Mouse dietary 2yr car (Cont.)

The table below from the sponsor presents the incidence of liver tumors and # of animals with these tumors. It can be seen that there is no difference between # of males in drug grs and those of the cont, whereas, a **dose-dependent incr in # of females dosed 100&200mg/kg** was seen compared to the corresponding cont.

INCIDENCE OF HEPATOCELLULAR TUMORS BY NUMBERS OF ANIMALS WITH TUMORS/GROUP

	MALES						FEMALES					
Dose Group	1	2	3	4	5	6	1	2	3	4	5	6
N° Livers Examined*	75	74	75	74	75	75	74	75	75	73	75	75
Dose (mg/kg/day)	0	0	25	50	100	200	0	0	25	50	100	200
CARCINOMAS	3	7	5	5	1	5	3	0	0	1	1	0
ADENOMAS	15	10	6	10	10	13	2	0	3	1	4	9
TOTAL NUMBER OF ANIMALS WITH HEPATOCELLULAR NEOPLASMS**	18	16	10	15	14	18	2	0	3	2	5	9

* Excluded livers with severe autolysis

** Three males had both carcinomas and adenomas

TK: as indicated earlier, blood was collected on d7 and 1yr post dose at 5 times per day from 4mice/sex/dose/time point. Mean T_{max} values for the parent ranged between 0-18hr at d7 and 0-4hr at 1yr postdose, the corresponding values for the metabolite were 0-18hr on d7 and 0-8hr after 1yr after dosing. From the data in the table, in general, there seem to be no sex difference in conc or exposure of the parent or the metabolite (though some difference was seen for the metabolite AUC values at 200mg/kg on d7 and, for the parent AUC values of 200mg/kg gr at 1yr measurement). The conc incr linearly with dose for the parent and metabolite when measured on d7 but somewhat linear for samples collected at 1yr. Exposure was incr for both the parent and metabolite but the incr was not proportional to dose. There was no drug accumulation with time at the 25mg/kg however, both parent and metabolite in both sexes seemed to accumulate at ≥50mg/kg/d doses.

From the data, mean plasma levels of the parent ranged between 0.07±0.04 to 1.50±0.34ug/ml and the mean exposure ranged between 1.0 to 15.3ug.hr/ml. The mean plasma range for the metabolite were 0.03±0.01 to 1.51±0.95ug/ml and the mean exposure values ranged between 0.28 to 18.2ug.hr/ml. The range of plasma conc at the 200mg/kg for f was 0.02-0.62ug/ml and that for m 0.02 to 1.7ug/ml.

Mouse dietary 2yr car (Cont.)

The following table presents mean±s.d (not calculated for AUC); values in ( ) are for the desethyl metabolite (CL284-859).

Dose (mg/kg)	C_{max} (ug/ml)		AUC_{0-18} (ug.hr/ml)	
	m	f	m	f
DAY 7				
25	0.07±0.04 (0.03±0.01)	0.42±0.31 (0.38±0.33)	1.1 (0.28)	3.3 (2.44)
50	0.14±0.03 (0.07±0.02)	0.11±0.03 (0.06±0.02)	1.4 (0.62)	1.2 (0.59)
100	0.21±0.07 (0.18±0.06)	0.22±0.26 (0.17±0.30)	1.7 (1.32)	2.1 (1.65)
200	0.28±0.24 (0.32±0.35)	0.35±0.31 (0.45±0.45)	1.4 (0.88)	3.5 (5.74)
MONTH 12				
25	0.09±0.05 (0.07±0.04)	0.09±0.03 (0.07±0.02)	1.0 (0.82)	1.1 (0.78)
50	0.21±0.12 (0.23±0.14)	0.17±0.13 (0.18±0.14)	2.5 (2.73)	2.4 (2.14)
100	0.66±0.10 (0.77±0.09)	0.48±0.26 (0.71±0.33)	6.0 (8.17)	5.3 (7.35)
200	1.50±1.0 (1.50±0.34)	0.37±0.23 (1.51±0.95)	15.3 (18.2)	4.1 (13.7)

Mouse dietary 2yr car (Cont.)

Summary & Conclusions:

Oral dietary administration of CL-284,846 to mice at 25, 50, 100 & 200mg/kg/d caused a sig, dose- and drug-related incr in mortality in both sexes with more deaths in f than m. In both sexes, a sig trend in increased mortality was seen across the grs, however, when HD of 200mg/kg gr was excluded from analysis, a decreased trend of survival was not seen in males but a marginal decr was seen in f across the other dose grs (p=0.05). It was stated by the sponsor that these deaths were not related to adverse
clinical signs, marked changes in B.wt/food intake, or other drug related effects. It is

the **opinion of the reviewer that although a clear cause of death could not be established, a drug effect can NOT be excluded.** There were no clinical signs in any f gr or in m dosed ≤100mg/kg. The only drug related finding was in HDm where a small incr in frequency of wet and/or stained abdominal area and piloerection were seen. A sig and dose-dependent incr in mean B.wt was seen in m and f starting on wks 1-2 of dosing that lasted till wk54 in m and wk80 in f. These wt incr in m were mild ranging between 2-7% compared to the combined cont but greater incr noted in f specially those in higher doses: 2-6% at 25mg/kg and 12-15% at ≥ 50mg/kg. The increases in mean B.wt were drug related but not considered of biological sig in all m drug grs and f dosed 25mg/kg but biologically and toxicologically sig in f dosed 100&200mg/kg because they correlated with histopath findings (liver adenomas in f; see below). Increase in mean wt was accompanied by incr in food intake as seen in m dosed ≥100mg/kg/d and in f dosed ≥50mg/kg/d. These incr were drug related and occurred mainly during the 1st 15wks of dosing and ranged between 2-16% higher than the values for the combined cont. There were no drug related effect on hematology. The following drug related gross findings were seen (compared to the cont): Liver - mass: in 9/75 f dosed 200mg/kg vs. 3/75 of f cont. This was correlated with histopath finding of increased adenomas in this gr.

The main drug related histopath finding was a **statistically sig increase in incidence of hepatocellular adenomas in females dosed 200mg/kg** that exceeded the historical range. These findings in f dosed 200mg/kg/d occurred at **plasma conc that ranged between 0.02-0.62ug/ml and, mean exposure levels of 3.5-4ug.hr/ml.** Other female drug grs as well as all male grs were presented with this tumor but the incidence was not dose-dependent. The sponsor considered this lesion to be an ***epigenetic*** phenomenon and does not constitute a health risk to human. This conclusion was based on the following arguments presented by the sponsor:

1. The liver adenomas was only found in f mice compared to 2 cont grs, not dose dependent, and not found in either sex of the rat when the drug was administered daily for 2yrs. at comparable doses.

Mouse dietary 2yr car (Cont.)

