation of weekly food consumption, and calculated actual doses of administered CLB derived from these parameters, the intended doses were stated to have been achieved. Tap water and a complete rodent diet in powdered form (Spratts Laboratory-Diet No. 2), with or without RU 4723, were available to the mice ad libitum. The mice were between 31 and 35 days old at the time treatment commenced. During the first six weeks an additional 43 HD male mice were introduced to supplement losses resulting from severe fighting (animals group housed, 4/cage). Nine weeks after commencement of the study an additional group of 42 HD male mice was incorporated into the study (study Group 5). It was hoped that younger mice would be less aggressive when first caged together; these animals were said to be between 25 and 29 days old at the start of treatment. Observations included mortality, clinical signs, body weights, food consumption, and macroscopic and microscopic pathology. According to the report, a limited number of tissues (asterisks below) from all animals were evaluated for neoplastic changes and a more complete panel of tissues (listed below) from 20/sex in C and HD were examined for non-neoplastic (and presumably neoplastic) changes. All tissues showing macroscopic change were also preserved for microscopic examination.

*Adrenal glands	Mammary glands (caudal)
Brain	*Mesenteric lymph nodes
Caecum	*Ovaries
Cervical lymph nodes	Pancreas
Duodenum	*Pituitary gland
Epididymides	Prostate
Eye	Skin
Gall bladder	*Spleen
Heart	Stomach (antrum and pyloric fundus)
Ileum	*Testes
Jejunum	Thymus (if present)
Kidneys	*Thyroid glands
*Liver	Urinary bladder
*Lungs	Uterus.

It was stated that any tissues exhibiting treatment-related non-neoplastic change in HD mice were examined in all other mice of all groups. It is assumed that the same applied to neoplastic changes seen in this larger set of tissues, but this was not made explicit. TK was not performed. No justification for dose selection was provided in the study report. In the non-clinical summary, the sponsor stated that the HD was "33% to 40% of the lowest single oral lethal doses in mice, and on a body surface area (mg/m<sup>2</sup>) basis, and was 8- to 12-times the maximum dose studied in Phase II/III controlled trials."

b. Results

i. Clinical Signs, Mortality, Body Weight

During the first 11 weeks of the study, HD male mice exhibited a marked increase in fighting among cage mates. Wounds, said to be severe in many cases, were located predominantly around the scrotal region, the dorsal lumbar region, and the forelimbs. According to the report, the level of aggression seemed to reach a peak during Week 6 with subsequent abatement and gradual reversion to the level normally found in young male mice of this strain. Behavior in male mice of the added HD group and in other male and female groups appeared not to be affected by treatment. No other T-R signs were recorded.

Mortality attributed primarily to injury associated with severe fighting among males in the HD group necessitated replacement of 43 animals in this group during the first 6 weeks of the study. Nine weeks after study initiation, an additional group of 42 males was added to the study and received 100 mg/kg/day for 80 weeks. These animals were said to be several days younger at initiation than the original animals, and to have "displayed fewer behavioral abnormalities on initiation of treatment" and "fought less." This was thought to have improved survival compared to the original group of HD males, none of which survived to study termination. Given that the mortality in the original HD male group was thought to be due primarily to fighting combined with the fact that survival of the added HD group was greater than that in the control group, it was concluded that there were no treatment-related changes in survival in the males. However, females also displayed a dose-related decrease in survival (SS at HD; **Table IVC.1.1**).

There was no clear effect of treatment on male BWs (**Table IVC.1.3; Figures IVC.1.1-2**). However, in male mice in the additional HD group (said to be slightly younger at the onset of treatment) BWs were lower than those of their non-contemporaneous controls throughout the treatment period, although growth-rates appeared to parallel those of the control group. Peak BW was about 5 g lower in this added group. There was no disparity in achieved dose to account for this difference. It was concluded in the report that there was no consistent adverse effect on the growth-rate of the older mice (males or females), and that the decreased peak adult bodyweight (10%) in the added HD males was due to the younger age at start of treatment ("before the mice were 29 days old"). However, it appears that BWs were also reduced somewhat throughout much of the dosing period in HD females.

**Table IVC.1.1**

Mortality distribution\*

Group	:	1	2	3	4
Compound	:	Control	-----	RU 4723	-----
Dosage (mg/kg/day)	:	0	4	20	100

Treatment period (weeks)	Group and sex								
	1 ♂	2 ♂	3 ♂	4 ♂	5 ♂	1 ♀	2 ♀	3 ♀	4 ♀
1-16	1/60	0/60	2/60	66/103 <sup>c</sup>	0/42	1/60	0/60	1/60	4/60
17-32	2/59	1/60	1/58	13/37 <sup>c</sup>	4/41	1/59	2/56 <sup>†</sup>	0/59	1/56
33-48	3/57	1/59	3/57	7/24 <sup>b</sup>	5/37	5/58	2/54	0/59	7/55
49-64	13/54	14/58	11/54	10/17 <sup>a</sup>	8/32	6/53	5/52	11/59	17/48 <sup>b</sup>
65-80	28/41	28/44	33/43	7/7	11/24	26/47	31/47	35/48	23/31
1-80	47/60	44/60	50/60	103/103 <sup>c</sup>	28/42	39/60	40/56 <sup>†</sup>	47/60	52/60 <sup>a</sup>

† Excluding 4 animals which drowned during Week 26.  
a Significantly different from controls, P < 0.05.  
b Significantly different from controls, P < 0.01.  
c Significantly different from controls, P < 0.001.  
\* Expressed as  $\frac{\text{number of mice dying during the period}}{\text{number of mice alive at onset of the period}}$

**Table IVC.1.2** Survival Data

Doses (mg/kg)	0		4		20		100		
No. of animals	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60 <sup>a</sup>	M: 42 <sup>b</sup>	F: 60
Died/Sacrificed Moribund	47	39	44	44	50	47	60	28	52
Terminal Sacrifice	13	21	16	16	10	13	0	14	8
Survival (%)	22	35	27	27	17	22	0	33	13

M = Male, F = Female

<sup>a</sup> Excludes animals replaced through Week 6. A total of 43 replacement animals were introduced to this group to replace animals lost due to fighting (animals were group housed).

<sup>b</sup> Group added and treated for 80 weeks to insure that sufficient numbers of males were treated at this dose for a sufficient period of time.

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**Table IVC.1.3**

Bodyweight - group mean values (g)

Group	:	1	2	3	4	5
Compound	:	Control	-----	RU 4723	-----	
Dosage (mg/kg/day)	:	0	4	20	100	100

Week number	Group and sex								
	1 ♂	2 ♂	3 ♂	4 ♂	5 ♂	1 ♀	2 ♀	3 ♀	4 ♀
0	27	27	27	27	19	22	23	23	22
1	32	32	32	33	26	26	26	27	26
2	35	36	34	35	31	27	27	28	28
3	36	37	36	37	32	28	28	28	28
4	37	38	36	37	33	29	29	30	30
5	38	39	38	38	33	29	30	30	30
6	39	40	39	39	35	30	30	31	31
7	40	41	40	40	37	32	32	33	32
8	40	41	40	40	37	31	31	33	31
9	41	41	41	40	38	32	31	32	32
10	41	43	42	42	37	32	33	34	33
11	41	42	41	41	38	33	33	33	33
12	42	42	42	42	38	33	33	34	33
13	41	42	42	41	39	34	33	34	32
14	41	41	41	40	39	34	33	35	32
15	41	42	41	40	38	33	33	35	33
16	43	44	44	42	40	35	35	36	34
17	43	45	44	42	38	35	34	36	35
18	44	46	44	43	39	36	35	36	35
19	41	43	42	41	40	34	34	35	34
20	44	46	45	43	39	36	35	36	35
24	42	44	43	41	41	36	36	36	34
28	45	47	46	45	42	37	37	37	36
32	46	49	47	45	43	38	37	38	35
36	45	48	47	44	43	39	38	38	36
40	47	50	49	47	42	39	38	39	37
44	49	52	51	48	43	40	40	40	39
48	49	51	49	47	44	40	39	40	37
52	47	50	49	47	44	40	39	40	38

56	47	50	48	45	44	39	39	40	37
60	48	50	48	44	42	39	39	40	38
64	46	49 <sup>a</sup>	47 <sup>φ</sup>	46 <sup>φ</sup>	43	40	39 <sup>φ</sup>	39 <sup>φ</sup>	37 <sup>c</sup>
68	45	47	45	42	43	38	38	38	36
72	43	47	44	-	38	37	39	37	36
76	45	45	45	-	42	38	38	36	37
80	44	43	45	-	43	36	35	36	36
Bodyweight change Weeks 0-40	20	23	22	20	23	17	15	16	15
Weeks 41-80	-3	-7	-4	-	+1	-3	-2	-3	-1

a Significantly different from controls, P < 0.05.

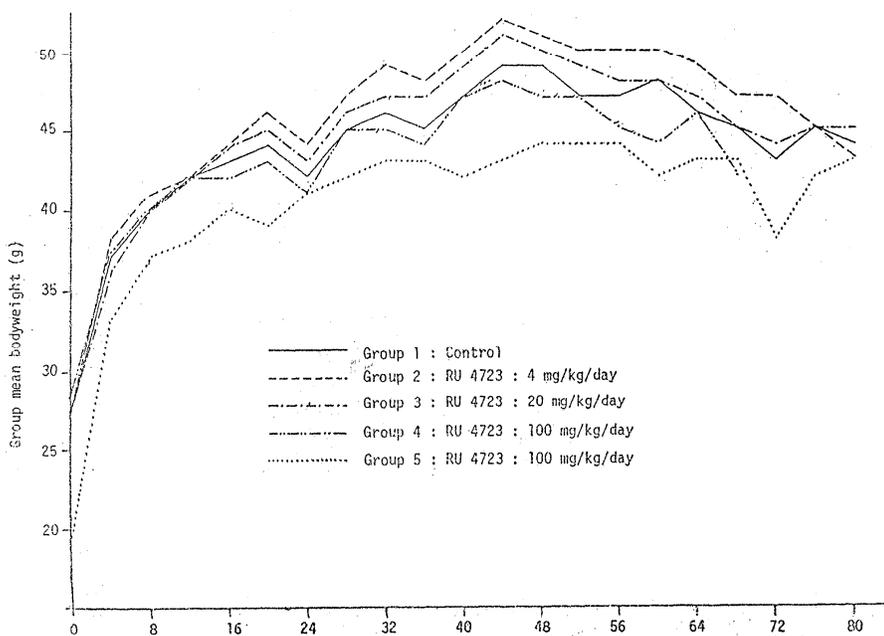
c Significantly different from controls, P < 0.001.

φ Not significantly different from controls, P > 0.05.

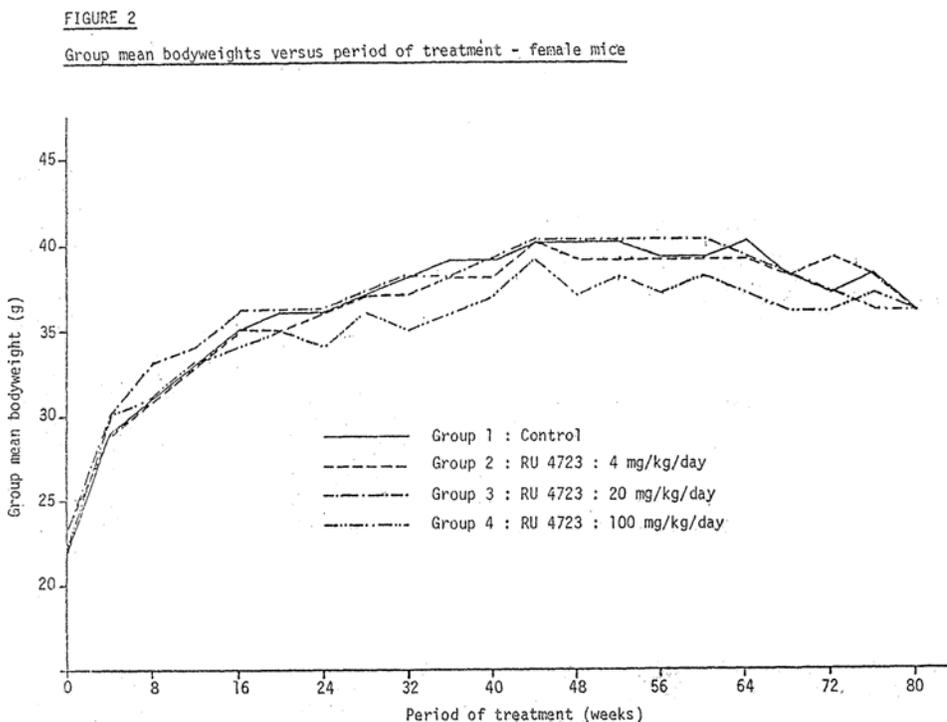
**Figure IVC.1.1**

FIGURE 1

Group mean bodyweights versus period of treatment - male mice



**Figure IVC.1.2**



ii. Non-neoplastic

A wide range of degenerative and inflammatory lesions were present among both control and treated animals; however, these changes appeared to occur in a random fashion and were not considered to be treatment-related.

iii. Neoplastic (Tables IVC.1.4, 1.5 and 1.6; FDA statistical review)

Among HD female mice, there were fewer tumor-bearing mice than in controls (Table IVC.1.4) due to a lower frequency of all tumors except pulmonary adenoma in this group (Table IVC.1.5). According to the report, the lower tumor incidences were due to the fact that mortality was significantly increased in both sexes at the HD (original HD males; Table IVC.1.1), so that the number of treatment weeks was too low for the full expression of the normal tumor frequency to occur, especially lymphoma. However, incidences of lymphoma appeared to be D-D decreased in treated females compared to C, both in animals dying during the course of the study and in those killed at termination, although the differences did not reach SS. The incidence of malignant lymphoma in controls was 7/59 (11.9%), which was slightly, but not significantly, higher than the cumulative incidence of this tumor in control female mice of the same strain in nine recent carcinogenicity studies in the conducting laboratory (43/485; 8.9%, range 3.8 - 22.5%). Hepatomas (later classified as hepatocellular adenomas) were increased (NS) in Group 5 males compared to controls. The other tumors identified were of types commonly found in this strain of mouse, and they also were distributed in random fashion across the groups. Tumor multiplicity was not clearly affected by treatment, although more than one tumor type was only seen in (2) replacement HD males (Table IVC.1.6).

**Table IVC.1.4**

Group distribution of benign and malignant tumours

Group : 1 2 3 4 5  
 Compound : Control ----- RU 4723 -----  
 Dosage (mg/kg/day) : 0 4 20 100 100

Number of:	Group and sex									
	1 ♂	2 ♂	3 ♂	4 ♂	5 ♂	1 ♀	2 ♀	3 ♀	4 ♀	5 ♀
animals bearing benign tumours	4	9	6	1	7	3	4	5	2	
animals bearing malignant tumours	3	3	0	0	1	10	6	5	1 <sup>a</sup>	
animals bearing at least one tumour of any type	7	12	6	1	8	13	10	11	3 <sup>a</sup>	
Number of animals examined:	60	60	59	58	42	59	60	60	60	
Number of cadavers lost <sup>†</sup> :	0	0	1	2	0	1	0	0	0	
Number of animals commencing treatment:	60	60	60	60*	42	60	60	60	60	

a Probability of distribution arising by chance,  $P < 0.05$  (Chi<sup>2</sup> test, two-tailed).  
 † Includes losses due to autolysis and/or cannibalism.  
 \* Excluding animals replaced before or during Week 6.

**Table IVC.1.5**

Group distribution of different tumour types

Group : 1 2 3 4 5  
 Compound : Control ----- RU 4723 -----  
 Dosage (mg/kg/day) : 0 4 20 100 100

Organ and neoplasm	Group and sex									
	1 ♂	2 ♂	3 ♂	4 ♂	5 ♂	1 ♀	2 ♀	3 ♀	4 ♀	5 ♀
<u>Mammary gland</u>										
Benign epithelial adenoma	0	0	0	0	0	0	1	0	0	
Undifferentiated adenocarcinoma (M)	0	0	0	0	0	0	0	1	0	
<u>Lungs</u>										
Pulmonary adenoma	2	5	2	0	2	1	2	1	2	
Pulmonary adenocarcinoma (M)	1	0	0	0	1	1	1	1	0	
<u>Liver</u>										
Benign hepatoma	1	3	3	1	5 <sup>‡</sup>	0	0	0	0	
<u>Pituitary gland</u>										
Adenoma	0	0	0	0	0	0	0	1	0	
<u>Adrenal gland</u>										
Benign pheochromocytoma	0	0	0	0	0	0	1	0	0	
<u>Stomach</u>										
Squamous carcinoma (M)	0	0	0	0	0	1	0	0	0	
<u>Ovary</u>										
Ovarian cyst adenoma	-	-	-	-	-	0	0	1	0	
<u>Skin and subcutis</u>										
Sarcoma (M)	0	1	0	0	0	0	0	0	0	
Osteosarcoma (M)	0	0	0	0	0	1	0	0	0	
<u>Uterus</u>										
Leiomyoma	-	-	-	-	-	1	0	0	0	
<u>Liver</u>										
Haemangioma	1	1	1	0	0	0	0	0	0	
<u>Ovary</u>										
Haemangioma	-	-	-	-	-	1	0	1	0	
<u>Lymph nodes</u>										
Haemangioma	0	0	0	0	0	0	0	1	0	
Pleomorphic cell sarcoma (M)	0	0	0	0	0	0	1	0	0	
Lymphoma (M)	2	2	0	0	0	7	4	4	1	
Number of animals examined:	60	60	59	58	42	59	60	60	60	
Number of cadavers lost <sup>†</sup> :	0	0	1	2	0	1	0	0	0	
Number of animals commencing treatment:	60	60	60	60*	42	60	60	60	60	

‡ Malignant tumour.

