1yr rat (Cont.)

Results:

Mortality: table below from the sponsor presents mortality incidence and time of death in all groups. A total of 5/30 rats died in the control gr, 8/30 in LD, 13/30 MD, and 16/30 in HD; all these deaths except as follows, occurred later in the study (wk 23 and up); 3HD rats died following the first dose. Two of the female control rats were mistakingly dosed with 50mg/kg on day347 and died within 24hr of dosing. Three of 30 rats each in treated groups were found dead on day196 shortly after ocular application of 1% atropine; additional studies failed to reveal any interaction between zaleplon and atropine.

Dose (mg/kg/day)	Time of Death (hours postdose)	Mortality Males	Mortality Females	Days of Death[a] Males	Days of Death[a] Females
0	0-4	1/15[b]	0/15	162	
	4-8	0/15	0/15		
	≥8	2/15[b]	2/15[c]	291,369	348(2)
5	0-4	5/15	2/15	168,196(2)[d], 213,325	184,196[d]
	4-8	0/15	0/15		
	≥8	0/15	1/15		363
20	0-4	6/15	2/15	176,196(3)[d], 258,349	74,96
	4-8	0/15	1/15		230[e]
	≥8	2/15	2/15	343,363	246,297
50	0-4	5/15	3/15	0(2), 196(2)[d], 216	141,196, 274
	4-8	1/15	1/15	204[e]	231
	≥8	1/15	5/15	263	0,61,122[e], 250,309

[a] Unless otherwise indicated, animals were found dead. When more than one animal died on the same day, the number of animals that died is shown in parentheses.
[b] Deaths attributed to underlying pathological conditions.
[c] These two controls were inadvertently dosed with 50 mg/kg on day 347.
[d] On Day 196, 1% atropine (a mydriatic) was applied followed by dosing. With the exception of one male at 5 mg/kg/day, animals that died on Day 196 were found dead prior to the ocular examination. Therefore, additional studies were subsequently conducted in rats to determine a potential interaction between CL 284,846 and atropine; results of these studies suggest no evidence of any apparent interaction between CL 284,846 and atropine.
[e] Sacrificed moribund.

Clinical signs: those of general CNS depression: ataxia, inactivity, and, hyperactivity occurred at all doses, prostration noted at ≥20mg/kg and, dose-dependent incr in convulsions seen at ≥20mg/kg grs. Convulsions started after d63 of dosing in both survivors and non-survivors. There were 1-33 episodes of convulsions and they occurred daily prior to dosing, this suggests withdrwal. Ataxia (4/7), inactivity (1/7), and prostration (1/7) were also seen in cont animals that were inadevertently dosed with 50mg/kg dose on study d347.

Mean B.wt & Food intake: except for a small but sig decr in mean wt gain in males of all drug grs between -1 to d6 of dosing, mean wt gain was increased in both sexes reaching statistical sig at some intervvals.. The changes in mena wt and wt gain correlated with changes in mean food intake in both sexes. Mean wt gain in males dosed 5&20mg/kg was comparable to the cont after study d6 whereas, the decr continued in males dosed 50mg/kg until d27. Mean wt gain was slightly but sig elevated in all male and female drug grs from d43-41 with the incr continued in HDf till end of study: all relative to the cont gr.

Ophthalmoscopy: eyes were examined predrug and at 6 and 12 months by focal illumination and indirect ophthalmoscopy. Atropine was used to produce mydriasis. Corneal opacity was noted in 1HDm and Panophthalmitis in 1HDf. Other findings were contributed to trauma from blood sampling. Therefore, except for the corneal retinopathy, there was no drug-related effects.
Hematology & Clin Chemistry: blood was sampled from the retro-orbital sinus on days 94, 185, & 366 from 10/sex/dose. There were no drug related findings. Some values reached statistical sig however, either they were not dose dependent, seen in a single sex, or due to large individual variations.
Urinalysis: the only drug effect is an apparent decr in specific gravity in males dosed 20&50mg/kg/d during the 182d of sampling. At the end of the yr, the decr was seen in both males and females of the 2 high doses with the decr being less in f. Since no other factors were affected such as electrolyte, this finding may not be of a tox significance.
TK: quantitation limit=5ng/ml HPLC

Dose (mg/kg/d)		Mean C_{max}±s.d. (ug/ml)			
		Parent		Des-ethyl metabolite	
		M	F	M	F
5	D0	0.7±0.01	1.0±0.25	0.40±0.02	0.51±0.2
	D366	1.8±0.3	2.6±0.6	1.3±0.3	1.1±0.2
20	D0	3.3±1.0	5.0±1.0	2.3±1.1	2.5±0.8
	D366	9±1.7	12.0±0.8	6.0±1.6	6.1±0.5
50	D0	7.0±1.0	10.4±5.0	7.5±0.3	5.6±0.6
	D366	15±2.1	37±NA	16.5±1.3	30±NA

NA only 1rat/time point/gr

APPEARS THIS WAY ON ORIGINAL

1yr rat (Cont.)

Organ wts: table below from sponsor:

	Males				Females			
Group:	1	2	3	4	1	2	3	4
Dose: (mg/kg/day)	0	5	20	50	0	5	20	50
No. Examined:	12	10	7	8	13	12	10	6
Liver:								
% Increase								
Absolute		5.8	23.0*	27.9*		4.0	26.2*	33.1*
Relative		-1.5	8.1	23.0*		4.3	8.9	23.8*
Kidneys:								
% Increase								
Absolute		2.3	21.0*	25.4*		-1.4	15.4*	25.7*
Relative		-4.4	5.6	19.2*		-2.0	-1.8	15.1
Adrenals:								
% Increase								
Absolute		0.2	8.3	17.0*		2.8	43.5*	56.4*
Relative		-4.5	-4.0	16.5		2.7	21.1	43.6*

* = Significantly different than control at p=0.05
- = % decrease

from the above table, it is clear that mean liver wt (absol & rel) in both sexes, incr dose-dependently with incr in dose. Mean absol and rel wt of kidneys also incr dose dependently in m&f. Similarly, mean wt of adrenals incr dose dependently in m&f (except a loss was seen in rel wt of the kidneys in males rel to the cont); all rel tot he corresponding cont values.
Gross & Histopath: the following gross findings in animals that were found dead or killed in moribund, were considered drug related: distended esophagus with remnants of ingested food, found in 67% of all drug animals that were found dead including the 2 female controls that were inadevertently given the 50mg/kg dose. This finding was seen in rats that were found dead on d0 as well as those found dead on d348. No drug related gross findings including esophageal distensions, were found in terminal-sacrifice animals.

The incr in liver wts were accompanied by mild hepatocyte centrilobular hypertrophy noted in 5m and 1m dosed 50mg/kg (6/14 survivors). No corresponding histopath findings were seen for the kidneys and the adrenals.

Summary & Conclusion:
Oral gavage administration of zaleplon to male and female rats for 1yr caused dose-dependent incr in mortality and clinical signs including convulsions. The convulsions were consistent with withdrwal syndrome since they occurred prior to daily dosing. No drug related effects were noted on clin chem, hematology or ophthalmoscopy. Mean wt gain in general was increased in both sexes and the incr corresponded to similar change in food intake. Mean liver, kidney, and adrenals wts were incr sig and dose dependently. However, only the incr in liver wt was accompanied with changes in histopath (hepatocyte hypertrophy). A **NOEL is <5mg/kg/d dose.**

1yr Dog tox study/study# 91028/MIRACL-25705/American Cyanamid Co., NY.

Route/Dose/Duration: oral caps/5, 20, 40mg/kg/d*/control group was administered the empty gel cap.
Species/strain/No./sex/gr: beagle dogs/5/sex/gr;

* the 40mg/kg dose was selected because it is expected to affect RBC parameters and incr liver wt based on a 3mo tox study. The 5mg/kg dose is a small multiple of proposed clinical dose.

The following parameters were assessed: mortality, clinical signs, B.wt, food intake, EKG, ophthalmoscopy, clin chem, hematology, urinalysis, TK, organ wt, gross, and histopath.

