Nurrsing behavior: the following were assessed: licking of pups for care, retrieving pups, and crouching with pups (table below from sponsor). All of these parameters were observed in all grs. drug and vehicle. However, there was an increased # of dams in drug grs that did not exhibit one or more of these nursing behaviors. Absence of lactation was seen at higher incidences in dams on drug that were not fostered than in any other gr. The sponsor related these nursing and lactation behaviors to the sedative effect of the drug.
There were no drug effect on nursing or lactation after ppd10 (except for crouching with pups on ppd21&13 in gr with drug-fostered pups and vehicle-pups).
Autopsy: no drug related finding in any gr.

ZAL-846: Oral Perinatal/Postnatal Toxicity Study with Cross-fostering in Rats
Summary of Nursing Behavior and Lactating Condition in Dams

Compound		Dose		Day after delivery										
Dams	Pups	(mg/kg/day)		0	1	2	3	4	5	6	7	8	9	10
Vehicle	NF	0	Licking pups for care	20/0	19/1	19/1	18/1	19/0	19/0	18/0	19/0	19/0	19/0	19/0
			Retrieving pups	20/0	19/1	19/1	18/1	19/0	19/0	19/0	19/0	19/0	19/0	19/0
			Crouching with pups	20/0	19/1	19/1	19/0	19/0	19/0	18/0	19/0	19/0	19/0	19/0
			Lactating	18/2	19/1	19/1	19/0	19/0						
			(N)	(20)	(20)	(20)	(19)	(19)	(19)	(19)	(19)	(19)	(19)	(19)
Vehicle	Vehicle	0	Licking pups for care	20/0	20/0	19/1	20/0	20/0	20/0	20/0	20/0	20/0	20/0	20/0
			Retrieving pups	19/1	20/0	20/0	19/1	19/1	19/1	20/0	20/0	20/0	20/0	20/0
			Crouching with pups	19/1	20/0	19/1	20/0	20/0	20/0	20/0	20/0	20/0	20/0	20/0
			Lactating	20/0	20/0	20/0	20/0	20/0						
			(N)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)
Vehicle	ZAL-846	0	Licking pups for care	18/0	18/0	18/0	18/0	18/0	17/0	17/0	17/0	17/0	17/0	17/0
			Retrieving pups	17/1	17/1	17/1	17/1	17/1	17/0	17/0	17/0	17/0	17/0	17/0
			Crouching with pups	17/1	18/0	16/2	18/0	16/2	17/0	17/0	17/0	17/0	17/0	17/0
			Lactating	18/0	17/1	18/0	18/0	17/1						
			(N)	(18)	(18)	(18)	(18)	(18)	(17)	(17)	(17)	(17)	(17)	(17)
ZAL-846	NF	50	Licking pups for care	16/1	15/1	12/1	11/1	9/1	9/0	9/0	9/0	9/0	9/0	9/0
			Retrieving pups	13/4	11/5	11/2	11/1	9/1	9/0	9/0	9/0	9/0	9/0	9/0
			Crouching with pups	14/3	11/5	10/3	11/1	9/1	9/0	8/1	9/0	9/0	9/0	9/0
			Lactating	12/5	12/4	10/3	10/2	9/1						
			(N)	(17)	(16)	(13)	(12)	(10)	(9)	(9)	(9)	(9)	(9)	(9)
ZAL-846	ZAL-846	50	Licking pups for care	16/0	16/0	16/0	16/0	16/0	16/0	16/0	16/0	16/0	16/0	16/0
			Retrieving pups	15/1	15/1	16/0	14/2	15/1	16/0	16/0	16/0	16/0	16/0	16/0
			Crouching with pups	16/0	15/1	16/0	15/1	13/3	15/1	15/1	14/2	16/0	16/0	16/0
			Lactating	16/0	16/0	16/0	14/2	16/0						
			(N)	(16)	(16)	(16)	(16)	(16)	(16)	(16)	(16)	(16)	(16)	(16)
ZAL-846	Vehicle	50	Licking pups for care	18/0	18/0	18/0	18/0	18/0	18/0	18/0	18/0	18/0	18/0	18/0
			Retrieving pups	18/0	17/1	17/1	18/0	17/1	18/0	18/0	18/0	18/0	18/0	18/0
			Crouching with pups	18/0	18/0	17/1	17/1	13/5	18/0	18/0	18/0	18/0	18/0	18/0
			Lactating	18/0	18/0	18/0	17/1	18/0						
			(N)	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(18)

Effects on Pups:
Mortality: there was an incr in the total # of dead pups during the 1st 5days after delivery in drug grs with not-fostered pups and those fostered with drug (tables below from sponsor). Viability indices in these 2grs on ppd4 were 47&79% respectively, rel to 94, 99, & 89% in cont grs. A total of 8/16 litter with total litter loss during the 1st 5d after delivery in the drug gr with not-fostered pups. In the drug-drug gr with fostered pups, the # of litters with partial pup loss was 11/14 or 79% compared with 15, 20, and 44% in cont grs. From ppd5 and on, there was no drug effect on pup loss in any gr. Weaninig index was comparable in all grs. The greatest pup

loss occurred in those drug treated dams that were not cross-fostered (100% litters with at least 1 loss or 17.7% pup loss/litter), or cross-fostered to a drug treated dam (~79% litters with at least 1loss or 23% pup loss/litter) as compared to the other grs (tables below).

Compound		Dose	Sex	No. of pups after	Number of dead pups				Viability index on	Number of dead pups								
					Day after delivery					Day after delivery								
Dams	Pups	(mg/kg/day)		Surviving	1	2	3	4	day 4 (%)	5	6	7	8	9	10	11	12	13
Vehicle	NP	0	Male	141	0	12	0	1	94.1	1	0	0	0	0	0	0	0	0
			Female	135	0	6	0	1	94.0	0	0	0	0	0	0	0	1	0
			Total	286	0	18	0	2		1	0	0	0	0	0	0	1	0
			Mean						94.1									
			(N)	(20)	(20)	(20)	(20)	(20)		(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)
Vehicle	Vehicle	0	Male	129	0	2	0	0	98.9	0	0	1	0	1	0	0	0	0
			Female	164	0	2	0	0	99.0	0	0	0	0	0	0	0	0	0
			Total	293	0	4	0	0		0	0	1	0	1	0	0	0	0
			Mean						99.0									
			(N)	(20)	(20)	(20)	(20)	(20)		(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)
Vehicle	ZAL-846	0	Male	130	7	6	1	1	88.4	2	2	0	1	0	0	1	0	0
			Female	132	7	5	0	1	89.9	1	0	1	1	1	0	2	0	0
			Total	262	14	11	1	2		3	2	1	2	1	0	3	0	0
			Mean						89.2									
			(N)	(18)	(18)	(18)	(18)	(18)		(18)	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(18)
ZAL-846	NP	50	Male	123	44	14	0	1	69.5	1	0	0	0	0	1	1	0	0
			Female	119	43	17	0	6	45.2	1	1	1	0	1	0	0	0	1
			Total	242	87	31	0	7		2	1	1	0	1	1	1	0	1
			Mean						67.4*									
			(N)	(17)	(17)	(17)	(17)	(17)		(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
ZAL-846	ZAL-846	50	Male	123	24	2	2	0	75.6	0	2	0	0	0	1	0	0	0
			Female	101	19	1	1	0	82.5	0	2	0	0	0	0	1	0	0
			Total	224	43	3	3	0		0	4	0	0	0	1	1	0	0
			Mean						79.1*									
			(N)	(16)	(16)	(16)	(16)	(16)		(16)	(16)	(16)	(16)	(16)	(16)	(16)	(16)	(16)
ZAL-846	Vehicle	50	Male	137	1	3	1	1	96.3	1	0	0	0	0	0	0	0	0
			Female	127	2	0	2	0	98.6	1	0	1	0	0	0	0	0	0
			Total	264	3	3	3	1		2	0	1	0	0	0	0	0	0
			Mean						96.5									
			(N)	(15)	(15)	(15)	(15)	(15)		(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)

Compound		Dose	Sex	Number of dead pups								Weaning index (%)	No. of litters with total litter loss (%)	No. of litters with partial pup loss (%)
				Day after delivery										
Dams	Pups	(mg/kg/day)		14	15	16	17	18	19	20	21			
Vehicle	NP	0	Male	0	0	0	0	0	1	0	0	98.8	1/20	3/20
			Female	0	0	0	0	0	0	0	0	98.9	(5.0)	(15.0)
			Total	0	0	0	0	0	1	0	0			
			Mean									98.9		
			(N)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)			
Vehicle	Vehicle	0	Male	0	1	0	0	0	0	0	0	97.3	0/20	4/20
			Female	0	0	0	0	0	0	0	0	100.0	(0.0)	(20.0)
			Total	0	1	0	0	0	0	0	0			
			Mean									98.7		
			(N)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)			
Vehicle	ZAL-846	0	Male	0	0	0	0	0	0	0	0	99.4	1/18	8/18
			Female	0	0	0	1	0	0	0	0	99.6	(5.6)	(44.4)
			Total	0	0	0	1	0	0	0	0			
			Mean									99.7		
			(N)	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(18)			
ZAL-846	NP	50	Male	0	0	0	0	0	0	0	0	84.3	8/16	8/17
			Female	0	0	0	0	0	0	0	0	90.8	(50.0)	(47.1)
			Total	0	0	0	0	0	0	0	0			
			Mean									87.6		
			(N)	(17)	(17)	(17)	(17)	(16)	(16)	(16)	(16)			
ZAL-846	ZAL-846	50	Male	0	0	0	0	0	0	0	0	98.2	0/14	11/14
			Female	0	0	0	0	0	0	0	0	98.2	(0.0)	(78.6)
			Total	0	0	0	0	0	0	0	0			
			Mean									98.2		
			(N)	(16)	(16)	(16)	(16)	(14)	(14)	(14)	(14)			
ZAL-846	Vehicle	50	Male	0	0	0	0	0	0	0	0	98.8	0/16	7/16
			Female	0	0	0	0	0	0	0	0	98.1	(0.0)	(43.8)
			Total	0	0	0	0	0	0	0	0			
			Mean									98.5		
			(N)	(17)	(17)	(17)	(16)	(16)	(16)	(16)	(16)			

Pup mortality continued:

Compound		Dose	No. of litters	No. of litters with total litter loss	% total litter loss	No. of litters with partial pup loss	% partial litter loss	% pup loss per litter
Dams	Pups	(mg/kg/day)	(a)	(b)	(b/a x 100)	(c)	a/(a-b) x 100	(d)
Vehicle	NF	0	20	1	5	5	26.3	2.1
Vehicle	Vehicle	0	20	0	0	6	30	2.2
Vehicle	ZAL-846	0	18	1	5.6	11	84.7	11.4
ZAL-846	NF	50	16	8	50	8	100	17.7
ZAL-846	ZAL-846	50	14	0	0	11	78.6	22.6
ZAL-846	Vehicle	50	16	0	0	8	50	5.1

(b), (c), (d) : Excluding those litters from dams that were found dead.
Viability index on day 4 was calculated as follows : (No. of live pups on day 4 after birth/No. of pups after fostering) X 100.
Weaning index was calculated as follows : (No. of live pups on day 21 after birth/No. of pups on day 4 after birth) X 100.
NF : Not fostered. (N) : Number of dams. * : Significantly different from the vehicle group at p<0.05

Clinical Signs in Pups: pups dosed with the drug inutero had small bodies, were emaciated and hypothermic compared with cont pups. In this same litter of drug gr, pups showed decr in locomotion in 10m & 4f.