† Includes losses due to autolysis and/or cannibalism.

‡ Probability of distribution arising by chance,  $P > 0.05$  (Fisher's exact test, two-tailed).

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**Table IVC.1.6**

Multiplicity of tumour types

Group	:	1	2	3	4	5
Compound	:	Control	-----	RU 4723	-----	
Dosage (mg/kg/day)	:	0	4	20	100	100

Number of:	Group and sex								
	1 ♂	2 ♂	3 ♂	4 ♂	5 ♂	1 ♀	2 ♀	3 ♀	4 ♀
animals bearing no tumour:	53	48	53	57	36	46	50	50	57 <sup>a</sup>
animals bearing tumours of one type	7	12	6	1	4	13	10	9	3 <sup>a</sup>
animals bearing tumours of two types	0	0	0	0	2	0	0	1	0
Number of animals examined:	60	60	59	58	42	59	60	60	60
Number of cadavers lost <sup>†</sup> :	0	0	1	2	0	1	0	0	0
Number of animals commencing treatment:	60	60	60	60*	42	60	60	60	60

† Includes losses due to autolysis and/or cannibalism.

\* Excluding those animals replaced before or during Week 6.

<sup>a</sup> Probability of distribution arising by chance, P < 0.05.

c. Conclusion

Chronic oral (dietary) administration of CLB to CF-1 mice at doses of 4, 25, or 100 mg/kg was associated with slightly decreased BW (HD females), increased mortality (first HD males and HD females), and increased incidences (NS) of hepatocellular adenomas (males). The unacceptably high mortality rate in the initial HD male group was attributed to fighting by the sponsor. There was no increase in mortality in a second (younger) group of males treated with the same HD compared to C. When this group is included, similar numbers remained at the end of the study compared to controls; however, given the difference in response and the uncertainty about the cause of mortality it is not clear what effect inclusion of this additional group has on study validity in terms of study population characteristics. The study duration of 80 weeks is less than currently recommended, and there are a number of other concerns about the adequacy of the study based on deviations from the current documentation and observational requirements. For example, it is not clear that an adequate number of tissues were examined microscopically in an adequate number of animals, and lack of TK data makes the accuracy of dosing questionable.

2. RU 4723: ONCOGENIC RESPONSE IN RATS TO DIETARY ADMINISTRATION FOR 104 WEEKS FINAL REPORT 0-104 WEEKS (Study: LNCT-020 [79/RUC027/367]; conducted by (b) (4); report dated 10/24/79; non-GLP [GLP introduced 6/79])

a. Methods

CLB (RU 4723; lot nos.: 29 and 36) was administered in the diet (ad libitum) to rats (CD, (b) (4) 60 group/sex) at doses of 0, 4, 20 or 100 mg/kg/day for 104 weeks. Drug was incorporated in the diet at levels to yield the above doses; dietary concentrations were adjusted weekly during the first 20 weeks and every 4 weeks thereafter. Achieved doses were close to expected based on analysis of drug concentration in the diet, quantitation of weekly food consumption, and calculated actual doses derived from these parameters. Animals were group housed, 5/cage. Observations included mortality, clinical signs, body weights, food consumption, and macroscopic and microscopic pathology. A limited number of tissues (asterisks below) from all animals were evaluated for neoplastic changes and a more complete panel of tissues (listed below) from 20/sex in C and HD were examined for non-neoplastic (and presumably neoplastic) changes. All tissues showing macroscopic change were also preserved for microscopic examination.

*Adrenal glands	Mammary glands (caudal)
Brain	*Mesenteric lymph nodes
Caecum	*Ovaries
Cervical lymph nodes	Pancreas
Duodenum	*Pituitary gland
Epididymides	Prostate
Eye	Skin
Gall bladder	*Spleen
Heart	Stomach (antrum and pyloric fundus)
Ileum	*Testes
Jejunum	Thymus (if present)
Kidneys	*Thyroid glands
*Liver	Urinary bladder
*Lungs	Uterus.

It was stated that any tissues exhibiting treatment-related non-neoplastic change in HD rats were examined in all other rats of all groups. It is assumed that the same applied to neoplastic changes seen in this larger set of tissues, but this was not made explicit. TK was not performed. No justification for dose selection was provided in the report, but according the nonclinical summary: "In the 6-month study, transient sedation and/or piloerection and mortality were observed in males at 100 mg/kg; thus 100 mg/kg/day was selected as the highest dose level for this carcinogenicity study."

b. Results

i. Mortality, body weight

There were no treatment-related (T-R) clinical observations and no clearly T-R differences in mortality, which was nonetheless high in males (**Table IVC.2.1**). According to the report, a ventilation failure in Week 74 resulted in the death of 8 males (2 each at LD and HD, and 4 MD) and 1 HD female (6 of these animals were not analyzed for macroscopic or microscopic changes due to cannibalism or severe autolysis). Food consumption was not significantly affected by CLB treatment. BW was SS decreased in HD males (not at the end of the study) and females (14% compared to C at termination) at several time points (**Fig IVC.2.1 Table IVC.2.2**).

ii. Gross pathology

Macroscopic findings at necropsy included liver pallor and thyroid gland enlargement in HD males, a decreased incidence of prominent mammary tissue, and an increase in the incidence of intra-uterine nodules in MD and HD females.

iii. Non-neoplastic histopathology

T-R non-neoplastic histopathologic changes included increased incidences of endometrial hyperplasia, cystic endometrial hyperplasia, and endometrial polyps/polypoid areas in HD females (**Table IVC.2.3**); a dose-related (D-R) decrease in the incidence of marked mammary gland development in females; decreased incidences of myocardial fibrosis in both males and females at all doses; and a dose-related decreased incidence of chronic inflammation of the lungs in both sexes.

iv. Neoplastic histopathology

There was a dose-related increased incidence of benign thyroid gland follicular cell adenomas in male rats that was statistically significant at the HD (**Table IVC.2.4**). The types, incidences, and distribution across treatment groups of other neoplastic changes, including malignant neoplasms, did not appear to be affected by treatment with the possible exceptions of increased incidences of epithelial carcinoma and pituitary adenoma in MD females (**Tables IVC.2.5** and **IVC.2.6**). Tumor multiplicity also appeared to be increased in this group (**Table IVC.2.7**).

c. Conclusion

Dietary administration of CLB to rats at doses of approximately 4, 20, or 100 mg/kg induced a D-R increased incidence of benign thyroid gland follicular cell adenomas in male rats that was statistically significant at the HD (in both sponsor's and FDA analyses). There was no justification of dose selection in the report, and it is not clear that doses were adequate based on BW and survival effects. As in the mouse study, there are significant deficiencies in the provision and/or reporting of data and questions about dosing accuracy in the absence of TK information.

**Table IVC.2.1**

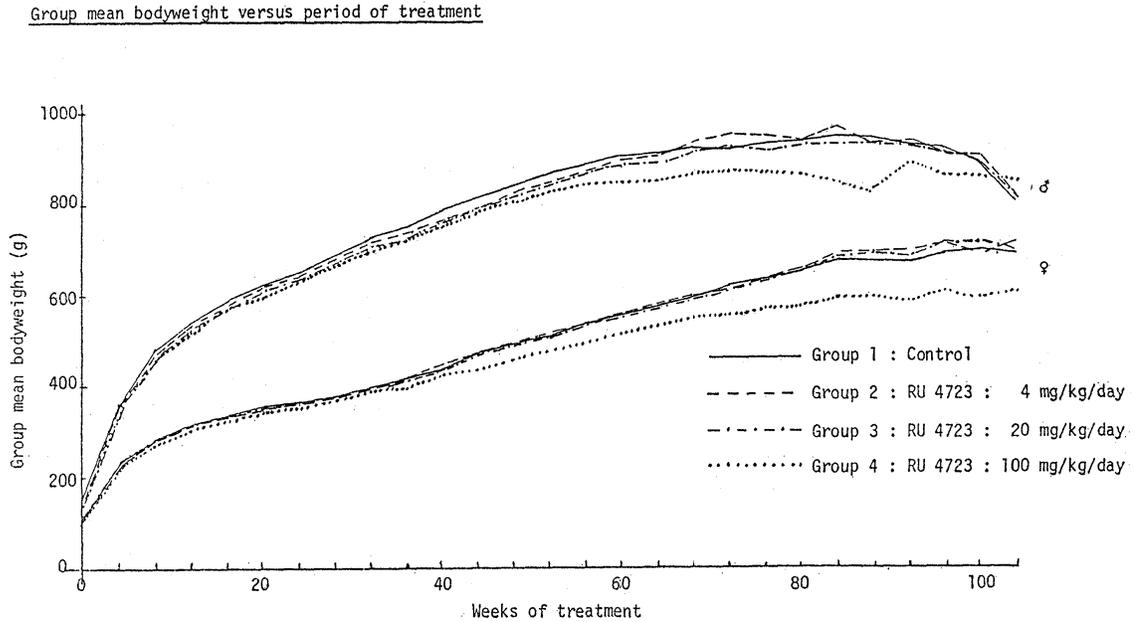
Mortality distribution\*

Group	:	1	2	3	4
Compound	:	Control	---	RU 4723	---
Dosage (mg/kg/day)	:	0	4	20	100

Weeks	Group and sex							
	1 ♂	2 ♂	3 ♂	4 ♂	1 ♀	2 ♀	3 ♀	4 ♀
1-24	0/60	1/60	0/60	1/60	0/60	0/60	0/60	1/60
25-48	2/60	2/59	2/60	2/59	0/60	2/60	1/60	1/59
49-72	17/58	9/57	11/58	9/57	5/60	3/58	3/59	10/58
73-104	29/41	41/48	40/47	39/48	28/55	33/55	29/56	29/48
Total Weeks 1-104	48/60	53/60	53/60	51/60	33/60	38/60	33/60	41/60
As % of control	(100)	110	110	106	(100)	115	100	124
†	0	2	4	2	0	0	0	1

\* Incidences expressed as  $\frac{\text{No. of animals dying in specified period}}{\text{No. of animals alive at beginning of period}}$   
 † Deaths occurring as a result of ventilation failure during Week 74.

**Figure IVC.2.1**



**Table IVC.2.2**

Bodyweight (g) - group mean values

Group	:	1	2	3	4
Compound	:	Control	- - -	RU 4723	- - -
Dosage (mg/kg/day)	:	0	4	20	100

Week number	Group and sex							
	1 ♂	2 ♂	3 ♂	4 ♂	1 ♀	2 ♀	3 ♀	4 ♀
0	140	140	140	139	116	116	117	117
1	207	208	210	205	157	157	156	156
2	262	263	267	260	184	183	180	180
3	313	314	318	308	207	206	204	203
4	357	358 <sup>♠</sup>	364 <sup>♠</sup>	350 <sup>♠</sup>	228	228 <sup>♠</sup>	225 <sup>♠</sup>	223 <sup>♠</sup>
5	392	394	400	384	246	245	243	241
6	425	423	432	414	259	259	257	252
7	451	447	457	439	272	274	271	268
8	470	467 <sup>♠</sup>	473 <sup>♠</sup>	454 <sup>a</sup>	280	282 <sup>♠</sup>	276 <sup>♠</sup>	273 <sup>♠</sup>
9	489	485	493	474	290	293	288	286
10	508	502	510	493	298	302	296	295
11	524	515	525	507	306	307	303	302
12	540	529	538	520	312	312	309	307
13	554	545	553	534	316	316	314	310
14	565	555	566	547	321	318	317	314
15	577	570	575	556	324	324	322	315
16	593	579 <sup>♠</sup>	587 <sup>♠</sup>	568 <sup>a</sup>	331	329 <sup>♠</sup>	328 <sup>♠</sup>	323 <sup>♠</sup>
17	602	590	596	577	334	333	331	327
18	615	600	605	585	338	336	335	331
19	624	608	615	596	342	339	338	334
20	621	607 <sup>♠</sup>	611 <sup>♠</sup>	596 <sup>a</sup>	349	348 <sup>♠</sup>	348 <sup>♠</sup>	345 <sup>♠</sup>
24	650	637	644	626	360	360	359	351
28	691	675	679	664	377	376	375	370
32	726	708 <sup>♠</sup>	709 <sup>♠</sup>	696 <sup>a</sup>	398	398 <sup>♠</sup>	398 <sup>♠</sup>	387 <sup>♠</sup>
36	755	734	737	720	412	413	411	393
40	790	765	770	753	440	442	436	420
44	815	796 <sup>♠</sup>	799 <sup>♠</sup>	779 <sup>♠</sup>	463	465 <sup>♠</sup>	462 <sup>♠</sup>	437 <sup>♠</sup>
48	840	821	823	804	485	487	483	457
52	863	848	844	826	505	509	506	475

56	886	876 <sup>φ</sup>	867 <sup>φ</sup>	845 <sup>φ</sup>	532	532 <sup>φ</sup>	528 <sup>φ</sup>	490 <sup>a</sup>
60	903	895	885	851	553	557	546	513
64	906	907	898	855	570	573	568	524
68	922	922 <sup>φ</sup>	916 <sup>φ</sup>	871 <sup>φ</sup>	590	598 <sup>φ</sup>	594 <sup>φ</sup>	543 <sup>a</sup>
72	920	933	925	877	618	613	614	555
76	933	955	920	865	627	633	632	566 <sup>b</sup>
80	939	948 <sup>φ</sup>	917 <sup>φ</sup>	869 <sup>φ</sup>	648	652 <sup>φ</sup>	651 <sup>φ</sup>	577 <sup>b</sup>
84	950	968	936	856	675	694	682	597
88	947	937	943	837	673	695	694	599 <sup>b</sup>
92	930	940 <sup>φ</sup>	934 <sup>φ</sup>	897 <sup>φ</sup>	671	698 <sup>φ</sup>	713 <sup>φ</sup>	586 <sup>b</sup>
96	927	913	911	869	690	711	715	606
100	900	907	900	862	699	686	718	598
104	812	822 <sup>φ</sup>	822 <sup>φ</sup>	859 <sup>φ</sup>	690	712 <sup>φ</sup>	701 <sup>φ</sup>	604 <sup>a</sup>
Bodyweight gain	672	682	682	720	574	596	584	487
As % of controls	-	101	101	107	-	104	102	85

a Significantly different from controls, P < 0.05.  
b Significantly different from controls, P < 0.01.  
φ Not significantly different from controls.

**Table IVC.2.3** Percent incidence of uterine lesions in female rats

Lesion	Group			
	1	2	3	4
Endometrial hyperplasia	-	-	-	12
Cystic endometrial hyperplasia	22	22	25	44
Endometrial polyps and polypoid areas	5	5	15	17

**Table IVC.2.4** Thyroid tumor incidences in rat carcinogenicity study

Doses (mg/kg)	0		4		20		100	
No. of animals examined	M	F	M	F	M	F	M	F
Died/Sacrificed Moribund	48	33	51	38	52	33	49	40
Terminal Sacrifice	12	27	7	22	7	27	9	19
Incidence of Thyroid Follicular Cell Adenoma								
Died/Sacrificed Moribund	3	0	2	1	4	0	11*	3
Terminal Sacrifice	0	1	0	1	1	3	4*	0

M = Male; F = Female

\* Significantly different from controls, p<0.05 (Chi Square or Fisher's Exact test, as appropriate)

**Table IVC.2.5**

Group distribution of tumours, segregated by tumour-type, in rats dying during the treatment period

Group : 1 2 3 4  
 Compound : Control --- RU 4723 ---  
 Dosage (mg/kg/day) : 0 4 20 100

Organ and neoplasm	Group and sex							
	1 ♂	2 ♂	3 ♂	4 ♂	1 ♀	2 ♀	3 ♀	4 ♀
Number of animals examined:	48	51	52	49	33	38	33	40
<u>Skin and subcutis</u>								
Epithelial adenoma	0	1	2	2	0	0	1	0
Carcinoma (M)	1	1	0	1	0	0	1	0
<u>Mammary gland</u>								
Epithelial adenoma	1	4	1	3	29	28	23	28
Epithelial carcinoma (M)	1	0	0	0	4	5	5	4
Basal cell carcinoma (M)	0	0	0	0	1	0	0	0
<u>Lungs</u>								
Papillary adenoma	1	0	0	1	0	0	0	0
Alveolar adenoma	0	0	0	0	1	0	0	0
<u>Liver</u>								
Hepatocellular carcinoma (M)	0	0	0	0	0	0	1	0
<u>Urinary bladder</u>								
Mucosal papilloma	0	0	0	0	0	0	1	0
<u>Uterus</u>								
Endometrial adenoma	-	-	-	-	0	0	2	2
Adenocarcinoma (M)	-	-	-	-	0	2	0	0
<u>Intestines</u>								
Polypoid adenoma	0	0	0	1	0	0	0	0
Gastric adenoma	0	0	0	0	1	0	0	0
<u>Pancreas</u>								
Islet cell adenoma	1	1	1	0	1	0	0	0
<u>Liver</u>								
Hepatoma	0	0	1	0	0	0	0	0
<u>Kidney</u>								
Clear cell carcinoma (M)	0	1	0	0	0	1	0	0
<u>Pituitary</u>								
Adenoma	14	15	22	16	17	22	19	20

<u>Parathyroid gland</u>								
Adenoma	1	0	2	0	0	0	0	0
<u>Thyroid gland</u>								
Follicular cell adenoma	3	2	4	11 <sup>a</sup>	0	1	0	3
Parafollicular cell adenoma	2	2	2	0	1	1	4	6 <sup>φ</sup>
<u>Adrenal gland</u>								
Cortical adenoma	1	1	2	0	1	1	0	0
<u>Testes</u>								
Leydig cell adenoma	0	0	0	1	-	-	-	-
<u>Skin and subcutis</u>								
Mesenchymal	5	7	7	4	2	1	3	3
Sarcoma (M)	2	1	0	0	0	0	0	0
<u>Kidney</u>								
Lipoma	0	0	0	1	0	0	0	0
<u>Prostate gland</u>								
Fibrosarcoma (M)	0	0	0	1	-	-	-	-
Abdominal fibrosarcoma (M)	0	0	0	0	1	0	0	0
<u>Uterus</u>								
Fibrosarcoma (M)	-	-	-	-	0	0	0	1
<u>Cervix</u>								
Fibroma (M)	-	-	-	-	0	0	0	2
Osteogenic sarcoma (M)	-	-	-	-	0	0	0	1
Leiomyoma	-	-	-	-	0	0	0	1
<u>Uterus</u>								
Leiomyofibroma	-	-	-	-	0	1	0	0
<u>Liver</u>								
Haemangiosarcoma (M)	0	0	0	0	0	1	0	0
<u>Liver - continued</u>								
Granulocytic leukaemia (M)	0	0	0	0	0	0	0	1
Lymphoma (M)	2	2	1	2	1	0	2	1
<b>Total neoplasms</b>	<b>35</b>	<b>38</b>	<b>46</b>	<b>44</b>	<b>60</b>	<b>64</b>	<b>62</b>	<b>73</b>

(M) Denotes malignant neoplasm.