Results:
Mortality & Clinical signs: no animals died in the 5mg/kg gr or the cont. At 20mg/kg gr, a total of 3/10 were dead (2m were sacrificed on days 124&207 & 1f was found dead on d257), and at 40mg/kg gr, total of 5/10 were dead (2m found dead on days 81&203, and 2f found dead on days 150&293, 1f killed in moribund on d154). In all of these dogs except for 1, convulsions prior to next day dosing were seen and considered withdrwal, the eiths dog was found dead prior to daily observation.. Other clinical signs in all dose grs included CNS depression (ataxia, inactivity, prostration, sleeping, and eyes closed). Other signs included tremors, rigidity, aggressiveness, salivation, and soft/loose stool (this and salivation were seen in all 10/10 dogs in each dose rel to 2/10 & 9/10 in cont respectively).
B.wt. & Food intake: mean wt was decr in all drug grs 2wks postdose rel to the cont. Mean wt in males was decr in all 3 grs in a dose dependent manner after wk30 of dosing, and in females, a dose-dependent decr noted after wk5. In general, the mean wt was 11-16% less than the cont in the 40mg/kg gr and 8-12% less than the cont in 5&20mg/kg grs. The decr in wt persisted till end of study in 20&40mg/kg grs but was comparable to the cont in the 5mg/kg gr. The decr in wt did not correlate to changes in food intake. There was no drug effect on food intake in any gr.
EKG: done pre-dose and on study days: 160/161, 279, & 342. There were no sig drug related findings in any gr. In a single HDf, a 2nd-degree AV block was noted on a single occassion but considered normal variation and not drug related.
Ophthalmoscopy: both indirect ophthalmoscopy and focal illumination were done done pre-dose and on study days: 161, 273, and 343. There were no drug related findings.
Hematology, Clin Chem, Urinalysis: no drug related findings on any of these parameters. Blood was collected via jugular puncture predose and on days: 22/23, 84, 175, 275, and 358. Urine was collected predose and on days 177/178, 275/276, and 359/360. A single f dosed 20mg/kg had macrocytic anemia from d175 till end of study. This dog showed presence of macrocytes, MCV, and circulating metarubricytes. The sponsor dismissed this as a drug effect because it was seen only in this f dog and not in any HD gr dogs.

1yr dog (Cont.)

TK: was done for the parent and its des-ethyl metabolite, blood was collected on days: 0, 86, 176, & 356 between 1 and upto 24hr postdose; ql = 5ng/ml. Tables below from sponsor for parent and des-ethyl metabolite:

Parent drug:

		Day 0			Day 86			Day 176			Day 356		
Dose (mg/kg/day)	Sex	Cmax (ug/mL)	Tmax (hr)	AUC 0->24 (ug.hr/mL)	Cmax (ug/mL)	Tmax (hr)	AUC 0->24 (ug.hr/mL)	Cmax (ug/mL)	Tmax (hr)	AUC 0->24 (ug.hr/mL)	Cmax (ug/mL)	Tmax (hr)	AUC 0->24 (ug.hr/mL)
5	Male (CV%)	1.8 (22)	1	5.9 (32)	1.9 (11)	1-2	6.5 (8)	2.3 (7)	1-2	8.8 (13)	2.1 (13)	1	8.8 (18)
	Female (CV%)	1.7 (35)	1-2	6.2 (5)	2.9 (45)	1-2	11.6 (47)	1.5 (30)	1-2	6.3 (42)	1.9 (36)	1-2	6.7 (55)
20	Male (CV%)	4.0 (35)	1-4	33.3 (50)	8.0 (75)	2	43.1 (58)	8.1 (29)	2-4	50.4 (27)	7.3 (38)	2-4	52.1 (20)
	Female (CV%)	3.1 (16)	1-2	14.8 (42)	7.5 (32)	2	59.7 (9)	10.2 (18)	2	68.9 (18)	8.1 (20)	2-4	68.7 (22)
40	Male (CV%)	8.0 (51)	2-6	93.9 (60)	13.8 (23)	2-4	107.2 (10)	13.4 (53)	2-4	103.4 (41)	10.5 (55)	2	87.5 (47)
	Female (CV%)	9.5 (51)	4-8	122.2 (60)	19.2 (8)	2	131.9 (1)	15.2 (43)	2	127.5 (35)	16.0 NA	4	159 NA

Metabolite- CL-284-859

		Day 0			Day 86			Day 176			Day 356		
Dose (mg/kg/day)	Sex	Cmax (ug/mL)	Tmax (hr)	AUC 0->24 (ug.hr/mL)	Cmax (ug/mL)	Tmax (hr)	AUC 0->24 (ug.hr/mL)	Cmax (ug/mL)	Tmax (hr)	AUC 0->24 (ug.hr/mL)	Cmax (ug/mL)	Tmax (hr)	AUC 0->24 (ug.hr/mL)
5	Male (CV%)	0.7 (14)	2	2.8 (29)	0.6 (9)	2	2.6 (6)	0.7 (0)	2	3.6 (8)	0.6 (5)	2	3.7 (11)
	Female (CV%)	0.6 (33)	2-4	2.9 (7)	0.9 (44)	2	4.7 (47)	0.5 (40)	2	2.3 (30)	0.6 (27)	2-4	3.0 (50)
20	Male (CV%)	1.5 (53)	2-6	14.1 (60)	1.7 (83)	2-4	15.5 (48)	2.2 (14)	2-6	19.2 (16)	2.1 (34)	4	18.0 (18)
	Female (CV%)	1.1 (18)	2	8.7 (38)	2.05 (34)	2-6	28.3 (9)	2.5 (16)	2	32.1 (6)	2.2 (25)	4-6	21.8 (23)
40	Male (CV%)	2.5 (40)	4-6	33.9 (46)	3.8 (21)	4-6	40.1 (4)	3.5 (23)	4-6	37.4 (40)	2.8 (81)	2	29.9 (46)
	Female (CV%)	3.2 (25)	4-12	46.0 (28)	4.9 (11)	4	56.8 (9)	4.9 (57)	4-6	49.8 (27)	4.0 (NA)	4	53.6 (NA)

C_{max} = Maximun Drug Concentration
T_{max} = Time to reach C_{max}
AUC = Area under the plasma concentration time curve
* = Mean (CV%) of 3 dogs/sex/group, unless indicated otherwise
NA = Not Applicable (2 dogs/group)

1yr dog (Cont.)

were no drug effect on hematology, clin chem, ophthalmoscopy, urinalysis, or EKG. A sig incr noted in absol and rel wt of the liver and adrenals of 20&40mg/kg grs. No drug related histopath or gross findings in any drug gr. Plasma conc and exposure increased non-proportional with dose for both the parent and the metabolite (des-ethyl), no sex difference, and, no drug accumulation with repeated dosing. A NOEL for this study can not be determined due to clinical signs and B.wt changes at the lowest dose of 5mg/kg dose.

REPRODUCTIVE AND DEVELOPMENTAL TOX STUDIES:

Segment I - Fertility in rats using CL 284-846 orally (#LJT-1787 MIRACL-26300)/Study initiation Date: May 1992/ [redacted] / conducted under Japanese GLP guidelines.

Doses: **1,10, 100mg/kg/d oral gavage**; control received the vehicle (0.5% Na-CMC + 0.1% Tween 80. Dose selection was based on results of 1mo tox study in rats.
Strain/No./Sex/Dose: Sprague Dawley/30/sex/dose (vol. 10ml/kg).
Duration: male rats (6wks old) were dosed 9wks before the start of dosing and continuing till sacrifice (total of 14wks); females (11wks old) dosed 2wk prior to dosing and continuing till gd7.
Parameters assessed: for both sexes: clinical signs, mortality, B.wt, and food intake. Males: on d98 of dosing, rats were killed and examined grossly, organ wts were measured (heart, liver, kidneys, thymus, spleen, adrenals, testes, epididymides, seminal vesicles, and prostates). Females: estrous cycle (vaginal smears were examined for 2wks pre-dosing till successful copulation), mating (copulation index and fertility index; 1 each m+f placed together in a single cage for no more than 2wks), and necropsy: pregnant f were killed on gd20 detailed gross exam done and organ wts determined, standard reproductive parameters were assessed (# corpora lutea, # implantations, etc..). Fetuses: no., location in uterine horn, live/dead, early/late resorptions, B.wt, sex ratio, and 2/3 of fetuses per litter were fixed in ethanol for skeletal exam and the remaining 1/3 fixed in formalin for soft tissue exam.

Results:
Mortality: 2m dosed 100mg/kg (1 died after a single dose and the 2nd m died after the 2nd dose). Three HDf were found dead (2 died after a single dose and the 3rd after the 3rd dose). All deaths were considered drug related. Additional 1 MDf was found dead due to "technical error".
Clinical signs: in m & f: decreased activity, abnormal gait, decreased muscle tone (those 3 parameters were significantly increased in 10&100mg/kg; $p<0.05$), decreased grip strength, salivation, prone posture, and convulsions (these parameters were significantly increased in 100mg/kg dose group relative to the control; $p<0.05$).
B.wt & Food intake: both parameters were decreased in both sexes at various times during treatment relative to the vehicle control: sometimes reaching statistical significance ($p<0.05$). Mean wt was significantly and dose dependently decreased (6-10% less than the control) in MD&HDm for the periods 7-70 & 7-98 of dosing respectively. Mean food intake was also decreased and correlated with the decrease in mean wt in these 2 groups at 0-7, 7-14, & 14-21d for the MD and days 0-7 in HDm (5-6.6% less than control). Mean wt in females premating, was significantly decreased in MD & HD during d7&14 (5.7-6% less than control). Mean wt was also decreased in pregnant f during gd7-20 (6-7.6% less than control). Mean wt gain was significantly decreased in MDf during gd 2-3 and in HDf during gd3-4, 6-7, & 8-9. The decrease in mean wt gain in f ranged between 50g/d to no gain or, mean loss of 7g/d in HD during gd8-9. Mean food intake was significantly decreased in all f drug groups during gd0-7, in LD&MD during gd6-12 but increased in HD during gd12-20 (15% over the control); all relative to the vehicle control. The decrease in food in f ranged between 7-13.6% less than control.
Organ wt: In dams, absolute wt of the heart, kidneys, liver, and thymus in MD were

significantly ($p<0.05$) decreased relative to control, and relative wt of liver, kidney, and thymus in this group were also decreased in MD; no organ wt changes in non-pregnant dams. In males, the mean relative and absolute wt of the adrenals in HDm was significantly increased relative to the control and, both relative and absolute wts of liver in MD males were significantly less than those of the vehicle control; no other organ wt changes.