B.wt: mean wt of pups (both sexes) in vehicle inter-cross gr was sig decr (~78%) on ppds 0-2 rel tot hose of cont (vehicle nont-fostered gr). Mean wt in all drug grs was sig decr rel to the cont at ppd0-10 or 14 and from ppd4-17 in dosed dam and vehicle-treated pups (tables below from sponsor for mean wt changes in m & f rats:

Summary of Body Weight (g) in Pups (Male)

Compound		Dose		Mean body weight											
				Day after birth											
Dams	Pups	(mg/kg/day)		0	1	2	3	4	5	6	7	10	14	17	21
Vehicle	NF	0	Mean	6.6	7.1	8.0	9.0	10.4	11.8	13.5	15.2	21.3	27.9	32.7	46.5
			±S.D.	0.4	0.6	0.8	1.0	1.2	1.3	1.7	2.0	2.3	3.0	3.4	5.7
			(N)	(20)	(20)	(19)	(19)	(19)	(18)	(19)	(19)	(19)	(19)	(19)	(19)
Vehicle	Vehicle	0	Mean	6.4	6.9	7.6	8.6	9.9	11.3	12.9	14.7	20.6	27.6	32.6	45.5
			±S.D.	0.5	0.7	0.8	0.9	1.1	1.4	1.6	1.7	2.4	3.5	4.1	7.0
			(N)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)
			%	96.8	96.2	95.1	95.6	95.6	95.5	95.6	96.6	97.0	98.9	99.5	97.7
Vehicle	ZAL-846	0	Mean	6.1*	6.4*	7.2*	8.3	9.6	11.5	13.4	15.2	21.5	29.3	34.8	48.4
			±S.D.	0.3	0.5	0.9	1.3	1.7	1.4	1.8	2.1	2.8	2.4	3.7	4.3
			(N)	(18)	(18)	(18)	(18)	(18)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
			%	92.7	89.6	90.0	91.6	92.5	97.3	99.5	100.0	101.3	105.1	106.4	104.1
ZAL-846	NF	50	Mean	6.0*	6.0*	6.8*	7.5*	8.4*	10.0*	11.4*	12.7*	17.9*	25.8	31.0	43.0
			±S.D.	0.3	0.8	1.1	1.5	2.1	1.6	1.7	1.9	1.9	3.8	4.9	7.1
			(N)	(17)	(15)	(10)	(10)	(10)	(9)	(9)	(9)	(9)	(9)	(9)	(8)
			%	91.2	84.0	85.0	83.1	81.2	84.7	84.8	83.5	84.2	92.4	94.7	92.3
ZAL-846	ZAL-846	50	Mean	6.0*	6.2*	6.5*	7.3*	8.2*	9.3*	10.5*	11.9*	17.2*	25.0*	30.5	41.9
			±S.D.	0.4	0.8	1.0	1.4	1.6	1.9	2.2	2.3	3.1	4.3	4.7	6.3
			(N)	(15)	(15)	(16)	(16)	(16)	(16)	(16)	(16)	(16)	(16)	(14)	(14)
			%	91.2	86.8	81.3	80.9	79.2	78.8	78.1	78.2	80.9	89.6	93.2	90.1
ZAL-846	Vehicle	50	Mean	6.7	7.1	7.6	8.5	9.4*	10.6*	11.7*	12.9*	18.0*	24.6*	29.2*	42.6
			±S.D.	0.6	0.7	0.8	0.9	0.9	1.0	1.2	1.3	1.7	2.8	3.3	4.4
			(N)	(18)	(18)	(18)	(18)	(18)	(17)	(18)	(18)	(18)	(17)	(16)	(16)
			%	102.1	99.2	95.4	93.9	90.8	89.8	87.0	84.8	84.7	88.1	89.2	91.5

Summary of Body Weight (g) in Pups (Female)

Compound		Dose		Mean body weight											
				Day after birth											
Dams	Pups	(mg/kg/day)		0	1	2	3	4	5	6	7	10	14	17	21
Vehicle	NF	0	Mean	6.2	6.8	7.5	8.6	10.0	11.3	13.1	14.9	20.3	27.3	31.4	44.8
			±S.D.	0.4	0.6	1.0	0.9	1.1	1.3	1.5	1.6	2.3	2.8	3.1	4.9
			(N)	(20)	(20)	(20)	(19)	(19)	(18)	(19)	(19)	(19)	(19)	(19)	(19)
Vehicle	Vehicle	0	Mean	6.0	6.4	7.2	8.1	9.5	10.8	12.4	14.1	19.6	26.3	30.9	43.5
			±S.D.	0.4	0.6	0.8	0.8	1.0	1.3	1.5	1.6	2.4	3.5	3.9	6.0
			(N)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)
			%[a]	96.0	95.0	96.0	94.2	95.2	95.2	94.7	94.6	96.7	96.7	98.3	97.2
Vehicle	ZAL-846	0	Mean	5.7*	6.0*	6.8*	7.8	9.0	10.8	12.5	14.3	20.5	28.0	33.3	46.3
			±S.D.	0.3	0.4	0.7	1.1	1.5	1.2	1.5	1.7	2.2	2.4	3.5	4.2
			(N)	(18)	(18)	(18)	(18)	(18)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
			%[a]	91.9	88.5	91.2	90.0	90.6	95.4	95.8	96.3	101.2	102.6	105.9	103.4
ZAL-846	NF	50	Mean	5.7*	5.5*	5.9*	6.3*	8.3*	9.4*	10.6*	12.0*	17.2*	24.6	29.4	41.2
			±S.D.	0.4	0.7	1.3	1.9	1.4	1.6	1.9	1.9	2.7	3.9	5.5	7.8
			(N)	(17)	(15)	(13)	(12)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(8)
			%[a]	91.9	81.1	79.1	73.0	83.4	83.2	81.2	80.6	84.9	90.3	93.6	91.9
ZAL-846	ZAL-846	50	Mean	5.7*	5.8*	6.2*	6.9*	7.9*	8.8*	10.1*	11.3*	16.3*	24.1*	29.8	41.3
			±S.D.	0.3	0.7	0.9	1.2	1.4	1.7	1.9	2.0	2.8	4.0	4.6	6.2
			(N)	(15)	(15)	(16)	(16)	(16)	(16)	(16)	(16)	(16)	(16)	(14)	(14)
			%[a]	91.9	85.5	83.1	80.0	79.4	77.9	77.4	75.9	80.4	88.4	94.8	92.2
ZAL-846	Vehicle	50	Mean	6.4	6.7	7.2	8.1	9.0*	10.0*	11.1*	12.4*	17.2*	23.7*	28.3*	41.1
			±S.D.	0.4	0.5	0.6	0.7	0.8	1.0	1.1	1.3	1.8	3.0	3.5	4.4
			(N)	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(17)	(16)	(16)
			%[a]	102.7	99.0	97.1	93.9	90.5	88.5	85.1	83.3	84.9	87.0	90.0	91.8

NF : Not fostered. (N) : Number of dams.
a : As compared to the vehicle treated non-cross-fostering group.
* : Significantly different from the vehicle group at $p<0.05$.

Summary & Conclusion:

Oral gavage administration of CL 284-846 to pregnant rats at 50mg/kg during gd17 to ppd21 caused dam mortality, clinical signs, decr mean wt and food intake, increase in onset time to parturition and duration of delivery. The drug caused an incr in pup mortality, decrease in mean pup wt, and clinical signs. The pup mortality occurred in drug treated dams irrespective whether the litters were cross fostered to treated or cont dams.

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SUMMARY AND CONCLUSION FOR REPRO & DEVELOPMENTAL STUDIES:

CL 284-846 was tested in 5 male and female fertility (Segment I) studies, rat and rabbit Segment II teratology studies, rat Segment III per- and post-natal - lactation study, and rat cross-fostering study. The maximu dose tested for repro-developmental studies in the rat was 200mg/kg/d and in the rabbit, 50mg/kg/d; these doses represent 1176x and 294x the maximum propsed clinical dose on a mg/kg basis. In a separate PK study, pregnant rats were administered a single oral gavage dose of CL 284-846 at 1, 10, 100mg/kg on gd15 and the parent and its metabolites (des-ethyl, M1, M2. And M2 glucuronide) were measured. A similar PK study was done in pregnant rabbits at doses 2, 10, 50mg/kg administered on gd17. The **mean ($\pm$CV) C_{max} and AUC_{0-inf} at 100mg/kg dose in the rat were 19$\pm$23ug/ml and 163$\pm$8ug.hr/ml respectively and the corresponding values for the rabbit at 50mg/kg dose were 3$\pm$39ug/ml and 21$\pm$31ug.hr/ml respectively. The mean C_{max} in the rat is 475 times, and that in the rabbit is 75 times the mean C_{max} measured in humans at the maximum recomended dose of 10mg/d.**

The NOEL in the rat for maternal toxicity is 1mg/kg/d and for repro parameters in males and females and fetal development as well as teratogenicity, is 100mg/kg. The NOEL in the rabbit for teratogenicity is 50mg/kg/d and for general toxicity is 2mg/kg/d.

