

Dog

52-week dog:

Pure-bred beagles, 4/sex/dose, age 5.75 to 7.25 months (mean 6.25*) at study initiation, weight range 8.5-11.1 kg m and 6.9-10.9 kg f.

* these dogs seem to be fairly young, in general, mean age is 9 months.

Study No. DS-93-011/Laboratory: [REDACTED]

Route: oral gelatin capsules.

Doses: 10, 20, 40 mg/kg/d for 1 yr. The control group received similar number of empty gelatin capsules.

Results:

Clinical Signs: motor excitability in 2 HD f wk1 and 1 HD f wk3 of dosing.

Mortality: none

Body weight/food and water consumption: body weight gain significantly decreased in all doses and both sexes starting at end of treatment wk1 onward (Table below from sponsor). The decrease was greater for MD and HD males and HD females compared with the controls during wks 1-7 (dogs were fed 400 g/d diet). Increasing the food to 500 g/d during wks 8-52 did not reverse the weight loss. When the sexes were combined, the mean gain remained significantly reduced for mid and high dosed dogs compared with the control group. MDF had no effect on food consumption. MD and HD dogs (m+f) drank on the average significantly more water than the control dogs during wks 1-4, 21-49. These animals showed some tendency to drink more water during the pre-dosing period relative to the control.

Initial weight (g) and weight change after 7 & 52wks

Dose mg/kg/day	Animal No./Sex	Bodyweight (g)			Weight change (g)		
		Initial weight	Weight after 7 weeks	Weight after 52 weeks	0-7 weeks	8-52 weeks	0-52 weeks
Control	301r 302r 303r 307r 302f 304f 305f 306f						
	Mean of ♂	9525	11050	14650	+1525	+3600	+5125
	Mean of ♀	9025	9925	12525	+900	+2400	+3500
	Mean of ♂+♀	9275	10488	13588	+1213	+3100	+4313
10	309r 311r 313r 315r 310f 312f 314f 316f						
	Mean of ♂	9425	10600	13325	+1175	+2625	+3800
	Mean of ♀	9025	9650	11225	+625	+1575	+2200
	Mean of ♂+♀	9225	10125	12225	+900	+2100	+3000

BEST POSSIBLE

Dose mg/kg/day	Animal No./Sex	Bodyweight (g)			Weight change (g)		
		Initial weight	Weight after 7 weeks	Weight after 52 weeks	0-7 weeks	8-52 weeks	0-52 weeks
20	317r 319r 321r 323r 318f 320f 322f 324f						
	Mean of ♂	9475	9775	11950	+300	-2175	+2475
	Mean of ♀	8700	9475	11025	+775	-1550	+3225
	Mean of ♂+♀	9088	9625	11488	+538	-1863	+2400
40	325r 327r 329r 331r 326f 328f 330f 332f						
	Mean of ♂	9750	10100	11750	+350	-1650	+2000
	Mean of ♀	8550	8900	10750	+250	-1950	+2200
	Mean of ♂+♀	9150	9450	11250	+300	-1800	+2100

Ophthalmoscopy: no drug-related findings.

Hematology: Hb, PCV, and RBC count were slightly but significantly reduced in mid and high dosed dogs relative to the control (6-13%). Total WBC count, lymphocytes count was slightly reduced in mid and high dosed dogs (21-26%), eosinophil count was reduced 63% in MD but not in HD dogs, and platelet count was increased in HD. There was interanimal variation and the levels for RBC, Hb, and PCV were low during pre-dose in drug groups relative to the control. These effects make it difficult to conclude with certainty that these changes are drug-induced. It can be assumed that the changes in HD animals are drug-related.

Urinalysis: no drug-related findings.

Blood Chemistry: serum alkaline phosphatase (SAP) level was significantly increased in HD wks 6 and 12 (1.7-1.8 fold) relative to the control but no significance was noted on wk 50. On wk 25, SAP levels were elevated (insignificantly) in all three groups. Also on wk 50, Na level was significantly decreased in all treated groups, creatinine significantly decreased in males of MD and HD, and a slight insignificant increase in total protein of HD dogs.