Reproductive Parameters: the drug had no effect on dam parameters i.e. estrus cycle, mating behavior, copulation/fertility indices, # corpora lutea, # of implantations, dead fetuses, or resorptions, in any drug group relative to the control. No drug related gross or histopath findings in any group. In males, presence of normal sperm in testes was checked in HD m that failed to fertilize f and normal sperms were seen in all of these m. There were no histopath findings in any organs including male reproductive organs. The fertility indices in m & f of HD were 79&78% respectively, which are lower than those in control m&f at 93% (did not reach statistical significance).

Effect on fetuses: mean fetal wt of m+f of MD were significantly higher than those of the control, but no drug effect noted on no. of fetuses or sex ratio. Viability indices were 90.6, 92, 93, & 94.7% in control, 1, 10, & 100mg/kg groups respectively, (viability in 100mg/kg reached statistical significance relative to the control).

External Exam: there were no significant differences between drug and control groups in external malformations. The frequencies of external malformations (e.g. atresia, cleft palate, kinky tail, edema, agnathia, & aglossia, etc.) were 1.0, 0.7, 0.5, and 1.2% in control and 3 drug groups respectively.

Visceral Exam: there were no significant differences between drug and control groups in visceral malformations, the frequencies were 3.4, 4.0, 3.0, and 1.2% in vehicle and drug groups respectively. A significant ($p<0.05$) increase in frequency of visceral variations was seen in MD fetuses as follows: 4.5, 13.3., 12.2, & 12.0% in vehicle and drug groups respectively, the corresponding no. of fetuses with variations was: 6, 18, 16, & 13 respectively. The variations that were present at higher frequency in the MD relative to the control are: thymic remnant in the neck, left umbilical artery, dilation of renal pelvis, and dilation of the ureter.

Skeletal Exam: there were no significant difference in skeletal malformations between drug groups and control, the frequencies of skeletal malformations were: 1.2, 0, 0.4, and 1.5% respectively. No significant difference beween drug grs and control regarding skeletal variations the frequencies were 0, 1.6, 3.6, and 1.5% respectively. However, the no. of ossified sacral and caudal vertebrae in MD reached statistical significance relative to the control (8 ± 0.8 vs. 7.4 ± 0.6 in MD & control respectively). The no. of ossified sternebrae was not significant between vehicle and drug grs.

Summary & Conclusion:

Oral administration of CL 284-846 at 1, 10, or 100mg/kg to male rats 9wks pre-mating and to females 2wk pre-mating and through gd7 caused death in HD males and females and clinical signs at $\geq$10mg/kg that included hypoactivity, abnormal gait. decreased muscle tone, decreased grip strength, salivation, prone posture. and convulsions. Mean wt and/or wt gain and food intake were decreased significantly in m & f (5.6-10% of control), at different periods of dosing sometimes, reaching statistical significance. In dams, absolute wt of the heart, kidneys, liver, and

thymus in MD were significantly ($p<0.05$) decreased relative to control, and relative wt of liver, kidney, and thymus in this group were also decreased in MD; no organ wt changes in non-pregnant dams. In males, the mean relative and absolute wt of the adrenals in HDm was significantly increased relative to the control and, both relative and absolute wts of liver in MD males were significantly less than those of the vehicle control; no other organ wt changes. In general, CL 284-846 had no significant effect on fetal parameters including external, visceral, and skeletal exams (malformations and variations) relative to the control. Some of the findings that reached statistical significance were small and not dose dependent. It was concluded that the **NOEL for the toxicity of the drug is 1mg/kg and the NOEL for the reproductive parameters in males and females and fetal development was 100mg/kg.**

In the above study, there were 2 mating trials for those males and females that did not copulate on the 1st try. Few males in HD failed to fertilize females but normal sperm was confirmed in all males. Also, histopath exam of a no. of tissues/organs including the testes, epididymides, seminal vesicles, and prostate did not reveal any unusal findings. The sponsor conducted another study (non-GLP; study# LJT2094/GTR 29668/study initiation date: Aug 94/Wyeth-Ayerst labs) to investigate the apparent lower fertility of male and female rats noted at the 100mg/kg.

Male SD rats (n=14-16) were orally dosed via gavage 0 or 100mg/kg CL 284-846 for 4wk- (exp1) or 9wk- (exp2) pre-mating (to un-treated f) and continued through mating and confirmation of pregnancy in f on gd13. The control group dosed with the vehicle.

Parameters assessed: mortality, clinical signs, B.wt, copulation/fertility indices, hormone assays (FSH, LH, and testosterone in 5m/gr), wts of selected organs, gross exam of thorasic and abdominal cavities, sperm head count, and histopath of selected organs/tissues (testis, epididymides, seminal vesicles, and prostate). The untreated f that were mated to these drug males were examined for # of corpora lutea, # of fetuses, viability index, location of fetus in the uterine horn, and implantation index.

Results:
Mortality: accidental death on d2 of dosing in 1 male of the 9wk dosing group; no other deaths.
Clinical signs: clonic convulsions in 6/15 m of 9wk gr after 44-76d of dosing.
B.wt: significant decrease in mean wt (4-9% of control) throughout the study of both periods.
Organ wt: small but significant increase noted in both periods in mean wts of the liver, kidneys, spleen, adrenals, and, seminal vesicles (wk4 only). These mean increases ranged between 9-16.7% over the control (except the wt of the adrenals increased 32% over the control).
Hormone level: significant increase in mean FSH was seen (160 ± 62ng/ml vs. 202 ± 74ng/ml in control at 9wk and at 4wk: 43 ± 10 in drug groups vs. 24 ± 2.5ng/ml in control); mean LH level was slightly decreased at 9wk period but unaffected at 4wk period. The testosterone level was elevated ~1.5x over the control at both periods relative to the control; no histopath findings in the testes.

Segment I male rat (Cont.)

Gross and Histopth.: no drug related findings.
Reproductive parameters: no drug effect on copulation and fertility indices (93-100% in control and drug group). No drug effect on time to copulation or other reproductive parameters.

Summary and Conclusion: oral gavage dosing of CL 284-846 to male rats for 4 or 9wks prior to mating to non-treated females and through mating and gd13 in pregnant f, did not cause death. Clinical signs included clonic convulsions, decrease in mean wt, and changes in organ wts without any gross or histopath findings. The drug had no effect on sperm count but hormone levels were affected.

- Male fertility study in rats using CL 284-846 orally (LJT# 2422/GTR#29936/Study initiation Date: Jul 1996/[redacted] conducted under Japanese GLP guidelines.

This study is similar to the above with regard to dose, design and objective. It was done to assess the apparent decrease in fertility seen in the Segment I rat study above (LJT1787). In the opinion of the reviewer, this and the above experiment are the same except for the GLP status and hormone levels were not assessed in this study. In addition, the results were the same as those in the non GLP study#LJT2094/GTR 29668.

Dose: **100mg/kg/d oral gavage**; control received the vehicle (0.5% Na-CMC + 0.1% Tween 80.
Strain/No./Sex/Dose: Sprague Dawley/20 (vol. 10ml/kg).
Duration: male rats (6wks old) were dosed 4wks before the start of dosing and continuing through mating to non treated f and till confirmation of pregnancy on gd14(total of 49-51d).
Parameters assessed: mortality, clinical signs, B.wt, food intake, copulation/fertility indices, wts of selected organs (testes, epididymides, seminal vesicles, and prostate), gross exam of thorasic and abdominal cavities, sperm head count, and histopath of selected organs/tissues (testis, epididymides, seminal vesicles, and prostate). The untreated f that were mated to these drug males were examined for # of corpora lutea, # of fetuses, viability index, location of fetus in the uterine horn, and implantation index.

Results:
Mortality: one male of drug group found dead on d1 of dosing; no other deaths.
Clinical signs: clonic convulsions in 1m on d51 of dosing. Other signs included decreased motor activity, abnormal gait, prone position, and salivation.
B.wt/Food intake: small but significant decrease in mean wt (4-12% of cont) from d3-49 of study. Mean food intake was slightly but significantly decreased 8-10% of control during 1st wk of dosing but increased on d35-38 & 42-49 of dosing. The sponsor suggested the decrease in wt during early dosing period was due to sedation and the latter increase was random variation since no corresponding B.wt change was seen. Pregnant f had normal wt and food intake irrespective of treatment given to their males.