Decrease in fertility was noted in the 1st Segment 1 rat study. To further assess this finding, additional 4 male & female fertility studies were conducted. In the 2nd&3rd studies in male rats, 100mg/kg dose, caused changes in hormone levels (⇑ FSH & testosterone, & ⇓ in LH) but no drug effect on sperm count, fertility or copulation indices, organ wt and histopath, in females, there were no drug effects on # corpora lutea, # fetuses, viability & implantation indices, and fetal location in the uterine horn. In 1 of the 2 female fertility studies, the results from this study could not be utilized because the fertility index of the vehicle cont gr was unusually low compared to historical data (60% vs. 71-100%). The fertility indices in females in this study dosed CL 284-846 at 100&200mg/kg were 90&67%. In the 2nd female fertility study at 100mg/kg dose administered at 3 different periods of the female cycle, CL 294-846 **shortened the estrus cycle and decreased fertility index**; it is noted though. that these effects were seen at maternally toxic dose of 100mg/kg as 5/40 dams dosed 100mg/kg/d were found dead.

CL 284-846 at 1, 10, and 100mg/kg/d, was administered orally to rats during gd6-17 to assess its teratogenicity. Clinical signs and decreases in mean B.wt and food intake were observed at the 2 high doses during dosing period. There was no drug effect on any repro parameters of dams, however, **mean fetal wt was sig reduced** at the 100mg/kg gr. At 100mg/kg/d dose, F0 fetuses showed a sig incr in alterations (delayed ossification of ribs, sternum, caudal vertebrae, metacarpals, and hindpaw phalanges). The drug had no effect on F1 deaths, clinical signs, or necropsy findings. **Mean B. wt/wt gain was sig reduced** in F1 males of the 100mg/kg gr from postweaning d36 till beginning of cohabitation; no drug effect in females. CL 284-846 had no effect on physical, sexual maturation, behavior, mating/fertility, c-section observations, or gross external malformation of F2 generation. Rabbits were administered CL 284-846 at 2, 10, or 50mg/kg/d during gd6-18. The drug caused clinical signs and decrease in mean wt and food

intake of 10&50mg/kg/d grs during the dosing period. Mean delayed resorprtions were incr in 10&50mg/kg grs rel to the cont but these values were within historical data. The drug caused no malformations and was not teratogenic in rabbits up to 50mg/kg/d dose.

CL 284-846 at 1, 7, & 50mg/kg/d, administered orally to pregnant rats during gd17 to ppd21 (Segment III) caused no mortality but clinical signs observed in all drug grs and were dose dependent in frequency and seen mostly during the 1st 1hr of dosing. Mean B.wt and food intake were affected by drug administration (no correlation between the 2 parameters). **Duration of pregnancy seemed slightly longer in MD&HD** (sig only in MD) rel to the cont. A single MD f delivered on gd26 whereas most deliveries in both doses occurred between gd21-23. During lactation & nursing periods, there were **4 dams in HD with all newborn pups dead** compared with only 1 dam each in cont, LD& MD. In F1 generation, there was a **non-dose-dependent incr in # of stillbirths: 36, 26, & 11 in MD, HD, & cont respectively**. Moreover, **# of dead pups before culling was incr dose-dependently** but did not reach statistical sig. (48, 82, and 24 in MD, HD, & cont respectively). Viability index of pups on d4 of delivery was therefore, decreased in HD to 91% compared with 100% in cont, 99% in LD & 97% in MD. Mean wt of F1 pups was sig decr in HD gr. The drug had no effect on F1 physical development, growth, reflexes & response to stimuli, motor function, learning, memory, estrus cycle, fertility, or nursing behavior. The only drug effect on F2 pups was a sig 11-14% decr in mean wt of HD m&f at wks 1, 2, & 3 after birth.
The NOEL for maternal repro effects is 1mg/kg and that for functional, behavioral, and repro performance of F1 generation is >50mg/kg/d.

In a cross-fostering study at 50mg/kg/d dose administered from gd17 to ppd20, CL 284-846 caused **death in 5/51 dams** (ppd17 & 1 death on ppd14), however, death did not occur at this dose in previous rat tox studies. The sponsor contributed the deaths in this study, to increased stress in these dams due to cross fostering and the 24hr continued observation during delivery. The drug caused an **incr in pup mortality, decrease in mean pup wt, and clinical signs.** The pup mortality occurred in drug treated dams irrespective of whether the litters were cross fostered to treated or cont dams.

GENETIC TOXICOLOGY

The potential of CL 284-846 to induce gene mutation was assessed in the following assays:

1. 5 in vitro bacterial gene mutation assays,
2. in vitro MCGM (mammalian cell gene mutation) - HGPRT in CHO (Chinese hamster ovary) cells,
3. 2 UDS assays in rat hepatocytes and in vivo - in vitro rat liver UDS,
4. chromosomal aberrations (chrom abs) in CHO,
5. chrom abs in human lymphocytes,
6. in vivo mouse bone marrow micronucleus (MN), and,
7. in vivo rat bone marrow chrom abs.

1. Ames bacterial reverse gene mutation - plate incorporation method: Study#89126 MIRACL-22787/and #90036 MIRACL 23186/ [redacted] study date: 1990/GLP FDA&EPA & OECD guidelines.

- Salmonella strains tested: TA1535, TA1537, TA1538, TA98, & TA100 in -/+ S9.
- Appropriate positive controls for each strain were tested, the negative control included the vehicle: DMSO and, untreated cells; all tests done in triplicates.
- Preliminary tox assay was done in duplicates using TA1538 & TA100 in -S9. Background lawn and/or frequency of spontaneous revertants were used to assess tox. Doses tested ranged from 50-5000ug/plate in -S9 and, appropriate positive controls as well as vehicle and untreated negative controls were used.
- The assay was repeated 3 times because in the 1st assay the concentration tested ranged between 16.7-3330ug/plate in -/+S9 but a ppt was not seen upto 3330ug/plate so the assay was repeated using concentrations upto 10,000ug/plate. However. a ppt was seen at ≥5000ug/plate, so the assay was repeated using 5 strains with the top concentration at 3330ug/plate in -/+S9. During the 48hr incubation, the ppt observed at ≥5000ug/plate was partially dissolved so the assay was repeated for the 3rd time upto 10,000ug/plate in all 5 strains at 167, 500, 1670, 3330, 6670, and 10,000ug/plate in -/+S9. In all of the 3 assays, the treated cultures did not exceed the 2x incr in # of revertants over the concurrent negative control (vehicle and untreated cultures). The positive controls produced the expected positive response in - & + S9.
- It was concluded that CL 284-846 was **not mutagenic** in the Ames upto 10,000ug/plate in either presence or absence of metabolic activation.

Bacterial E.coli with a confirmatory assay - Plate incorporation/Study#94114 MIRACL-27193/study date: Sep 1994/GLP FDA & EPA & OECD guidelines. [redacted]

- CL 284-846 was tested in E.coli WP2 uvrA in-/+S9
- Appropriate positive control was tested, the negative control included the vehicle: DMSO
- Preliminary tox assay was done in -/+S9 at least 8 concentrations tested, 1 plate per concentration. Background lawn was used to assess tox. Doses tested ranged from 6.7-5000ug/plate in -/+S9 and, appropriate positive controls as well as vehicle neg controls were used.
- The main assay was tested upto 5000ug/plate since no tox or ppt were observed in the preliminary tox assay at this concentration in either -/+S9. All concentrations were tested in triplicates.
- It was concluded that CL 284-846 was **not mutagenic** in E.coli WP2 uvrA in either -/+S9 and in main and confirmatory assays upto 5000ug/plate.

Bacterial Ames Salmonella & E.coli - plate incorporation method/Study#93193/ MIRACL-26520/[redacted]/study date: Oct 1993/GLP FDA&EPA & OECD guidelines.

- CL 284-846 was tested in Salmonella strains TA98, TA100, TA1535, TA1537, & TA1538 together with E.coli WP2 uvrA in-/+S9
- Appropriate positive controls for each strain were tested, the negative control included the vehicle: DMSO.
- Preliminary tox assay was done in -/+S9 at least 8 concentrations tested, 1 plate per concentration using TA100 & E.coli WP2 uvrA. Background lawn was used to assess tox. Doses tested ranged from 6.7-5000ug/plate in -/+S9 and, vehicle negative control was also tested however, **no positive control were assessed.** A moderate ppt at 3333 & 5000ug/plate was observed in -/+S9 (only at 5000ug/plate in WP2 uvrA in +S9).
- The main assay was tested upto 3333ug/plate in -/+S9, higher concentration was not tested due to presence of ppt at ≥3333ug/plate. All concentrations were tested in triplicates.
- It was concluded that CL 284-846 was **not mutagenic** in the 5 Salmonella strains or E.coli WP2 uvrA in either -/+S9 and in the main and confirmatory assays upto 3333ug/plate.

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Bacterial Ames Salmonella & E.coli - plate incorporation method for M2 metabolite (CL 345-905)/Study#93194/ MIRACL-26635/ [redacted] study date: Oct 1993/GLP FDA&EPA & OECD guidelines.

- CL 345-905 was tested in Salmonella strains TA98, TA100, TA1535, TA1537, & TA1538 together with E.coli WP2 uvrA in-/+S9
- Appropriate positive controls for each strain were tested, the negative control included the vehicle: DMSO.
- Preliminary tox assay was done in -/+S9 at least 8 concentrations tested, 1 plate per concentration using TA100 & E.coli WP2 uvrA. Background lawn and revertant frequency were used to assess tox. Doses tested ranged from 6.7-5000ug/plate in -/+S9 and, vehicle negative control was also tested however, **no positive controls were assessed.** No cytotoxicity or ppt occurred upto 5000ug/plate.
- The main assay was tested upto 5000ug/plate in -/+S9. All concentrations were tested in triplicates.
- It was concluded that CL 345-905 was **not mutagenic** in the 5 Salmonella strains or E.coli WP2 uvrA in either -/+S9 and in the main and confirmatory assays upto 5000ug/plate.