Organ weights: increase in liver weight in 7/8 HD and 5/8 MD dogs over the 4% of body weight normal upper limit. Liver weight was significantly increased in all three groups and relative kidney weight increased significantly in mid and high dose dogs (see Table). The absolute and relative mean weights of prostate in MD and HD treated dogs are reduced relative to those of the control (13g control, 6 and 7g MD and HD respectively for the absolute wt and the corresponding values for the relative wts are 0.09, 0.05 and 0.06g). The absolute thymus weight was reduced in HD relative to the control (8.8 and 13g respectively, or 32%).

Organ wts-results of statistical analysis for male and female rats combined

Organ	Dosage level mg/kg/day	Absolute weight	Weight adjusted for final bodyweight	Results of statistical analysis
Liver	Control	362.4	323.4	
	10	403.5	401.3	**
	20	440.0	457.0	**
	40	544.9	569.1	**
Kidneys	Control	66.2	58.2	
	10	61.2	60.8	
	20	64.2	67.7	*
	40	43.3	68.2	*

BEST POSSIBLE

* Significant at the 5% level of probability (William's test)
 ** Significant at the 1% level of probability (William's test)

Macroscopic examination: no drug-related changes.

Histopathology: 4/4 HD male dogs had slightly increased hemosiderin deposition in hepatic Kupffer cells relative to the control. However, no other changes noted that could account for the weight change or hematology and clinical chemistry effects. Minimal reactive hyperplasia of lymph nodes was found in 1/4 each f and m MD and HD respectively and none noted in the control. An extracapsular nodule of cortical tissue in adrenals and focal vacuolation of the zona glomerulosa were found in 2/4 LD, 2/4 MD, and 2/4 HD male dogs and to a lesser extent in female dogs.

Comments: Basis for dose selection in this study was not provided. It is assumed by the reviewer that it might have been based on the dog 12-wk oral toxicity study (non GLP). In that study, doses tested were 20, 50, 100/75 mg/kg. MDF produced motor excitation and stereotypy at high doses, at all doses dogs lost weight despite normal appetite, heart rate was decreased in HD, increased QT without effect on QRS, irreversible corneal opacity, and changes in hematology and clinical chemistry parameters. The bradycardia was considered as a compensatory mechanism for the motor-excitability-induced tachycardia, and the clinical chemistry parameters included increases in cholesterol, total lipids, and SAP levels. Liver, kidney, and thyroid weights were increased.

Summary and Conclusion:

Oral administration of 10, 20, or 40 mg/kg MDF to dogs for 52 wks did not cause any deaths or clinical signs of toxicity except some motor excitation in high doses. Body weight gain was reduced although appetite seem to be normal, water intake was increased in HD relative to the control the toxicological significance of which is unclear. RBC, Hb, and PCV values were slightly but significantly reduced in high doses. On wk 50, Na level was significantly decreased in all treated groups. Liver, and kidney weights were significantly increased in high doses and mean weight of prostate in MD and HD treated dogs were reduced relative to the control. There were no macroscopic findings, and histopathology revealed slightly increased incidence of hemosiderin deposition in hepatic Kupffer cells relative to the control in high doses. An extracapsular nodule of cortical tissue in adrenals and focal vacuolation of the zona glomerulosa were found in one half of all treated male groups and to a lesser extent in female dogs. A NOEL could not be determined. 10 mg/kg is the LOEL based on body weight loss, clinical chemistry, and histopathology findings.