2. There was no incr in liver carcinomas of either sex relative to the cont.

3. The incr in adenomas in f was associated with the incr in mean B.wt. The sponsor supported this argument by referencing the literature. Studies have shown a correlation between incr in B.wt specially during wk52 of dosing, and increased incidence of liver tumors in mice (Ward et al., 1996 Toxicol Pathol#24; Hart et al., 1996 Exp Toxicol Pathol# 48; Fu et al., 1994 Carcinogenesis#15; Sheldon et al., 1995 Toxicol Pathol# 23; Hart & turturro, 1990 J. Nutr Biochem#1). In the present study, mean B.wt was incr in m & f mice of all drug grs with a 13-15% incr over the cont in f mice dosed 100&200mg/kg/d. These incr in wt were seen from wk1 of dosing and persisted throughout the 1st 18mo of dosing.

4. The sponsor referred to the 2 genetic tox assays conducted with CL,284-846: the UDS & in vivo rat BM MN. Based on the negative findings in these assays, the sponsor suggested that the mechanism of the liver adenomas in f is indirect, caused by non-genotoxic changes in liver homeostasis that can lead to development of these tumors. **However,** based on the reviewer's conclusions, CL-284,846 was **clastogenic both in presence and/or absence of S9, causing structural and numerical aberrations (polyploidy and endoreduplication) when tested in Chinese hamster ovary cells and human lymphocyte chromosomal aberration assays.** Therefore, an epigenetic mechanism of action for the liver adenomas can not be proposed. The sponsor stated that these sequence of findings (incr wt, incr liver wt and hypertrophy, induction of liver enz) and the presence of liver tumors are similar to those seen with phenobarb.

5. The sponsor arguments continued and a reference was made to a study that indicated epigenetic mechanisms leading to tumors that occur *only* at high doses with long continued exposure to the cpd and that these types of tumors have not been associated with human carcinogenesis (Williams, 1987 In: nongenotoxic mechanisms in carcinogenesis, Banbury report 25, Cold Spring Harbor, NY). The liver adenomas in the present study occurred in f administered 100&200mg/kg for 2years at plasma levels that represent at least 59x higher than those measured in humans dosed 10mg/d.

6. The sponsor refers to the position of the NTP (National Toxicology Program) that concludes. "findings of liver tumors in mice only, do not carry the same wt for human risk as a trans-species, multiple site carcinogenic response".

7. A recently published article (Alden et al., 1996, Tox Path# 24) concluded that "a positive hepatic tumorigenic response in mice, if not accompanied by a tumor response in other tissues or in the rat, is generally not considered predictive of a human cancer risk".

Mouse dietary 2yr car (Cont.)

8. The EWG of the ICH, has commented on the decr usefullness of the mouse 2yr bioassay to predict human risk (ICH: draft guidelines on testing for car of pharmaceuticals; notice FR, Aug 21 1996). A report on marketed human drugs known to be carcingenic in rodents, demonstrated the poor predictability of mouse car study for detection of human cancer risk (Davies & Monro, 1995, J. Am. Coll. Toxicol. #14).

Based on all of the above arguments, the sponsor concluded that the increased incidence of liver adenomas in f mice dosed 200mg/kg is an epigenetic mechanism of the drug and that CL 284, 846 is not a direct carcinogen and does not constitute a risk to human health.

The reviewer agrees with most of the above arguments by the sponsor that explain the cause of liver adenomas in f to be perhaps, through the indirect effect of the drug causing enz induction (which is by the way, not a large effect), incr liver wt and hypertrophy, and cell proliferation that caused the development of these pre-neoplastic lesions. Also, the absence of any tumors in the rat lifetime bioassay and no similar or dose dependent findings in m mice and no increase in incidence of liver carcinomas in these mice, argues that the liver adenomas induced by CL-284,846 may have been induced via an indirect mechanism. However, the reviewer disagrees with the sponsor that the drug is not mutagenic based on 2 negative genetic tox assays referrenced by the sponsor. CL 284-846 was positive clastogen in CHO and human lymphocyte in vitro cytogenetic assays. Therefore, a non-genotoxic mechanism can not be ruled out.

APPEARS THIS WAY ON ORIGINAL

Dietary Administration: 65 week study (# 94176; GTR# 29669)
Study Date: Dec 1994
Report Date: Jun 1997 Lab: Wyeth-Ayerst

This study was originally designed to be a 2 year car study however, in view of another (the above study) dietary mouse 2 yr study that was ongoing uneventfully and close to completion, this study was considered as a chronic tox study and terminated at wk65. Also, liver samples for potential PCNA assessment and liver, kidney, and lung samples for potential ^{32}P postlabelling were collected from a given no. of mice/gr at end of study and end of 4wk recovery; however, these analyses were not done.

Strain/# per sex/dose: CD-1; 65/sex/dose; there were 2 cont grs; one gr had 65mice/sex the other had 50/sex; the cont grs were administered the diet alone.

Doses (mg/kg/d)/duration: 0, 0, 25, 50, 100, 200 for 65wks; at end of 65wks, 10/sex/dose were kept for a 4wk recovery (wk 69).

Doses were selected based on 2wk and 3m dietary dose range finder studies. Death occurred at 1500mg/kg/d in the 3mo study and hepatic histopath was seen at ≥240mg/kg in the 2wk study. Therefore, the HD for this study was selected as 200mg/kg.

Parameters assessed: mortality, clinical signs, B.wt, food intake, palpable mass assessment, and clinical lab parameters (hematology and clin chem done only for moribund-kill mice). Postmortem was done on all mice (scheduled sacrifice, moribund, and found dead). Ten mice per sex/dose were randomly selected for postmortem exam. Organ wt (liver, kidneys, heart, adrenals, brain, testes, or ovaries), gross exam and histopath of approximately 50 tissues per mouse per sex per gr was done.

Results:
Mortality & Clinical Signs: no drug related deaths in any gr except for a single death in a male dosed 50mg/kg/d that was killed in moribund during wk60 of dosing. The sponsor indicated that the cause of death in this mouse was unknown since no blood samples were taken for evaluation of hematology and no necropsy records or tissues taken for histopath. The sponsor considered this to be an isolated incidence. *It is the opinion of the reviewer that although the death of a single mouse may not have had an overall effect on the quality of the study, a cause of death was not determined, therefore, a drug related effect can not be ruled out.* Clinical signs were minimal and included staining of urogenital area and swelling or perianal area, these were seen in all grs with slightly higher incidence in mice treated vs. the cont.

Mouse 65wk dietary (Cont.)

Table below presents survival (%) at end of study.