2. MCGM - HGPRT in CHO cells/Study#90031/MIRACL-23185/ [redacted] study date: Apr 1990/GLP FDA.

- Appropriate positive controls in +/- S9 were tested, the negative control was the vehicle: DMSO.
- Preliminary tox assay was done in -/+S9, 10 concentrations were tested, they ranged from 0.1-1250ug/ml in -/+S9. Concentrations higher than 1250ug/ml showed ppt in the solvent DMSO. Drug concentrations at 500, 1000, and 1250ug/ml showed white ppt in culture medium therefore, limiting the use of concentrations higher than 1250ug/ml. **No positive controls were included in either -/+S9.** All concentrations including the negative control, were tested in duplicates. Cytotoxicity was not seen upto 1250ug/ml (relative cloning efficiency (RCE) was 74&33% in - & + S9 respectively). However, the drug ppt out of solution at ≥500ug/ml. Based on solubility limits, the top concentration for the main assay was 1000ug/ml for both -/+S9.
- In a cytotox assay conducted concurrently with the main assay, the RCE for the positive cont in - & +S9 were 25&50% respectively. The OECD guidelines as well as ICH recommends relative survival of 10-20% but n.l.t 10%; this was not achieved in this assay. All concentrations were tested in duplicates.
- Concentrations of the drug tested in the main assay were 100, 250, 500, 750, 1000ug/ml in -&+ S9, also a solvent control and a solution control were included.
- It was concluded that CL 345-905 was **not mutagenic** in the HGPRT assay, it did not incr the number mutant colonies in either -/+S9 upto 1000ug/plate.

3. UDS in rat primary hepatocytes/Study#90033/MIRACL-23040/ [redacted] study date: Apr 1990/GLP FDA.

- Appropriate positive control, 2-acetylaminofluorine (AAF), was tested. DMSO served as the negative control and was the vehicle for CL 284-846 whereas, ethanol was the solvent for 2-AAF.
- One each, male Sprague Dawley rat was used for the range finder and the main UDS assay.
- Ten concentrations of CL 284-846 were used in the range finder, 0.1-1250ug/ml; concentrations >1250 ppt out of sol. Duplicate cultures were used; exposure to drug or solvent was carried out for 18hr. Relative cell survival (RCS) for each duplicate was determined by comparing treated to solvent control and, relative tox was assessed by subtracting RCS from 100%. Below are the results of the range finder (table from the sponsor):

TREATMENT ug/ml	NO. OF PLATES COUNTED	AVE. VIABLE CELLS PER PLATE	SURVIVAL INDEX (a)	RELATIVE CELL SURVIVAL (b)	RELATIVE TOXICITY (c)
WTE (d)	2	111,000	44.4	100.0%	0.0%
SOLVENT	2	111,000	44.4	100.0%	0.0%
0.1	2	111,000	44.4	100.0%	0.0%
0.5	2	111,000	44.4	100.0%	0.0%
1.0	2	105,000	42.0	94.6%	5.4%
5.0	2	111,000	44.4	100.0%	0.0%
10.0	2	87,000	34.8	78.4%	21.6%
50	2	99,000	39.6	89.2%	10.8%
100	2	12,000	4.8	10.8%	89.2%
500	2	0	0.0	0.0%	100.0%
1000	2	0	0.0	0.0%	100.0%
1250	2	0	0.0	0.0%	100.0%

CELLS SEEDED PER PLATE: 250000

(a) Survival Index = $\frac{\text{Average Viable Cells per Plate}}{\text{Cells Seeded per Plate}} \times 100$

(b) Relative Cell Survival = $\frac{\text{Survival Index}}{\text{Survival Index of Solvent Control}} \times 100$

(c) Relative Toxicity = 100 - Relative Survival

(d) Untreated Control

UDS in rat hepatocytes (Cont.)

- A parallel cytotox assay was done with the main UDS assay, triplicate cultures were used. Results were as follows (table from sponsor):

TREATMENT MICROGRAM PER ML	NO. OF PLATES COUNTED	AVE. VIABLE CELLS PER PLATE	SURVIVAL INDEX (a)	RELATIVE CELL SURVIVAL (b)	RELATIVE TOXICITY (c)
MMS (d)	3	117,000	46.8	97.5%	2.5%
ETHANOL	3	120,000	48.0	100.0%	0.0%
DMSO	3	120,000	48.0	100.0%	0.0%
1.0	3	111,000	44.4	92.5%	7.5%
5.0	3	117,000	46.8	97.5%	2.5%
10.0	3	117,000	46.8	97.5%	2.5%
25	3	93,000	37.2	77.5%	22.5%
50	3	66,000	26.4	55.0%	45.0%
75	3	66,000	26.4	55.0%	45.0%
2AAF 2.0	3	45,000	18.0	37.5%	62.5%
2AAF 10.0	3	36,000	14.4	30.0%	70.0%

same footnote as range finder table above

Based on the results from the range finder, the max concentration used in the parallel cytotox and UDS assays was 75ug/ml. It is noted though that a relatively large difference in RCS and relative tox is seen between the range finder and the concurrent cytotox assays at close concentrations (100&75ug/ml); such difference was not explained.

- In the main assay, CL 284-846 was not toxic at 10ug/ml with RCS of 97.5% whereas, at 75ug/ml, the RCS was 55%.

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UDS in rat hepatocytes (Cont.)

- CL 284-846 did not cause DNA damage at any concentration as evidanced by absence of significant increase in mean net nuclear grain counts rel to the that in the negative or solvent controls. A clear increase was seen with the positive control. (Table below from sponsor):

TREATMENT MICROGRAM PER ML	RCS	SLIDES COUNTED	NUCLEI COUNTED	AVERAGE NET NUCLEAR GRAIN COUNT	STANDARD DEVIATION	NUCLEI WITH ≥ 5 NET NUCLEAR GRAINS	% NUCLEI WITH ≥ 5 NET NUCLEAR GRAINS	% CELLS IN S PHASE
VHE	97.5%	3	75	0.48	3.25	5	6.7%	1.78%
Ethanol	100.0%	3	75	-0.51	2.33	4	5.3%	1.0%
SOLVENT	100.0%	3	75	-0.35	3.41	6	8.0%	1.67%
10.0	77.5%	3	75	0.33	3.72	11	14.7%	0.67%
25	77.5%	3	75	0.28	2.66	3	4.0%	0.56%
50	55.0%	3	75	-0.92	3.17	3	4.0%	0.56%
75	55.0%	3	75	0.09	2.26	2	2.7%	1.22%
2AAF 2.0ug/ml	37.5%	3	75	40.14	14.85	75	100.0%	1.44%

RCS = Relative Cell Survival
VHE = Untreated Control

- It is noted that only 75 cells were counted, OECD guidelines recommends at least 100 cells per concentration.
- It was concluded that CL 284-846 **did not cause DNA damage** in primary rat hepatocytes under these experimental conditions.

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3. Chromosomal Aberration assay in CHO cells/Study#90032/MIRACL-23365/ [redacted] /study date: Apr 1990/GLP FDA.

- Appropriate positive controls in +/- S9 were tested, the negative control was the vehicle: DMSO.
- Preliminary tox assay was done in -/+S9, 7 concentrations tested, they ranged from 5-1250ug/ml in -/+S9. Concentrations higher than 1250ug/ml showed ppt in the solvent DMSO. Drug concentrations at 500, 1000, and 1250ug/ml showed white ppt in culture medium therefore, limiting the use of higher concentrations. The vehicle was used as the negative control, **No positive controls in either -/+S9 were tested.** All concentrations including the negative control, were tested in duplicates.
- A parallel cytotox assay was done with the main assay following the same treatment durations as the main assay. Duplicate cultures were used for the main assay. Cytotox for the range finder and parallel assay, was assessed based on relative cell growth (RCG).
- The assay was conducted properly according to OECD guidelines (incubation times, pH, osmolality checks, S9 preps, etc..).
- The main assay was conducted with 3 harvest times: 4, 8, & 12hr following initiation of exposure and for the 12hr, RCG was also determined. There were 8 replicate cultures, 2 for determining RCG and the other 6 for cell harvest at the 3 time periods (2 cultures per time point). Only 2 cultures were used for the positive control, harvested at 12hr only. For the chrom abs, 300 metaphases per concentration (150/culture) were assessed.
- The concentrations tested in the main assay were: 250, 500, 650, 800, 1000, and 1250ug/ml in -S9 and those in +S9 were: 100, 200, 350, 500, 650, and 800ug/ml. Chrom ab analysis was done only for the 3 top concentrations for both -/+S9.
- Results for the range finder and the parallel cytotox were acceptable. The RCG for the range finder was 101% at 5ug/ml and 8% at 1250ug/ml in +S9 and, 97 & 47% at these 2 concentrations in -S9. The RCG for the parallel cytotox was 89 & 54% in +S9 at 100&800ug/ml respectively, and 106 & 60% at 250 & 1250ug/ml in -S9 respectively.
- **A significant and dose dependent increase in chrom abs was seen in both - & + S9 in the main assay. Stronger signal was seen in +S9 than that in -S9.**
- In -S9 at the 4hr, no. of aberrations per cell and % of cells with aberrations was significantly increased over the negative control at the 1000ug/ml (2.7 & 2 fold respectively). At the 8hr, these values at the same concentrations were 1.75 & 2 times higher than the solvent control, respectively, and at 12hr, the corresponding values were 2 & 1.7 times respectively, (statistical significance was reached only at the 8hr period). Also at the 12hr harvest time in -S9, polyploidy was increased at the low and high concentrations relative to the negative control and was much higher than the positive control: 6 & 5% in low and high concentrations vs. 2.5&1.5% in untreated and solvent control respectively, and 2.5% in positive control. (3% at the 800ug/ml mid concentration).

CHO chrom abs (Cont.)