Summary and Conclusions of Chronic Studies:

Oral administration of modafinil to rats and dogs was well tolerated upto 52wks of repeate dosing. No mortality was seen in rats upto 60mg/kg dosed for 52wks and in dogs at 40mg/kg dosed for 52wks. In the rat 6mo study, one male rat dosed 200mg/kg was killed on wk23 due to 22g loss in wt and necropsy showed enlarged spleen and small flaccid testes, another male in this dose gr was found dead on wk1 and prior to death it had severe respiratory distress, convulsions, and salivation; necropsy did not reveal any sig finding except for a dark liver. Also in the 6mo rat study, one f dosed 200mg/kg was found dead and necropsy showed enlarged and cystic kidneys with subcapsular discoloration, one m dosed the low dose of 20mg/kg died on wk2 and found to have enlarged liver. The sponsor did not related any of these 4 deaths to modafinil. The following findings were recorded:

- ◆ decr in wt gain in rat dosed \geq 50mg/kg and dogs dosed 20&40mg/kg.
- ◆ decr in Hb, PCV, RBC in female rats dosed 200mg/kg for 6mo and dogs dosed 20&40mg/kg for 1yr. Also in the dog, the total WBC & lymphocyte count was decr.
- ◆ cholesterol level was incr in the rat dosed 200mg/kg for 6mo and rats dosed 30&60mg/kg for 1yr.
- ◆ ALP level was incr in the dog dosed 40mg/kg during mid study, serum Na decr in all 3 drug grs, total protein incr in dogs dosed 40mg/kg.
- ◆ liver wt (absol and/or rel) was incr in rats dosed \geq 30mg/kg dosed for 6mo and 1yr and in dogs the incr was dose-dependent in all 3 drug grs.
- ◆ weight of spleen and kidneys was incr in 200mg/kg rats dosed for 6mo and incr in rats dosed 60mg/kg; kidney wt was also incr but only in rats dosed 60mg/kg for 1yr.
- ◆ the wt of the thymus was decr in dogs dosed 40mg/kg and the prostate wt was decr in 20&40mg/kg dose gr.
- ◆ there was an incr in hemosiderin deposition in hepatic Kupffer cells in dogs dosed 40mg/kg.
- ◆ NOEL could not be determined in the rat 6mo study or the dog 1yr; in the rat 1yr the NOEL was 6mg/kg.

REPRODUCTIVE AND DEVELOPMENTAL STUDIES

Seg I rat fertility study/Study conducted in 1984-1985/ [REDACTED] non-GLP/study# DS-93-014.

Modafinil was administered by oral gavage to Sparague-Dawley rats (24m/gr and 27f/gr) at 20, 50, or 100mg/kg/d. The cont gr was administered the vehicle. Modafinil was prepared as a suspension in 0.5% CMC and shaken well before administration. Rats were 9wks old at study initiation and weighed 174-224g m and 194-261g f. Males were treated for 9wk prior to mating and females for 2wks prior to mating. Dosing continued throughout mating period for both sexes (mating period was 11d) and gestation period for the f. One half of females were killed at end of gestation (gd20) for teratology study and, the remaining

half was allowed to give birth to F1 generation and lactate. The latter half of the females did not meet the requirement of having a minimum of 10 pregnant f by end of study, therefore, 3 additional confirmed-pregnant f (therefore, the total of 27f/gr noted above) was added. These females were, treated throughout gestation, birth, and lactation until weaning of F1 generation at which time they were killed. At weaning, 1-2 males and 1-2 females from each litter of F1 rats, were retained and mated when they reached 13wks of age. These F1 females were allowed to give birth spontaneously and feed their young (F2 generation). The remaining F1 rats were kept and killed on ppd21-23 for autopsy and organ wts; no histopath was done. F2 rats (4/sex/litter) were observed till weaning.

The following parameters were assessed:

F0 generation: Growth, signs, B.wt, food intake, repro parameters for females included: mating index, mating interval, fertility index, repro index, wt.gain and food intake during gestation. For F0 females killed on gd20: embryotox, no. of corpora lutea, implantation sites, fetal viability, sex ratio, resorptions, fetal exam for skeletal and visceral anomalies. For F0 females giving birth: no. of pregnant females, gestation length, B.wt, food intake during lactation, gestation index, resorption index, no. and sex ratio upto ppd21, viability index, lactation index, and necropsy.