Male Fertility in male rats (Cont.)

Wt of repro organs: absolute and not relative wt of the epididymides and prostate were significantly decreased compared to the control. Therefore, this decrease was related to the decrease in mean wt of these animals. Relative wt of the testis was significantly higher than that of the control, but no effect on absolute wt. This change was considered incidental and not related to the drug.
Gross and Histopth.: no drug related findings.
Repro parameters: no drug effect on copulation (100% drug and control), and fertility indices (90% control and 100% in drug group). Sperm head count was comparable in drug and control groups. No drug effect on any reproductive parameter in f.

Summary and Conclusion: oral gavage dosing of 100mg/kg CL 284-846 to male rats for a total of 49-51d to include time prior to mating to non-treated females, through mating, and gd14 in pregnant f, had no effect on male fertility under these experimental conditions.

- Female fertility study in rats using CL 284-846 orally (LJT#1944/GTR#29665/Study initiation Date: Aug 1993/[redacted] conducted under Japanese GLP guidelines.

Similar to the above 2 male fertility studies, the objective of this study was to address the cause of the lower f fertility noted in Seg I study (LJT1787).

Doses: **100&200mg/kg/d oral gavage**; control received the vehicle (0.5% Na-CMC + 0.1% Tween 80.
Strain/No./Sex/Dose: Sprague Dawley/10/dose (vol. 10ml/kg).
Duration: female rats (10wks old) were dosed for 14d before mating with untreated m and continuing through mating and till gd7.
Parameters assessed: mortality, clinical signs, B.wt, food & water intake, copulation/fertility indices, gross exam of thorasic and abdominal cavities (with detailed exam of uterus & ovaries), # corpora lutea, # of fetuses, fetal location in uterine horn, dead/live fetuses, # of resorptions, implantation sites, viability & mortality indices. Fetuses were weighed, sexed, and examined for external abnormalities.

Results:
Mortality: one f dosed 200mg/kg found dead on d2 of dosing; no other deaths.
Clinical signs: in both doses: decr motor activity, abnormal gait, and lacrimation. At 200mg/kg: low body temp, red gums, and prone position.
B.wt/Food & Water intake: small but sig incr noted in mean food, water intake, and B.wt in both dose grs at various periods of dosing starting during the premating period. These incr ranged between 7-8% for B.wt and 3-11% for food intake, rel to the cont.
Gross Exam: no drug findings.
Repro Parameters: the copulation index for cont and drug grs was 100%. The fertility index for

the cont was exceptionally low at 60% compared to historical cont range of 71-100%. The fertility indices for the 100&200mg/kg grs were 90&67% respectively. The fertility index of 90% in the 100mg/kg is higher than that of the concurrent cont and the value reported in the previous study (LJT1787) of 60&78% respectively.

Summary & Conclusion: CL 284-846 was orally administered at 100 & 200mg/kg to female rats during premating, throughout mating, and till gd7. The assessment of the drug effect on female fertility could not be determined in this study because the fertility index in the cont gr was unusually low.

- Female fertility study in rats using CL 284-846 orally (LJT#2407/GTR#29667Study initiation Date: Jun 1996/ Lederle - Japan/conducted under Japanese GLP guidelines.

Similar to the above male & female fertility studies, the objective of this study was to address the cause of the lower f fertility noted in Seg I study (LJT1787).

Doses: **100mg/kg/d oral gavage**; control received the vehicle (0.5% Na-CMC + 0.1% Tween 80.
Strain/No./Sex/Dose: Sprague Dawley/20/gr (vol. 10ml/kg).
Duration: female rats (11wks old) were dosed as follows (table from sponsor):

Group	Compound	Dose (mg/kg/day)	Route of dosing	Dosing volume (ml/kg)	Administration period	Number of females	Number of Dosing days
1	Vehicle	0	Oral	10	From 2 weeks before mating, through mating (2 weeks) and until day 7 of pregnancy	20	23~26
2	ZAL-846	100	Oral	10	From day 0 to day 7 of pregnancy	20	8
3	ZAL-846	100	Oral	10	From 2 weeks before mating until the day before copulation	20	1~19
4	ZAL-846	100	Oral	10	From 2 weeks before mating, through mating (2 weeks) and until day 7 of pregnancy	20	1~39

Vehicle : 0.5% Sodium carboxymethyl cellulose + 0.1% polysorbate 80 in water for injection.

Parameters assessed: mortality, clinical signs, B.wt, food intake, estrus cycle, copulation/fertility indices, gross exam of thorasic and abdominal cavities (with detailed exam of uterus & ovaries), # corpora lutea, # of fetuses, fetal location in uterine horn, dead/live fetuses, # of resorptions, implantation sites, viability & mortality indices. All fetuses were discarded without exam after determination of viability.

Results:
Mortality: 5/40 of gr3&4 were found dead after the 1st dose on d1; no other deaths in any gr. The cause of death in these rats was not reported.
Clinical signs: the usual clinical signs seen with CL 284-846 in other studies were also observed here during the 1st few days of dosing in all grs: decr motor activity, abnormal gait (seen till later dosing), abnormal posture, and decr muscle tone. In addition, some rats experienced the following signs: bradypnea, shivering, hypothermia, piloerection, and soiled perianal region. No clinical signs noted in the cont rats.
B.wt & Food intake: mean wt was sig decr in the drug grs rel to the cont but this decr did not always correspond to a comparable decr in food intake. Mean wt was sig decr in gr4 during d14 of dosing (pre-mating period)(4% of cont), no change in wt during pregnancy till gd9&10 where mean wt in this gr was sig decr ~6% rel to the cont. The only other decr in mean wt was in gr2 during gd9-14 (6.6-8.5% of cont). Mean food intake was not affected pre-pregnancy, but during

gd0-3, sig and dose-dependently decr recorded in gr2&3 (25&16% of cont), but sig incr in gr4 (16% over the cont). The only other decr in food intake was in gr2 (18-20% less than the cont), throughout gestation period except gd12-14.

Estrous Cycle: table below from sponsor presents the drug effect on estrus cycle:

Group	Compound and Dose (mg/kg/day)	No. of animals		No. of estrous stages	No. of estrous cycle			
					Length of estrous cycle (days)			
					4 and 5	Frequency (%)	Others	Frequency (%)
1	Vehicle 0	20	Total	52	49	94.2	3	5.8
2	ZAL-846 100	20	Total	52	51	98.1	1	1.9
3	ZAL-846 100	15	Total	34	26	76.5	8	23.5
4	ZAL-846 100	16	Total	34	28	82.4	6	17.6

CL 284-846 decr the cycle 76.5&82% in gr3&4 rel to that in the cont. The sponsor stated that since similar finding was not seen in other studies and the outcome of pregnancy in these grs in the present study was not affected, the decr in estrus length is considered incidental. This rational is unacceptable, and that this shortening of estrus is a drug related effect. The normal pregnancy outcome in this study has no direct impact on estrus cycle, since a change in estrus cycle clearly can affect the ability and the chance of getting pregnant.

Mating Performance: copulation index was 100% in all drug grs and the cont. Copulation occurred within 5d in almost all rats except in 1f in gr4 where it copulated on 2nd day of 2nd mating and became pregnant. Fertility index was 90% in cont and gr2 and 74&81% in gr3&4 respectively. The low fertility indices in grs3&4 were considered drug related.

Reproductive Parameters in Dams:

Summary of Reproductive Data of Dams on Day 14 of Pregnancy

Group	Compound and Dose (mg/kg/day)	No. of dams		No. of corpora lutea	No. of implants	Rate of implantation (%)	Live fetuses No. of fetuses	Viability (%)	Intrauterine deaths No. of deaths	Mortality (%)	Classification Implantation sites	Resorptions	Macerated fetuses
1	Vehicle 0	18	Total	345	311		295		16		0	16	0
			Mean	19.2	17.3	91.6	16.4	94.6	0.9	5.4	0	0.9	0
			±S.D.	3.4	1.7	10.3	2.2	6.1	1	6.1	0	1	0
2	ZAL-846 100	18	Total	314	274*		255		19		1	18	0
			Mean	17.4	15.2	88.0	14.2	93.2	1.1	6.8	0.1	1	0
			±S.D.	2.7	2.3	13.3	2.5	9.1	1.4	9.1	0.2	1.3	0
3	ZAL-846 100	14	Total	260	219		201		18		6	12	0
			Mean	18.6	15.6	81.8	14.4	89.4	1.3	10.6	0.4	0.9	0
			±S.D.	3.0	5.9	25.1	6.0	14.8	1.4	14.8	0.8	1.0	0
4	ZAL-846 100	13	Total	237	192		180		12		1	11	0
			Mean	18.2	14.8	80.1	13.8	90.6	0.9	9.4	0.1	0.8	0
			±S.D.	2.7	4.6	21.9	5.0	13.4	0.8	13.4	0.3	0.8	0

From the above table, the total # of implants was decr dose dependently (sig only in gr2) rel to the cont. Rate of implantation was decr though not sig in gr3&4 but this decline was considered "not consistent with findings in previous studies". Fetal mortality seemed incr over the cont in grs3&4 with large s.d.