**Table IVC.2.6**

Group distribution of tumours, segregated by tumour-type, in rats killed after 104 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	---	RU 4723	---
Dosage (mg/kg/day)	:	0	4	20	100

Organ and neoplasm	Group and sex							
	1 ♂	2 ♂	3 ♂	4 ♂	1 ♀	2 ♀	3 ♀	4 ♀
Number of animals examined:	12	7	7	9	27	22	27	19
<u>Skin and subcutis</u>								
Adenoma	0	0	1	0	0	0	1	0
<u>Mammary gland</u>								
Epithelial adenoma	3	1	0	0	19	20	26	17
Epithelial carcinoma (M)	1	0	0	0	4	0	8	2
<u>Lungs</u>								
Papillary adenoma	0	0	0	0	0	0	1	1
<u>Pancreas</u>								
Islet cell adenoma	1	2	0	2	2	0	0	0
<u>Stomach</u>								
Squamous carcinoma (M)	0	1	0	0	0	0	0	0
<u>Liver</u>								
Haemangiosarcoma (M)	0	0	0	0	0	0	1	0
Carcinoma (M)	1	0	0	0	0	0	0	0
<u>Pituitary gland</u>								
Adenoma	5	3	3	4	13	10	17	4
<u>Parathyroid gland</u>								
Adenoma	0	1	0	0	0	0	0	0
<u>Thyroid gland</u>								
Follicular cell adenoma	0	0	1	4 <sup>a</sup>	1	1	3	0
Squamous carcinoma (M)	0	0	0	0	0	0	1	0
Parafollicular cell adenoma	1	2	1	0	8	4	3	2
<u>Adrenal gland</u>								
Cortical adenoma	1	0	0	0	0	0	1	0
<u>Ovaries</u>								
Granulosa cell tumour	-	-	-	-	0	0	1	1
<u>Salivary gland</u>								
Fibroma	0	0	0	0	1	0	0	0
<u>Skin and subcutis</u>								
Mesenchymal	1	1	2	1	1	1	3	1
<u>Testes</u>								
Interstitial cell tumour	1	0	0	0	-	-	-	-
<u>Uterus</u>								
Polypoid cell adenoma	-	-	-	-	1	0	2	0
Leiomyofibroma	0	0	0	0	0	1	0	1
Haemangioma	0	0	0	0	0	1	0	0
Lymphoma (M)	0	0	0	1	0	0	0	0
Myoma	0	0	1	0	0	0	0	0
Total neoplasms	15	11	9	12	50	38	68	29

(M) Denotes malignant neoplasm.

**Table IVC.2.7**

Group distribution of animals bearing one or more tumours<sup>†</sup>  
in decedents or in animals killed after 104 weeks of treatment

Group : 1 2 3 4  
Compound : Control --- RU 4723 ---  
Dosage (mg/kg/day) : 0 4 20 100

Tumour multiplicity	Group and sex							
	1 ♂	2 ♂	3 ♂	4 ♂	1 ♀	2 ♀	3 ♀	4 ♀
<u>Animals killed at termination</u>								
0 tumours	1	1	2	2	2	0	1	1
1 tumour	7	2	2	3	8	11	5	9
2 tumours	4	3	2	3	11	6	12	7
3 tumours	0	1	1	1	5	5	4	2
4 or more tumours	0	0	0	0	1	0	5	0
Total tumours	15	11	9	12	50	38	68	29
Number of animals examined:	12	7	7	9	27	22	27	19
<u>Decedent animals</u>								
0 tumours	25	22	20	21	2	3	0	4
1 tumour	12	20	20	15	11	12	14	13
2 tumours	10	9	13	11	13	17	11	12
3 tumours	1	0	0	1	5	6	6	8
4 or more tumours	0	0	0	1	2	0	2	3
Total tumours	35	38	46	44	60	64	62	73
Number of animals examined:	48	51	52	49	33	38	33	40
<u>Animals killed at termination and decedent animals</u>								
0 tumours	26	23	22	23	4	3	1	5
1 tumour	19	22	22	18	19	23	19	22
2 tumours	14	12	15	14	24	23	23	19
3 tumours	1	1	1	2	10	11	10	10
4 or more tumours	0	0	0	1	3	0	7	3
Total tumours	50	49	55	56	110	102	130	102
Total number of animals examined:	60	58	59	58	60	60	60	59

<sup>†</sup> Expressed as the number of individuals bearing the indicated number of tumours, regardless of tumour-type.

### 3. Mechanistic Studies

Two mechanistic studies were conducted in juvenile rats (~3 weeks old) to examine effects of CLB on circulating and/or tissue pituitary and thyroid hormone levels that could be associated with thyroid changes observed in the rat chronic toxicity and carcinogenicity studies. In the first study (LNCT-031), rats (10/sex/grp) received a oral doses of CLB (0, 50, or 200 mg/kg) for 2 or 4 weeks. Serum T3 was increased at 2 and 4 weeks and serum T4 was increased at 4 weeks. Increases in thyroid weights were greater in males than in females. Pituitary TSH decreased and serum TSH increased in females. Pituitary ACTH and vasopressin increased in both sexes. *In vitro*, CLB at 1 and 10 mg/mL caused a concentration-dependent displacement of T3 from serum proteins. In the follow-up study (LNCT-032), rats (10/sex/grp) received oral doses of CLB (0, 12.5, 50 or 200 mg/kg) for 4 weeks. Adrenal weights were increased and seminal vesicle and prostate weights were decreased in HD males. Serum TSH was increased in males at ≥50 mg/kg/day, and serum T3 was increased at both doses in males and in HD females. There were no significant changes in thyroxine (T4). The results indicate that CLB can alter the pituitary-thyroid axis leading to changes in thyroid function and size in the juvenile rat.

#### D. REPRODUCTIVE TOXICITY

1. Report on reproduction tests with HR 376 (A 50 376) in NMRI-mice (Influence on fertility, pregnancy and postnatal development) (Report. No. LNCT-024; dated 6/27/74; conducted by (b) (4) non-GLP)

##### a. Methods

Male and female mice (NMRI, 20/sex/grp) received oral (dietary admixture) doses (batch number not listed) of approximately 0 (control diet), 8, 40, or 200 mg/kg (40, 200, or 1000 ppm; food consumption could not be measured due to the design of the food containers and so doses were calculated based on an estimated consumption of 6 g/day) for up to 120 days (60 days pretreatment, mating for up to 2 weeks, pregnancy, lactation). No justification for dose selection was given. Pregnant females were allowed to deliver and rear their young during a 21-day lactation period. Dams were observed daily and weighed once a week until the end of the study. According to the report, "an exact account of the food consumption could not be made because the animals could scatter the food through the cage, this being due to the design of the food containers." Litter parameters were assessed at parturition and offspring were observed daily and weighed weekly during lactation. Parental animals were sacrificed at the end of the dosing (males after successful mating and females at weaning) and offspring were sacrificed at weaning. All dead and sacrificed animals were examined grossly.

##### b. Results

###### i. Mortality and Clinical Observations

There were no T-R differences in mortality or clinical observations among treated males and females.

###### ii. Body Weight

Body weight gain was increased in LD and MD males during the first 6 weeks of the study, but there no effects on BW gain in HD males or treated females.

###### iii. Fertility and Litter Data

In the mating trial after 60 days of treatment, all females showed a vaginal plug within 13 days. The fertility of the male animals was also not impaired by treatment; with exception of 1 MD animal, all males mated successfully. There was no treatment effect on maintenance and duration of pregnancy. Offspring death (stillbirth or neonatal death) was dose-dependently increased in treated litters and body weights at birth and during the first PN week were decreased in a dose-dependent manner (**Table IVD.1.1**). There was a marked dose-dependent decrease in rate of rearing in all treatment groups during lactation compared to controls: 2 C, 7 LD, 14 MD, and 18 HD dams failed to rear their litters. The pups from these litters died in the neonatal period “after the dams had neither bitten through the umbilical cord nor cleaned or nursed them.” It was concluded by the sponsor that this increased postnatal mortality rate was not due to an impairment of the viability of the offspring, but to a compound-induced abnormality of the dams after delivery. No external anomalies were observed in the offspring.

iv. Necropsy

Upon autopsy of the parent animals, increased liver weights (SS) were found at the HD. There were no macroscopic changes attributed to treatment.

c. Conclusions

Treatment of male and female mice with CLB (8, 40, or 200 mg/kg in the diet) prior to and during mating, gestation, and lactation produced no parental toxicity or adverse effects on fertility but resulted in markedly increased neonatal mortality as well as decreased BW in the offspring. Although decreased offspring survival was attributed to maternal toxicity in the report, no evidence was provided to support this contention. Maternal neglect can also be a consequence of offspring abnormality, i.e., direct developmental toxicity.

**Table IVD.1.1**

Dose	Control	40 ppm	200 ppm	1000 ppm
Used females	20	20	20	20
Pregnant	20	20	20	20
Non-pregnant	-	-	-	-
Litters	20	20	20	20
Rate of conception (%)	100	100	100	100
Body weight gain of the pregnant females over days 1-18 after mating	20.7 ± 2.9	22.2 ± 3.3	21.6 ± 3.9	23.8 ± 3.5
Living offsprings per litter	9.6 ± 2.6	9.2 ± 3.3	8.3 ± 3.0	8.1 ± 4.0
Dead offsprings per litter	0.5 ± 0.8	0.9 ± 1.1	0.9 ± 1.0	2.0 ± 2.7*
Supernumerary implantation sites per dam	0.7 ± 1.0	1.1 ± 1.7	1.3 ± 2.0	0.6 ± 0.8
Body weight of the young (g)				
after delivery and after				
7 days	1.46 ± 0.15	1.44 ± 0.19	1.42 ± 0.20	1.35 ± 0.16
14 days	3.94 ± 0.67	3.66 ± 0.75	3.65 ± 0.64	2.74 ± 0.40
21 days	7.08 ± 1.18	7.16 ± 1.15	7.63 ± 0.64	6.85 ± 1.16
Sex of the young (%) male	57	52	56	59
female	43	48	44	41
Rate of rearing (%)	76.3	57.4**	23.3**	7.2**
Non-reared litters	2	7	14	18
Externally perceptible abnormalities	-	-	-	-
Abnormalities of the inner organs	-	-	-	-
Special findings	-	-	-	-

\* significantly higher than standard (P<0.05)

\*\* significantly less than standard (P<0.001)

BEST  
POSSIBLE  
COPY

2. Report on Reproduction Test with HR 376 on Wistar Rats (Influence on Fertility, Pregnancy, Post-natal Development) (Report. No. LNCT-026; dated 1/29/73; conducted by (b) (4) non-GLP)

b. Methods

Male and female rats (in house-bred SPF Wistar, 20/sex/grp) received oral (dietary admixture) doses of CLB (batch no. 1 E 0884) of approximately 0 (control diet), 3.49, 17.42, or 85.4 mg/kg (40, 200, or 1000 ppm) for 60 days prior to mating for up to 2 weeks and continuing through pregnancy and lactation in females. No justification for dose selection was given. Pregnant females were allowed to deliver and rear their young during a 21-day lactation period. Dams were observed daily and weighed once a week until the end of the study. Litter parameters were assessed at parturition; offspring were observed daily and weighed weekly during lactation. Parental animals were sacrificed at the end of the dosing (males after successful mating and females at weaning) and offspring were sacrificed at weaning. All dead and sacrificed animals were examined grossly. Toxicokinetic analysis was not conducted.

b. Results

i. Mortality, Clinical Observations, and Body Weight

There were no T-R differences in mortality, clinical observations, food consumption, or BW gain among treated males and females (**Table IVD.2.1**).

ii. Fertility and Litter Data

In the mating trial after 60 days of treatment, treated females took longer to show sperm positive (**Table IVD.2.2**; mistake showing LD and MD infertile instead of MD and HD) and 1 MD and 1 HD female failed to mate (**Table IVD.2.3**). This was attributed to disturbance of estrous cyclicity. The fertility of the male animals was apparently not impaired by treatment; all males mated successfully. There was no treatment effect on maintenance and duration of pregnancy (dystocia in 1 LD dam resulted in death and litter loss). There were no apparent effects on litter parameters at birth or during lactation. No external anomalies were observed in the offspring.

iv. Necropsy

There were no macroscopic findings in the parental animals or offspring.

c. Conclusions

Treatment of male and female rats with CLB (approximately 3.49, 17.42, or 85.4 mg/kg in the diet) prior to and during mating, gestation, and lactation produced no parental toxicity but may have resulted in slight impairment of fertility at the MD and HD (single animal failed to mate in each group). There were no adverse effects on the offspring at these doses. The HD cannot be considered adequate based on the lack of parental toxicity and justification for dose selection.

**Table IVD.2.1**

Table 1                      Reproduction Test with HR 376 in Wistar Rats  
Survey of the Breeding-Findings

Dose	Control	40 ppm	200 ppm	1000 ppm
Used females	20	20	20	20
Pregnant	20	20	19	19
Non-pregnant (infertile)	-	-	1	1
Litters	20	20	19	19
Rate of conception (%)	100	100	95	95
Bodyweight gain of the pregnant females from day 1-21 p.c. (g)	103 ± 15.5	98 ± 10.6	102 ± 14.9	100 ± 11.4
No. of living young per litter	11.7 ± 1.3	10.8 ± 3.0	11.0 ± 1.8	11.1 ± 1.3
No. of dead young per litter	0.1 ± 0.3	0.6 ± 2.6	0.05	0.2 ± 0.5
Supernumerary implantation sites per dam	0.6 ± 0.8	0.3 ± 0.5	0.6 ± 0.8	0.4 ± 0.6
Bodyweight of the young (g) after delivery	5.53 ± 0.52	5.55 ± 0.59	5.83 ± 0.62	5.65 ± 0.59
7 days after	11.66 ± 2.24	12.24 ± 2.08	13.44 ± 1.82	12.81 ± 1.84
14 days after	21.61 ± 3.67	22.18 ± 2.61	23.21 ± 3.03	22.85 ± 2.73
21 days after	33.80 ± 6.08	35.31 ± 5.05	36.85 ± 4.76	36.59 ± 5.39
Sex (%) male	42	50	49	48
female	58	50	51	52
Rate of rearing (%)	84.3	86.0	97.1	93.4
Non-reared litters	1	1	-	-
External abnormalities	-	-	-	-
Special findings: dystocia, death	-	1	-	-

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**Table IVD.2.2**

Number of days from beginning of pairing attempts up to detection of spermatozoa in the vaginal smear:

Days	Controls	40 ppm	200 ppm	1 000 ppm
	Number of animals			
1 - 5	16	18	14	16
6 - 10	4	1	2	3
11 - 15	-	-	2	1
16 - 20	-	-	-	-
21 - 25	-	-	1	-
26 - 50	-	1+	1+	-
				+ infertile

**Table IVD.2.3**

Number of attempts at pairing until pregnancy occurred:

Pairing attempts	Controls Number of animals	40 ppm	200 ppm	1 000 ppm
1	18	14	16	18
2	2	6	3	1
3 weeks	-	-	1	1

3. Teratological Study of 1-phenyl-5-methyl-8-chloro-1,2,4,5-tetrahydro,2-4-di-keto-3H-1,5-BDZ (UR 376) in Mouse Fetuses (Study No. LNCT-21; conducted by (b) (4); report dated 7/23/74; non-GLP)

a. Methods

HR 376 (CLB) was administered orally (by gavage) to pregnant (ICR-JCL) mice from GD 7 to 12 at dose levels of 6, 25, or 100 mg/kg. Sixteen/group were sacrificed on GD18 and implantations, resorptions, and dead and living fetuses in the uterus were counted. BW, crown-rump length, placental weight and anogenital distance were measured in live fetuses. After examination for external malformations with a dissecting microscope, half the living fetuses were cleared and the skeletons were stained with alizarin red S for examination of skeletal abnormalities. The remaining fetuses were fixed with Bouin's solution and examined for visceral abnormalities with the freehand razor-blade sectioning (Wilson's) method. Six/grp were allowed to deliver, and the live born and stillborn were counted. The offspring were reared to PND 21, and BWs and numbers of live and dead were recording weekly during the lactation period. The living young were observed daily for clinical signs and abnormal behavior. On day 21, the dams and reared young were sacrificed and the dams were autopsied and their uteri and other viscera were examined macroscopically. The reared young were examined for body weight, body length, sex, and external malformations; they were then autopsied and examined for visceral abnormalities, and the heart, lung, liver, spleen, and kidney were each weighed. The skeletal specimens of about two-thirds were examined for skeletal malformations.