Summary of Survival at Study Termination

Dosage Level (mg/kg/day)	Males	Females
0	41/50 (82%)	42/50 (84%)
0	57/65 (88%)	59/65 (91%)
25	57/65 (88%)[a]	60/65 (92%)
50	55/65 (85%)	53/65 (82%)
100	60/65 (92%)	54/65 (83%)
200	56/65 (86%)[b]	58/65 (89%)

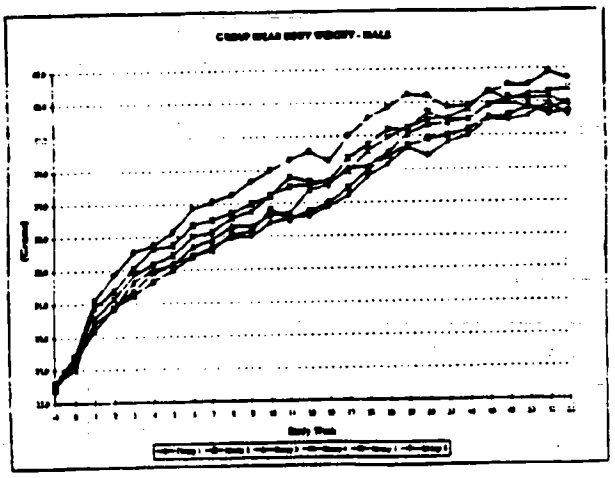
a: One additional male was found dead during week 66.
b: One additional male was found dead during week 69

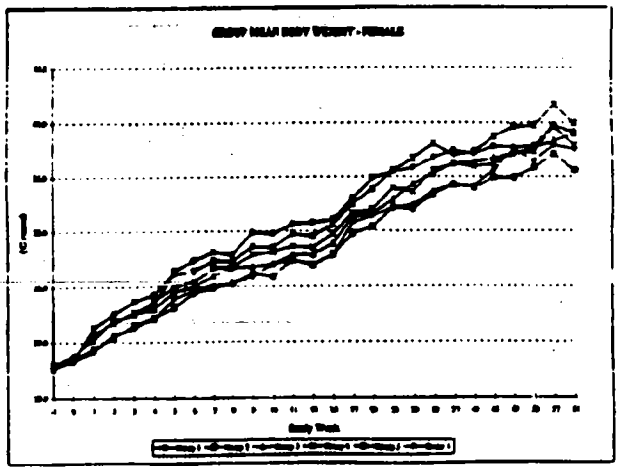
Hematology, Clin Chemistry, Palpable Mass, & Gross Necropsy: there were no drug related changes in these parameters. Any findings that reached statistical sig were considered normal variations for these mice, small, and not dose dependent.

APPEARS THIS WAY ON ORIGINAL

Mouse 65wk dietary (Cont.)

B.wt & Food Intake: mean B.wt was incr in treated mice throughout the study compared with the combined cont. The incr in wt was dose dependent, seen at ≥25mg/kg/d doses starting from wk1 till end of study. The incr in mean wt (rel to the combined cont values), ranged between 3-5% in m&f dosed 25&50mg/kg, from 3-7% in m and 4-11% in f dosed 100mg/kg, and from 4-10% in m and 6-10% in f dosed
200mg/kg. These incr in wt did not correspond to an incr in food intake. There were some changes in food intake that reached statistical sig when compared to consumption in the cont grs, however, these changes were considered by the sponsor to be normal variations. Figures below from sponsor.





Organ wt: there were no drug related changes in any organ except the liver in treated males. Table below from sponsor presents the changes in mean liver wt of treated male mice rel to that in the cont gr.

Liver Weight: Increases in Male Mice

Dosage Level (mg/kg/day)	Absolute Liver Weight	% Liver to Brain Weight Ratio	% Liver to Body Weight Ratio
0[a]	-	-	-
25	+4%	+4%	+2%
50	+14%[b]	+18%[c]	+9%
100	+11%[b]	+12%[b]	+15%[c]
200	+25%[c]	+24%[c]	+18%[b]

a: The percentage increases were calculated using the mean from the pooled control group.
b: $P \leq 0.05$. c: $P \leq 0.01$.

Mouse 65wk dietary (Cont.)

Liver wt (absol or rel to B.wt or brain wt) was not affected in any treated female grs nor in males dosed 25mg/kg. As discussed in the histopath section, these liver wt incr in males were accompanied by centrilobular hepatocellular hypertrophy [the latter was also seen in f dosed ≥100mg/kg; see below].
Histopath: the following was observed in males and/or females:

Non-Neoplastic:

- **Hepatocyte Centrilobular Hypertrophy** in m dosed ≥25mg/kg and in f dosed ≥100mg/kg. Hyperplasia was not seen with the hypertrophy however, though hypertrophy was associated with incr in liver wt in males dosed ≥50mg/kg, but not in females. This liver microscopical change was not evident in mice at the end of the 4wk recovery period. *The sponsor considered the liver hypertrophy as an adaptive physiological response to the drug's effect on liver enz i.e. induction.* Noted however, that NO explanation was provided for the liver hypertrophy noted in f since mean liver wt was not affected in f.

- **Liver vacuolation** was seen in m dosed 200mg/kg and in f dosed ≥50mg/kg. The sponsor described the vacuolation as large, irregular, clear vacuolation in the cytoplasm that were distinct from fatty vacuoles. The vacuolation was focal to multifocal scattered in the lobules with slight to moderate severity. The sponsor could not assess the tox sig of this finding but suggested it may represent "hydropic degeneration".

Neoplastic Lesions:

- **Hepatocellular Adenoma** was seen in **m dosed 200mg/kg/d** but not seen in any f gr or males dosed at ≤100mg/kg. These liver adenomas were not accompanied by liver carcinomas or hyperplasia or hepatic cell proliferation. The incidence in m dosed 200mg/kg was 9/63 mice or 14% this is compared to 6/115 or 5% incidence in the combined controls. This incidence was within the historical range obtained from 78wk mouse car studies done at Wyeth-Ayerst in NY at 4-18% and that from 78wk mouse car studies done at [redacted] labs of 4-19%. *Noted is that the incidence of liver adenomas was similar among drug gr and the cont at the end of 4wk recovery period.* The liver carcinoma incidence was 0 in the 2control grs, 25 & 50mg/kg dose grs but 1/65 & 1/63 mice in 100&200mg/kg male grs; none in females. The combined incidence of liver adenomas + carcinomas was 5, 11, 8, 8, & 16% in 2cont, 25, 50, 100, & 200mg/kg grs respectively. The incidence of malignant liver tumors or any other tumors was not incr in these mice.

Mouse 65wk dietary (Cont.)

The sponsor explained that the increased incidence of liver adenomas in m dosed 200mg/kg does not impose a human cancer risk for the following reasons:

- these and other tumors were not seen in a 2yr rat dietary car study, they were not seen in female mice in this study, or in male mice dosed ≤100mg/kg. **However, the incidence of liver adenomas was sig incr and over that of historical range in f mice dosed 200mg/kg in a 2yr mouse dietary car study (see above)*.**

- there was no incr in incidence of pre-neoplastic lesions in these male mice or incr in any other malignant liver or other organ/tissue tumors.

- the sponsor referrence the literature including studies by the NTP, to indicate that findings of liver tumors in mice of a single sex and in a single species with no other tumors, do not carry the same weight of evidence to human risk when compared to findings in more than one species, in both sexes, and multiple tumor types (see above 2yr mouse car study for more detail).

- with regard to the mechanism of the liver adenomas in male mice in this study, the sponsor suggests a **non-mutagenic** and indirect/epigenetic mechanism with changes in liver homeostasis and incr mean B.wt. CL 284-846 is considered a weak enz inducer in animals and together with incr in liver wt, and liver hypertrophy are effects similar to those of phenobarb. Therefore, these changes in cell proliferation may have caused changes in hormone levels and other changes in homeostasis that led to development of neoplasia. **Again, the reviewer disagrees with the sponsor that a genetically-induced mechanism of action is ruled out, and feels that such mechanism is possible because CL 284-846 was a calstogen in the CHO and human lymphocyte assays.**

*** although the increase in liver adenomas was seen only in male mice of HD in the present study, a similar increased incidence of liver adenomas in female mice was seen in the above 2yr dietary car study. Therefore, one can argue that liver adenomas were observed in both sexes of mice though in 2 different studies. Such finding in both sexes at the same dose of 200mg/kg though in 2 different assays, may have more weight than fidnings in a single sex as, suggested by the sponsor.**

APPEARS THIS WAY ON ORIGINAL

Mouse 65wk dietary (Cont.)