Summary & Conclusion: oral administration of CL 284-846 to female rats at specific periods of premating, through mating, and during early gestation caused death in 5/40 rats and clinical signs. Mean wt was sig decr in some rats during mating and/or gestation; some decr in food intake was also seen but did not correlate with decr mean wt. CL 284-846 shortened estrus cycle in grs3&4 and decr fertility index in these gr (74&81% respectively)(drug dosed 2wk premating and 2wks premating, through mating and till gd7, respectively) rel to the cont. Total # of implantations was decr dose dependently in all drug grs reaching sig only in gr2 and rate of implantation was decr though not sig in grs3&4, all rel to the cont. It is concluded that CL 284-846 affects dam fertility index and ability to get pregnant (change in estrus cycle) in addition, some effect may be on fetal mortality though the variability in the mean was too high to make a clear conclusion. It is noted that these drug effects are occurring at maternally toxic doses (death in this dose of 100mg/kg).

APPEARS THIS WAY ON ORIGINAL

- Segment II Teratology - Developmental tox - embryo/fetal tox and teratogenic potential study in rats including behavioral postnatal evaluation using CL 284-846 orally via gavage (study#91146/Study initiation Date: Oct 1991/ [redacted]

Doses: **1, 10, & 100mg/kg/d oral gavage***; control received the vehicle (0.5% Na-CMC + 0.1% Tween 80; (vol. 10ml/kg).
* actual doses administered based on analysis of test article activity done after end of in-life portion of the study, were 1.03, 10.26. & 102.6mg/kg/d based on 99.5% activity whereas, that determined at beginning of study was 96.9%.

Strain/No./Sex/Dose: Sprague Dawley/35/dose F0 generatrion.
Duration: pregnant f rats (10wks old) were dosed during gd6-17. Twenty three dams were killled on gd20 and the remainig 12/gr were allowed to deliver* and rear till ppd21 at which day dams were killed. Dams that did not deliver by gd25 were killed.

* F1 generation litters were culled to 8 pups (4/sex) per litter on ppd7. On ppd21, a minimum of 24 rats per sex per gr were selected to continue the study and observed beginning on ppd22 for B.wt, feed, survival, growth, and sexual maturation. Starting on ppd23-25, 1/sex/litter of the 24 rats, were tested for 2 behavioral tests: passive avoidance and water maze (the latter was tested at 69-71d of age). At 86-91 days of age, F1 m+f were randomly assigned and cohabited. Following the 21d cohabitation, F1 males were killed and necropsied with testes and epididymides weighed and preserved in formalin for possible evaluation. F1 females, were killed on gd20 and F2 fetuses examined externally.
Parameters assessed: mortality, clinical signs, B.wt, food intake, standard repro indices, gross exam, fetal exams*. Behavioral evaluation was done on F1 generation.

* ½ of fetuses of F0 per litter were examined for soft tissue alterations and the other ½ examined for skeletal changes (stained with alizarin red).

Results:

F0

Mortality: a single f in 100mg/kg dose was killed in moribund on gd17 and a 2nd dam was found dead on d4 of lactation. The first dam showed decr motor activity on gd6, impaired righting reflex, and ataxia on gd6-17, and few days before sacrifice, lost righting reflex, muscle falccidity, decr motor activity, salivation, labored breathing, ptosis, and lacrimation. In addition, this dam lost wt and ate little starting on gd15 till sacrifice on gd17. Necropsy showed distended bladder with urine, blood clot in trigone area of the bladder, and a cyst in the r.ovary. the litter had 15 dead fetuses with 1 later resorption. The dam that was found dead on d4 of lactation was ataxic on gd6-17. decr motor activity on gd16&17, no effect on wt or food intake and no gross findings. The litter of this dam had 16 liveborn pups that were killed after the dam was found dead.

Clinical signs: the following noted in 10&100mg/kg grs: ataxia, decr motor activity, impaired and/or loss of righting reflex; severity of these effects incr with dose. Hyperactivity was seen in 0, 2, 6, and 3 rats in cont, 1, 10, and 100mg/kg grs respectively. No clinical signs were seen in any gr during lactation.

B.wt & Feed: maternal mean wt, wt gain, and food intake were sig decr at various times during and after dosing in 10&100mg/kg dose grs. A sig decr in maternal mean wt was seen in 10mg/kg gr during gd7-20 and a loss after the 1st 2 doses and in the 100mg/kg gr, there was a mean wt loss during gd6-7 & a sig decr in wt on gd7. In addition, in 10mg/kg gr, wt gain was also sig decr during gd8-9 & 9-12 which lead to overall decr in wt for the entire dosing period (viewed as gd6-18, 6-20, and 0-20). In the 100mg/kg gr, aside from the sig decr mentioned above, mean wt gain was sig incr on gd7-8 and was comparable to cont for the rest of the dosing period. The sig loss in mean wt in 100mg/kg gr reflected the sig decr in mean fetal wt in this gr.

Dams wt were also sig and dose-dependently decr in the 10&100mg/kg during d1 of lactation. After ppd1, mean wts were comparable among all drug and cont grs; a single time (ppd5) decr ($p \leq 0.05$) in mean wt of 10mg/kg dams, was considered incidental; all other changes in wt, wt gain, and feed are considered drug related.

The changes in wt of 10&100mg/kg grs, were paralleled by decr in absol and rel food intake (g/d and g/kg/d respectively) in these grs. Both of these parameters were decr throughout the dosing period in the 10mg/kg gr being most remarkable at the beginning of dosing. In the 100mg/kg gr, both parameters were sig decr after the dosing period. No food intake changes in any gr during lactation.

Repro Parameter in Dams: the results were based on observations from 21, 23, 20, 20 pregnant dams in cont, 1, 10, and 100mg/kg grs respectively. Table below from sponsor, These results indicate no drug effects on any repro parameters of dams.

CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0(VEHICLE)	II 1	III 10	IV 100
RATS TESTED	N	35	35	35	35
PREGNANT	N(%)	32(91.4)	33(94.3)	33(94.3)	31(88.6)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 20 OF GESTATION	N	21	23	20	20
CORPORA LUTEA	MEAN±S.D.	18.6 ± 2.2	18.5 ± 2.1	17.8 ± 3.6	17.8 ± 1.6
IMPLANTATIONS	MEAN±S.D.	16.9 ± 2.9	16.7 ± 1.9	15.8 ± 3.6	16.7 ± 1.9
LITTER SIZES	MEAN±S.D.	15.4 ± 2.6	15.9 ± 2.2	14.4 ± 3.5	15.4 ± 1.6
LIVE FETUSES	N MEAN±S.D.	323 15.4 ± 2.6	365 15.9 ± 2.2	288 14.4 ± 3.5	308 15.4 ± 1.6
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	1.5 ± 1.6	0.8 ± 0.9	1.1 ± 1.2	1.3 ± 1.3
EARLY RESORPTIONS	N MEAN±S.D.	31 1.5 ± 1.6	19 0.8 ± 0.9	21 1.0 ± 1.3	24 1.2 ± 1.3
LATE RESORPTIONS	N MEAN±S.D.	0 0.0 ± 0.0	0 0.0 ± 0.0	1 0.0 ± 0.2	2 0.1 ± 0.3
DAMS WITH ANY RESORPTIONS	N(%)	15(71.4)	10(60.9)	12(60.0)	14(70.0)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	21(100.0)	23(100.0)	20(100.0)	20(100.0)

a. Dosage occurred on days 6 through 17 of gestation.

Table below from the sponsor presents data for the litter:

LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY - F1 GENERATION LITTERS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	1	10	100
LITTERS WITH ONE OR MORE LIVE FETUSES	N	21	23	20	20
IMPLANTATIONS	MEAN±S.D.	16.9 ± 2.5	15.7 ± 1.9	15.5 ± 3.6	16.7 ± 1.5
LIVE FETUSES	N	323	365	288	308
	MEAN±S.D.	15.4 ± 2.6	15.9 ± 2.2	14.4 ± 3.5	15.4 ± 1.6
LIVE MALE FETUSES	N	169	170	156	143
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	52.4 ± 13.1	46.6 ± 13.0	54.0 ± 18.7	46.5 ± 12.6
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	3.61 ± 0.22	3.53 ± 0.32	3.66 ± 0.23	3.01 ± 0.16**
MALE FETUSES	MEAN±S.D.	3.71 ± 0.22	3.68 ± 0.35	3.72 ± 0.24	3.13 ± 0.16**
FEMALE FETUSES	MEAN±S.D.	3.50 ± 0.23	3.43 ± 0.31	3.35 ± 0.23	2.92 ± 0.16**
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	8.8 ± 9.8	5.1 ± 5.7	6.8 ± 7.8	7.6 ± 7.4

a. Dosage occurred on days 6 through 17 of gestation.
** Significantly different from the vehicle control group value (P≤0.01).