Dose selection: Doses were based on a 2-week toxicology study in which oral doses of 50, 200, or 400 mg/kg were given to adult female mice. 3/10 HD and 2/20 MD mice died, while no effects on mortality or BW were seen at the LD.

b. Results

i. Maternal effects

There was no difference in body weight gains of the dams during pregnancy between the drug-treated and control groups, and no abnormalities were macroscopically observed in the limited number of organs examined at necropsy.

ii. Developmental effects

- (1) There were no treatment-related differences in number of implantations, resorptions, dead and living fetuses, placental weights, body weights, crown-rump length, or sex proportion of the living fetuses (**Table IVD.3.1**).

- (2) External malformations were increased at the HD (**Table IVD.3.1**); there were 5 cases of cleft palate, 4 cases of open eyelid, 1 case of kinked tail, and 1 case of complication with cleft palate and open eyelid out of the total of 195 fetuses in the HD group. According to the report, there were no visceral abnormalities in any group.
- (3) There was no effect on skeletal malformations; skeletal variations appeared to be increased in treated litters but not in a clearly D-R manner (**Table IVD.3.2**).
- (4) There were no clear differences in offspring parameters during lactation (**Table IVD.3.3**). At necropsy, there were no external abnormalities and no T-R differences in incidences of visceral or skeletal abnormalities.

c. Conclusions

Treatment of pregnant mice with CLB (oral gavage doses of 0, 6, 25, or 100 mg/kg) from GD 7 to 12 produced increased incidences of external malformations at the HD in the absence of maternal toxicity. This study cannot be considered adequate for a variety of reasons, including the non-standard dosing period.

**Table IVD.3.1**

Effects of HR 376 on the Fetuses When Administered Orally to Maternal Mice on Days 7 - 12 of Pregnancy

Group (mg/kg)	Maternal animals			Fetuses												
	No. of animals	Body weight (g) at pregnancy period		No. of implants	No. of resorptions	No. of dead fetuses	No. of living fetuses	Average No. of fetuses/placenta/mother	Average weight of placenta (g)	Average body weight (g)	Average crown-rump length (cm)	Sex (%)		No. of malformations		
		day 0	day 18									Increase	M	F	External	Internal
100	16	30.2 ±2.2	57.2 ±4.9	27.0 ±4.4	204	5 (2.5)	4 (2.0)	195 (95.6)	12.2 ±2.8	0.11 ±0.02	1.38 ±0.09	2.55 ±0.15	108 (55.4)	87 (44.6)	11 <sup>a</sup> (5.6)	0
25	16	29.6 ±1.7	54.7 ±5.1	25.1 ±5.1	185	14 (7.6)	1 (0.5)	170 (91.9)	10.6 ±2.9	0.11 ±0.01	1.43 ±0.10	2.59 ±0.12	95 (55.9)	75 (44.1)	2 <sup>b</sup> (1.2)	0
6	16	30.5 ±2.0	57.1 ±4.3	26.6 ±3.8	209	15 (7.2)	4 (1.9)	190 (90.9)	11.9 ±2.1	0.11 ±0.02	1.39 ±0.02	2.55 ±0.11	96 (50.5)	94 (49.5)	3 <sup>c</sup> (1.6)	0
Control (2 % Starch)	16	29.4 ±2.2	56.9 ±3.7	27.5 ±3.1	206	11 (5.3)	4 (1.9)	191 (92.7)	11.9 ±1.7	0.11 ±0.01	1.41 ±0.10	2.56 ±0.12	108 (56.5)	83 (43.5)	5 <sup>d</sup> (2.6)	0

Notes: Figures in parentheses show percentage:  
a: 5 cleft palate, 4 open eyelid, 1 kinked tail and 1 complication of cleft palate and open eyelid  
b: 1 cleft palate and 1 open eyelid  
c: 2 cleft palate and 1 open eyelid  
d: 5 open eyelid

**Table IVD.3.2**

Skeletal Development in the Fetuses of Maternal Mice Orally Administered HR 376 on Days 7 - 12 of Pregnancy

Group (mg/kg)	No. of maternal animals	No. of fetuses examined	Malformations	Variations					Total average No. of sacral and caudal vertebrae
				Y-shaped cervical vertebral arches	Vertebral centra*	Dislocated sternebrae	Cervical rib	Lumbar rib	
100	16	103	1 <sup>a</sup> (1.0)	25 (24.3)	0	6 (5.8)	32 (31.1)	29 (28.2)	13.3 ±1.5
25	16	88	0	14 (15.9)	0	4 (4.5)	23 (26.1)	23 (26.1)	12.8 ±1.6
6	16	100	1 <sup>b</sup> (1.0)	26 (26.0)	0	3 (3.0)	27 (27.0)	24 (24.0)	12.4 ±1.1
Control (2 % Starch)	16	97	1 <sup>c</sup> (1.0)	16 (16.5)	0	1 (1.0)	19 (19.6)	27 (27.8)	13.4 ±1.4

Notes: Figures in parentheses show percentage.  
\*: Dumbbell- and/or bipartite-shaped vertebral centra  
a: Transformation of tail bone  
b: Fused sternebrae  
c: Bifurcation of metatarsus

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Table IVD.3.3

Effects of HR 376 on the Young When Administered Orally  
to Maternal Mice on Days 7 - 12 of Pregnancy

Group (mg/kg)	100	25	6	Control (2 % starch)
<u>Maternal animal</u>				
No. of mice	6	6	6	6
Average period of pregnancy (day)	19.0 ±0.0	19.0 ±0.0	18.8 ±0.4	19.0 ±0.6
Average body weight gains during pregnancy (g)	24.4 ±2.5	25.3 ±4.2	28.1 ±6.3	24.6 ±5.8
<u>Young born</u>				
Average litter size	10.2 ±0.8	11.8 ±2.5	11.5 ±3.6	9.5 ±3.9
No. of stillborn	0	0	0	0
Average body weight (g)	1.8 ±0.2	1.6 ±0.1	1.7 ±0.2	1.7 ±0.2
<u>Young</u>				
7th day				
Average No. of living young/ litter	9.8 ±0.8	11.7 ±2.5	11.3 ±3.3	9.0 ±3.2
Total average body weight (g)	4.3 ±0.7	3.5 ±0.7	4.1 ±1.0	4.6 ±1.1
14th day				
Average No. of living young/ litter	9.8 ±0.8	10.8 ±2.4	11.3 ±3.3	8.8 ±3.0
Total average body weight (g)	7.1 ±0.8	5.6 ±1.8	6.3 ±1.4	7.8 ±1.8
21st day				
Average No. of living young/ litter	9.2 ±1.7	10.7 ±2.6	11.2 ±3.3	8.8 ±3.0
Average body weight (g)	10.2 ±1.9	7.6 ±2.8	9.4 ±2.4	12.1 ±3.0
Average body length (cm)	6.5 ±0.5	5.7 ±0.7	6.0 ±0.5	6.7 ±0.6
Total No. of dead young	6	7	2	4
No. of survived young	55	64	67	53
Male	23 (41.8)	22 (34.4)	30 (44.8)	29 (54.7)
Female	32 (58.2)	42 (65.6)	37 (55.2)	24 (45.3)
Average percent of reared	90.3 ±6.5	91.0 ±14.9	97.6 ±3.7	95.6 ±10.9
No. of internal and external malformations	0	0	2 <sup>a</sup>	1 <sup>a</sup>

Notes: Figures in parenthesis show percentage.  
a: Hydronephrosis

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Table IVD.3.4

Skeletal Development in the Young of Maternal Mice Orally  
Administered BR 376 on Days 7 - 12 of Pregnancy

Group (mg/kg)	100	25	6	Control (2 % Starch)
No. of maternal animals	6	6	6	6
No. of young examined	38	45	46	39
Abnormalities	1 (2.6) <sup>a</sup>	1 (2.2) <sup>a</sup>	1 (2.2) <sup>b</sup>	1 (2.6) <sup>c</sup>
<u>Bifurcation of vertebral arches</u>				
Cervical vertebrae				
1	4 (10.5)	26 (57.8)	18 (39.1)	9 (23.1)
2	3 (7.9)	12 (26.7)	4 (8.7)	6 (15.4)
3	0	6 (13.3)	1 (2.3)	0
4	0	4 (8.9)	0	0
5	0	6 (13.3)	0	0
6	0	8 (17.8)	1 (2.3)	0
7	0	11 (24.4)	2 (4.3)	0
Thoracic vertebrae				
1	2 (5.3)	20 (44.4)	2 (4.3)	4 (10.3)
2	0	16 (35.6)	8 (17.4)	1 (2.3)
3	0	6 (13.3)	0	0
4	0	5 (11.1)	0	0
5	0	3 (6.7)	0	0
6-9	0	0	0	0
10	1 (2.6)	5 (11.1)	3 (6.5)	0
11	2 (5.3)	7 (15.6)	2 (4.3)	0
12	0	6 (13.3)	0	0
13	0	3 (6.7)	0	0
Lumbar vertebrae				
1	0	2 (4.4)	0	0
2	0	2 (4.4)	0	0
3	0	3 (6.7)	0	0
4-6	0	0	0	0
Bifurcation of vertebral centra	0	0	0	0
Y-shaped cervical vertebral arches	1 (2.6)	5 (11.1)	1 (2.3)	1 (2.3)
Cervical rib	1 (2.6)	0	1 (2.3)	1 (2.3)
Lumbar rib	11 (28.9)	8 (17.8)	15 (32.6)	13 (33.3)
Unstained kneecap	0	0	0	0
Total average No. of caudal vertebrae	28.7 +0.5	28.2 +0.7	28.7 +1.0	29.3 +0.9

Notes: Figures in parentheses show percentage.

a: Transformation of the 5th sternebra, b: Fused ribs,  
c: Fused sternebrae and bilateral asymmetry of the  
sternocostal articulation

4. H4723 Teratological study (Study No. LNCT-23; conducted by (b) (4) report dated 7/10/72; non-GLP)
- a. Methods

H 4723 (CLB; 0.25% aqueous solution in carboxymethyl cellulose) was administered orally (by gavage) to Swiss CD-1 mice (24/group), Sprague-Dawley rats (25-30/group), or Small Russian rabbits (20/group) starting on GD 6 until GD 17 (mice), 20 (rats), or 27 (rabbits) at doses of 4, 20, or 100 mg/kg. Pregnant females were sacrificed on GD 18, 21, or 28 and implantations, resorptions, and dead and living fetuses in the uterus were counted. After examination for external malformations with a dissecting microscope and measurement of fetal weights, half the living fetuses were cleared and the skeletons were stained with alizarin red S for examination of skeletal abnormalities. The remaining fetuses were fixed with Bouin's solution and examined for visceral abnormalities with the freehand razor-blade sectioning (Wilson's) method.

Dose selection: Doses were based on preliminary studies (only brief summaries provided). According to the study report, when administered orally to mice for 12 days, 400 mg/kg resulted in mortality and 200 mg/kg in loss of weight and hyperexcitability; when administered orally to male and female rats for 21 days, 300 mg/kg was excessively toxic (not described); and when administered to female rabbits for 21 days, 100 mg/kg produced only a slight reduction in BW in 2 out of 9 animals. The summaries did not indicate whether the same strains were used.

b. Results

i. Mice

The results are summarized in **Table IVD.4.1**.

- (1) Only limited maternal toxicity information was provided indicating that there were no deaths and that “on the whole gestation progressed without any incident.”
- (2) Embryo-fetal mortality was increased in a non-D-R manner in all treated groups compared to controls; however, the number of viable fetuses was decreased only at the HD.
- (3) There was no apparent effect on fetal abnormalities. Although cleft palate was observed in some fetuses, there was no relationship to treatment.

ii. Rats

The results are summarized in **Table IVD.4.2**.

- (1) No maternal mortality was observed.
- (2) Although there was no clear effect on fetal BWs (slight non-D-R decreases at all doses), increased incidences of retarded ossification were observed in treated fetuses at all doses (also non-D-R).

iii. Rabbits

The results are summarized in **Table IVD.4.3**.

- (1) Maternal mortality was increased at the HD, but these doses were not considered treatment-related based on necropsy findings of lung infection. There was a dose-related increase in total litter loss: 1, 1, 2, and 4 in C, LD, MD, and HD.
- (2) Fetal BWs were decreased and embryo-fetal loss increased at the HD. An increased incidence of extra rib was also seen in this group.

c. Conclusions

Treatment of mice, rats, or rabbits throughout organogenesis at doses of 4, 20, or 100 mg/kg produced developmental toxicity in all three species (increased embryo-fetal mortality in mice and rabbits, decreased fetal BWs in rabbits, and increased incidences of skeletal variations in rats and rabbits), primarily at the HD. There was insufficient information to assess maternal toxicity, so the adequacy of the high doses cannot be determined. In addition, fetal line listings were incomplete.

Table IVD.4.1

Groups	I	II	III	IV
Doses in mg/kg	0	4	20	100
Mated females	24	24	24	24
Pregnant females	23	20	22	24
Females died during the test	0	0	0	0
Females killed on day 18	23	20	22	24
<u>Examination of the killed females</u>				
- Gestations completely interrupted	0	0	0	0
- Total number of implantations	301	272	297	309
- Total number of foetal losses	9	21	19	22
- Total number of viable foetuses	292	251	278	287
<u>Mean per female</u>				
- Implantations	13,08	13,60	13,50	12,87
- Foetal losses	0,39	1,05	0,86	0,91
- Viable foetuses	12,69	12,55	12,63	11,95
<u>Rate of foetal losses %</u>				
Rate of foetal losses %	2,99	7,72	6,39	7,11
<u>Mean weight of a foetus (g)</u>				
Mean weight of a foetus (g)	1,34	1,26	1,22	1,37
Total number of foetuses	292	251	278	287
<u>External examination</u>				
- Examined foetuses	292	251	278	287
- Abnormalities	1 bilateral exophthalmos	0	0	0
<u>Internal examination</u>				
- Examined foetuses	141	128	135	136
- Abnormalities (cleft palate)	1	1	0	1
<u>Examination of the skeleton</u>				
- Examined foetuses	151	123	143	151
- Abnormalities				
. Retarded ossification at the level of sternum	19	10	29	4
. Retarded ossification at the level of cranium	1	3	9	1
. Excess ribs	4	7	6	7
. Missing ribs		1	6	

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Table IVD.4.2

Groups	I	II	III	IV
Doses in mg/kg	0	4	20	100
Mated females	29	24	34	30
Pregnant females	19	17	24	21
Females died during the test	0	0	0	0
Females killed on day 21	19	17	24	21
<u>Examination of the killed females</u>				
- Gestations completely interrupted	0	0	1	0
- Total number of implantations	204	223	270	237
- Total number of foetal losses	25	6	23	8
- Total number of viable foetuses	179	217	247	229
Mean per female				
- Implantations	10,73	13,11	11,25	11,28
- Foetal losses	1,31	0,35	0,95	0,38
- Viable foetuses	9,42	12,76	10,29	10,90
Rate of foetal losses %	12,2	2,69	8,51	3,37
Mean weight of a foetus (g)	5,55	5,34	5,30	5,48
Total number of foetuses	179	217	247	229
<u>External examination</u>				
- Examined foetuses	179	217	247	229
- Abnormalities	0	0	0	0
<u>Internal examination</u>				
- Examined foetuses	84	107	124	110
- Abnormalities	0	0	0	0
<u>Examination of the skeleton</u>				
- Examined foetuses	95	110	123	119
- Abnormalities				
. Non ossification of the sternal vertebrae	1	1	1	1
. Retarded ossification of the sternal vertebrae	7	11	10	12
. Retarded ossification of the cranium		6	2	8
. Excessive number of ribs	1			1

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Table IVD.4.3

Groups	I	II	III	IV
Doses in mg/kg	0	4	20	100
Mated females	20	19	20	23
Pregnant females	18	19	18	20
Females died during test	2	1	1	4
Females killed on day 28	16	18	17	16
<u>Examination of the killed females</u>				
- Gestations completely interrupted	1	1	2	4
- Total number of implantations	109	135	117	100
- Total number of foetal losses	15	4	20	26
- Total number of viable foetuses	94	131	97	74
Mean per female				
- Implantations	6,81	7,50	6,88	6,25
- Foetal losses	0,93	0,22	1,17	1,44
- Viable foetuses	5,87	7,27	5,70	4,11
Rate of foetal losses %	13,8	2,96	17,1	26 *
Mean weight of a foetus (g)	27,7	27,4	27,6	26,6
Degree of significance: * 0.01 < p < 0.05				
Total number of foetuses	94	131	97	74
<u>External examination</u>				
- Examined foetuses	94	131	97	74
- Abnormalities	0	1 exophthalmos 1 exencephalus*	0	0
<u>Internal examination</u>				
- Examined foetuses	42	67	51	35
- Abnormalities	0	0	1 hydrocéphalus*	0
<u>Examination of the skeleton</u>				
- Examined foetuses	52	64	46	39
- Abnormalities				
. Non ossification of the sternal vertebrae	11	7	7	7
. Retarded ossification of the sternal vertebrae	6	7	5	7
. Excess ribs	1	5	1	11
. Ribs joint together			1	
. Retarded ossification of the cranium		1		
* Malformations combined with others in the same foetus (see text)				

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5. Teratological Study of 1-Phenyl-5-methyl-8-chloro-1,2,4,5-tetrahydro,2-4-diketo-3H-1,5-BDZ (HR 376) in Rat Fetuses (Report No. LNCT-022; dated 7/23/74; conducted by (b) (4); non-GLP)

a. Methods

CLB was administered to pregnant rats (SD-JCL, 16/group cesarean, 6/group delivery) from GD 9-14 at oral gavage doses of 0 (vehicle: 2% starch), 25, 100, or 400 mg/kg. Sixteen dams in each experimental group were sacrificed on day 21 of pregnancy, and implantations, resorptions, and dead and living fetuses in the uterus were counted. In the living fetuses, body weight, crown-rump length, placental weight, and anogenital distance were measured. After examination for external malformations, about half the fetuses were cleared and the skeletons stained with alizarin red S for examination on skeletal malformations. The remaining fetuses were fixed with Bouin's and examined for visceral abnormalities with the free-hand razor blade section method. Six pregnant/group were allowed to delivered, the liveborn and stillborn were counted and newborn rats were reared until day 21 after birth, and clinical observations were observed daily, body weight, and number of the dead weekly. On PND 21, dams were autopsied and examined macroscopically; and BW, body length, sex, and external, visceral, and skeletal abnormalities were assessed in the offspring.