F1 generation: prior to mating: physico-behavioral development were examined: righting reflex (ppd0), startle response (ppd8), traction reflex (ppd3), reaction to noise (ppd9), opening of eye lids, eruption of incisors, etc.. Post mating: B.wt, food intake, signs, repro index, gestation index, no. and morphology of implantation sites, no., wt, and sex ratio of F2 generation up to ppd21, and, viability and lactation index.

Results:

There were no drug related findings on the assessed parameters except as noted. Most of the findings occurred at comparable rates between the cont and drug grs. Mating interval for F0 (the only drug treated generation) was 2.54d in control and 2.04-2.22d in drug grs indicating no drug effect. Resorption index (RI) was incr in HDF0 f at 12% vs. 6% in the cont, the RI was similar in LD&MDf to that of the cont. RI was also incr in HDF1 f at 15% vs. 9% in cont and again, similar incidence in LD&MD to the cont. Also in F1 females, one MDf had 9 stillborns but the incidence in all the rest in this gr and in LD&Hdf was similar to that in the cont. Therefore, this finding was not considered drug related due to its randomness.

It is concluded that oral administration of modafinil to rats had no effect on male or female fertility or any other effects on repro parameters including F1 and F2 generations except for 1.7-2% incr in resorptions in HDF0&F1 compared with the cont. This incr is small and unclear if it is drug related, more info are needed to verify this finding. Therefore, the NOEL for fertility and repro in this study is 100mg/kg/d.

Seg II teratology study in rats/Study conducted at end of 1981 [REDACTED] GLP/study# DS-93-015.

Modafinil was administered by oral gavage to pregnant Sparague-Dawley rats (25f/gr) at 50, 100, 200mg/kg/d during gd6-15. Pregnant females were killed on gd 20. The cont gr was administered the vehicle. Modafinil was prepared as a suspension in 10% gum arabic. B.wt at study initiation was not provided.

The following parameters were assessed:

During gestation: mortality, signs, B.wt and food intake. The following repro parameters were assessed on pregnant females killed on gd20: no. of corpora lutea, implantation sites, and early and late resorptions. The fetuses were divided into 2 grs, one examined for skeletal and the other gr for visceral anomalies.

Results:

There was an incr in resorptions in HD: the number of females with resorptions was 19 vs. 10 in cont, and, the total no. of resorptions was 42 vs. 28 in cont. These parameters in LD&MD were comparable to the cont. However, the mean no. of gestating females at term was similar among the drug grs and the cont. Also, the no. of resorptions per female presented with resorptions, was not different:

Resorptions:	Dose:	cont	50	100	200
total no.		28	28	30	42
mean no. \pm s.d of gestating f		1.33 \pm 2.15	1.17 \pm 1.81	1.43 \pm 1.40	1.68 \pm 1.63
no. f presenting resorptions		10	13	13	19
mean no. of resorp. per f presenting with resorptions		2.55	2.15	2.30	2.20

BEST POSSIBLE

For the fetal visceral exam, there was an incr in hydronephrosis (HS) in fetuses from HD was as follows:

	Cont	HD
Left HS	4/126(3.2%)	9/150(6%)
L&R HS	3/126(2.4%)	12/150(8%)

No difference in LD&MD.

The only skeletal effect was an incr in incomp ossification (head bones) in fetuses from LD&HD rel to the cont (28/150(18.5%), 27/150(18%) vs. 8/120(6.7%) in cont, respectively).

These skeletal and visceral changes are relatively small and may not be drug related.