From the above table and as indicated above, mean fetal wt was sig decr in 100mg/kg gr rel to that of the cont (15.6-17% less than the cont); there were no other effects.

Fetal Findings: CL 284-846 at 100mg/kg caused a sig incr in litters with fetuses with alterations, in fetuses with *any* alterations and, the % of fetuses with *any* alterations, rel to the cont. (Table below from sponsor):

FETAL ALTERATIONS - SUMMARY - F1 GENERATION LITTERS/FETUSES

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	1	10	100
Litters Evaluated	N	21	23	20	20
Fetuses Evaluated	N	323	365	288	308
Live Fetuses	N	323	365	288	308
Dead Fetuses	N	0	0	0	0
Litters with Fetuses with any Alteration Observed	N(I)	9(42.8)	10(43.5)	5(25.0)	15(75.0)**
Fetuses with any Alteration Observed	N(I)	15(4.6)	22(6.0)	10(3.5)	33(10.7)**
% Fetuses with any Alteration/Litter	X±S.D.	6.47 ± 8.05	5.84 ± 10.04	3.15 ± 6.13	11.30 ± 10.35*

a. Dosage occurred on days 6 through 17 of gestation.
* Significantly different from the vehicle control group value (P≤0.05).
** Significantly different from the vehicle control group value (P≤0.01).

The sig alterations in the 100mg/kg gr in litters and fetuses were delayed ossification of ribs, sternum, caudal vertebrae, metacarpals, and hindpaw phalanges (see detail below).

Ossification size/fetus/litter (Values are means±s.d.); doses are in mg/kg/d

	Vehicle	1	10	100
caudal vertebrae	5.11±0.5	4.72±0.4*	5.12±0.4	4.4±0.3*
Sternal cntr	4±0.2	3.7±0.4	4±0.2	3.3±0.4*
Metacarpals	3.6±0.3	3.5±0.4	3.6±0.3	3.2±0.2*
Phalanges	5±0	5±0	5±0	4.9±0.*

* $p \leq 0.05$ or 0.01

There were 21, 23, 20, and 20 litters examined and 166, 190, 149, and 161 fetuses examined in cont, 1, 10, and 100mg/kg respectively.

F1

CL 284-846 caused no deaths, clinical signs, or necropsy findings upto 100mg/kg dose. A decr (sometimes sig) was observed in absol wt of testes and epididymides of 100mg/kg m; no effect on rel wt, therefore, this finding was considered a result of the sig decr in terminal mean wt in this gr. The drug caused a sig decr in mean wt and wt gain in males dosed 100mg/kg from postweaning d36 till cohabitation for the B.wt and, wt gain was sig decr during the entire postweaning, entire growth period (postweaning d1 - till cohabitation begun), and several postweaning days (the decr ranged between 4-9% less than the cont). There was no drug effect on wt in females.
Mean rel food intake (g/kg/d) in m dosed 100mg/kg was sig incr during d1-64 postweaning and the absol feed (g/d) were unaffected by the drug in any gr. Food intake was also sig incr in f treated with the drug including low dose gr. However, the incr in feed in 1mg/kg was not considered drug related since only a single value of absol feed was sig and the incr in rel feed was within 5% of cont. The sig incr in absol and rel feed in 10&100mg/kg grs recorded through the entire postweaning period d1-64 and they were dose dependent. Absol and rel feed intake continued to incr sig in 100mg/kg F1 gestating females rel to the cont throughout gestation or during specific days of gestation.

CL 284-846 had no effect on physical, sexual maturation (assessed as age at testes descent & preputial separation, age at vaginal patency), behavior (learning, short- and long-term memory retention), mating and fertility, or c-section observation, and no gross external malformation in F2 generation.

Summary and Conclusion: oral gavage administration of Cl 284-846 to pregnant f rats during gd6-17 caused no malformations in rats upto 100mg/kg/d dose and no effect in F1 generation on behavior or standard repro parameters and no gross external findings in F2 generation. The 100mg/kg dose represents 588 fold higher than the propsed clinical dose of 10mg/d on a mg/kg basis or 95 folds on a mg/m^2 basis.

- Segment II Teratology - Developmental tox - embryo/fetal tox and teratogenic potential study in New Zealand white rabbits using CL 284-846 administered orally via gavage (study#91147/Study initiation Date: Nov 1991/ [redacted]

Doses: **2, 10, & 50mg/kg/d oral gavage***; control received the vehicle (0.5% Na-CMC + 0.1% Tween 80; (vol. 10ml/kg).

* actual doses administered based on analysis of test article activity done after end of in-life portion of the study at 99.5%, were 2.05, 10.26, 51.3mg/kg/d whereas, doses determined at beginning of study were based on 96.9% activity.

Strain/No./Sex/Dose: New zealand white rabbits/20/dose.
Duration: pregnant f rabbits (3-3.9kg) were dosed during gd6-18.
Parameters assessed: mortality, clinical signs, B.wt, food intake, standard repro indices (implantation sites and #, # corpora lutea, early/late resorptions, live/dead fetuses, gross exam, & fetal exams (wt, external exam, visceral/skeletal exam, live/dead, & sex ratio) .

Rabbits were killed on gd29 and gross necropsy of abdominal and thorasic cavities was done. Any lesions were retained in buffered formalin for examination, all other tissues discarded.

Rabbits were artificially inseminated 3hrs after injection of 20USP units of HCG (6 million spermatozoa/0.25ml saline). The spermatozoa were obtained from 3 proven male breeders. The day of insemination was considered d0 of presumed gestation. Artificial insemination was done once on 1 of 3 consecutive days.

Results:
Mortality: no deaths in any gr and no premature deliveries. There were 2 cont does that aborted. One dam aborted on gd21: red material in cage was seen on gd20, this doe lost wt and had reduced food intake after gd8, there were 5 early resorptions. The 2nd doe, aborted on gd20, it did not show remarkable change in B.wt or food intake and it had 6 early resorptins; no gross findings in either doe.
Clinical Signs: the only sign in LD was dry feces in a single doe on gd19-21. Several does in MD&HD showed ataxia, decr motor activity, dried feces, green urine, and impaired/loss of righting reflex.
B.wt & Food Intake: both parameters were sig reduced in MD&HD throughout the entire dosing period (gd6-19), the decr ranged between . Mean wt loss and/or decr wt gain was

seen during various intervals of the dosing period in these 2 dose grs. During gd19-29 (postdose period), maternal mean wt gain and rel feed were sig incr in MD but these isolated findings were considered incidental and unrelated to the drug. In general, mean wt gain and feed intake were sig reduced in MD&HD during dosing period that they led to a sig decr in these 2 parameters in these 2 grs during gd6-29 & 0-29. Mean wts were sig decr in MD&HD during gd11-28. The decr in mean wt and/or wt gain in the MD&HD were paralled by a decr in absol (g/d) and rel (g/kg/d) food intake during the entire dosing period (gd6-19) and intervals within the dosing period. There was no effect in LD gr.

Repro Parameters: tables below from sponsor presents c-section observations for does and fetuses, the mean litter late resorptions was incr in MD&HD rel to the cont, however, the values were within historical range from this lab (data provided) and such finding was not observed in a pilot study done by the lab at doses of 30&100mg/kg. Therefore, this effect was not considered to be drug related. Table below from sponsor:

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	3	10	30
RABBITS TESTED	N	20	20	20	20
PREGNANT	N(%)	17(85.0)	18(90.0)	20(100.0)	18(90.0)
ABORTED	N(%)	2(11.8)	0(0.0)	0(0.0)	0(0.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	15	18	20	18
CORPORA LUTEA	MEAN±S.D.	9.9 ± 2.3	9.8 ± 1.6	9.0 ± 2.0	9.6 ± 1.5
IMPLANTATIONS	MEAN±S.D.	7.7 ± 2.2	8.3 ± 2.1	7.0 ± 2.6	7.5 ± 1.9
LITTER SIZES	MEAN±S.D.	7.8 ± 2.4	7.5 ± 2.0	6.6 ± 2.6	6.7 ± 1.6
LIVE FETUSES	N	105	135	121	121
	MEAN±S.D.	7.0 ± 2.4	7.5 ± 2.0	6.6 ± 2.6	6.7 ± 1.6
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.7 ± 1.0	0.8 ± 2.3	0.4 ± 0.7	0.8 ± 1.1
EARLY RESORPTIONS	N	9	12	1	5
	MEAN±S.D.	0.6 ± 0.9	0.7 ± 2.4	0.0 ± 0.2	0.3 ± 0.5
LATE RESORPTIONS	N	2	2	6	9
	MEAN±S.D.	0.1 ± 0.4	0.1 ± 0.3	0.3 ± 0.6	0.5 ± 0.9
DOES WITH ANY RESORPTIONS	N(%)	7(46.7)	5(27.8)	5(25.0)	8(44.4)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	1(5.6)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	15(100.0)	17(94.4)	20(100.0)	18(100.0)