- Tables below from the sponsor present the results in +S9 at the 3 harvest times:

Test Article Conc. (ug/ml)	No. of Metaphases Scored	+S9 4hr Type and Frequency of Aberrations														No. of Aberrations per Cell	% of Cells with Aberrations
		PP	* tg	* Isg	tb	Isb	tf	Isf	d	r	qr	tr	Cr	** pu	** sd	* e.	
Solvent	300	1.0%	38	4	7	9		5	2	1							0.09	5.3
500	300	1.5%	59	10	15	15	1	9		1	1					1	0.14	11.3
650	175	9.1%	8	7	21	13		12	1			1			1		0.33	21.1
800	124	12.0%	4		13	9		7			2				1		0.33	16.9

Test Articl Conc. (ug/)	No. of Metaphases Scored	+S9 8hr Type and Frequency of Aberrations														No. of Aberrations per Cell	% of Cells with Aberrations
		PP	* tg	* Isg	tb	Isb	tf	Isf	d	r	qr	tr	Cr	** pu	** sd	* e.	
Solvent	300	2.0%	17	17	5	2		6									0.043	4.3
500	300	3.0%	25	8	22	7		8									0.123	10.3
650	300	3.5%	56	33	30	19		14									0.27	15.3
800	300	2.0%	37	29	21	29	1	14	2								0.223	16.0

Test Article Conc. (ug/ml)	No. of Metaphases Scored	+S9 12hr Type and Frequency of Aberrations														No. of Aberrations per Cell	% of Cells with Aberrations
		PP	* tg	* Isg	tb	Isb	tf	Isf	d	r	qr	tr	Cr	** pu	** sd	* e.	
H_2O	300	2.5%	20	1	3			5	1			1					0.033	2.0
Solvent	300	2.0%	17	4	4	2		7									0.043	4.0
500	300	5.0%	7	1	7		1	14	2		2	5	1		1		0.15	9.7
650	300	6.0%	24	5	11	4	1	11	2	1		1				1	0.10	9.7
800	300	6.0%	19	30	12	3	1	4	2		1	1	2			1	0.887	7.3
Positive Control (CP 30)	300	2.5%	19	11	25	20	6	26		1	2	11	3		1		0.41	28

* Gaps and endoreduplications are scored, but not included in calculations.

** pu and sd are considered as = 10 aberrations in calculations.

Chrom abs (Cont.)

- In +S9, from the tables above, **polyploidy increased significantly, dose dependently, and was above the values in the positive control,** at the 4&12hr harvest (9.1 & 12% at 4hr and 6&6% at the 12hr at the 650&800ug/ml respectively). **The no. of cells with abs and % of cells with abs were significantly increased in all 3 concentrations at all 3 harvest times with 1.75-5 fold increase over the solvent and/or water negative control values.**
- It was concluded that CL 284-846 **is clastogenic in CHO and causes polyploidy at the 4&12hr in +S9 and at the 12hr in -S9.**

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4. Chrom abs in CHO cells/Study#94095/MIRACL-27063/ [redacted] /study date: Jul 1994/GLP FDA & EPA, and OECD.

The CHO chrom ab assay was repeated by a different contracting lab. [redacted] incubating for longer harvest times upto 44hr, the results remained the same as those in the above study by [redacted] CL 284-846 is clastogenic in CHO cells, an in vitro mammalian cell system, in both - & + S9. Below are the tables from the sponsor with brief discussion of the assay protocols.

- Appropriate positive controls in +/- S9 were tested, the negative control was the vehicle:DMSO
- Preliminary tox assay was done in -/+S9. The cells were exposed to the drug for 20hr in -S9 and for 4hr with a total of 20hr from start of cpd exposure. Tables below from sponsor:

PRELIMINARY TOXICITY IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Treatment[1]	Mitotic Index[2] (%)	Percent Change[3]	Cell Cycle Kinetics: Percentage of cells in M_1	M_2	M_3	Average Generation Time[4] (AGT)
DMSO	6.0		65	35	0	14.8
CL 284,846 (Sedative/Hypnotic) Batch: PC 1420						
0.152 µg/mL	7.0	17	64	36	0	14.7
0.457 µg/mL	8.6	43	66	34	0	14.9
1.52 µg/mL	8.8	47	73	27	0	15.7
4.57 µg/mL	7.6	27	70	30	0	15.4
15.2 µg/mL	8.2	37	67	33	0	15.0
45.7 µg/mL	7.4	23	67	33	0	15.0
152 µg/mL	8.8	47	62	38	0	14.5
456 µg/mL	9.0	50	100	0	0	20.0
1520 µg/mL[5]	0.2	-97	100[6]	0	0	20.0

CHO cells were treated in +S9 for 4hr, metaphase cells were collected after 20hr growth period in BrdU.

PRELIMINARY TOXICITY IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Treatment[1]	Mitotic Index[2] (%)	Percent Change[3]	Cell Cycle Kinetics: Percentage of cells in M_1	M_2	M_3	Average Generation Time[4] (AGT)
DMSO	8.6		38	62	0	12.3
CL 284,846 (Sedative/Hypnotic) Batch: PC 1420						
0.152 µg/mL	7.8	-9	39	61	0	12.4
0.457 µg/mL	8.0	-7	45	55	0	12.9
1.52 µg/mL	8.8	2	48	52	0	13.2
4.57 µg/mL	8.0	-7	34	66	0	12.0
15.2 µg/mL	9.2	7	30	70	0	11.8
45.7 µg/mL	7.0	-19	34	66	0	12.0
152 µg/mL	5.2	-40	100	0	0	20.0
456 µg/mL	0.4	-95	100	0	0	20.0
1520 µg/mL[5]	0.0	-100	0	0	0	N/C[6]

[1]CHO cells were treated in the absence of an exogenous source of metabolic activation for 20 hours at 37±1°C. Metaphase cells were collected following a 20 hour growth period in BrdU.
[2]Mitotic Index = (Cells in mitosis/500 cells scored) x 100.
[3]Percent change = (Treatment mitotic index - control mitotic index)/control mitotic index, expressed as a percentage.
[4]Average Generation Time:
20 hours of BrdU exposure
((1 x frequency M1 cells) + (2 x frequency M2 cells) + (3 x frequency M3 cells))
[6]N/C = not calculated.
[5]Partially soluble (i.e., visible precipitate) in treatment medium at this concentration.

CHO Chrom abs - MA (Cont.)

- Two main assays were done, an initial and a repeate assay.
- The initial assay, cells were exposed to 4 concentrations of the drug using duplicate cultures. These were **38, 76, 152, 304ug/ml in -&+S9.** In the -S9, exposure to the drug continued upto the addition of colcemid (20hr), and in +S9 treatment was for 4hr (total 20hr harvest). In the initial assay, cells were harvested at a single time of 20hr from start of treatment. The top concentration gave 50% inhibition of MI (mitotic index); 3 lower concentrations were assessed for chrom abs.
- The concentrations for the repeate assay, were selected based on the initial assay and were the same i.e. **38, 76, 152, & 304ug/ml in -/+S9.** For both assays, positive and negative controls were assessed (solvent and untreated). Cells were exposed/treated for 20&44hr after start of a continuous treatement in -S9 and for 20&44hr after 4hr treatment in +S9. 200 metaphases per dose were examined for chrom abs and the concentrations were selected so that the highest concentration would have about 50% inhibition of MI, 3 additional concentrations were also tested.
- Statistical analysis was done using Fisher's exact test, if a positive result was found, the Cochran-Armitage test was used to detect dose-response.

CHO Chrom abs - MA (Cont.)

- Results from the initial assay are as follows (tables from sponsor):

Initial assay 20hr harvest - absence of S9

Treatment[1]	Flask	Mitotic Index[2] (%)	Cells Scored	Aberrant Cells[3] (%)	Total Number of Structural Aberrations: Gaps	Chromatid-type[4]: Breaks	Exch	Chromosome-type[5]: Breaks	Dic	Ring	Severely Damaged Cells[6]	Average Aberrations Per Cell[6,7]
Untreated cells	A	8.4	100	4	1	3	0	0	1	0	0	0.040
	B	8.0	100	3	0	1	0	0	2	0	0	0.030
DMSO	A	7.2	100	5	2	4	0	0	2	0	0	0.060
	B	8.4	100	2	2	1	0	0	1	0	0	0.020
CL 284,846 (Sedative/Hypnotic) Batch: PC 1420												
38 µg/mL	A	8.6	100	3	1	1	0	0	2	0	0	0.030
	B	6.2	100	6	1	2	3	0	2	0	0	0.070
76 µg/mL	A	5.8	100	4	1	3	0	3	1	0	0	0.070
	B	4.8	100	1	0	1	0	0	0	0	0	0.010
152 µg/mL	A	6.6	100	0	0	0	0	0	0	0	0	0.000
	B	5.2	100	0	0	0	0	0	0	0	0	0.000
304 µg/mL	A	4.0	100	1	0	1	0	0	0	0	0	0.010
	B	3.0	100	4	1	2	0	3	1	0	0	0.060
MMC 0.08 µg/mL	A	6.8	100	30	5	30	15	0	2	0	0	0.470
	B	6.4	100	27	4	18	15	0	2	0	0	0.350

[1]CHO cells were treated for 20 hours at 37±1°C in the absence of an exogenous source of metabolic activation.

Initial assay 20hr harvest +S9

Treatment[1]	Flask	Mitotic Index[2] (%)	Cells Scored	Aberrant Cells[3] (%)	Total Number of Structural Aberrations: Gaps	Chromatid-type[4]: Breaks	Exch	Chromosome-type[5]: Breaks	Dic	Ring	Severely Damaged Cells[6]	Average Aberrations Per Cell[6,7]
Untreated cells	A	7.6	100	2	1	2	0	0	0	0	0	0.020
	B	6.6	100	1	0	0	0	0	1	0	0	0.010
DMSO	A	7.0	100	2	0	1	0	0	0	1	0	0.020
	B	7.4	100	1	0	2	0	0	0	0	0	0.020
CL 284,846 (Sedative/Hypnotic) Batch: PC 1420												
500 µg/mL	A	4.8	100	1	0	1	0	0	0	0	0	0.010
	B	4.6	100	4	0	4	1	0	0	0	0	0.050
550 µg/mL	A	5.0	100	9	0	0	0	1	0	0	0	0.090
	B	6.4	100	8	1	9	1	0	0	0	0	0.100
600 µg/mL	A	1.8	100	21	2	22	10	0	0	0	0	0.320
	B	0.6	99	20	0	10	0	1	0	0	1	0.354
650 µg/mL	A	3.0	100	16	1	12	10	0	1	0	0	0.230
	B	0.8	100	10	0	12	7	1	0	0	0	0.200
CP 25 µg/mL	A	1.2	100	47	1	73	10	7	0	0	2	1.100
	B	1.4	100	47	0	77	17	4	0	0	1	1.080

[1]CHO cells were treated for 6 hours at 37±1°C in the presence of an exogenous source of metabolic activation.
[2]Mitotic index = number mitotic figures x 100/500 cells counted.
[3]Excluding cells with only gaps.
[4]Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Exch) include quadriradials, triradials and complex rearrangements.
[5]Chromosome breaks include breaks and acentric fragments; dic, dicentric chromosome.
[6]Severely damaged cells include cells with one or more pulverized chromosomes and cells with 10 or more aberrations.
[7]Severely damaged cells and pulverizations were counted as 10 aberrations.