Summary & Conclusions:

Oral dietary administration of CL-284,846 to mice at 25, 50, 100 & 200mg/kg/d for 65wks, did not cause death or clinical signs, nor did it affect food intake, hematology, clin chem, or gross exam. An incr in the incidence of liver adenomas without incr in liver carcinomas was seen in males dosed 200mg/kg. Non-neoplastic findings included incr in mean absol and rel liver wt and liver hypertrophy in males and females at doses ≥25mg/kg and ≥50mg/kg respectively. Mean B.wt was incr dose-dependently in both sexes at ≥25mg/kg/d doses starting from wk1 till end of study. This incr ranged between 3-11%. The incr in wt was not accompanied by an incr food intake. The incr in the incidence of liver adenomas in male mice dosed 200mg/kg was not considered by the sponsor, relevant to human cancer risk for the reasons stated above.

APPEARS THIS WAY ON ORIGINAL

Mouse Gavage Carcinogenicity study /Study# 440
Study Initiation Date Dec 2 1993 Lab: [redacted]

THE OBJECTIVES OF THIS STUDY WERE NOT MET, DUE TO EXCESSIVE DEATHS IN MALE AND FEMALE MICE OF ALL DOSES INCLUDING THE CONTROL GROUP, THE STUDY WAS TERMINATED AT WEEK 42 OF DOSING.

Doses:* **0, 1, 5, 20mg/kg/d for 2yrs.** cont groups (2) received the vehicle: 0.5% methylcellulose and 0.1% Tween 80.

* on d42 (wk7) of dosing the doses were increased to **10, 40, 80mg/kg/d in m and 25, 100, 200mg/kg/d in f.**

The initial doses were selected based on results of 1, 3, and 5mo gavage dose range finding studies. Centrilobular hypertrophy was seen at ≥40mg/kg/d, mean wt decr seen at ≥20mg/kg/d administered for 5mo.

Strain/No./Sex/Dose: CD-1/65/sex/dose with 42/sex/dose for the TK. Drug blood levels (TK) for the parent and desethyl metabolite were evaluated on day7 from 4/sex/dose at 0, 0.5, 1, 2, 4, and 6hr postdose; blood collected from retro-orbital sinus. The TK mice were killed after blood collection with no further exam.

Parameters Assessed: mortality, clinical signs, B.wt, food intake, palpable mass assessment, hematology, TK, organ wt, gross exam, and histopath.

Results:

Mortality and Clinical signs: many mice in all grs were found dead. The cause of death in these animals appears to be due to gavage error as determined in 90% of the deaths in m and 88% of the deaths in f. Tables below from the sponsor presents the % of animals with evidence of gavage injury and ratio and % mortality per gr:

ratio of mortality and the percentage for each dosage group

Group*	1	2	3	4	5
Toxicology Aspect					
Males					
N° of FD %	43/65 66.2	47/65 72.3	24/65 36.9	37/66 56.9	55/65 84.6
Females					
N° of FD %	29/65 44.6	32/65 49.2	16/65 24.6	51/66 78.5	49/65 75.4
Toxicokinetic Aspect					
Males					
N° of FD %	--- ---	--- ---	13/18 72.2	15/18 83.3	15/18 83.3
Females					
N° of FD %	--- ---	--- ---	12/18 66.7	15/18 83.3	17/18 94.4

FD = Found dead animals
* Surviving animals from group 5 were sacrificed after 31 weeks of study, and the surviving animals from the remaining groups were sacrificed after 42 weeks of study.

Group	1	2	3	4	5
Males					
% of animals with evidence of gavage trauma	89.4	87.2	87.5	97.3	92.7
Females					
% of animals with evidence of gavage trauma	86.2	75.0	81.3	96.1	91.8

Mouse 42wk gavage (Cont.)

B.wt & Food intake: there was no drug effect on either parameter. A small decr in mean wt gain was seen in m dosed 80mg/kg (2wks after the dose was incr from 20 to 80mg/kg), that lasted till end of study. Also, in m dosed 40mg/kg random changes (decr) in wt gain were seen during the same period for the 80mg/kg m gr.
Hematology: accurate assessment could not be made due to high variation within each gr and values for HD mice were determined at different time points rel to the cont.
Organ wt.: no drug related findings in any gr.
Gross Findings: no apparent drug related findings in any gr at terminal kill. Mice that were found dead, finding in many of these mice showed evidence of gavage trauma such as pulmonary discoloration, pulmonary, esophageal, or diaphramatic muscle perforation, and, blood or fluid in chest cavity. There was a single mass in 1 LDf in the r.ovary, it was firm, cystic, yellow, and contained yellow material.
Histopath: done only on found dead mice not on mice at terminal sacrifice. The number of mice found dead prior to terminal kill and their distribution among the grs is as follows (table from sponsor):

Group*	1	2	3	4	5
Males					
N° of FD %	43/65 66.2	47/65 72.3	24/65 36.9	37/65 56.9	55/65 84.6
Females					
N° of FD %	29/65 44.6	32/65 49.2	16/65 24.6	51/65 78.5	49/65 75.4

FD = Found dead animals
*** Surviving animals from group 5 were sacrificed after 31 weeks of study, and the surviving animals from the remaining groups were sacrificed after 42 weeks of study.**

From the above, the # and percent with evidence of gavage trauma are as follows (table from sponsor):

Group	1	2	3	4	5
Males					
N° of animals %	30 69.8	37 78.7	15 62.5	28 75.7	48 87.3
Females					
N° of animals %	23 79.3	22 68.8	11 68.8	44 86.3	42 85.7

Mouse 42wk gavage (Cont.)

Respiratory pathology and/or pericarditis were seen in these mice, in addition to tracheitis, pleuritis, or pulmonary hemorrhage. Microscopic evaluation in tissues/organs other than those associated with gavage trauma were not done. The sponsor stated that any increases in incidences of microscopical findings in these mice specially those dosed the 2 higher doses, was contributed to the increased number of deaths.

The sponsor indicated that the microscopic findings were peer reviewed. Appendix IX of the submission (vol 1.050), presents the peer review "certification" and in it, is stated that "all tissues from SELECTED animals from all groups" were peer reviewed and 2 individuals signed. It is unknown to the reviewer the basis for the selection of these mice over others, it is also unknown whether the process was done blindly (though it does not appear to be) and, whether the peer reviewers were from outside or within Wyeth-Ayerst?

TK: the following table from the sponsor presents the TK parameters:

TK of CL 284-846 and its desethyl metabolite (CL 284-859) in mice after oral gavage dosing.