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

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Fetal Findings: tables below from sponsor summarizes the findings of alterations, malformations, and variations:

FETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	2	10	50
Litters Evaluated	N	15	17	20	18
Fetuses Evaluated	N	105	135	133	121
Live Fetuses	N	105	135	133	121
Dead Fetuses	N	0	0	0	0
Litters with Fetuses with any Alteration Observed	N(%)	14(93.3)	17(100.0)	20(100.0)	18(100.0)
Fetuses with any Alteration Observed	N(%)	44(41.9)	57(42.2)	64(48.1)	69(57.0)
% Fetuses with any Alteration/Litter	$\bar{X}\pm$S.D.	47.22 ± 26.92	44.15 ± 28.16	50.82 ± 20.59	56.27 ± 21.05
Litters with Fetuses with any Malformation Observed	N(%)	1(6.7)	3(17.6)	3(15.0)	2(11.1)
Fetuses with any Malformation Observed	N(%)	1(1.0)	3(2.2)	3(2.2)	8(6.6)
% Fetuses with any Malformation/ Litter	$\bar{X}\pm$S.D.	0.83 ± 3.23	2.29 ± 5.22	2.08 ± 5.39	6.25 ± 23.58
Litters with Fetuses with any Variation Observed	N(%)	14(93.3)	17(100.0)	20(100.0)	18(100.0)
Fetuses with any Variation Observed	N(%)	44(41.9)	56(41.5)	62(46.6)	63(53.7)
% Fetuses with any Variation/Litter	$\bar{X}\pm$S.D.	47.22 ± 26.92	43.18 ± 28.84	48.74 ± 20.57	53.09 ± 18.18

From this table there was no sig incr in any litter or fetal alterations rel to the cont. A statistical sig incr in lung agenesis of intermediate lobe (a variation) was seen in 13 LD fetuses (from 6 litters) compared with 3, 2, and 6 fetuses with this variation in cont, MD, & HD respectively, from 3, 2, and 5 litters respectively. This variation was considered by the sponsor not drug related because it was not dose-dependent. Other variations included skull, hyoid, sternum and, ribs. In general, there were no statistically or biologically sig drug related variations or

malformations of soft or skeletal tissues in any gr. It was concluded that CL 284-846 is not a teratogen in rabbits upto 50mg/kg/d dose. This dose represents 294 fold higher than the propsed clinical dose of 10mg/d on a mg/kg basis or 94 folds on a mg/m^2 basis.

- Segment III -Peri- & Post-natal/lactation repro study in SD rats using CL 284-846 administered orally via gavage (study#LJT1936 GTR#29583/Study initiation Date: Jul 1994/ [redacted]

Doses: **1, 7, 50mg/kg/d oral gavage**; control received the vehicle (0.5% Na-CMC + 0.1% Tween 80; (vol. 10ml/kg).
Strain/No./Sex/Dose: SD females/25/dose.
Duration: pregnant f (14wks old at start of study (231-293g)) were dosed during gd17 to ppd21; ten f/dose were then placed on drug free recovery period from ppd22 to ppd28. Dams were killed on lactation day 28.
Parameters assessed: F0: mortality, clinical signs, B.wt, food intake, delivery & nursing behaviors. F1: mortality, sex ratio, clinical signs, wt, growth & physical differentiation, reflexes and responses, spontaneous motor activity, learning performance, & repro performance. F2 pups: mortality, sex, general condition, B.wt, autopsy at 3wks after birth.

In addition, the following parameters were assessed for F0 dams: gross exam of thoracic and abdominal cavities, selected organ wt (heart, liver, kidneys, thymus, spleen, and adrenals), & # of implantation sites. F1: skeletal exam of all fetuses or pups (dead or culled), for all and selected m+f: gross exam of thoracic and abdominal cavities and wts of selected organs/tissues from selected m+f. F2: external, visceral, and skeletal exams of all fetuses (except those culled or dead) and, gross exam of thoracic and abdominal cavities of pups.

Results:

F0

Mortality: no deaths in any gr.
Clinical Signs: the usual signs associated with CL 284-846 and reported in other tox studies, were observed in all drug grs and were dose dependent in frequency they occurred mainly during the 1st hr postdose and lasted as long as 4hr postdose (in HD). These signs included: ataxia, decr motor activity, abnormal gait, etc.. No clinical signs were seen during recovery period.
B.wt & Food intake: a small but sig incr (6% over the cont) in mean wt of dams dosed 50mg/kg on day of delivery (within dosing period), rel to the cont however, a small (3% less than cont), but sig decr in mean wt noted in dams dosed 7mg/kg from days 7-21 after delivery. No wt changes in any gr during recovery or days post delivery. The changes in mean wt did not correspond to changes in food intake. Mean food intake was decr in HD dams during gd15-18 (11.5% less than cont) and, days 0-7 & 7-14 post delivery (12-13.5% less than cont); also mean wt decr in MD during gd18-20 (8% less than cont). During recovery period days 21-28, mean food intake was sig incr, 12%, in LD&HD dams.

Delivery & Nursing: table below from sponsor:
From this table, there were 1 dam in cont, 4 in MD, and 1 in HD that needed >4hr to complete delivery. All dams delivered between d21-23 of gestation with the exception of **1 dam in MD**

Seg III Rat - F0 obsevation of delivery

Treatment (mg/kg/day)	No. of pregnant females	No. of females with live offspring	Delivery index (%)	Duration of pregnancy(day) 21	22	23	Mean ±S.D.		No. of implantations	No. of offspring(pups) born alive	No. of stillbirth	Birth index (%)	Delivery status and nursing behavior (No. of dams)
				No. of dams									
Vehicle (0)	25	25	100	7	17	1		Total	381	366	11		
							21.8	Mean	16.3	14.2	0.4	91.8	P(1), D(1), N(1)
							0.5	±S.D.	2.2	3.3	1.0	11.6	
CL 284,846 (1)	25	25	100	5	20	0		Total	404	381	7		
							21.8	Mean	16.2	15.2	0.3	94.4	D, N(1)
							0.4	±S.D.	1.1	1.3	0.6	5.9	
CL 284,846 (7)	25	24	96	1	18	5		Total	402	334	36		
							22.2*	Mean	16.1	13.4	1.5	80.5	P(3), P, N(1), D, N(1)
							0.5	±S.D.	3.6	4.1	1.8	24.5	
CL 284,846 (50)	25	25	100	2	22	1		Total	392	334	26		
							22.0	Mean	15.7	13.4	1.0	84.6	P, D(1), D(1), D, N(2)
							0.4	±S.D.	2.4	4.1	1.3	20.9	

Vehicle: 0.5% carboxymethyl cellulose sodium + 0.1% Tween 80 in water for injection.
Delivery index was calculated as follows: [No. of females with live offspring(pups) / No. of pregnant females]x100
Birth index was calculated as follows: [No. of offspring(pups) born alive / No. of implantations]x100
Prolonged : It took more than 4 hours to delivery.
Delivery status and nursing behavior :
P : Prolonged D : All offspring(pups) died during the nursing period N : Poor nursing behavior
* : Significantly different from the vehicle group, P<0.05

that did not deliver till d26 of gestation. This dam had soiled perianal region on gd23 with vulval bleeding on gd24; this dam was killed on gd26 and pregnancy was confirmed and implantation sites seen without any abnormal findings. Except for this dam, the mean duration of gestation was similar among drug and cont grs. The birth indices were 92, 94, 81, and 85% in cont, LD, MD, & HD respectively. During lactation and nursing period, there were a total of 7 dams with **all newborn pups dead** (1, 1, 1, & 4 dams in cont, LD, MD, & HD respectively). The cause of death of these pups was **related to abnormal nursing behavior**, as stated by the sponsor.

There were some small, non-dose dependent but sig changes in mean absol organ wts of dams that were killed on days 21&28 postdelivery. These included, in dams killed on d21: decr in MD liver (also in LD), thymus, and spleen, and a small incr in adrenal wt in HD rats. These changes ranged between 6-27% of the cont. The only statistically sig effects in dams killed on d28 postdelivery were a 1x incr in mean adrenal wt of HD and a 5% decr in mean kidney wt in MD. These organ wt changes did not correlate with autopsy findings.