CHO Chrom abs - MA (Cont.)

- CL 284-846 did not induce chrom abs in -S9 in the initial assay. However, in +S9, there was a **significant increase in structural abs** (chromatid breaks and exchanges) and average abs per cell (tables above). Both Fisher's exact test ($p \leq 0.01$) and Cochran Armitage test ($p \leq 0.05$) for dose response were positive. At harvest time, microscopic exam of cell monolayer in +S9 showed 25-50% cytotoxicity at 600&650ug/ml and, 0-25% cytotox at the other dose levels. In -S9, cytotoxicity of cell monolayer was 0-25% at ≤152ug/ml and 25-50% at 304ug/ml. Table below from sponsor presents the summary of results:

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Summary Table - Initial Assay

Treatment	S-9 Activation	Harvest Time (Hours)	Mitotic Index	Cells Scored	Aberrations Per Cell[1] (Mean ± SD)	Cells With Aberrations[2] (%)
Untreated	-	20	8.2	200	0.035 ± 0.184	3.5
DMSO	-	20	7.8	200	0.040 ± 0.221	3.5
CL 284,846 (Sedative/Hypnotic) Batch: PC 1420						
38 µg/mL	-	20	7.4	200	0.050 ± 0.240	4.5
76 µg/mL	-	20	5.3	200	0.060 ± 0.314	2.5
152 µg/mL	-	20	5.9	200	0.000 ± 0.000	0.0
304 µg/mL	-	20	3.5	200	0.035 ± 0.253	2.5
MMC 0.08 µg/mL	-	20	6.6	200	0.410 ± 0.758	28.5**
Untreated	+	20	7.1	200	0.015 ± 0.122	1.5
DMSO	+	20	7.2	200	0.020 ± 0.172	1.5
CL 284,846 (Sedative/Hypnotic) Batch:PC 1420						
500 µg/mL	+	20	4.7	200	0.030 ± 0.196	2.5
550 µg/mL	+	20	3.7	200	0.095 ± 0.326	8.5**
600 µg/mL	+	20	0.8	199	0.393 ± 1.039	20.6**
650 µg/mL	+	20	1.9	200	0.215 ± 0.529	17.0**
CP 25 µg/mL	+	20	1.3	200	1.130 ± 1.886	46.5**

[1]Severely damaged cells were counted as 10 aberrations.
[2]**, p≤0.01; Fisher's exact test.

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CHO Chrom abs - MA (Cont.)

- Tables below from sponsor present the results of the main assay in +/- S9 with the types of structural aberrations:

INDEPENDENT REPEAT ASSAY: 20 HOUR HARVEST

Treatment[1]	Flask	Mitotic Index[2] (%)	Cells Scored	Aberrant Cells[3] (%)	Total Number of Structural Aberrations: Gaps	Chromatid-type[4]: Breaks	Exch	Chromosome-type[5]: Breaks	Dic	Ring	Severely Damaged Cells[6]	Average Aberrations Per Cell[3,7]
Untreated cells	A	8.6	100	1	0	0	0	1	0	0	0	0.010
	B	8.4	100	4	1	2	0	2	0	0	0	0.040
DMSO	A	6.0	100	2	0	2	0	0	0	0	0	0.020
	B	9.0	100	2	0	1	0	1	0	0	0	0.020
CL 284,846 (Sedative/Hypnotic) Batch: PC 1420												
500 µg/mL	A	5.0	100	8	0	7	0	4	0	0	0	0.110
	B	5.6	100	6	0	4	0	3	0	0	0	0.070
550 µg/mL	A	8.0	100	23	1	4	0	22	0	0	0	0.260
	B	6.2	100	18	0	5	1	15	0	0	0	0.210
600 µg/mL	A	5.6	100	33	1	9	1	35	0	0	0	0.450
	B	4.6	100	42	0	16	2	39	0	0	0	0.570
650 µg/mL	A	3.0	100	37	2	9	3	44	0	0	0	0.580
	B	3.6	100	16	0	9	1	10	0	0	0	0.200
CP 12.5 µg/mL	A	3.6	100	44	1	40	4	62	0	0	0	1.060
	B	2.0	100	47	0	29	3	84	0	0	0	1.160

INDEPENDENT REPEAT ASSAY: 44 HOUR HARVEST

Treatment[1]	Flask	Mitotic Index[2]	Cells Scored	Cells with Aberrations[3] (%): Numerical	Structural	Number of Structural Aberrations: Chromatid-type[4]: Gaps	Breaks	Exch	Chromosome-type[5]: Breaks	Dic	Ring	Severely Damaged Cells[6]	Average Structural Aberrations Per Cell[3,7]
Untreated cells	A	7.2	100	1	1	0	1	0	0	0	0	0	0.010
	B	8.4	100	0	2	0	1	0	0	1	0	0	0.020
DMSO	A	8.8	100	3	3	0	3	0	0	0	0	0	0.030
	B	9.2	100	2	1	0	1	0	0	0	0	0	0.010
CL 284,846 (Sedative/Hypnotic) Batch: PC 1420													
500 µg/mL	A	8.2	100	3	0	0	0	0	0	0	0	0	0.000
	B	8.0	100	0	0	0	0	0	0	0	0	0	0.000
550 µg/mL	A	7.6	100	3	3	0	0	1	0	2	0	0	0.030
	B	8.0	100	3	4	0	2	3	0	0	0	0	0.070
600 µg/mL	A	6.8	100	3	11	2	11	0	0	2	0	0	0.220
	B	8.6	100	7	9	1	14	3	0	1	0	0	0.180
650 µg/mL	A	4.6	100	8	18	3	7	13	5	3	0	1	0.380
	B	5.2	100	8	12	1	9	6	2	2	0	0	0.190
CP 25 µg/mL	A	5.0	100	1	100	0	0	0	0	0	0	100	10.000
	B	4.2	100	1	100	0	0	0	0	0	0	100	10.000

[1]CHO cells were treated for 4 hours at 37±1°C in the presence of an exogenous source of metabolic activation.
[2]Mitotic index = number mitotic figures x 100/500 cells counted.
[3]Excluding cells with only gaps; numerical aberrations include polyploid and endoreduplicated cells.
[4]Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Exch) include quadriradials, triradials and complex rearrangements.
[5]Chromosome breaks include breaks and acentric fragments; dic, dicentric chromosome.
[6]Severely damaged cells includes cells with one or more pulverized chromosomes and cells with 10 or more aberrations.
[7]Severely damaged cells and pulverizations were counted as 10 aberrations.

CHO Chrom abs - MA (Cont.)

Summary Table for Repeate Assay

Treatment	S-9 Activation	Treatment/ Harvest Time (Hours)	Mitotic Index	Cells Scored	Aberrations Per Cell[1] (Mean ± SD)	Cells With Aberrations[2] (%) Numerical[3]	Structural
Untreated	-	4/20	9.1	200	0.010 ± 0.100		1.0
DMSO	-	4/20	9.4	200	0.000 ± 0.000		0.0
CL 284,846 (Sedative/Hypnotic) Batch: PC 1420							
38 µg/mL	-	4/20	7.4	200	0.005 ± 0.071		0.5
76 µg/mL	-	4/20	6.8	200	0.000 ± 0.000		0.0
152 µg/mL	-	4/20	8.3	200	0.010 ± 0.100		1.0
304 µg/mL	-	4/20	5.1	200	0.045 ± 0.716		2.0
MMC 0.08 µg/mL	-	4/20	8.4	200	0.165 ± 0.457		14.0**
Untreated	-	20/20	4.7	200	0.010 ± 0.100		1.0
DMSO	-	20/20	6.2	200	0.010 ± 0.100		1.0
CL 284,846 (Sedative/Hypnotic) Batch: PC 1420							
38 µg/mL	-	20/20	4.9	200	0.030 ± 0.198		2.5
76 µg/mL	-	20/20	4.6	200	0.020 ± 0.172		1.5
152 µg/mL	-	20/20	3.2	200	0.020 ± 0.223		1.0
304 µg/mL	-	20/20	2.5	200	0.025 ± 0.234		1.5
MMC 0.08 µg/mL	-	20/20	3.8	200	0.435 ± 0.985		28.5**
Untreated	-	44/44	5.4	200	0.010 ± 0.141	0.0	0.5
DMSO	-	44/44	4.2	200	0.000 ± 0.000	0.0	0.0
CL 284,846 (Sedative/Hypnotic) Batch: PC 1420							
38 µg/mL	-	44/44	2.5	200	0.015 ± 0.122	1.0	1.5
76 µg/mL	-	44/44	2.9	200	0.010 ± 0.100	1.5	1.0
152 µg/mL	-	44/44	1.8	200	0.020 ± 0.172	1.0	1.5
304 µg/mL	-	44/44	0.7[4]				
MMC 0.08 µg/mL	-	44/44	2.1	200	0.810 ± 1.398	0.0	45.0**
Untreated	+	4/20	8.5	200	0.025 ± 0.157		2.5
DMSO	+	4/20	7.5	200	0.020 ± 0.140		2.0
CL 284,846 (Sedative/Hypnotic) Batch: PC 1420							
300 µg/mL	+	4/20	5.3	200	0.090 ± 0.344		7.0*
350 µg/mL	+	4/20	7.1	200	0.255 ± 0.491		20.5**
400 µg/mL	+	4/20	3.0	200	0.310 ± 0.750		27.5**
450 µg/mL	+	4/20	3.3	200	0.390 ± 0.728		36.5**
CP 12.5 µg/mL	+	4/20	2.8	200	1.110 ± 1.445		66.5**
Untreated	+	4/44	7.8	200	0.015 ± 0.122	0.5	1.5
DMSO	+	4/44	9.0	200	0.020 ± 0.140	2.5	2.0
CL 284,846 (Sedative/Hypnotic) Batch: PC 1420							
300 µg/mL	+	4/44	8.1	200	0.000 ± 0.000	1.5	0.0
350 µg/mL	+	4/44	7.8	200	0.050 ± 0.297	3.0	3.5
400 µg/mL	+	4/44	7.7	200	0.200 ± 0.743	5.0	10.0**
450 µg/mL	+	4/44	4.9	200	0.285 ± 0.948	8.0**	13.0**
CP 25 µg/mL	+	4/44	4.6	200	10.000 ± 0.000	1.0	100.0**

[1]Severely damaged cells were counted as 10 aberrations.
*, $p \leq 0.05$; **, $p \leq 0.01$; Fisher's exact test.
[2]Data not collected for 20 hour harvest time.
[3]Numerical aberrations include polyploid and endoreduplicated cells.
[4]Not evaluated due to excessive toxicity.