Dose selection: Doses were said to be based on study in which doses of 100, 400, or 1000 mg/kg were given to female rats for 28 days. There were no deaths, but an increase in liver weights at the MD or greater was considered a basis for setting the HD in the current study.

b. Results

i. Maternal effects

At the HD, BW gain was decreased (29% compared to C) during the dosing period, but final BWs were only slightly (4%) lower (**Table IVD.5.1**).

ii. Developmental effects

(1) There was no difference between the drug-treated and control groups in the number of implantations, resorptions, and dead and living fetuses; and placental weight, body weight, crown-rump length, and sex proportion of fetuses were similar among groups (**Table IVD.5.2**). There was no apparent effect of treatment on incidences of external or visceral malformations (only found in 2 MD fetuses). There was a slight increase in skeletal variations (bipartite vertebral centra, lumbar rib) at the HD (**Table IVD.5.3**).

(2) There were no clear T-R differences in numbers of liveborn or stillborn (there was a slight decrease in avg litter size at the HD, but without measurement of implantations no conclusions can be drawn), body weight at birth, survival and body weight during the rearing period, or on body weight, body length, and sex proportion at weaning (**Table IVD.5.4**). No external malformations were observed, and visceral abnormalities were found in only 1 HD fetus (hydronephrosis). There was also no clear effect of treatment on skeletal abnormalities, although fused cervical arches were only seen at the MD and HD (**Table IVD.5.5**).

c. Conclusions

Treatment of pregnant rats with CLB (oral gavage doses of 25, 100, or 400 mg/kg) from GD 9-14 produced slightly increased incidences of skeletal variations at HD. This study does not provide

an adequate assessment of effects on embryo-fetal development because of the short dosing period.

**Table IV.D.5.1**

Body Weight Gains (g) of Pregnant Rats Treated with HR 376 Orally on Days 9 - 14 of Pregnancy

Group (mg/kg)	No. of animals	Average body weight (g)		Average body weight gains (g) during pregnancy (Days of pregnancy)			
		0	21st	0-9th	9-15th	15-21st	0-21st
400	16	259	363	35	20	49	103
		+25	+34	+9	+6	+15	+17
100	16	256	357	32	27	42	101
		+23	+31	+10	+6	+17	+20
25	16	260	374	38	30	47	115
		+16	+24	+8	+5	+12	+18
Control (3 % Starch)	16	267	377	33	28	49	110
		+21	+35	+11	+6	+13	+22

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**Table IV.D.5.2**

Effect of HR 376 on the Fetuses when Administered Orally to Maternal Rats on Days 9 - 14 of Pregnancy

Group (mg/kg)	Maternal animals			Fetuses										
	No. of animals	Body weight (g) at pregnancy period	No. of implantations	No. of resorptions	No. of dead fetuses	No. of living fetuses	Average No. of living fetuses/mother	Average placental weight (g)	Average body weight (g)	Average crown-rump length (cm)	Sex (%)		No. of malformations	
											M	F	External	Internal
400	16	259 363 103	219	9	0	210	13.1	0.54	5.05	4.14	109	101	0	0
		+25 +34 +17												
100	16	256 357 101	222	13	0	209	13.1	0.57	4.87	4.07	117	92	1 <sup>a</sup>	2 <sup>b</sup>
		+23 +31 +20												
25	16	260 374 115	242	9	0	233	14.6	0.54	4.91	4.10	129	104	0	0
		+16 +24 +18												
Control (3 % Starch)	16	267 377 110	220	19	1	200	12.5	0.60	5.01	4.12	105	95	0	0
		+21 +35 +22												

Notes: Figures in parentheses show percentage.

a: Edema

b: One case was hypertrophic adrenal gland and the other hydrocephalus.

**Table IVD.5.3**

Skeletal Development in the Fetuses of Maternal Rats Orally Administered HR 376 on Days 9 - 14 of Pregnancy

Group (mg/kg)	No. of maternal animals	No. of fetuses examined	Malformations	Variations					Average No. of sacral and caudal vertebrae
				Y-shaped cervical vertebral arches	Vertebral centra*	Dislocated sternbrae	Cervical rib	Lumbar rib	
400	16	111	0	0	4 (3.6)	0	0	82 (73.9)	10.8 ±0.9
100	16	108	0	0	2 (1.9)	0	0	61 (56.5)	10.4 ±0.7
25	16	121	0	0	3 (2.5)	0	0	79 (65.3)	10.3 ±0.6
Control (2 % Starch)	16	103	0	0	1 (1.0)	0	0	70 (68.0)	10.8 ±0.8

Notes: Figures in parentheses show percentage.  
\* Dumbbell- or bipartite-shaped thoracic vertebral centra

**Table IVD.5.4**

Effect of HR 376 on the Young when Administered Orally to Maternal Rats on Days 9 - 14 of Pregnancy

Group (mg/kg)	400	100	25	Control (2 % Starch)
<u>Maternal animal</u>				
No. of rats	6	6	6	6
Average period of pregnancy (day)	22.2 ±0.4	22.0 ±0	22.0 ±0	22.0 ±0
Average body weight gains during pregnancy (g)	97.5 ±12.9	100.5 ±14.7	103.3 ±10.2	103.0 ±15.3
<u>Young born</u>				
Average litter size	12.5 ±1.9	13.0 ±1.8	13.0 ±2.2	10.8 ±1.0
No. of stillborn	2 (2.7)	0	0	0
Average body weight (g)	5.6 ±0.3	5.6 ±0.4	5.4 ±0.4	5.9 ±0.4
<u>Young</u>				
7th day				
Average living young/litter	11.7 ±2.2	12.8 ±1.8	12.2 ±2.4	10.7 ±1.0
Average body weight (g)	11.9 ±1.5	11.8 ±1.6	11.4 ±1.4	13.1 ±1.0
14th day				
Average living young/litter	11.3 ±2.0	12.7 ±1.8	11.8 ±2.5	10.7 ±1.0
Average body weight (g)	24.2 ±1.5	23.1 ±2.8	23.7 ±2.1	25.5 ±1.0
21st day				
Average living young/litter	11.3 ±2.0	12.5 ±1.4	11.8 ±2.5	10.7 ±1.0
Average body weight (g)	38.4 ±4.0	35.3 ±5.5	37.3 ±3.5	40.4 ±2.0
Average body length (cm)	10.5 ±0.3	10.4 ±0.6	10.4 ±0.3	10.6 ±0.2
No. of dead young	7 (9.3)	3 (3.8)	7 (9.0)	1 (1.5)
Average No. of survived young/litter	11.3 ±2.0	12.5 ±1.4	11.8 ±2.5	10.7 ±1.0
Sex (%)	Male 23 (33.8)	Female 43 (57.3)	Male 38 (53.5)	Female 29 (45.5)
Average percent of reared young	91.1 ±12.0	96.5 ±3.9	90.9 ±9.9	98.5 ±3.7
No. of external or internal malformations	1 <sup>a</sup> (1.5)	0	0	0

Notes: Figures in parentheses show percentage.  
a: Hydronephrosis

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**Table IVD.5.5**

Skeletal Development in the Reared Young of Maternal Rats  
Orally Administered HR 376 on Days 9 - 14 of Pregnancy

Group (mg/kg)	400	100	25	Control (2 % Starch)
No. of maternal animals	6	6	6	6
No. of young examined	47	51	49	45
Abnormalities	6 (12.3)	4 (7.8)	11 (22.4)	2 (4.4)
Fused cervical arches	2	1	0	0
Bifurcated xiphoid processes	1	0	1	0
Nodular ribs	0	2	2	1
Lumbarization of sacral vertebral arches	3	1	8	1
Bifurcation of vertebral arches				
Cervical vertebrae				
1	0	1 (2.0)	0	0
2	1 (2.1)	1 (2.0)	0	0
3	1 (2.1)	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	3 (6.1)	0
7	2 (4.3)	3 (5.9)	2 (4.1)	0
Thoracic vertebrae				
1	14 (29.8)	13 (25.5)	12 (24.5)	14 (31.1)
2-9	0	0	0	0
10	27 (57.4)	28 (54.9)	29 (59.2)	22 (48.9)
11	21 (44.7)	19 (37.3)	15 (30.6)	9 (20.0)
12	14 (29.8)	13 (25.5)	8 (16.3)	4 (8.9)
13	6 (12.3)	11 (21.6)	5 (10.2)	1 (2.2)
Lumbar vertebrae				
1	0	6 (11.8)	0	0
2	0	2 (3.9)	0	0
3	0	1 (2.0)	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
Bifurcation of vertebral centra	7 (14.8)	7 (13.7)	8 (16.3)	8 (17.7)
Y-shaped cervical vertebral arches	0	0	0	0
Cervical rib	0	0	0	1 (2.2)
Lumbar rib	25 (53.2)	38 (74.5)	32 (65.3)	23 (51.1)
Unstained kneepan	44 (93.6)	45 (88.2)	48 (98.0)	38 (84.4)
Average No. of caudal vertebrae	27.9 ±0.2	27.9 ±0.7	27.9 ±0.5	28.1 ±0.3

Notes: Figures in parentheses show percentage.

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6. Report on an oral peri- and postnatal toxicity study of HR 376 in Wistar rats (Exp. No. LNCT-025; report dated 9/15/76; conducted by (b) (4) non-GLP)

1. Methods

CLB (HR 376; Batch 29) was given by oral gavage to female Wistar rats (Hoe:WISKf (SPF 71); 20/group) from GD 17 to PND21 at doses of 0 (2% starch mucilage), 5, 32, or 200 mg/kg. The dams were allowed to litter and rear their offspring. During the lactation period the development and survival rate of the offspring were recorded, and auditory and visual function tests and a simple behavioral test (swimming test in which righting reflex, motor activity, coordination, and balance were evaluated) were carried out. No justification of dose selection was provided.

2. Results

a. Maternal effects

According to the report, "no dam showed any disturbance of behavior and of the general condition." There was a small D-R decrease (9% over PND 0-21 at HD; earlier data not given) in maternal BW gain (**Table IVD.6.1**). There were no T-R effects on reproductive parameters.

b. Developmental effects

- i. There was a small D-R increase in postimplantation loss (supernumerary implantation sites) and decrease in live offspring (**Table IVD.6.1**). BW at birth was similar among groups (or inversely related to litter size). Postnatal pup survival and BW gain appeared unaffected by treatment, except for one HD unreared litter.
- ii. Although the data were not provided, the report stated that "The auditory and visual tests suggested no noxious effect of HR 376 in the surviving offspring" and "in the behavioral and activity studies (swimming test) no difference in various reactions was noticed between the drug-treated and the control animals."
- iii. Autopsy of the dams and offspring revealed no macroscopic changes.

3. Conclusions

Treatment of female rats with clonazepam (oral gavage doses of 5, 32, or 200 mg/kg) from GD 17 to PND 21 produced slight maternal toxicity at the HD (decreased BW gain) but had no clear effects on development in offspring based on the data provided. This study does not provide an adequate assessment of pre- and postnatal developmental toxicity due to the dosing period, endpoints, and lack of documentation.

**Table IVD.6.1**

PERI-/POST-NATAL STUDY OF HR 376 IN WISTAR RATS  
Survey of findings

Dosed on days 17 p.c. <sup>1)</sup> - 21 p.p. <sup>2)</sup>	Control	5 mg/kg	32 mg/kg	200 mg/kg
Experimental females with sperm	20	21	22	22
Pregnant females	20	20	20	20
Females with dead fetuses only	-	-	-	-
Females with live fetuses	20	20	20	20
Body weight gain(g) in dams (day 0 - 21)	116 ± 15.6	114 ± 15.4	108 ± 15.1	106 ± 19.4
Live offspring per litter	10.4 ± 2.4	10.8 ± 2.4	9.8 ± 2.0	9.8 ± 2.3
Dead offspring per litter	0.4	0.05	-	0.1
Supernumerary implantation sites per dam	0.6	0.6	0.9	1.0
Body weight (g) of offspring				
at birth	5.61 ± 0.43	5.58 ± 0.56	5.86 ± 0.52	5.79 ± 0.69
7 days after birth	12.52 ± 1.52	12.80 ± 1.72	13.77 ± 1.76	13.27 ± 2.13
14 days after birth	22.71 ± 2.58	22.23 ± 2.56	24.41 ± 3.18	23.37 ± 3.43
21 days after birth	36.00 ± 4.15	35.50 ± 4.77	38.79 ± 5.88	37.27 ± 6.90
Sex of offspring (♂) - male	47	52	52	53
- female	53	48	48	47
Survival rate (♂) at 21 days	94.4	97.7	98.9	92.4
Unreared litters	-	-	-	1

1) post copulationem  
2) post partum

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E. JUVENILE ANIMAL TOXICITY

1. Oral Gavage Repeated-Dose Toxicity Study of CLB in Rats (Study No. OVNC-9005, conducted by (b) (4)); Report dated 11/21/08; GLP

a. Methods

CLB (Lot number 317712) was administered by oral gavage to juvenile rats (CrI:CD(SD); 40/sex/group from 40 litters; split litter design) from postnatal day (PND) 14 to PND 53 (subset 1) or 49 (subset 2) at doses of 0 (corn oil vehicle), 4, 36, or 120 mg/kg (2 ml/kg). Endpoints for all animals included clinical observations, body weights, sexual maturation, FOB, hematology, and clinical chemistry. Half of the animals in each group (Subset 1) were assessed for motor activity (during the fourth week of treatment), auditory startle habituation (during the fourth week of treatment), and Morris water maze (during the fourth and fifth week of treatment). The remaining half (Subset 2) were evaluated for motor activity (day 61 ± 2 days postpartum), auditory startle habituation (day 61 ± 2 days postpartum), Morris water maze (beginning at 70 ± 3 days postpartum), and reproductive performance. On PND 54 (Subset 1 male and female rats), DG 13 (Subset 2 female rats) or PND 117 to 119 (Subset 2 male rats), rats were sacrificed and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Blood samples were collected from Subset 1 rats, processed for hematology or clinical chemistry evaluations and shipped for analysis. Both femurs were excised, the lengths recorded and retained. Organ weights were recorded and a complete set of tissues were retained for possible histopathological evaluation. Histological examination was performed on all tissues from 10/sex/group from each sacrifice timepoint. All gross lesions were saved and examined histologically. Left femurs were used for analysis of bone density. Sperm concentration and motility were evaluated for Subset 2 male rats. Rats that died or were sacrificed before scheduled termination were examined for the cause of death or condition on the day the observation was made and a gross necropsy was performed. When possible, blood samples were collected and tissues were weighed and retained, as previously described, for possible histopathological evaluation. Additional animals (30/sex/grp from 20 litters) were included for TK measurements on PNDs 14 and 49. Doses were said to be based on previously conducted toxicity studies in adult rats in which decreases in body weight gain, decreased white blood cell counts, increased liver, thyroid, adrenal and ovary weights and histopathology changes in the kidney and liver were observed at doses of 100 mg/kg/day or greater.

b. Results

i. Mortality, Clinical signs, Body weight

No T-R mortality occurred. Mortality that occurred was most often the result of intubation errors, with the number of deaths being similar across groups and the clinical signs consistent with accidental deaths. T-R clinical signs (decreased motor activity, limited use of both hindlimbs, impaired/lost righting reflex, ataxia, ptosis, cold to touch, apparent dehydration and urine-stained abdominal fur) were only observed during the first two weeks at the MD and HD. BW gain, BW, and feed consumption were unaffected by treatment in the TK groups, but in the main study animals, SS reductions in BW gain occurred transiently in HD males and throughout treatment in MD and HD females (**Table IVE.1.1**). Body weight gains in the affected groups rebounded during the postdosing period, and gestational BW gains in the mated females (Subset 2) and terminal BWs in both sexes were unaffected.

ii. Pre- and Post-weaning development

There were no T-R effects on reflex or morphological development.

iii. Behavioral testing

During the motor activity testing, statistically significant (SS) increases in numbers of movements were recorded for HD group subset 1 females during Blocks 5 and 6, which resulted in a SS increased total number of movements for the females in this dose group (**Table IVE.1.2**). This effect was not seen in the recovery (Subset 2) animals. Although not SS, a trend for increased acoustic startle response magnitude was seen in Subset 2 males and females (**Table IVE.1.3-4**). In the Morris water maze, the average time it took to reach the platform in trials 1 to 3, 4 to 6 and 7 to 11 during the postdosing testing session was increased in HD (Subset 2) females, resulting in an overall increased time to reach the platform for trials 1 to 11 in this group (**Table IVE.1.5**).

iv. Mating and fertility and pregnancy parameters

Reproductive performance and sperm parameters were unaffected by treatment.

v. Clinical Pathology and Necropsy

BUN levels were significantly reduced in the HD female on DG 13. However, no changes were noted in the ureters, kidneys or bladders (no gross lesions or microscopic findings) that would indicate malfunction of the urinary tract and the value for this parameter was within the historical control range. There were no other T-R effects on clinical pathology parameters. The ratio of thymus to terminal body weights was increased (SS) in MD and HD Subset 1 male rats and thyroid to terminal body weight ratio was significantly increased in HD Subset 1 males. These differences were considered possibly T-R in the report. Femur lengths were decreased (SS) in HD Subset 1 females (**Table IVE.1.6**). On PND 54, decreases in area, bone mineral content (BMC) and bone mineral density (BMD) were noted for all treated groups for the whole femur and at subregions of interest at all dosage levels relative to controls, reaching SS in HD females (**Figure IVE.1.1**). No consistent results were obtained for males or females assigned to Subset 2, suggesting the effects on bone density parameters are reversible after a drug-free period.