DOSE (mg/kg/day)	DAY	SEX	C_{max} (ug/mL)	AUC$_{0-24}$* (ug-hr/mL)	T_{max} (hr)
		PARENT			
1	7	MALE	0.042±0.01	0.078	0.5
1	7	FEMALE	0.033±0.01	0.091	0.5
5	7	MALE	0.093±0.03	0.191	0.5
5	7	FEMALE	0.200±0.06	0.284	0.5
20	7	MALE	3.08±1.55	2.608	0.5
20	7	FEMALE	3.20±1.62	2.807	0.5
		METABOLITE			
1	7	MALE	NA	NA	NA
1	7	FEMALE	NA	NA	NA
5	7	MALE	0.104±0.03	0.085	0.5
5	7	FEMALE	0.231±0.07	0.172	0.5
20	7	MALE	1.124±0.24	1.469	1.0
20	7	FEMALE	2.31±1.10	2.474	0.5

N = 4 [illegible]

* AUC$_{0-24}$ was treated as AUC$_{0-24}$ as the plasma concentrations after 4 hours were BQL and the animals were dosed once daily

NA= Concentrations were BQL after 0.5 hours of dosing

Conc and exposure for the parent (except at 5mg/kg) and the metabolite increased more than proportional to dose. At 5mg/kg, the incr in conc and exposure was less than proportional to dose. There was no sex difference in the values for the parent however, females seem to have higher conc and exposure than m for the metabolite.

Mouse 42wk gavage (Cont.)

Summary and Conclusions:

The carcinogenic potential of CL 284-846 could not be assessed in this study due to the excessive deaths in all groups including the controls, that led to terminating the study after 42wks. The sponsor determined that the majority of the deaths (90%) were due to gavage trauma as was supported by gross and microscopical findings. Therefore, a drug related effect seemed to have been ruled out by the sponsor as the cause of death. No hitopath was done on any mice that survived and killed at end of study.

The reviewer believes that assessment of histopath on terminal kill mice would have been useful and informative. It is also unclear why so many gavage errors occurred in spite of the experienced technical staff involved in the study (as stated by the sponsor). It is the reviewer's opinion that the info presented in this study are inconclusive, and it can not be stated that these deaths are ENTIRELY not-drug related, specially since no histopath was done on terminal sacrifice mice and not all the deaths in mice that found dead prior to study end, were caused by gavage trauma.

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♦ Rat Dietary Carcinogenicity study /Study# 419
Study Initiation Date Dec 4 1992 Lab: [redacted]

Doses:* **0, 1, 10, 20mg/kg/d for 104 wks.** cont gr received the drug-free diet.
Strain/No./Sex/Dose: Sprague-Dawley/60/sex/dose with 120/sex/dose for the cont; additional 24/sex/dose were used for TK. Drug blood levels (TK) were evaluated on day7 and months 6&12 of the study. There were additional 16/sex/dose and in cont rats to assess hepatic function. In addition to these "hepatic" gr, 15rats/sex were added to the cont gr and 35rats/sex/dose were included in each CL 284-846 gr in case of excessive deaths in the original animal grs. The same dosing schedule was followed for these satellite rats and they were killed at end of study.

* doses were selected based on results of dietary 2wk and 13wk range finding studies and the results obtained in a 1yr rat gavage study. Rats tolerated doses upto 20mg/kg/d for 2wk administered via the diet, with no sig drug related effects. In the 3mo dietary study, there were drug related toxicities at 100mg/kg and the NOEL was 32mg/kg/d. In the 1yr gavage study, a high incidence of mortality was seen at ≥20mg/kg/d (13/30) and 50mg/kg/d (16/30). Based on these findings the high dose in the 2yr car study was selected as 20mg/kg/d via the diet.

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Rat dieatary 2yr (Cont.)

Parameters Assessed: mortality, clinical signs, B.wt, food intake, palpable mass assessment, ophthalmology, hematology, TK, organ wt, gross exam, and histopath.
Below is a table from the sponsor showing disposition of the animals:

	NECROPSY	TISSUES COLLECTED	ORGAN WEIGHTS	HISTO-PATHOLOGY
PI/HEPATIC ASPECT				
Study Day 7	4 rats/sex/group	Liver and gross lesions only	Liver 4 rats/sex/group	None
Study Month 6	4 rats/sex/group	Liver and gross lesions only	Liver 4 rats/sex/group	None
Study Month 12	4 rats/sex/group	Liver and gross lesions only	Liver 4 rats/sex/group	4 rats/sex/group
Study Month 24	All animals	Full tissue	All animals	None
Animals FD from 28 Nov 94	All animals	Full tissue	None	None
Animals SE from 28 Nov 94	All animals	Full tissue	None	None
TOXICOLOGY ASPECT	All animals	Full tissue	24 month scheduled sacrifice only	All animals

FD: Found dead
SE: Sacrificed in extremis

Results:
Mortality & Clinical Signs: there were no drug related effect on survival nor was there a trend in mortality between drug and cont grs. Similarly, there were no drug related clinical signs in any gr. Table below from sponsor shows # and percent of rats surviving till end of study:

GROUP	FEMALES	MALES
1: Control Diet	38/120[a] (32%)	53/120 (44%)
2: 1 mg/kg/day	23/60 (38%)	30/60 (50%)
3: 10 mg/kg/day	17/60 (28%)	30/60 (50%)
4: 20 mg/kg/day	23/60 (38%)	28/60 (47%)
a= denominator indicates initial group size.		

Rat dieatary 2yr (Cont.)

Ophthalmology & Palpable Mass: no drug effect on either parameter.
B.wt & Food Intake: there was no clear trend in any female drug gr during the first 18mo of dosing however, mean wt of MD&HDf was sig lower than the cont at 10%14% lower values by end of study respectively. In treated males, mean wt gain was slightly incr in HD during the 1st year of dosing and similar gain to the cont, noted thereafter. In MDm, mean wt was slightly lower than the cont during the last few wks of the study. However, this decr in MDm in absence of dose response was not considered of any toxicological sig. Mean food intake was incr in MD&HDf during the 12&18mo of dosing with similar intake thereafter. No food effect in treated males.
Hematology: there was no drug related effect on any parameter. The following findings were not dose dependent, scattered, and relatively small compared with the cont. A sig decr in mean RBC, Hct, and Hb of all f drug grs at 12mo, HDf at 18mo, and at 24mo (though at this time, the differences were not dose dependent). These decr ranged between 4-7.7% lower than the cont. In HDm at 12mo, mean RBC, HcT, and Hb values were decr 5.5-6.3% lower than the cont. however, these values were incr over the cont at 18mo, and similar values were measured at 24mo. A small and not always dose dependent but statistically sig decr noted in Hb of MDm and both Hb and HcT were decr in LDm. In HDm&f, a sig decr noted in mean WBC count (contributed to decr decr in lymphocyte #; WBC count in m 7-17% & 11% in f. It was concluded that the hematology findings though may be drug related, are of no tox consequence.
Organ wts/Gross, & Histopath:
There were about 55 tissues from each rat at end of study in the main grs that were examined for gross and histopath. Three liver samples per rat from 5/sex in the hepatic gr, were collected and stored in phosphate buffered 10% formalin to: (1) examine cell proliferation as deemed neccessary; (2) stored at -70°C for enz induction assessment; and/or (3) stored in phosphate buffered 10% formalin for LM. ***None of these evaluations were done since they were considered un-neccessary.***

1 year kill for hepatic/TK animals: no organ wt effects in f. In m, only liver wt was affected as follows:

Liver wt.	Dose (mg/kg)			
	0	1	10	20
Absol (g)	17.6	20	21.2*	22.5**
Rel/B.wt (%)	2.8	2.6	3.0	3.1*

* $p \leq 0.05$ ** $p \leq 0.01$

The incr in liver wt in males did not correlate to any gross or histopath findings. There were no neoplastic findings in any of these rats.