- From the above tables, it is clear that CL 284-846 was **clastogenic causing both structural and numerical aberrations in +S9.** This positive response occurred mainly at the higher concentration although at the 44hr harvest time, a concentration-dependent response was seen in all 4 concentrations tested for the numerical abs and a concentration-dependent increase in structural abs seen for the 3 higher concentrations, all responses are relative to the negative control. A significant increase in abs was also seen at the 20hr harvest in all 4 concentrations. The Cochran Armitage test for dose response was positive for both harvest times and for the numerical abs at the 44hr.
- CL 284-846 was not clastogenic in -S9 regardless of harvest time.
- It is concluded that CL 284-846 **is clastogenic in +S9 (Fisher's exact test and Cochran Armitage for dose response). Both structural and numerical abs were induced relative to the negative control.**

5. Chrom abs in Human lymphocytes with a confirmatory assay with multiple harvest times/GTR#30416/ [redacted] /study date: Mar 1997/GLP FDA, OECD, and Japanese guidelines.

- Appropriate positive controls in +/- S9 were tested, the negative control was the vehicle: DMSO.
- Dose range finder/cytotoxicity assay was done with CL 284-846 in -/+S9. The concentrations tested with S9 were 174, 580, 1740ug/ml and in -S9: 58, 174, 580, 1740ug/ml. DMSO and negative controls (medium and cells only) were used as drug free controls; no positive controls were tested in the range finder. Cultures were harvested 22hr after initiation of treatment. Cytotoxicity was assessed using MI. The drug was incubated with cells for 19.4hr in -S9 and 3hr in +S9. Single cultures per concentration were tested. Mitotic inhibition at 1740ug/ml in +S9 was 54% and only 6% at the lowest concentration of 174ug/ml. Based on these results, concentrations selected for the main assay in +S9 were113, 227, 452, 903, 1360, & 1810ug/ml for 22hr harvest time. The MI inhibition in -S9 for the dose range finder were 18, 45, 55, & 94% at 58, 174, 580, and 1740ug/ml respectively, compared with solvent control. Based on these results, the concentrations selected for the main assay in -S9 were 28.4, 56.7, 113, 227, 452, 903, & 1810ug/ml for 22hr harvest.
- Two assays for the chrom abs were done: an initial one and a repeate.
- In the **initial assay** in -S9 at **22.1hr** assay, a precipitate (ppt) was seen at ≥903ug/ml. Inhibition of MI were 5, 19, 43, 52, 93, & 74% at 56.7, 113, 227, 452, 903, & 1810ug/ml respectively. Chrom abs analysis was done only for 56.7, 113, 227, and 452ug/ml. CL 284-846 **did not induce structural or numerical abs at any of these concentration.**
- In the initial assay in +S9, a ppt was seen at ≥903ug/ml. Inhibition of MI at **22.1hr** assay, were 2, 10, 15, 50, 81, & 81% at 113, 227, 452, 903, 1360, & 1810ug/ml respectively. Chrom abs analysis was done only for 113, 227, 452, and 903ug/ml. CL 284-846 **did not induce structural or numerical abs at any of these concentrations.**
- Based on the results of the initial assay, the following concentrations were evaluated for the repeate assay: +S9: 113, 225, 300, 450, 600, 900, & 1200ug/ml in 21.9 & 46hr assays: in -S9 the concentrations for the confirmatory assay were: 113, 225, 300, 450, 600, 900, & 1200ug/ml for the 21.9hr assay and, 61.5, 113, 225, 300, 450, 600, 900, & 1200ug/ml for the 46hr assay.

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Chrom abs in humans (Cont.)

- In the **confirmatory assay in -S9**, a ppt was seen at ≥600ug/ml at the 21.9hr, inhibitions of MI of 33, 40, 40, 65, 72, 84, & 79% were observed relative to the solvent control in the cultures dosed at 113, 225, 300, 450, 600, 900, & 1200ug/ml respectively. Chrom ab analysis was done only for the 113, 225, 300, & 450ug/ml. CL 284-846 **did not induce structural or numerical abs at any of these conc.** In the 46hr assay, a ppt was also seen at concentrations ≥600ug/ml with inhibition of MI of 33, 32, 56, 71, 84, 94, 97, & 98% at 61.5, 113, 225, 300, 450, 600, 900, & 1200ug/ml respectively. Chrom ab analysis was done only for 61.5, 113, and 225ug/ml. CL 284-846 **did not induce structural or numerical aberrations at any of these concentrations.**
- In the **confirmatory assay in +S9**, a ppt was seen at ≥600ug/ml at the 21.9hr assay with reduction in MI of 6, 3, 21, and 67% rel to the solvent control at 450, 600, 900, & 1200ug/ml. Chrom ab anaylsis was done for concentrations ≥450. CL 284-846 **did not induce structural or numerical aberrations at any of these concentrations.** In the 46hr assay, a ppt was seen at ≥600ug/ml with MI inhibitions of 31, 39, & 78% for 450, 900, & 1200ug/ml. Chrom ab analysis was done for 450, 600, 900, & 1200ug/ml respectively. CL 284-846 **did not induce structural or numerical aberrationss at any of these concentrations. However, at 1200ug/ml in the 46hr and at 900ug/ml at 21.9hr, incidence of cells with endoreduplication was 1.5 & 0.5% respectively. The concurrent negative control incidence was 0. The sponsor referred to historical control data from 1995 where the solvent control range of 0-2% was reported therefore, the sponsor indicated that CL-284-846 did not induce endoreduplication since these values were within the historical control range. However, it is interesting to note that the upper range of 2% is in -S9, in presence of S9, the range is 0-0.5%. Moreover, in the historical solvent control data of 1996, the range in + and - S9 was 0-0. Therefore, it is the reviewer's opinion that CL 284-846 caused endoreduplication that was outside the concurrent and one set of the historical range values.**

Also, the levels of cytotoxicity indicated by the sponsor are not that high to prevent analysis of chrom abs at these concentrations. It is a common knowledge that the important factor in chrom ab assays that determine the concentration at which chrom ab analysis can be done is having adequate number of cells (100/culture or 200 cells/concentration) and not neccessarily, the level of cytotoxicity. Therefore, higher concentration of CL 284-846 should have been tested for chrom ab analysis.

Based on the above arguments, it can be concluded that CL 284-846 caused endoreduplication in human lymphocyte and that higher concentrations both in - & + S9, could and should have been studied for chrom abs.

6. In vivo Micronucleus in mice/Study#90030/MIRACL-23442/[redacted] study date: Aug 1990/GLP FDA and OECD.

- Male CD-1 mice were used (n=10/dose/time point except for 300mg/kg there were 9 males each for the 24 & 48hr harvest and 10 for the 72hr). The vehicle was 0.5% MC + 0.1% Tween 80 in water. The positive control was cyclophosphamide (CP) at 60mg/kg.
- Doses were 10, 100, & 1000mg/kg. Excessive mortality, 16/30, was seen at 1000mg/kg therefore, this dose was excluded from the study. Another dose of 300mg/kg was used as the high dose.
- CL 284-846 was administered via oral gavage as a single dose at 10ml/kg. Mice were killed at 24, 48, and 72hr post dose. The vehicle control mice were also killed at these times however, the positive control group was killed at a single time point of 24hr. Mice were killed by cervical dislocation and femur removed and processed.
- Clinical signs were seen in all doses: ataxia seen immediately after dosing, decreased body tone, body drop with abnormal gait. No signs in control.
- There was no significant increase in micronucleated polychromatic erythrocytes (MPCE) at the 24 & 48hr harvests relative to the control. A significant increase ($p=0.02$) in MPCE was seen at the mid dose of 100mg/kg at the 72hr harvest (2.00 ± 1.33 vs. 0.900 ± 1.10 for the control) however, no increase noted at the low and high doses. The sponsor stated that this mean increase in MPCE fell within the historical control range of 0-2.00 for mean MPCE/1000 PCE/mouse. **However, when the range and not the mean for the 100mg/kg dose, was compared to historical data, this range was 0-4 MPCE which is 2x higher than the upper range of 2 for historical data; though this may not change the sponsor's conclusion dramatically, it should be noted.** The sponsor indicated that because of absence of dose response trend and no positive respense in at least one dose, this finding was considered not to meet the criteria for a positive finding (a significant positive response in at least one dose and a significant positive dose response trend).
- The PCE/NCE ratio was significantly increased in low and mid dose groups at the 48hr and in low dose at the 72hr harvest time. However, these values fell within historical range.
- the sponsor concluded that CL 284-846 did not induce MN in mouse bone marrow upto 300mg/kg oral dose.
- According to OECD guidelines, at least 2000 PCE per animal is counted for presence of micronuclei; the sponsor did only 1000 cells. Clearly, scoring one half of the cells reduces the chance of detecting any positive finding by 1/2.