There were no T-R microscopic observations.

vi. Plasma drug levels

Mean concentration-time profiles for plasma CLB and N-CLB on PNDs 14 and 48 are shown in **Table IVE.1.7**. Increases for each were greater than the relative increases in dose and levels fell dramatically over the course of treatment such that profiles on PND 48 were not well characterized due to the fact that a large fraction of mean concentrations were below the quantitation limit.

c. Conclusions

Oral administration of CLB (4, 36, or 120 mg/kg) to rats during the juvenile period of development (postnatal days 14-48) resulted in adverse effects on growth (decreased bone length and density) and behavior (altered motor activity and auditory startle response, learning deficit) at the high dose. The plasma exposures (AUC) to CLB and its major active metabolite N-CLB were very low at the end of treatment, presumably due to development of metabolizing systems and/or induction.

**Table IVE.1.1 Body Weight Change**

**Males - Main Study**

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0	4	36	120
SUBSET 1					
RATS TESTED	N	20	20	20	20
BODY WEIGHT CHANGE (G)					
DAYS 14 - 21	MEAN±S.D.	+16.5 ± 2.8	+16.0 ± 4.7	+14.9 ± 3.6	+12.0 ± 4.1**
DAYS 21 - 28	MEAN±S.D.	+35.8 ± 3.8	+36.2 ± 5.3	+36.6 ± 5.3	+34.6 ± 5.2
DAYS 28 - 35	MEAN±S.D.	+56.9 ± 5.7	+57.1 ± 6.8 [ 19]b	+53.8 ± 7.4	+52.4 ± 6.6
DAYS 35 - 42	MEAN±S.D.	+63.0 ± 7.2	+65.2 ± 9.4 [ 19]b	+62.6 ± 10.3	+63.0 ± 9.0
DAYS 42 - 48	MEAN±S.D.	+47.8 ± 20.7	+54.0 ± 8.3 [ 19]b	+50.4 ± 10.2	+48.9 ± 7.3
DAYS 48 - 54	MEAN±S.D.	+59.1 ± 18.2	+63.0 ± 13.2 [ 18]b	+54.9 ± 9.0	+54.1 ± 9.0
DAYS 14 - 54	MEAN±S.D.	+279.1 ± 17.9	+291.9 ± 36.4 [ 18]b	+273.2 ± 32.4	+265.0 ± 28.4

**Females - Main Study**

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0	4	36	120
SUBSET 1					
RATS TESTED	N	20	20	20	20
BODY WEIGHT CHANGE (G)					
DAYS 14 - 21	MEAN±S.D.	+16.5 ± 3.9	+15.7 ± 3.0	+13.6 ± 2.4**	+10.5 ± 3.4**
DAYS 21 - 28	MEAN±S.D.	+33.8 ± 4.5	+32.6 ± 2.9	+33.1 ± 2.8	+30.7 ± 3.0**
DAYS 28 - 35	MEAN±S.D.	+44.2 ± 9.1	+45.1 ± 3.2 [ 19]b	+44.3 ± 3.6	+40.5 ± 4.3
DAYS 35 - 42	MEAN±S.D.	+41.6 ± 8.1	+40.3 ± 5.6 [ 19]b	+40.3 ± 2.9	+38.0 ± 5.7
DAYS 42 - 48	MEAN±S.D.	+28.7 ± 6.7	+25.3 ± 4.2 [ 19]b	+22.0 ± 8.8**	+22.3 ± 4.5**
DAYS 48 - 54	MEAN±S.D.	+28.3 ± 6.9	+27.1 ± 6.8 [ 19]b	+25.8 ± 7.0 [ 19]b	+21.8 ± 6.0**
DAYS 14 - 54	MEAN±S.D.	+193.2 ± 22.3	+186.4 ± 11.1 [ 19]b	+181.0 ± 16.0* [ 19]b	+163.9 ± 14.7**

DAYS = POSTNATAL DAYS

[ ] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on postnatal days 14 through 53 (subset 1) and on postnatal days 14 through 49 (subset 2).

b. Excludes values for rats that were found dead.

\* Significantly different from the control group value (p≤0.05).

\*\* Significantly different from the control group value (p≤0.01).

**Table IVE.1.2**

TABLE B20 (PAGE 1): MOTOR ACTIVITY - SUMMARY - F1 GENERATION FEMALE RATS - MAIN STUDY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0	4	36	120
SUBSET 1					
DURING THE FOURTH WEEK OF TREATMENT					
NUMBER OF RATS	N	20	19b	20	20
NUMBER OF MOVEMENTS					
BLOCK 1	MEAN ± S.D.	135.8 ± 15.9	142.4 ± 16.5	139.0 ± 14.9	139.5 ± 10.3
BLOCK 2	MEAN ± S.D.	140.4 ± 13.4	151.3 ± 18.3	149.7 ± 15.8	150.2 ± 10.5
BLOCK 3	MEAN ± S.D.	138.6 ± 17.2	134.1 ± 31.9	130.2 ± 41.1	150.7 ± 18.6
BLOCK 4	MEAN ± S.D.	121.4 ± 49.2	115.5 ± 53.4	128.7 ± 42.3	145.2 ± 31.8
BLOCK 5	MEAN ± S.D.	99.0 ± 53.9	114.3 ± 54.9	128.6 ± 45.9	138.3 ± 40.0*
BLOCK 6	MEAN ± S.D.	80.7 ± 62.6	120.2 ± 49.0*	122.0 ± 39.6*	126.5 ± 43.7*
TOTAL	MEAN ± S.D.	715.9 ± 140.0	777.6 ± 144.7	798.1 ± 165.2	850.2 ± 108.7**
TIME (SECONDS) SPENT IN MOVEMENT					
BLOCK 1	MEAN ± S.D.	354.7 ± 65.1	359.6 ± 49.9	368.3 ± 48.8	374.5 ± 55.8
BLOCK 2	MEAN ± S.D.	300.0 ± 62.2	304.7 ± 55.4	274.4 ± 48.0	313.1 ± 68.0
BLOCK 3	MEAN ± S.D.	245.9 ± 72.8	245.2 ± 97.8	223.1 ± 97.4	283.9 ± 60.3
BLOCK 4	MEAN ± S.D.	197.6 ± 105.3	183.6 ± 122.1	223.0 ± 96.5	235.3 ± 65.4
BLOCK 5	MEAN ± S.D.	166.4 ± 116.3	189.5 ± 113.2	222.7 ± 102.5	223.5 ± 86.4
BLOCK 6	MEAN ± S.D.	140.0 ± 125.1	207.6 ± 102.6	191.7 ± 87.5	198.9 ± 92.6
TOTAL	MEAN ± S.D.	1404.4 ± 396.3	1490.2 ± 410.9	1503.1 ± 370.2	1629.0 ± 290.8

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 10 MINUTE PERIOD.

a. Dosage occurred on postnatal days 14 through 53 (subset 1) and on postnatal days 14 through 49 (subset 2).

b. Excludes values for rat 3104, which was found dead on postnatal day 35.

\* Significantly different from the control group value (p≤0.05).

\*\* Significantly different from the control group value (p≤0.01).

**Table IV.E.1.3**

TABLE B21 (PAGE 1): ACOUSTIC STARTLE HABITUATION - SUMMARY - F1 GENERATION MALE RATS - MAIN STUDY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0	4	36	120
RESPONSE MAGNITUDE					
DURING THE FOURTH WEEK OF TREATMENT (SUBSET 1):					
NUMBER OF RATS	N	20	19 <sup>b</sup>	20	20
BLOCK 1	MEAN ± S.D.	31.63 ± 19.53	37.44 ± 32.24	28.66 ± 19.70	25.45 ± 17.65
BLOCK 2	MEAN ± S.D.	24.30 ± 27.04	26.96 ± 27.95	18.04 ± 16.88	15.63 ± 12.15
BLOCK 3	MEAN ± S.D.	18.68 ± 18.08	22.59 ± 24.44	15.19 ± 12.32	12.22 ± 8.24
BLOCK 4	MEAN ± S.D.	17.76 ± 17.02	22.53 ± 20.99	15.48 ± 12.87	13.73 ± 10.56
BLOCK 5	MEAN ± S.D.	17.01 ± 15.42	19.49 ± 21.09	15.79 ± 13.20	11.21 ± 8.82
AVERAGE	MEAN ± S.D.	21.870 ± 17.884	25.805 ± 24.336	18.620 ± 13.694	15.645 ± 9.841
POSTNATAL DAY 61 ± 2 (SUBSET 2):					
NUMBER OF RATS	N	20	20	20	19 <sup>b</sup>
BLOCK 1	MEAN ± S.D.	104.59 ± 71.18	106.85 ± 58.64	110.71 ± 67.36	117.78 ± 68.92
BLOCK 2	MEAN ± S.D.	60.49 ± 48.24	67.26 ± 38.54	72.66 ± 48.80	75.96 ± 60.11
BLOCK 3	MEAN ± S.D.	46.75 ± 43.08	55.50 ± 38.61	60.04 ± 35.42	56.05 ± 34.52
BLOCK 4	MEAN ± S.D.	40.31 ± 41.87	46.73 ± 34.14	52.22 ± 32.92	59.90 ± 45.66
BLOCK 5	MEAN ± S.D.	39.13 ± 39.92	37.89 ± 28.16	46.17 ± 29.10	51.50 ± 34.32
AVERAGE	MEAN ± S.D.	58.260 ± 44.157	62.855 ± 36.233	68.355 ± 36.576	72.242 ± 42.368

DATA WERE RECORDED IN GRAMS (G).

BLOCK = TEN CONSECUTIVE TRIALS

RESPONSE MAGNITUDE = PEAK RESPONSE - BASELINE RESPONSE

AVERAGE = AVERAGE RESPONSE MAGNITUDE FOR 5 BLOCKS (50 TRIALS)

a. Dosage occurred on postnatal days 14 through 53 (subset 1) and on postnatal days 14 through 49 (subset 2).

b. Excludes values for rats that were found dead or sacrificed due to adverse clinical observations.

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**Table IV.E.1.4**

TABLE B22 (PAGE 1): ACOUSTIC STARTLE HABITUATION - SUMMARY - F1 GENERATION FEMALE RATS - MAIN STUDY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0	4	36	120
RESPONSE MAGNITUDE					
DURING THE FOURTH WEEK OF TREATMENT (SUBSET 1):					
NUMBER OF RATS	N	20	19 <sup>b</sup>	20	20
BLOCK 1	MEAN ± S.D.	25.78 ± 13.79	29.36 ± 23.04	25.66 ± 13.18	30.85 ± 17.16
BLOCK 2	MEAN ± S.D.	14.34 ± 8.03	19.10 ± 17.00	16.22 ± 8.05	17.30 ± 12.14
BLOCK 3	MEAN ± S.D.	15.50 ± 10.78	20.72 ± 11.35	13.52 ± 8.12	12.50 ± 9.34
BLOCK 4	MEAN ± S.D.	15.48 ± 14.26	16.04 ± 13.44	13.32 ± 8.78	13.18 ± 8.57
BLOCK 5	MEAN ± S.D.	16.08 ± 17.43	16.90 ± 12.31	12.56 ± 11.70	15.84 ± 13.67
AVERAGE	MEAN ± S.D.	17.440 ± 10.595	20.426 ± 13.150	16.255 ± 8.094	17.930 ± 10.358
POSTNATAL DAY 61 ± 2 (SUBSET 2):					
NUMBER OF RATS	N	17 <sup>b</sup>	19 <sup>b</sup>	19 <sup>b</sup>	19 <sup>b</sup>
BLOCK 1	MEAN ± S.D.	48.51 ± 23.45	57.01 ± 33.95	56.62 ± 43.78	56.63 ± 33.20
BLOCK 2	MEAN ± S.D.	31.30 ± 18.26	30.67 ± 21.27	34.53 ± 30.74	35.92 ± 21.85
BLOCK 3	MEAN ± S.D.	28.89 ± 18.68	25.44 ± 18.18	28.33 ± 29.77	33.21 ± 22.71
BLOCK 4	MEAN ± S.D.	21.57 ± 21.29	23.35 ± 19.67	28.07 ± 30.96	28.63 ± 21.44
BLOCK 5	MEAN ± S.D.	25.58 ± 24.20	20.89 ± 19.01	26.98 ± 31.67	23.92 ± 14.83
AVERAGE	MEAN ± S.D.	31.165 ± 18.927	31.474 ± 19.725	34.895 ± 28.458	35.658 ± 20.523

DATA WERE RECORDED IN GRAMS (G).

BLOCK = TEN CONSECUTIVE TRIALS

RESPONSE MAGNITUDE = PEAK RESPONSE - BASELINE RESPONSE

AVERAGE = AVERAGE RESPONSE MAGNITUDE FOR 5 BLOCKS (50 TRIALS)

a. Dosage occurred on postnatal days 14 through 53 (subset 1) and on postnatal days 14 through 49 (subset 2).

b. Excludes values for rats that were found dead or sacrificed due to adverse clinical observations.

**Table IVE.1.5**

TABLE B26 (PAGE 2): MORRIS WATERMAZE - SUMMARY - F1 GENERATION FEMALE RATS - MAIN STUDY

SUBSET 2 a		I	II	III	IV
DOSAGE GROUP					
DOSAGE (MG/KG/DAY) <sup>b</sup>		0	4	36	120
<b>SESSION 1</b>					
NUMBER OF RATS	N	17c	19c	19c	19c
AVERAGE TRIALS 1-3	MEAN ± S.D.	35.69 ± 9.13	36.19 ± 7.90	35.56 ± 9.03	38.73 ± 7.54
AVERAGE TRIALS 4-6	MEAN ± S.D.	17.79 ± 10.18	29.09 ± 11.68**	21.83 ± 10.13	28.93 ± 12.00**
AVERAGE TRIALS 7-11	MEAN ± S.D.	13.64 ± 5.37	15.60 ± 6.74	20.40 ± 9.87	21.19 ± 13.50
AVERAGE TRIALS 1-11	MEAN ± S.D.	20.79 ± 4.75	24.89 ± 6.59	24.93 ± 7.15	28.08 ± 9.33**
PROBE TRIAL (%) <sup>d</sup>	MEAN ± S.D.	53.8 ± 17.4	45.4 ± 13.9	42.9 ± 13.1	41.3 ± 13.5
<b>SESSION 2</b>					
NUMBER OF RATS	N	17c	19c	19c	19c
AVERAGE TRIALS 1-3	MEAN ± S.D.	22.26 ± 12.73	15.33 ± 10.13	26.27 ± 10.90	23.17 ± 11.02
AVERAGE TRIALS 4-6	MEAN ± S.D.	11.14 ± 9.79	13.18 ± 10.03	14.02 ± 8.62	10.15 ± 6.69
AVERAGE TRIALS 7-11	MEAN ± S.D.	8.02 ± 6.68	8.64 ± 4.71	8.73 ± 4.78	9.81 ± 8.25
AVERAGE TRIALS 1-11	MEAN ± S.D.	12.76 ± 7.45	11.71 ± 6.70	14.94 ± 5.06	13.55 ± 6.10
PROBE TRIAL (%) <sup>d</sup>	MEAN ± S.D.	52.4 ± 10.9	52.5 ± 10.0	50.7 ± 13.3	59.5 ± 14.9
<b>SESSION 3</b>					
NUMBER OF RATS	N	17c	19c	19c	19c
AVERAGE TRIALS 1-3	MEAN ± S.D.	12.86 ± 9.61	12.22 ± 9.00	13.48 ± 11.11	14.95 ± 11.10
AVERAGE TRIALS 4-6	MEAN ± S.D.	7.41 ± 4.96	6.47 ± 4.93	9.50 ± 4.70	7.31 ± 3.31
AVERAGE TRIALS 7-11	MEAN ± S.D.	6.18 ± 3.07	5.28 ± 2.32	6.83 ± 3.12	6.58 ± 2.60
AVERAGE TRIALS 1-11	MEAN ± S.D.	8.34 ± 4.81	7.51 ± 4.29	9.39 ± 4.27	9.07 ± 4.12
PROBE TRIAL (%) <sup>d</sup>	MEAN ± S.D.	56.1 ± 13.1 [ 14]e	54.9 ± 15.1 [ 16]e	54.3 ± 11.7 [ 16]e	57.0 ± 14.3 [ 16]e

AVERAGE TRIALS = AVERAGE TIME (SECONDS) TO REACH THE PLATFORM FOR ALL RATS IN A GROUP FOR THE SPECIFIED TRIALS

[ ] = NUMBER OF VALUES AVERAGED

a. Age of the rats on the first day of testing ranged from postnatal day 68 through 83; testing occurred over a three day period for each rat.

b. Dosage occurred on postnatal days 14 through 53 (subset 1) and on postnatal days 14 through 49 (subset 2).

c. Excludes values for rats that were found dead or sacrificed due to adverse clinical observations.

d. Probe trial was recorded in seconds and reported as a percentage of time the rat spent in the goal quadrant.

e. Excludes values for rats that could not have the probe trial (%) data retrieved due to defective media.

\*\* Significantly different from the control group value (p<0.01).