Rat dieatary 2yr (Cont.)

Organ wt changes noted in tox rats killed at end of study were small, occurred in 1 or both sexes, non-dose dependent, and, none correlated with any gross or histopath findings. Also, the s.d. was large in some wts due to the age-related spontaneous lesions/tumors noted in these organs. These changes included increases in the absol and/or rel wt of the: adrenals, liver, kidneys, ovaries, ptiuitary, heart, brain, thymus, and, spleen. Therefore, these wt changes were not considered drug related.

Tox Rats - Un-Scheduled Deaths:

Table below from sponsor presents incidences of non-neoplastic lesions in unscheduled deaths:

SELECTED NONNEOPLASTIC LESIONS WITH INCREASED INCIDENCE IN TREATED GROUPS, UNSCHEDULED DEATHS

SEX	MALE				FEMALE			
DOSAGE (mg/kg/day)	0	1	10	20	0	1	10	20
Adrenal Cortex								
Degeneration, Cystic	6% (4/70)	3% (1/30)	26% (8/31)	18% (6/33)	62% (28/45)	78% (28/37)	74% (23/43)	79% (28/36)
Liver								
Vacuolation, hepatocyte, random	0% (0/70)	17% (5/30)	13% (4/31)	42% (14/33)	-	-	-	-
Pancreas								
Hyperplasia, islet cell	20% (14/70)	27% (8/30)	10% (3/31)	12% (4/33)	7% (3/45)	22% (8/37)	14% (6/43)	16% (6/37)
Skeletal muscle, femoral								
Atrophy	-	-	-	-	2% (2/45)	3% (1/37)	14% (6/43)	19% (7/37)
Spleen								
Depletion, lymphoid	3% (2/70)	10% (3/30)	32% (10/31)	39% (13/33)	16% (7/45)	16% (6/37)	28% (12/43)	19% (7/37)
Testes								
Periarteritis, chronic	1% (1/70)	3% (1/30)	3% (1/31)	15% (5/33)	-	-	-	-
Tongue								
Atrophy	4% (3/69)	21% (6/28)	23% (7/31)	42% (13/31)	33% (15/45)	36% (13/36)	24% (10/41)	21% (8/37)

( ): Number of animals with lesion/Number of animals examined

From the above table, it can be seen that the following incidence of non-neoplastic lesions incr dose-dependently in either m or f drug grs:

- Skeletal muscle - femoral atrophy in MD&HDF.
- Spleen lymphoid depletion in MD&HDm.
- Tongue atrophy in all 3 m grs and MD&HDf ; though severity was incr in f it decr in m rel to the cont.
- Testes chronic periarteritis in all 3 m grs, severity incr with incr in dose as well as incidence.
- The incidence of liver hepatocyte vacuolation in m incr rel to the cont, but severity was similar among all drug grs.

Rat dieatary 2yr (Cont.)

The incidence of neoplastic findings in this gr of rats was as follows: incr in **carcinoma of pars distalis of pituitary gl** of HDf (11% or 4/37 vs. 2.5% or 2/81 rats for the cont); the incidence was 0/36 in LDf and 1/43 in mDf (2.3% of cont).

Table below from the sponsor presents selected NON-NEOPLASTIC lesions in UN-SCHEDULED deaths *AND* TERMINAL SACRIFICE rats:

SELECTED NONNEOPLASTIC LESIONS WITH INCREASED INCIDENCE IN TREATED GROUPS, UNSCHEDULED DEATHS AND FINAL SACRIFICE

SEX	MALE				FEMALE			
DOSAGE (mg/kg/day)	0	1	10	20	0	1	10	20
Adrenal Cortex:								
Degenerative cystic	8% (9/119)	7% (4/60)	25% (15/60)	25% (15/60)	69% (83/120)	85% (51/60)	73% (44/60)	85% (50/59)
Epididymides:								
Luminal debris, cellular	16% (19/120)	48% (29/60)	63% (38/60)	63% (38/60)	-	-	-	-
Lymph Node: Mandibular								
Hemosiderosis, sinus	33% (40/120)	80% (48/60)	78% (47/60)	56% (33/59)	-	-	-	-
Hyperplasia, lymphoid	10% (12/120)	2% (1/60)	10% (6/60)	14% (8/59)	5% (6/119)	2% (1/60)	3% (2/59)	10% (6/60)
Plasmacytosis, sinus	48% (58/120)	93% (56/60)	83% (50/60)	80% (47/59)	-	-	-	-

From the above table, these non-neoplastic lesions that occurred at higher incidences in treated rats rel to the cont, were considered by the sponsor not to be drug related because the morphology of these lesions was comparable to that seen in spontaneous lesions and they were not dose dependent and seen only in 1 sex.

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Rat dieatary 2yr (Cont.)

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SEX	MALE				FEMALE			
DOSAGE (mg/kg/day)	0	1	10	20	0	1	10	20
Lymph Node Mesenteric								
Histiocytosis, chron	29% (79/119)	93% (28/30)	100% (60/60)	97% (57/59)	-	-	-	-
Pancreas								
Basophilia, cytoplasm	6% (7/120)	13% (7/60)	5% (3/60)	15% (9/60)	-	-	-	-
Hyperplasia, islet cells	-	-	-	-	10% (12/120)	37% (22/60)	20% (12/60)	23% (14/60)
Pituitary gland								
Hyperplasia, pars distalis	7% (8/118)	17% (10/59)	15% (9/59)	15% (9/59)	-	-	-	-
Prostate								
Atrophy, diffuse	-	-	-	12% (7/60)	-	-	-	-
Skeletal Muscle Traumatic								
Atrophy	18% (22/119)	25% (21/85)	27% (16/59)	30% (18/60)	3% (3/120)	2% (1/60)	10% (6/60)	13% (8/60)
Spleen								
Depletion, lymphoid	2% (2/120)	5% (3/60)	17% (10/60)	20% (12/60)	7% (8/120)	10% (6/60)	20% (12/60)	12% (7/59)
Testes								
Atrophy, seminiferous tubules	22% (26/120)	17% (10/60)	17% (10/60)	27% (16/60)	-	-	-	-
Mineralization vascular/tubular	24% (29/120)	47% (28/60)	47% (28/60)	42% (25/60)	-	-	-	-
Periarteritis, chronic	6% (7/120)	3% (2/60)	5% (3/60)	20% (12/60)	-	-	-	-
Thyroid								
Hyperplasia, parafollicular	5% (6/120)	13% (8/60)	15% (9/60)	13% (8/60)	3% (4/120)	8% (5/60)	3% (2/60)	3% (2/60)

DOSAGE (mg/kg/day)	0	1	10	20	0	1	10	20
Tongue								
Atrophy	3% (3/118)	10% (6/60)	10% (6/59)	25% (14/56)	16% (18/115)	22% (13/59)	17% (10/58)	37% (22/59)
Vagina								
Exudate, purulent	-	-	-	-	18% (21/118)	25% (15/60)	42% (25/60)	30% (18/60)

( ): Number of animals with lesion/Number of animals examined

Rat dietary 2yr (Cont.)