[redacted] APPEARS THIS WAY ON ORIGINAL

7. In vivo chrom abs in rats/Study#90135/MIRACL-23308/ [redacted] /study date: Jul 1990/GLP FDA.

- Male SD rats (n=10/dose/time point) were administered a single gavage oral dose of CL 284-846 at 10, 40, and 170mg/kg (the high dose (HD) was selected to be 1/3 of the (MLD) median lethal dose). The vehicle was methocel 0.5% + 0.1% Tween 80 in water. The positive control was triethylenemelamine (TEM) at 0.5mg/kg p.o. Animals were killed at 24, 48, and 72hr post dose.
- In this assay only 100 cells per animal were assessed for MI; however, at least 1000 cells per animal for all treated and negative controls are recommended, according to OECD guidelines. This study is therefore, deficient and marginally, if at all accepted. It is noted that the minium # of cells for metaphase analysis according to OECD was done i.e. 100 cells per rat.
- CL 284-846 **did not induce chrom abs upto 170mg/kg p.o.** dose in rat bone marrow.

8. In vivo/in vitro rat hepatocyte UDS/[redacted] Study date: Aug 1994/GLP FDA, EPA, & OECD.

- SD male rats (5/dose gr) were administered CL 284-846 to assess the drug's potential to induce unscheduled DNA synthesis in primary rat hepatocyte cultures. Females were not tested because of similar metabolic and toxic profiles. The negative control was the vehicle, 0.5% methocel + 0.1% Tween 80 and the positive control was dimethylnitrosamine (DMN) 35mg/kg p.o.
- Table below presents study design including animal # and doses:

Group Number	Treatment Article	Dose Level	Number of Rats Treated	Clinical Signs after Treatment	Number of Rats Harvested	Clinical Signs at Harvest	Post Treatment Harvest Period
1	1% CMC	10 mL/kg	5	normal	3	normal	12 - 16 hours
2	CL 284,846	100 mg/kg	5	normal	3	lethargic; 1 nasal discharge	12 - 16 hours
3	CL 284,846	200 mg/kg	5	normal	3	lethargic and sneezing	12 - 16 hours
4	CL 284,846	400 mg/kg	5	normal	3	lethargic	12 - 16 hours
5	DMN	35 mg/kg	5	normal	3	lethargic	12 - 16 hours
6	1% CMC	10 mL/kg	5	normal	3	normal	1 - 3 hours
7	CL 284,846	100 mg/kg	5	normal	3	1 normal 2 lethargic	1 - 3 hours
8	CL 284,846	200 mg/kg	5	normal	3	lethargic	1 - 3 hours
9	CL 284,846	400 mg/kg	5	normal	3	lethargic	1 - 3 hours
10	DMN	35 mg/kg	5	normal	3	normal	1 - 3 hours

- Dose range finder was done at 100, 200, 400, 500, & 600mg/kg administered to 5/dose group rats as a single dose. Rats were observed for clinical signs for 3d postdose and, mean B.wt was monitored prior to dosing and at 1&3d postdose. Based on the results of the dose range finder the following doses were selected for the main assay: 100, 200, & 400mg/kg.
- Clinical signs noted were lethargy immediately postdose in all dose groups and crusty eyes. At ≥500mg/kg the following occurred: lethargy, paralysis, piloerection, prostration, diarrhea, crusty eyes, lacrimation, and excessive salivation. There were deaths in any gr.
- Clinical signs were not observed in the main assay immediately after dosing at both harvest times. Lethargy noted prior to sacrifice at both harvest times.
- In the UDS assay, hepatocytes were harvested at 1-3 and 12-16hr post dosing and the appropriate no. of cells were examined/counted per animal (150 cells per rat).
- CL 284-846 did not at any of the doses tested, caused a significant increase in mean net nuclear grain however, a sig incr noted with the positive control. It was concluded that CL 284-846 is negative in the in vivo UDS assay.

SUMMARY AND CONCLUSIONS FOR GENETIC TOXICOLOGY:

CL 284-846 was tested in a total of 12 assays (includes 5 bacterial assays, 2 CHO chrom abs. and, 2 UDS), for its gene mutation potential. The drug was not mutagenic in the Ames bacterial gene mutation assay, MCGM - HGPRT CHO cells for gene mutation, rat hepatocyte UDS, in vivo mouse bone marrow MN, and rat in vivo bone marrow chrom abs. The drug was **clearly clastogenic causing both structural and numerical aberrations (polyploidy and endoreduplication) in absence and/or presence of S9 in 2 CHO chrom ab assays and in human lymphocyte chrom ab assay.**

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CARCINOGENICITY

BACKGROUND:

[redacted] initially planned a **dietary** rat and mouse car studies with CL 284,846 in a protocol submitted May 12th 1992 (this submission also included 3mo rat and 3mo mouse dose range finding studies for dose selection). The doses proposed by the sponsor in that submission were **1, 5, 20mg/kg/d in the diet for both species**. A telecon between FDA (Fitzgerald and Osterberg) and the sponsor. took place on July 1992 to discuss dose selection for rodent dietary car studies. It was agreed that the doses in the **rat** car study be **1, 10, and 20mg/kg/d** (MD incr to 10mg/kg from the original proposal of 5mg/kg/d). The sponsor initiated the rat study on Dec 14 1992 but the mouse car study was postponed because the sponsor wanted to conduct more PK studies and dose range finding and the Division thought the doses for the mouse were too low. It was agreed that 4 dose groups will be tested in mice via the **diet** as follows: **25, 50, 100, and 200mg/kg/d**. In Nov 1993 (serial# 029), the sponsor submitted a proposal for: mouse ***gavage*** car study, a **3mo dietary- , 3mo gavage-** dose range finding studies, a **5mo gavage** dose range finding study, and, comparison of steady state AUC in mice and humans. In that amendment, (#029) the doses for the mouse **gavage** car study were **1, 5, 20mg/kg/d**. At the end of phase II meeting with the sponsor on Dec 14 1993, we learned that the mouse gavage car study has been initiated as of Dec 2 1993 (without concurrence from us or the Exec CAC) . Basis for dose selection for the mouse was AUC of parent which was determined at HD to be 100x the human AUC at 10mg oral dose. There were several memos and telecons between the Division and the sponsor regarding the basis for dose selection since the Division was not in favor of using AUC as the basis for dose selection and believed that MTD was the more appropriate basis for dose selection. On Jan 6 1994, the sponsor via a telecon, **proposed raising the HD from 20 to 50mg/kg** based on the similarity of AUC of the parent following 40mg/kg administered via gavage in a 3mo study, to that for 240mg/kg administered by diet (note that the Division's original recommendation for a HD was 200mg/kg).

On Jan 11 1994, the following was agreed to during a telecon between the Division and sponsor: AUC could not be used as basis for dose selection, the Division agreed that the doses be incr as follows: **m: 10, 40, 80mg/kg/d and f: 25, 100. 200mg/kg/d**, if any toxicity develops during dosing the sponsor is to inform the Division, and if mortality is not excessive, it may be recommended that the study continue beyond 24mo to compensate for the 6wk period where the doses were too low. Later in a Feb 25, 1994 correspondance (#040), the sponsor indicated that these higher doses have been implemented on d42 of study initiation.

On Jun 13 1994 (Amendment# 051) reference was made to the May 27 1994 telecon between the Division and sponsor regarding the progress of the mouse gavage car study. There were a number of gavage deaths and if that rate of death continued, the study could not last for 2yrs. The Division after consideration of the frequency of deaths, indicated that the study can proceed to 18mo and may still be considered a valid study. The sponsor. agreed that they will continue the gavage study for as long as possible. The sponsor at the same time, porposed to initiate a

"back up" **dietary** study in mice with a starting date of early June 1994. This study will have more animals per dose gr, 2 control grs instead of 1, and TK after 1yr and at end of study. The back up mouse dietary study was initiated on Jun 8 1994. In a Jun 30 telecon, the sponsor agreed with the Division to terminate HD gr in the gavage study due to high mortality and continue with the rest of the doses. However, it would have been preferable if possible, to obtain 50% survival rate at 18mo that might have rendered the study valid, but terminating the HD only, would make this study only as a supportive to the dietary study. A status report on the survival of the mouse gavage car study was sent by the sponsor on Aug 5 1994 (serial# 060). It was reported that mortality rate in the remaining drug grs continued to incr and recommended that the entire study be terminated; this study was terminated on Sep 1994.

All toxicology studies were conducted under GLP unless stated otherwise.
The following dose-range finding studies were conducted for the mouse and rat:

Rat:

3 month oral ***dietary*** study (#26070)
Doses: 1, 3, 10, 32, 100mg/kg/d

3 month oral **gavage** study (#23438)
Doses: 5, 50, 200mg/kg/d

3 month oral **gavage** study (#23441)
Dose: 100mg/kg/d

3 month oral **gavage** study with 1 month recovery (# 26170/[redacted]
Doses: 3, 10, 30, 100mg/kg/d

Mouse:

3 month oral ***dietary*** study (#26277)
Doses: 1. 5, 40, 240, 1500mg/kg/d

3 month oral ***dietary*** study (#27307 & 27333)
Doses: 25, 50, 100, 200mg/kg/d

5 month oral **gavage** study (#26929)
Doses: 20, 40, 80, 160, 240mg/kg/d

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RAT:

- 3 month oral ***dietary*** study (#388/Indexing# 26070)
  Study Initiation Date: Jun 1991 Lab: [redacted]

Doses: **1, 3, 10, 32, 100mg/kg/d**; control received the drug free diet.
Strain/No./Sex/Dose: Sprague Dawley/10/sex/dose; additional 20/sex/dose (3/sex/time point) for TK on days 7&86 at dose groups 1, 10, 100mg/kg/d.
(these TK rats were killed immediately after last time of blood collection and no gross or histopath was done). Each animal was bled 6 times over 24hr from the orbital sinus.

Parameters assessed: mortality, clinical signs, B.wt/wt gain, food intake/feed efficinecy, plasma levels/TK (blood collected from the oribital sinus on days 7&86 for TK), organ wts, **only the liver** was examined for histopath, and gross exam done on all tox animals.

Results:
Mortality & Clinical Signs: there were no deaths or clinical signs in any gr.
B.wt/Food Intake: a sig decr in mean B.wt gain was seen in HDm&f throughout the study (14&22% in m&f respectively, lower than the corresponding cont). Mean B.wt was also decr in HDm&f but reached statistical sig only in HDf (7% lower than the cont). These decreases did not correlate with a decr in food intake or food efficiency, in fact, an incr in these 2 parameters was noted in females dosed 10&32mg/kg, mean food intake was also incr in males dosed 10mg/kg and, food efficinecy was sig incr in females dosed ≥10mg/kg and males dosed 100mg/kg.

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