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**Table IVE.1.6**

TABLE B40 (PAGE 1): FEMUR LENGTH - SUMMARY - F1 GENERATION FEMALE RATS - MAIN STUDY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0	4	36	120
<b>SUBSET 1</b>					
NUMBER OF RATS	N	20	20	20	20
INCLUDED IN ANALYSES	N	20	19b	19b	19c
RIGHT FEMUR (MM)	MEAN±S.D.	31.0 ± 0.7 [ 19]c	30.6 ± 1.0	30.6 ± 0.7 [ 18]c	30.0 ± 0.7**
LEFT FEMUR (MM)	MEAN±S.D.	30.7 ± 1.1	30.6 ± 0.9 [ 17]c	30.3 ± 0.8	29.9 ± 0.9**
<b>SUBSET 2</b>					
NUMBER OF RATS	N	20	20	20	20
INCLUDED IN ANALYSES	N	17b	19b	19b	19b
RIGHT FEMUR (MM)	MEAN±S.D.	35.4 ± 0.9	35.4 ± 1.1	35.0 ± 1.0	35.0 ± 0.8
LEFT FEMUR (MM)	MEAN±S.D.	35.4 ± 1.0	35.4 ± 1.0	35.1 ± 1.1	34.8 ± 1.0

[ ] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on postnatal days 14 through 53 (subset 1) and on postnatal days 14 through 49 (subset 2).

b. Excludes values for rats that were found dead or sacrificed due to adverse clinical observations.

c. Excludes values for rats that had femurs damaged (length affected).

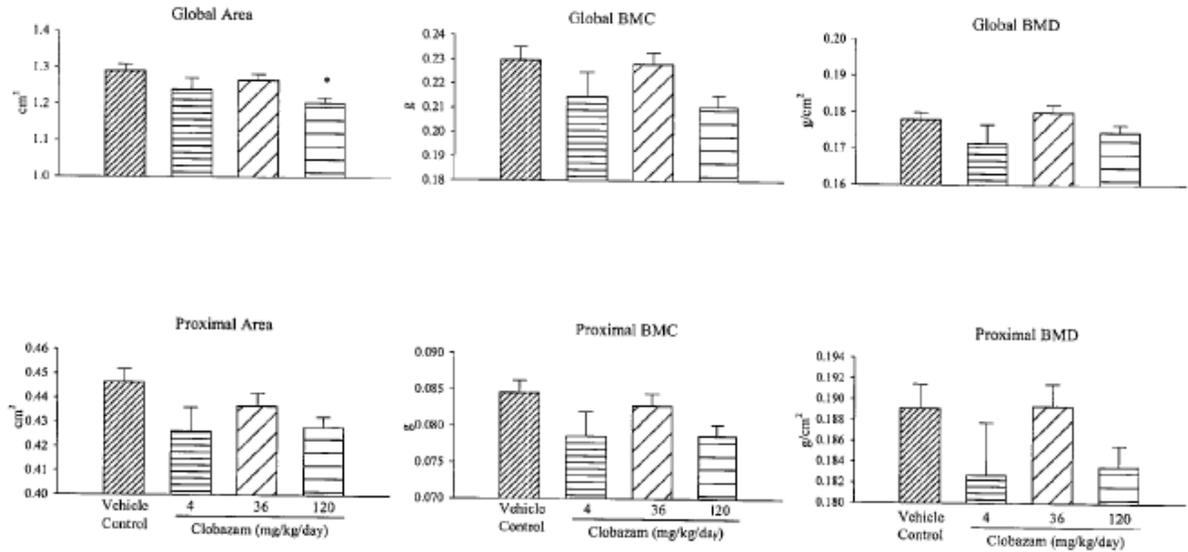
\*\* Significantly different from the control group value (p<0.01).

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**Figure IVE.1.1**

**Text Figure 3**

**Group Mean Bone Densitometry Values by Dual Energy X-ray Absorptiometry - Left Femur – Subset 1 Females - Ex Vivo**



Significantly different from Vehicle Control group value: \* -p<0.05 (Dunnett)

**Table IVE.1.7** Plasma levels of CLB and N-CLB in juvenile rats

Daily Dose (mg/kg):	0 (Control)		4		36		120	
No. of Animals: Toxicology	M: 40	F: 40	M: 40	F: 40	M: 40	F: 40	M: 40	F: 40
<b>Toxicokinetics:</b>								
No. of Toxicokinetic Animals	30	30	30	30	30	30	30	30
<b>Clobazam</b>								
<i>C</i> <sub>max</sub> (ng/mL)								
Postpartum Day 14	BQL <sup>b</sup>	BQL <sup>b</sup>	70.7	45.7	1350	1790	28000	9750
Postpartum Day 48	BQL <sup>b</sup>	BQL <sup>b</sup>	1.78	15.7	102	151	337	617
AUC (0-last) <sup>a</sup> (ng·hr/mL)								
Postpartum Day 14	BQL <sup>b</sup>	BQL <sup>b</sup>	300	459	6670	6920	217000	57200
Postpartum Day 48	BQL <sup>b</sup>	BQL <sup>b</sup>	0.89	32.7	51.0	75.5	756	1510
<b>N-Desmethyl Clobazam</b>								
<i>C</i> <sub>max</sub> (ng/mL)								
Postpartum Day 14	BQL <sup>b</sup>	BQL <sup>b</sup>	24.5	25.9	565	870	10200	11800
Postpartum Day 48	BQL <sup>b</sup>	BQL <sup>b</sup>	BQL	2.51	BQL	BQL	115	97.1
AUC (0-last) <sup>a</sup> (ng·hr/mL)								
Postpartum Day 14	BQL <sup>b</sup>	BQL <sup>b</sup>	128	184	3330	4000	156000	122000
Postpartum Day 48	BQL <sup>b</sup>	BQL <sup>b</sup>	BQL	1.26	BQL	BQL	57.5	48.6

## V. SUMMARY AND EVALUATION

### Pharmacology

In general, the mechanism of antiepileptic action of CLB (a 1,5-BDZ) is thought to be largely analogous to that of the 1,4-BDZs. This is supported by the results of the *in vitro* receptor binding screen, which demonstrated that CLB binding was limited to central and peripheral BDZ receptors as well as the GABA-gated chloride ion channel. Any differences between CLB and the 1,4-BDZs in terms of therapeutic efficacy and neurotoxicity are thought to be due to the variation in degree of agonist action at the high affinity BDZ receptor or to differing relative actions at the high and low affinity BDZ receptors (Meldrum and Chapman, *Epilepsia*, 27:S3–S13,1986). Modifications to the function of GABA as an inhibitory neurotransmitter underlie the pharmacological effects of the BDZs. Electrophysiologic studies have shown that BDZs potentiate GABA-ergic transmission at all levels of the neuroaxis. The changes induced by the interaction of GABA with its receptors are enhanced by BDZs, resulting in a decrease in the firing rate of neurons throughout the brain.

CLB was effective in preventing convulsions in various rodent models of chemically- or electroshock-induced convulsions (**Table IIA.1**). In these models, CLB ED<sub>50</sub> values (0.75 to 26 mg/kg p.o.) were similar to those of diazepam. CLB also demonstrated activity interpreted as anxiolytic in *in vivo* models in rat. Binding of N-CLB (N-desmethyclobazam) at the central BDZ receptor was similar to that of CLB (IC<sub>50</sub>s of 0.39 and 0.43  $\mu$ M for N-CLB and CLB, respectively), and when administered directly, N-CLB was active against PTZ-induced seizures in mice, although somewhat less potent than CLB (oral ED<sub>50</sub>s of 1.1 and 0.45 mg/kg for N-CLB and CLB, respectively). These data indicate that N-CLB contributes to the pharmacologic effects of CLB *in vivo*. The separation between doses associated with desired and untoward effects was greater for N-CLB than for CLB (**Table IIA.2**).

As with other BDZs, tolerance to the anticonvulant effects of CLB was observed in nonclinical seizure models (**Figures IIA.1-2**). According to the sponsor, “tolerance to CLB is not thought to be pharmacokinetically-based as maximum plasma concentrations of CLB and its pharmacologically active metabolite N-CLB in rats were similar following administration of a single oral dose or single daily oral doses for 10 days. As with other BDZs, tolerance to CLB is thought to primarily reflect pharmacodynamic (functional) tolerance resulting from a reduction in the allosteric interactions between GABA and the BDZ binding site of the GABAA receptor or down-regulation of the binding sites.” The PK data referred to by the sponsor do not apply, since the study in question only measured total radioactivity after a very low dose (0.57 mg/kg) of labeled drug. However, it is true that, in general, tolerance development to BDZs is thought to represent pharmacologic rather than pharmacokinetic tolerance. In a published study of these effects (Loscher and Schmidt, *Epilepsia* 47:1253-1284, 2006), the authors state, “In none of these experiments with BZD receptor ligands was tolerance associated with decreases in drug plasma levels.” On the other hand, marked decreases in plasma levels (attributed to enzyme induction but not demonstrated) were seen after repeated administration of CLB in the juvenile rat study and clinical signs disappeared after the first 2 weeks of dosing. In the 6-month rat general toxicity study (INCT-009; oral doses up to 400 mg/kg), clinical signs disappeared after between 2-4 weeks and liver weights were increased (no plasma level measurements). Plasma CLB and N-CLB levels also decreased along with clinical signs over the course of the 28-day TK study in dogs.

In a hERG assay both CLB and N-CLB displayed concentration-dependent inhibition of hERG currents, with estimated IC<sub>50</sub>s of approximately 296 and 110  $\mu$ M, respectively. According to the sponsor, based on the hERG assay alone, the inhibitory effects of CLB and N-CLB on I<sub>Kr</sub> suggest that if either compound alone or the two compounds in combination achieve free plasma concentrations in the range of 1 to 2.5  $\mu$ M ( $\geq$ 300 ng/mL), prolongation of the QT interval might be evident. However, in the rabbit Purkinje fiber study, both CLB and N-CLB were associated with minor shortening of the APD<sub>60</sub> and APD<sub>90</sub> consistent with activity at cardiac ion channels other than hERG. According to the sponsor, this may explain why changes in QT and QTc were not evident in the telemetered dog study as the activity of these compounds

at other ion channels may have mitigated their activity on IKr, resulting in no observable effect on cardiac conduction.

## ADME

Following administration of single or repeated oral doses of labeled CLB, oral absorption of total radioactivity was rapid and essentially complete in rats, dogs, and monkeys. The distribution of total radioactivity in rats and dogs was extensive in the liver and kidney in both species. Brain concentrations of total radioactivity were approximately 22% of plasma concentrations in rats and 60% to 75% of plasma concentrations in dogs. Negligible (<0.07%) or little residual radioactivity was present in tissues 14 days postdose. The *in vitro* binding to serum proteins was moderate. When the metabolism of CLB was assessed in rats, dogs, monkeys, and humans, N-dealkylation (N-CLB) represented the major metabolic pathway in all species (**Figure IIIB.2**). Direct hydroxylation was a minor pathway. The metabolites detected in human serum *in vivo* were M5, M7 and the metabolically active M9 (N-CLB). Only M9 represented greater than 10% of the CLB AUC<sub>0-24 hr</sub>, thus was the only major circulating metabolite. These metabolism studies indicated that there were no human unique metabolites. However, the only quantitative plasma metabolite data provided was for N-CLB in juvenile rats, dogs, monkeys, and humans. The majority of metabolites excreted in urine and feces were glucuronide conjugates of N-CLB and subsequent metabolic products in dogs and monkeys and sulfate conjugates of the same in rats. In rats, 98% of the administered dose was eliminated within 48 hours, and with both routes of administration, urinary and fecal excretion accounted for approximately 28% and 72%, respectively, of total radioactivity eliminated. In dogs, >98% of total radioactivity was eliminated within 3 days postdose. Urinary excretion of total radioactivity ranged from 73% to 78%, while fecal excretion accounted for 28% of the total radioactivity eliminated. Recovery of excreted radioactivity was incomplete in monkeys, limiting conclusions of the study, but urinary elimination was greater than fecal elimination. The only TK data provided in the submission are from a juvenile rat study and two 28-studies in the dog and monkey conducted in response to Division recommendations. These indicate that the animal toxicology studies provided little or no margin in terms of exposure to CLB or its major metabolite N-CLB (**Table IIIC.1**). The lack of TK data for adult rats is major deficiency in the nonclinical information provided for CLB.

## Toxicology

Rats received CLB orally for 28 days at doses of up to 1000 mg/kg; for 24 weeks at doses of up to 100 mg/kg; for 6 months at doses of up to 400 mg/kg/day; and for 18 months at doses of up to 600 mg/kg/day. Clinical signs were limited to transient sedation and piloerection at  $\geq 100$  mg/kg. A low incidence of mortality was observed in the 6-month study at  $\geq 100$  mg/kg/day, but there were no T-R deaths in the 18-month study at doses of up to 600 mg/kg. Body weight gain was reduced at 600 mg/kg in the 18-month study. Liver and thyroid weights were increased in all studies, and hepatocellular hypertrophy and thyroid atrophy (as evidenced by a decrease of the follicular colloid and cuboidal follicular cells) were noted in the 6-month study at  $\geq 100$  mg/kg/day. Increased eosinophilic granules and lipofuscin were noted in liver at the highest dose in the 18-month study (**Tables IVA.1.1-2**). The no observed adverse effect level (NOAEL) in the 18-month study was 35 mg/kg. Comparative TK data are not available in rats.

Dogs received CLB orally for 28 days at doses of up to 40 mg/kg; for 6 months at doses of up to 80 mg/kg; and for 1 year at doses of up to 40 mg/kg. Deaths were observed at  $\geq 20$  mg/kg/day in the 6-month study and at  $\geq 5$  mg/kg/day in the 1-year study. The cause(s) of deaths could not be determined but was thought to be related to withdrawal seizures. Clinical signs noted in all studies included somnolence, ataxia, and tremors; convulsions were observed in the 6-month and 1-year studies. Alkaline phosphatase was increased in the 6-month and 1-year studies, but histopathologic changes were limited to hepatocellular eosinophilic cytoplasmic inclusions in the 6-month study at 80 mg/kg. In the second 1-year study, the NOAEL was considered 5 mg/kg, although there was a slightly increased incidence of pigment accumulation in the liver and kidney (**Tables IVA.2.1-2**). Based on data from a separate 28-day GLP TK study recently conducted in beagle dogs, exposure to CLB at the highest doses in the 1-year study (40 mg/kg) is <0.1 that at the maximum recommended human daily dose (MRHD) of 40 mg, while exposure

to N-CLB is 2(F)-3(M)X that at the MRHD (**Tables IVA.2.3-4**). A 12-month oral toxicity study of N-CLB was also conducted in dogs, but only at a single dose of 40 mg/kg administered to only 1/sex. This study found evidence of withdrawal seizures, but no other toxicity was reported.

Monkeys received CLB for 10 weeks at oral doses of up to 20 mg/kg and for 1-year at oral doses of up to 20 mg/kg. In the 10 week study, T-R findings were limited to ataxia and tremors in 1 HD animal. In the 1-year study (2.5, 7.1, or 20 mg/kg), 1 MD male died during Week 38 and one HD male died during Week 17; ataxia and/or coma preceded death in both animals. Sedation occurred in all MD and HD animals during the treatment phase. Aggression and piloerection at  $\geq$  LD and restlessness at  $\geq$  MD occurred during the 3-day drug withdrawal phase that began 8 days prior to terminal necropsy. Withdrawal signs disappeared upon resuming dosing for the final 5 days of the study. There were no effects on body weight, food or water consumption, and no ophthalmic changes were noted. Significant decreases in heart rates of up to 20% compared to controls were seen at all doses. There were no T-R effects on hematology, clinical chemistry, or urinalysis parameters, and no noteworthy gross pathologic changes (**Table IVA.3.1**), but histopathology was only performed in animals that died. The NOAEL was determined to be 2.5 mg/kg/day. Systemic exposure to CLB and N-CLB was assessed in a 28-day non-GLP TK study in monkeys given a single oral dose of 2.5 or 20 mg/kg. Both HD males became moribund and were euthanized prior to scheduled termination. There were no apparent T-R gross findings, but histopathology was not conducted. TK parameters were determined from plasma CLB and N-CLB concentrations on Days 1, 14, and 28. Exposure to CLB at the highest dose (20 mg/kg) is  $<0.5$  that at the maximum recommended human daily dose (MRHD) of 40 mg, while exposure to N-CLB is 7 (F)-9(M)X that at the MRHD (**Tables IVA.3.2-3**).

#### Genetic Toxicology and Carcinogenicity

CLB and N-CLB were negative in valid Ames tests. In the *in vitro* chromosomal aberration study in CHO cells, CLB produced small but statistically significant increases in the percentage of cells with structural aberrations in the non-activated and activated 4-hour exposure groups (4.5 and 4%, respectively, compared to 0.5 and 0 in DMSO controls) at the highest concentrations tested (200 and 400  $\mu$ g/ml, associated with 58 and 31% cell growth inhibition) (**Table IVB.1**). However, based on pre-determined criteria, the response was considered negative (values fell within the historical control reference range of 0-5%). N-CLB was negative in the same assay. Both CLB and N-CLB were negative in the *in vivo* mouse micronucleus assay.

#### Mouse Carcinogenicity

CLB was administered in the diet to CD-1 mice at concentrations targeted to result in doses of 0, 4, 20, or 100 mg/kg for 80 weeks. No justification for dose selection was provided in the study report. In the non-clinical summary, the sponsor stated that the HD was "33% to 40% of the lowest single oral lethal doses in mice, and on a body surface area (mg/m<sup>2</sup>) basis, and was 8- to 12-times the maximum dose studied in Phase II/III controlled trials [10-times MRHD]." Due to a high rate of mortality, attributed primarily to injury associated with severe fighting, 43 HD males were replaced during the first 6 weeks of the study. Nine weeks after study initiation an additional 42 males were added and received 100 mg/kg/day for 80 weeks. These animals were said to be several days younger at initiation than the original animals, and apparently displayed fewer behavioral abnormalities on initiation of treatment, fought less, and as a result had improved survival relative to the original group of HD males, none of which survived to study termination. Although the sponsor attributed the HD mortality to fighting, females also showed a dose-related decrease in survival. Body weight gain and food consumption were unaffected by treatment in either sex. A D-R increase in the incidence of hepatocellular adenoma was observed in males when the added group of HD males was used (1/60, 3/60, 3/60, and 5/42 in C, LD, MD, and HD, respectively; **Table IVC.1.5**). Although the p-values were  $\leq 0.05$  for both the dose response and pair-wise comparisons, the FDA statistical reviewer (Min Min, Ph.D.) stated that "based on the criteria of adjustment for multiple testing of trends by Lin and Rahman, the incidence of none of the above [hepatomas] or any other tested tumor types in either sex was considered to have a statistically significant positive dose response relationship.