Neoplastic lesions:

There was no drug related tumors in treated male rats rel to the cont. In females, there were some findings that reached statistical significance (see below) but again the increased incidences of these tumors were considered not to be drug related because of 1 or more of the following reasons:

1. the findings were not dose related,
2. seen only in 1 sex,
3. severity equal to or less than that in the cont,
4. the frequency is within historical range for this strain as reported by [redacted]
5. morphologically these lesions were similar to those that develop spontaneously and are common in the aging rat.

Statistical significance was observed for the following tumors:

- **Carcinoma of the pituitary gl pars distalis in f** ($p \leq 0.05$; positive trend test). However, when the incidence of adenomas and carcinomas was combined, there was no statistical sig and this type of tumor was not seen in m. The incr in carcinoma of the pars distalis in f was dose related rel to the cont: **1.7% (2/118), 3.4% (2/59), 6.7% (4/60), and 10% (6/60) in 0, 1, 10, and 20mg/kg/d dose** respectively. [redacted] reported an overall historical control incidence for pars distalis carcinoma in f in 2yr studies of 10.47% with a range in f of 1.3-57.1%. [redacted] also reported the presence of carcinoma of pars distalis in f in 14 of the 19 studies conducted. The incidence in the present study was within the historical range. The spondor also reported that the incidence in the cont was unusually low that might have contributed to the positive trend.

- **Malignant lymphomas of hemolymphoreticular system in f** (Exact trend test) dosed **10mg/kg/d (1.7%, 1/60) and 20mg/kg/d (3.3%, 2/60).** This tumor was not seen in cont or in f dosed 1mg/kg/d nor in m dosed 10 or 20mg/kg/d. Historical cont data from [redacted] reports an overall incidence of 0.38% for this tumor in f from 2yr studies and a range of 2.0-4.2%. Another historical cont data for this tumor came from [redacted] with an incidence of 2.2% in 230 f SD rats from 4 car studies. The incidence in the present study is higher than that reported by the [redacted] but, within the historical range reported by [redacted] (4.2%).

- **Parafollicular thyroid carcinoma in HDf (Exact trend test) at 3.3% (2/60).** This tumor was not seen in cont or other dosed f gr nor in any treated m grs. Historical cont data from [redacted] reported an overall incidence of 3.38% in f in 2yr studies with a range of 2.1-13.1%. Moreover, when the incidence for adenomas and caecinomas was combined, analysis did not show statistical sig.

Rat dieatary 2yr (Cont.)

- With respect to palpable tumors, no positive trend was seen in m or f. However, Exact trend test showed statistical sig ($p \leq 0.05$) for **basal cell carcinoma of the skin in f (3.3%, 2/60)**. This tumor was not seen in cont or other treated f grs. Historical cont data from Charles River reported an overall incidence of 0.08% in f from 2yr studies with a range upto 1.4%. The present incidence is higher than the upper range however, the sponsor indicated that ≥1 tumors of this type would've been expected in the cont gr in this study but none was detected which might have contributed to the positive trend.

TK:

For the **parent:**

		Day 7		Day 180	
Sex	Dose (mg/kg/d)	C_{max} (ug/ml)	AUC_{0-24} (ug.hr/ml)	C_{max} (ug/ml)	AUC_{0-24hr} (ug.hr/ml)
m	1	0.03±0.007	0.35	0.03±0.014	0.42
f		0.04±0.009	0.51	0.02±0.009	0.33
m	10	0.26±0.057	3.43	0.40±0.135	5.10
f		0.47±0.124	4.26	0.32±0.175	4.00
m	20	0.78±0.142	9.46	0.88±0.317	10.60
f		1.00±0.149	10.71	1.05±0.178	12.36

Values for day 365 were:

Sex	Dose (mg/kg/d)	C_{max} (ug/ml)	AUC_{0-24} (ug.hr/ml)
m	1	0.04±0.004	0.44
f		0.03±0.01	0.40
m	10	0.46±0.176	6.56
f		0.41±0.100	5.40
m	20	1.17±0.519	15.70
f		1.23±0.944	16.86

Rat dieatary 2yr (Cont.)

The **metabolite (des-ethyl)(CL-284-859)**, TK parameters are as follows:

Sex	Dose (mg/kg/d)	Day 7 C_{max} (ug/ml)	Day 7 AUC_{0-24} (ug.hr/ml)	Day 180 C_{max} (ug/ml)	Day 180 AUC_{0-24hr} (ug.hr/ml)
m	1	ND	ND	0.02±0.004	0.23
f		ND	ND	0.01±0.006	0.10
m	10	0.16±0.0.25	2.00	0.52±0.026	7.00
f		0.18±0.041	2.00	0.24±0.168	2.82
m	20	0.54±0.206	6.56	1.62±0.234	19.00
f		0.46±0.086	5.07	0.75±0.279	9.00

Values for day 365 were:

Sex	Dose (mg/kg/d)	C_{max} (ug/ml)	AUC_{0-24} (ug.hr/ml)
m	1	0.04±0.013	0.50
f		0.01±0.004	0.21
m	10	1.00±0.217	14.00
f		0.32±0.150	4.17
m	20	3.16±1.300	45.01
f		1.00±0.242	12.56

The conc and exposure (except as noted) increased linearly with dose for both the parent and the metabolite (tables above). Exposure was more than proportional to dose for the metabolite at the 1yr measurment for the 10 and 20mg/kg doses.

Mean T_{max} for the parent was reached between 4-8hr (except in m dosed 10mg/kg at 6mo it was 12hr) for both sexes and for the parent, the mean ranged between 4-12hr for both sexes. There was no sex difference in conc and exposure for the parent but for the desethyl metabolite, the m showed generally higher conc and exposures than the f. The parent did not seem to accumulate with time however, at 10&20mg/kg for both sexes, there seem to be some accumulation of the metabolite as time went by (7x and 2.5x for m&f respectively for exposure: 1yr compared to d7 values).

SUMMARY AND CONCLUSIONS FOR THE RODENT CARCINOGENICITY STUDIES

Several dose-range finding studies were done using CL-284-846 in both the rat and mouse. There was a single 3mo dietary study in the rat, 2 in mice, 3 oral gavage 3mo studies in rat and 1 5mo oral gavage in mice. The strain of mice in 1 of the 2 mouse studies was C57BL46NCrlBr, the strain in the 2nd dietary and 5mo gavage mouse studies was CD-1 which is the strain used in the car studies. The rat strain in all studies including the car study was SD. There were 3 carcinogenicity studies in mice, 1 oral gavage and 2 dietary with 1 of the 2 dietary studies terminated at 64wk and considered a chronic tox study in mice; a single 2yr dietary car study was done in rats.