F1

There was a **moderate incr in # of stillbirths in MD (36) and HD (26) rel to the cont (11). Number of dead pups before culling was also incr dose-dependently in MD&HD** but did not reach statistical sig (total (m+f) 48&82 respectively) compared with 24 in cont. **The viability index of F1 pups on d4 was 90, 95, 84, & 72%** in cont, 1, 7, & 50mg/kg grs respectively, (sig in HD only). **There was a sig decr in weaning index in HD** (91%) compared to the cont (100%), and, 99&97% in LD&MD respectively.

Mean B.wt of pups of both sexes in HD was sig reduced rel to the cont from after birth till end of study (d70 after birth). Small but sig decr in wt of MD on 1st wk after delivery had recovered by end of that wk. These decr in mean wt ranged between 4-21% of the cont.

There were changes in mean absolute and/or relative wt of some organs. These changes were small, not dose related, did not correlate with any gross findings, and, possibly secondary to the decr in mean B.wts of the dams. There were no drug effects on autopsy of F1 pups on 3, 6, and 10wk of age.

Drug related effects were not seen in F1* on growth, physical differentitation, reflexes & responses to stimuli, motor activity, learning performance, emotionality, estrous cycle, or fertility upto 50mg/kg dose.

* physical development assessed by: ear unfurling, hair growth, tooth eruption, eyelid openning, testes descend.

Reflexes & responses: righting reflex, palmar grasp, pinna response, pivotting, startle response, pain response, walking, visual function, corneal reflex, & air righting.

Learning: multiple water T-maze & open field.

Mating performance of F1 pups did not seem to be affected by the drug. Copulation & fertility indices were 100% in all grs except fertility index was 95.5% in MD and pregnancy rate was similar among drug and cont grs. Mean B.wts of F1 dams were not affected by the drug except for LD gr where mean wt was sig incr over the cont during gd0-20. There were no abnormal deliveries or nursing behavior in any gr.

F2

Mean wt of F2 pups was sig reduced in HD m&f at 1,2,3 wks after birth these decr ranged between 11-14% less than the cont. There were no other drug related effects on any F2 parameters: pup viability, growth, weaning rate, soft or skeletal tissue gross exams.

Summary & Conclusion: CL 284-846 administered orally to pregnant rats during gd17 through ppd21 caused no deaths in F0 dams (the only dams that were dosed). There was an incr in stillbirths in dams dosed 7&50mg/kg. These deaths were contributed to poor and abnormal nursing behavior. Number of dead pups before culling was also incr dose-dependently in MD&HD but did not reach statistical sig (total (m+f) 48&82 respectively) compared with 24 in cont. The viability index of F1 pups on d4 was 90, 95, 84, & 72% in cont, 1, 7, & 50mg/kg grs respectively, (sig in HD only). There was a sig decr in weaning index in HD (91%) compared to the cont (100%), and, 99&97% in MD&HD respectively. Mean B.wt of F1 pups of both sexes in HD was sig reduced rel to the cont (4-21% less than the cont), from after birth till end of study (d70 after birth)

There were no drug related effects in F1 pups on growth, physical differentitation, reflexes & responses to stimuli, motor activity, learning performance, emotionality, estrous cycle, or fertility upto 50mg/kg dose. Mating performance of F1 pups did not seem to be affected by the drug. Copulation & fertility indices were similar in all grs as well as pregnancy rate. Mean B.wts of F1 dams were not affected except for LD gr where mean wt was sig incr over the cont during gd0-20. There were no abnormal deliveries or nursing behavior in any gr.

Mean wt of F2 pups was sig reduced in HD m&f at from 1-3wks of age, these decr ranged between 11-14% less than the cont. There were no other drug related effects on any F2 parameters: pup viability, growth, weaning rate, soft or skeletal tissue gross exams.

It was concluded that the NOEL for maternal repro effects and for F1 pups is **1mg/kg/d.** The NOEL for F1 functional and behavioral development and repro performance is **>50mg/kg/d.**

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- Cross-Fostering study of CL 284-846 in SD rats administered orally via gavage (study#ljt2408 GTR#29937/Study initiation Date: jUN 1996/ [redacted]

Dose: **50mg/kg/d oral gavage**; control received the vehicle (0.5% Na-CMC + 0.1% Tween 80; (vol. 10ml/kg).
Strain/No./Sex/Dose: SD females; see experimental design in table below from sponsor:

Compound		Dose	Route	Dose volume	Administration[1]	No. of dams
Dams	Pups	(mg/kg/day)		(ml/kg/day)	period	
Vehicle	NF	0	P.O.	10	From day 17 of pregnancy to day 20 after delivery	20
Vehicle	Vehicle	0	P.O.	10	From day 17 of pregnancy to day 20 after delivery	20
Vehicle	ZAL-846	0	P.O.	10	From day 17 of pregnancy to day 20 after delivery	18
ZAL-846	NF	50	P.O.	10	From day 17 of pregnancy to day 20 after delivery	17
ZAL-846	ZAL-846	50	P.O.	10	From day 17 of pregnancy to day 20 after delivery	16
ZAL-846	Vehicle	50	P.O.	10	From day 17 of pregnancy to day 20 after delivery	18

Vehicle : 0.5% Sodium carboxymethyl cellulose + 0.1% polysorbate 80 in water for injection.
NF : Not fostered.
1) : The day, on which a vaginal plug was found, was designated as day 0 of pregnancy, and the day of delivery was designated as day 0 after delivery.

Parameters assessed: F0: mortality, clinical signs, B.wt, food intake, delivery & nursing behaviors, gross exam of thoracic & abdominal cavities. # of corpora lutea, and implantation sites. F1: mortality, sex ratio, clinical signs, B.wt, general physical condition, gross exam of thoracic & abdominal cavities of all pups, and wt of selected organs/tissues (brain, heart, liver, & kidneys) from 1 surviving pup/sex/litter.

Results:
Mortality: 5/51 rats treated with the drug were found dead on ppd17 (one dam was dead on ppd14); no deaths in the cont gr. The cause of death in 4 dams is unclear as stated by the sponsor, the fifth dam had convulsions before death. It is noted that there have been no deaths in rats previously treated (in other repro studies) with 50mg/kg CL 284-846. The sponsor suggested that the deaths in this study might have been caused by the increased stress in these animals due to cross fostering and the 24hr continued delivery observation. However,

Clinical signs: same as those observed in other tox studies: abnormal gait, decr motor activity, and prone posture. No signs noted in cont gr.
B.wt & Food Intake: slight, transient, but sig decr in mean wt of about 5% on gd17 (start of dosing) noted in drug grs compared to cont-non-cross-fostered gr. A sig decr in food intake

noted in drug grs on gd17 to ppd21. In drug intercorss gr the decr in food was absent by ppd7.
Delivery: table below from sponsor:

Summary of Delivery in Dams before Cross-fostering

Compound		Dose	No. of	Delivery day (day of pregnancy) No. of dams			Onset of delivery	Duration of delivery	No. of implantation sites	No. of * live newborns	No. of * stillbirths	Pup viability
Dams	Pups	(mg/kg/day)	dams	21	22		(hour)	(minute)				(%)
Vehicle	NF	0	20	4	16	Total			320	296	10	
						Mean	15.80	150	16.0	14.8	0.5	92.5
						±S.D.	6.56	35.6	1.1	1.6	0.8	7.3
Vehicle	Vehicle	0	20	12	8	Total			319	293	4	
						Mean	10.14	140	16.0	14.7	0.2	92.1
						±S.D.	9.18	20.4	2.3	2.1	0.6	7.0
Vehicle	ZAL-846	0	18	0	18	Total			294	266	7	
						Mean	17.64	140	16.3	14.8	0.4	90.4
						±S.D.	3.15	27.6	1.6	1.9	0.5	7.3
ZAL-846	NF	50	17	0	17	Total			271	242	14	
						Mean	19.76	157	16.0	14.2	0.8	89.4
						±S.D.	3.43	42.8	2.7	2.7	1.1	10.3
ZAL-846	ZAL-846	50	16	0	16	Total			250	224	8	
						Mean	17.29	156	15.6	14.0	0.5	89.4
						±S.D.	4.14	37.8	2.0	2.3	0.7	9.2
ZAL-846	Vehicle	50	18	1	17	Total			281	262	9	
						Mean	16.90	161	15.6	14.6	0.5	93.6
						±S.D.	3.04	38.9	2.1	2.0	1.2	8.3

Vehicle : 0.5% Sodium carboxymethyl cellulose + 0.1% polysorbate 80 in water for injection.
NF : Not fostered.
Onset of delivery : This means relative onset time from 14:00 on day 21 of pregnancy.
* : Newborns which were found after cross-fostering were included in this count, but these were omitted from the cross-fostering study.
Fetal viability was calculated as follows : (No. of live newborns/No. of implantation sites) X 100.

From the above table, all CL 284-846 dams except for 1, delivered on d22 compared with some cont dams delivered on d21. The onset of delivery/parturition seemed longer in drug grs (mean ranged between 17-20hr) vs. the cont dams (10-18hrs). There were no other drug effects on implantation sites, # of live fetuses, # of stillbirths, or pup viability.

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