Also based on the criteria of Haseman, none of the pair-wise comparisons of treated groups with the control was considered to be statistically significant in either sex for increased tumor incidence in the treated group.”

Sex	Organ Name	Tumor Name	Cont	Low	Med	High	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
			0mg N= 60	4mg N=60	200mg N=60	100mg N=42				
Male	liver	adenoma, hepatocellu	1	3	3	5	0.020	0.337	0.317	0.041

Among HD female mice, there were fewer tumor-bearing mice than in controls due to a lower frequency of all tumors except pulmonary adenoma in this group. According to the report, the lower tumor incidences were due to the fact that mortality was significantly increased in both sexes at the HD (original HD males), so that the number of treatment weeks was too low for the full expression of the normal tumor frequency to occur, especially lymphoma. Dr. Min also stated that, “Based on the survival criterion Haseman proposed, it could be concluded that there were not enough mice in both sexes that were exposed to the high dose for a sufficient amount of time.”

In addition to its confounding effect on mortality, group housing would affect the reliability of dietary dosing, since food consumption was calculated per cage. This combined with the lack of TK data and failure to demonstrate stability of the drug in the diet makes the accuracy of dosing very questionable.

Because of the age of the mouse and rat carcinogenicity studies (final reports for these studies were issued in October, 1979, shortly after GLP regulations were issued [June 1979]), the sponsor provided an impact analysis of the deviations from GLP. According to the sponsor, “both studies were conducted by a reputable contract research laboratory in accordance with the state of the science at that time and are considered to represent scientifically sound and accurate presentations of valid data.” The following deviations from the current documentation and observational requirements were noted: study initiation date not provided; test article identity, confirmation of stability not performed; test article lots and expiration dates not provided; reserve sample was not taken; there was no indication of a Quality Assurance (QA) oversight; there was no QA statement; report not signed by study director; study protocol not available; protocol compliance cannot be assessed; method of animal identification was not provided; no description of circumstances that may have affected data quality or integrity; and no information regarding the location or archived specimens and data along with the final report.

In addition, due to the legacy nature of the studies, electronic tumor datasets (tumor.xpt) for these studies were not available and had to be created by the sponsor. Raw histopathology data from the respective appendices of each study report were data entered to create the tumor dataset for the CLB NDA. A description on how the mouse and rat tumor datasets were created and an updated statistical evaluation of survival and tumor incidence data from both studies using these datasets were provided in two additional reports. Both of these reports note the following:

The study report indicates that an extensive list of organs from all animals was examined at necropsy and saved in fixative. It further indicates that selected organs from all animals were examined microscopically while all other organs were only examined microscopically in a portion of the animals. The individual animal pathology tables in the study report only present organs in which macroscopic or microscopic findings were observed or organs that were missing or unsuitable for microscopic evaluation. For any organ that was present but for which no macro- or microscopic finding was observed, there is no statement in the report that the organ was examined or was without findings. Therefore, it is not possible to definitively determine the number and type of organs examined microscopically in each animal.

The report states that all organs were examined macroscopically at necropsy and any organ with a macroscopic finding that was considered to potentially represent a neoplastic change was

saved in fixative for microscopic examination. Therefore, it was assumed that any organ for which macroscopic and microscopic findings were not reported had no tumor. Although this assumption may not be true in all cases, it was considered unlikely that significant numbers of tumors could have been present in these organs that appeared macroscopically normal at necropsy.

This and the reduced number of tissues included originally (28 vs 50+ recommended) are major deficiencies impacting the adequacy of the study. It is not clear that adequate numbers of animals were exposed for an adequate length of time or that an adequate number of tissues in an adequate number of animals was examined histopathologically. Other problems include the high rate of mortality in the mouse study, such that the FDA statistician questioned the validity of the study; the uncertain contribution of age and the role fighting played in the mortality in male mice; questions about the comparability of the second HD male group; the lack of justification for dose selection; uncertainty about the accuracy of dosing given dietary administration with group housing, no drug stability, and no TK; and the deviations from the current documentation and observational requirements noted above.

### Rat Carcinogenicity

CLB was administered in the diet to S-D rats at doses of 0, 4, 20 or 100 mg/kg/day for 104 weeks. Treatment did not induce any notable clinical observations or T-R changes in mortality, although overall survival was low. There was a statistically significant (15%) decrease in body weight gain in HD females. A dose-related increased incidence of thyroid follicular cell adenomas was seen in male rats that was statistically significant at the HD in both the sponsor's and FDA analysis (**Table IVC.2.4**). The types, incidences, and distribution across treatment groups of other neoplastic changes, including malignant neoplasms, did not appear to be affected by treatment. The results of two mechanistic studies conducted in rats indicated that CLB can alter the pituitary-thyroid axis, presumably through enzyme induction, leading to changes in thyroid function and size in the rat.

Organ Name	Tumor Name	Cont N=60	Low N=60	Med N=60	High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
THYROID	ADENOMA, FOLLICULAR	3	2	5	15	0.000	0.803	0.355	0.002

There was no justification of dose selection in the report, and it is not clear that doses were adequate based on BW and survival effects. In addition, as in the mouse study, the potential effect of group housing on the accuracy of dietary dosing combined with the lack of TK and stability data present a serious problem for study adequacy. And as seen in the mouse study, there were significant deficiencies in the provision and/or reporting of data. The following deviations from the current requirements were noted: study initiation date not provided; test article identity, confirmation of stability not performed; test article lots and expiration dates not provided; reserve sample was not taken; there was no indication of a Quality Assurance (QA) oversight; there was no QA statement; report not signed by study director; study protocol not available; protocol compliance cannot be assessed; method of animal identification was not provided; no description of circumstances that may have affected data quality or integrity; and no information regarding the location or archived specimens and data along with the final report.

The rat tumor dataset report contained the same statement that “it is not possible to definitively determine the number and type of organs examined microscopically in each animal” and made the same assumption that “any organ for which macroscopic and microscopic findings were not reported had no tumor” as the mouse study report. This lack of documentation as well as the limited number of tissues included in the protocol constitute major deficiencies impacting the adequacy of the rat study.

The mouse and rat carcinogenicity studies of CLB were presented to the Exec-CAC; the committee concluded that neither study was adequate according to current study requirements due to the numerous deficiencies noted above (see CAC review and minutes dated 9/20/11). They also concluded that the increased incidence of thyroid tumors in CLB-treated rats was a drug-related finding.

The findings in mouse and rat carcinogenicity studies of CLB may be generally consistent with what has been reported for other BDZs, although any comparison would be tentative at this point given the insufficient data. For example, oxazepam, which is also a metabolite of both diazepam and chlordiazepoxide, produced markedly increased incidences of hepatocellular adenomas and carcinomas as well as smaller increases in thyroid follicular cell adenomas in mice in a 2-year NTP study (NTP, 1993; Griffen et al, *Fund Appl Tox* 29, 147-154, 1996). The mechanism is thought to involve phenobarbital-like effects including alteration of circulating thyroid hormone levels secondary to enzyme induction. The role of thyroid hormones in the induction of these rodent liver and thyroid tumors is important for human risk assessment, since it has been proposed that rodent tumors due to thyroid disturbances are of no relevance to humans because thyroid binding globulin acts as a buffer to maintain plasma thyroid hormone levels in humans (ibid). Based on mechanistic studies, CLB is thought to increase thyroxine clearance in male rats which produces decreased plasma thyroid hormones followed by compensatory increases in TSH biosynthesis and secretion (Miyawaki et al, *Toxicology Letters*, 145, 291-301, 2003). Chronic high levels of TSH then result in hypertrophic follicular cells which ultimately progress to neoplasms. However, while oxazepam produced clear increases in hepatocellular adenomas and carcinomas in mice, CLB only produced a trend for increased adenomas. And while thyroid tumors were seen in mice treated with oxazepam, in the NTP carcinogenicity study of oxazepam in rats, the only tumor finding was a small increase in the incidence of renal tubule adenomas (NTP 1998), indicating significant differences in tumorigenic response between this BDZ and CLB. In addition, the ability of diazepam to act as a strong tumor promoter in mouse liver in an *in vivo* mouse liver tumor promotion assay has been correlated with its inhibition of mouse hepatocyte gap junctional intercellular communication *in vitro*, suggesting that other mechanisms may be involved (Diwan et al, *Carcinogenesis* 10: 1719-1724, 1989). Another BDZ, clonazepam had no significant effects in either of these assays (ibid).

A recent survey of nonclinical genotoxicity and carcinogenicity data for BDZs found that the studies recommended by the current guidelines are not available for the majority of individual agents (Brambilla et al, *Pharmacol Res*, 56:443-458, 2007). Of the 51 drugs examined in this survey, 12 (23.5%) did not have retrievable genotoxicity or carcinogenicity data. Of the remaining 39 (76.5%) with at least one genotoxicity or carcinogenicity test result, 12 (30.8%) had at least one positive finding: 9 tested positive in at least one genotoxicity assay, 8 in at least one carcinogenicity assay, and 5 gave a positive result in both at least one genotoxicity assay and at least one carcinogenicity assay. 18 drugs had both genotoxicity and carcinogenicity data; of these 11 (61.1%) were neither genotoxic nor carcinogenic, 2 (11.1%) were carcinogenic in at least one sex of mice or rats but tested negative in genotoxicity assays, and 5 (27.8%) gave a positive response in at least one genotoxicity assay and in at least one carcinogenicity assay. Only 8 (19.5%) of the 41 marketed BDZs and BDZ analogs had all the data required by current guidelines for testing of pharmaceuticals. This highlights the need for better data for CLB.

### Reproductive and Developmental Toxicity

Like the carcinogenicity studies, the reproductive and developmental toxicity assessments conducted with CLB date back to the early 70's. According to the sponsor, they reflect the state of the science at the time. However, there are numerous deficiencies in the conduct and reporting of these studies, including dosing periods that do not cover the entire period of organogenesis, inadequate doses and lack of justification for dose selection, inadequate evaluation of some endpoints, e.g., neurobehavioral and immunological, and, again, specific information not documented or available. The findings reported do not indicate any potent teratogenic effects, but plasma levels are not available for rodents and the highest doses tested were often inadequate in terms of producing minimal maternal toxicity.

In the mouse fertility study, which was actually a combined “Segment 1, 2, and 3,” treatment of male and female mice with CLB (8, 40, or 200 mg/kg in the diet) prior to and during mating, gestation, and lactation produced no parental toxicity or adverse effects on fertility but resulted in markedly increased neonatal mortality as well as decreased BW in the offspring. A similar study in rats (approximately 3.49, 17.42, or 85.4 mg/kg in the diet prior to and during mating, gestation, and lactation) produced no parental toxicity or clear effects on fertility (single MD and HD animal failed to mate in each group) or offspring development, but the HD cannot be considered adequate.

In a mouse embryo-fetal development study in which dosing only covered part of organogenesis (oral gavage doses of 0, 6, 25, or 100 mg/kg from GD 7 to 12) increased incidences of external malformations (cleft palate) were seen at the HD in the absence of maternal toxicity (**Table IVD.3.1**). This study cannot be considered adequate due to the lack of documentation, inadequate HD, and the non-standard dosing period.

In a report that included data from embryo-fetal development studies in mice, rats, and rabbits (4, 20, or 100 mg/kg in all three species throughout organogenesis), developmental toxicity was seen in all three species (increased embryo-fetal mortality in mice and rabbits, decreased fetal BWs in rabbits, and increased incidences of skeletal variations in rats and rabbits), primarily at the HD, but there was insufficient information to assess maternal toxicity (among other things), so the adequacy of the high doses cannot be determined.

Another rat embryo-fetal development study was conducted at more appropriate doses (oral gavage doses of 25, 100, or 400 mg/kg) which produced maternal toxicity and slightly increased incidences of skeletal variations at HD, but the dosing period was only from GD 9-14, thus missing important events in organogenesis.

An old Segment III, peri- and postnatal study was submitted (oral gavage doses of 5, 32, or 200 mg/kg from GD 17 to PND 21) in which the HD produced slight maternal toxicity but there were no clear effects on offspring development. However, the offspring assessments were very minimal and incomplete and as with the other studies there was a lack of adequate documentation.

A juvenile rat study, conducted more recently according to Division recommendations (4, 36, or 120 mg/kg over postnatal days 14-48), resulted in adverse effects on growth (decreased bone length and density) at the end of treatment that seemed to reverse and behavioral changes (altered motor activity and auditory startle response, learning deficit) that seemed to be more persistent since they were seen after the recovery period (**Tables IVE.1.2-5**). Plasma exposures (AUC) to CLB and its major active metabolite N-CLB were very low at the end of treatment, presumably due to development or induction of metabolizing systems and/or induction (**Table IVE.1.7**). Since plasma levels were only measured at the beginning and end of treatment, it cannot be determined how long exposures were maintained.

Although the reproductive and developmental toxicity studies are inadequate according to current standards, they do provide information that indicates that the effects of CLB on development are consistent with what has been reported for other BDZs (see review of BDZ developmental toxicity dated 3/5/96). The CLB studies, which focused primarily on morphological development, found increased incidences of cleft palate in mice exposed during organogenesis, as has been reported for other agents such as diazepam. The significance of this effect in mice, which are known to be sensitive to the induction of cleft palate, is unclear and may be related to maternal stress, although this did not appear to be a confounding factor in the CLB studies. Overall, as with the BDZs in general, there was no evidence of a strong teratogenic potential for CLB in animals. However, as was true for the carcinogenicity data, the shortcomings of the CLB developmental toxicity studies are shared by other BDZs from the same era. Epidemiological data addressing the risk of malformations for children born to women treated with BDZs during pregnancy are inconsistent; initial indications of an increased incidence of facial clefts in the offspring of exposed mothers have not been confirmed by more recent studies (Wikner et al, *Pharmacoepidemiology and Drug Safety* 16: 1203–1210, 2007).

As discussed in the review, there is more evidence for long-term functional impairment associated with prenatal exposure to BDZs than for malformations. There is evidence that prenatal exposure to BDZs can cause behavioral dysfunction both in animals and humans. In addition, prolonged impairment of cellular immune functions was found in rats after low dose BDZ exposure (e.g., diazepam 1.25 mg/kg/day) during part of fetal life (GDs 14-20) (Schlumpf et al, Neurotoxicol 10:501-16, 1989). These endpoints were not evaluated in the CLB studies with gestational exposure, although there was some evidence of developmental neurotoxicity in the recent juvenile rat study, even with the very low exposures measured at the end of that study. Relatively few clinical studies detail the developmental outcome of children exposed to BDZs in utero. The evidence of neurobehavioral effects following prenatal BDZ exposure is largely anecdotal and is limited to effects, e.g., the “floppy baby syndrome,” seen soon after birth.

## VI. RECOMMENDATIONS

The nonclinical information provided in the application is inadequate to fully characterize the long-term effects of clobazam, particularly with respect to carcinogenic and developmental toxicity potential. However, given the extensive clinical experience with this drug and the clinical need, it is possible that approval could be based on human data. In that case, the nonclinical deficiencies should be addressed postmarketing.

cc:  
NDA (202-067)  
Div File  
HFD-120/FreedL/FisherE/SunS

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J.E. Fisher, Ph.D.

### Labeling Recommendations

#### 8.1 Pregnancy

Pregnancy Category C. There are no adequate and well-controlled studies of clobazam in pregnant women and no adequate developmental toxicity studies of clobazam in animals. (b) (4)

(b) (4) developmental toxicity, including increased incidences of fetal abnormalities, has been reported following oral administration of clobazam to pregnant animals at doses similar to those used clinically. (b) (4)

(b) (4) here have been reports of neonatal flaccidity, respiratory and feeding difficulties, and hypothermia in infants born to mothers who received benzodiazepines, including clobazam, late in pregnancy. In addition, infants born to mothers receiving benzodiazepines late in pregnancy may be at risk of experiencing withdrawal symptoms during the postnatal period. Therefore, Onfi should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

#### 8.4 Pediatric Use

The safety and effectiveness in patients under 2 years of age have not been established.

In a study in which clobazam (4, 36, or 120 mg/kg/day) was orally administered to rats during the juvenile period of development (postnatal days 14-48), adverse effects on growth (decreased bone length and density) and behavior (altered motor activity and auditory startle response, learning deficit) were observed

at the high dose. The no-effect level for juvenile toxicity (36 mg/kg/day) was associated with plasma exposures (AUC) to clobazam and its major active metabolite, N-desmethyl clobazam, less than those expected at therapeutic doses in pediatric patients.

#### 12.1 Mechanism of Action

The mechanism of action of clobazam, a 1,5-benzodiazepine, is not fully understood but is thought to involve potentiation of GABAergic neurotransmission resulting from binding at the benzodiazepine site of the GABA<sub>A</sub> receptor.

#### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

##### Carcinogenicity

The carcinogenic potential of clobazam has not been adequately assessed. In a study in rats, oral administration of clobazam doses of 4, 20, and 100 mg/kg/day for 2 years resulted in an increased incidence of thyroid follicular cell adenomas in males at the high dose.

##### Mutagenicity

[REDACTED] (b) (4)

##### Impairment of Fertility

There are no adequate studies of the effects of clobazam on fertility.

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/s/  
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J EDWARD FISHER  
10/14/2011

LOIS M FREED  
10/14/2011