The doses selected for the rat dietary car study were 1, 10, & 20mg/kg/d and the doses for the 2yr mouse dietary car study were 25, 50, 100, & 200mg/kg/d. Dose selection for the rat and mouse car studies was appropriate as findings were observed at the higher doses.

The main findings in the rat 2yr dietary car study were:

- B.wt & Food intake: sig decr 10-14% in mean wt f dosed 10&20mg/kg at end of study but no effect at the 1st 18mo and no effect on wt gain or food intake. Mean wt or wt gain changes were more sporadic in males, wt gain was slightly incr in 20mg/kg during the 1st yr of dosing but similar to the cont thereafter. In m dosed 10mg/kg/d, mean wt was slightly reduced during the last few wks of the study with no dose response; food intake was not affected in males.

- Liver wt (absol & rel) in the rat killed at 1yr (TK gr), was sig incr in m dosed 20mg/kg, only the absol wt was incr in m dosed 10mg/kg. This incr in liver wt did not correlate with any gross or histopath. In rats at terminal sacrifice, liver wt and other organs were incr. but the increases were small, non dose dependent, noted in 1 or 2 sexes, and, did not correlate to any gross or histopath findings.

- Several tumor incidences reached statistical significance these included:
  Carcinoma of pars distalis of the pituitary gl in HDf,
  Malignant lymphomas of the lymphoreticular system in MD&HDf,
  Carcinoma of the parafolicular thyroid in HDf,
  Carcinoma of the basal cell of the skin in HDf.

These tumors were not dose dependent, occurred in 1 sex, the incidence was within historical data range, and some had a morphology that was similat to that of spontaneously occurring tumors in the aging rat. Based on these arguments, these tumors were not considered to be drug related.

Rat 2yr car study (Cont.)

- The mean plasma C_{max} for the parent ranged between **0.8±0.14 to 1.23±1.0ug/ml** at 20mg/kg with corresponding **$AUC_{0\text{-}24hr}$ between 9.5-17ug.hr/ml**; the mean C_{max} at 1mg/kg was 0.02±0.01 to 0.04±0.004ug/ml and $AUC_{0\text{-}24hr}$ at 0.33-0.51ug.hr/ml. Values for the main metabolite, desethyl, C_{max} were 0.5±0.09 to 3.2±1.3ug/ml at 20mg/kg and corresponding mean $AUC_{0\text{-}24hr}$ were 5-45ug.hr/ml; mean C_{max} at 1mg/kg were non detectable to 0.04±0.01ug/ml and the corresponding values for $AUC_{0\text{-}24hr}$ were not detectable to 0.50ug.hr/ml. Range for **T_{max}** for the parent was **4-12hr** for both sexes. There was no sex difference in conc and exposure for the parent but these parameters were higher in m than in f for the metabolite. The parent did not seem to accumumlate with time however, **accumulation was noted for both sexes for the metabolite** at 10&20mg/kg/d doses (7x & 2.5x for m&f respectively for exposure at 1yr rel to exposure values at 7d).

The main findings in the mouse 2yr dietary car study are:

- **Mortality incr in both sexes (f > m) dose dependently and was drug related, a positive trend was seen across the groups.** At 200mg/kg dose, mortality was sig incr in m & f. The sponsor indicated that these deaths were not contributed to adverse clinical signs, sig effect on B.wt/food intake, or other drug effects. It is the reviewer's opinion that a drug effect can not be ruled out.

- Clinical signs: there were no clinical signs in any f gr or in m dosed ≤100mg/kg. The only drug related finding was in HDm where a small incr in frequency of wet and/or stained abdominal area and piloerection were seen.

- B.wt & Food intake: a sig **and dose dependent** ***increase*** noted in mean wt of both sexes from wk1-2 till wk54 in m and wk80 in f. This incr was mild in m at

- 2-7% and moderate at 12-15% in f dosed ≥50mg/kg. The increases in wt were paralleled by an incr in food intake in m dosed ≥100mg/kg and f dosed ≥50mg/kg. The increase ranged between 2-16% rel to the cont and occurred around the 1st 15wks of dosing.

- Organs or tissues were not weighed.

- Non-neoplastic lesions in all animals: incr incidence of centrilobular hypertrophy in m dosed 200mg/kg and liver eosinophilic foci in all f drug grs (dose dependently); liver hyperplasia incidence was within cont values.

Mouse 2yr car study (Cont.)

- Neoplastic lesions in all animals: incr incidence of **hepatocellular adenomas in f** dosed 200mg/kg. The incidence was as follows: 4/75 at 100mg/kg and 9/75 at 200mg/kg rel to 0/75 & 2/74 in the 2 cont grs respectively. These values exceeded those of historical data range. There was an incr in liver carcinomas in all m drug grs and in f dosed 50&100mg/kg, this incr was not dose-dependent and within acceptable historical cont range.
- The mean plasma C_{max} for the parent at 25mg/kg ranged between 0.07±0.04-0.42ug/ml, at 100mg/kg 0.21±0.07 to 0.66±0.1ug/ml, at **200mg/kg 0.3±0.24 to 1.5±1.0ug/ml.** The corresponding values for $AUC_{0\text{-}24hr}$ are 1.0-3.3, 1.7-6.0, and **1.4-15ug.hr/ml** for 25, 100, & 200mg/kg respectively. The mean C_{max} values for the desethyl metabolite at 25mg/kg were 0.03±0.01 to 0.4±0.33ug/ml, at 100mg/kg 0.17±0.3 to 0.8±0.1ug/ml, at 200mg/kg 0.32±0.35 to 1.51±1.0ug/ml. The corresponding values for $AUC_{0\text{-}24hr}$ are 0.3-2.44, 1.32-8.2, and 1.0-18ug.hr/ml for 25, 100, and 200mg/kg respectively.

The main findings in the mouse 64wk Dietary *Chronic* tox study are:

- mean B.wt was **incr** in m & f from beginning of study till end, the increases relative to the cont values ranged between 3-11%. There was no corresponding increase in food intake.

- Mean absol and/or rel liver wt were increased in males dosed ≥50mg/kg/d grs (rel to body and/or brain wt). No liver wt changes noted in females or males dosed 25mg/kg/d.

- Non-neoplastic lesions: **liver centrilobular hypertrophy** in all male groups including 25mg/kg/d gr. This finding was correlated with incr in liver wt in males dosed at ≥50mg/kg/d. Slight to moderate **liver vacuolation** in m dosed 200mg/kg and f dosed ≥50mg/kg. The sponsor could not assess the tox significance of this finding but proposed it to be "hydropic degeneration".

- Neoplastic lesions: **hepatocellular adenomas** in **m dosed 200mg/kg** at 14% of cont (9/63 rel to the combined cont grs) but not in any female gr or males dosed ≤100mg/kg. There was no increase in liver carcinoma or hyperplasia in these or other animals over the cont and historical range. Note that the incidence of liver adenomas was similar among all grs at end of recovery period. Also the incidence of malignant liver tumors or any other tumors were not increased in these mice.

- Although the incidence of liver adenomas in this study fell within historical control range, this was not the case in the 2yr mouse dietary car study noted at the same dose of 200mg/kg in female mice. The latter incidence exceeded the historical cont values.