It is concluded that oral administration of modafinil to pregnant rats during organogenesis did not cause maternal toxicity and was not teratogenic upto 200mg/kg. There was some embryotoxicity noted in the absence of maternal toxicity, however, it may not be of a true biological significance. The total no. of resorptions in HDf was incr and, litter hydronephrosis and, incomp ossification in fetuses from HDf were also incr. The incr in number of resorptions might have been due to the higher no. of implantations noted in this gr relative to the cont and other drug grs as noted in the above table from the sponsor. Although, an incr in resorptions was also seen in the above study at 100mg/kg (non-GLP). Because of the weak signal of embryotoxicity, the NOEL for the maternal repro parameters, embryotoxicity, and teratogenicity is 200mg/kg.

Seg II teratology study in rabbits/Study conducted in early 1982/ /non-GLP/study# DS-93-016.

Modafinil was administered by oral gavage to pregnant New Zealand white rabbits (21f/gr, age 16-19wks) at 25, 50, and 100mg/kg/d during gd6-18. The cont gr was administered the vehicle. Pregnant females were killed on gd 29. Modafinil was prepared as a suspension in 10% gum arabic. B.wt at study initiation was not provided.

The following parameters were assessed:

During gestation: mortality, signs, B.wt and food intake. The following repro parameters were assessed on pregnant females killed on gd29: no. of corpora lutea, implantation sites, resorptions. The fetuses were divided into 2 grs, one examined for skeletal and the other gr for visceral anomalies.

Results:

Mean wt gain during treatment (gd6-18) was reduced in HDf rel to the cont (2.4 vs. 7% cont). Mean wt gain in HDf was minimally decr throughout the study (gd0-29) rel to the cont (12.8% vs. 13.3% cont). Decline in food intake accompanied the decr in mean wt gain in HD during treatment (147g vs. 160g cont). An incr in no. of aborting females (those fertilized but did not have fetuses at c-section) noted in MD (3/21) vs. cont (0/20), also the no. of resorptions per female was elevated in MD at 37/21 (mean±sd 2.2±3.2) vs. the cont at 13/20 (mean±sd 0.9±1.2). The sponsor indicated that this was contributed to 2 MDf that together had a total of 17 resorptions. The number of live fetuses (total per f) was slightly reduced in HDf at 78 vs. 117 in cont. There were no drug effects on fetal skeletal or visceral parameters.

It is concluded that oral administration of modafinil to pregnant rabbits during organogenesis, caused a small decr in mean wt gain and food intake in HD. Only in MDf, there was an incr in no. of resorptions and no. of aborting females. These latter findings may not be drug related since they were non-dose dependent. The NOEL for maternal repro tox is 50mg/kg and that for embryotox and teratogenicity is 100mg/kg.

Seg III peri- and post-natal study in rat/Study conducted at end of 1984/ [REDACTED] GLP/study# DS-93-017.

Modafinil was administered by oral gavage to pregnant Sprague-Dawley rats (25f/gr, age 8-10wks) at 20, 50, and 100mg/kg/d from gd15 and through lactation (ppd21). Females were killed on ppd21. The cont gr was administered the vehicle. Modafinil was prepared as a suspension in 5% CMC. B.wt at study initiation was 192-290g.

The following parameters were assessed: mortality, signs, B.wt, food intake, water intake, nursing behavior, resorption index, implantation sites, and other repro parameters. For the F1 generation: viability and growth including standard clinical tests such as righting reflex, startle response, response to noise...etc. Necropsy was done on all females that did not give birth, gestating females, and their litter. The females that did not give birth and gestating females were sacrificed between ppd21-23. From each litter, 2/sex were necropsied with the following organs weighed and examined: thymus, heart, liver, spleen, kidneys, adrenals, and lungs; (no histopath was done but these organs were preserved in 10% formalin).

The sponsor indicated that the drug conc prepared at the start of lactation were lower than the proposed theoretical conc specially at the HD. This was due to the poor homogenization of the suspension immediately preceding sample withdrawal. However, the sponsor stated that these samples were stirred constantly throughout intubation. It is unclear if this affected the study results.

Results:

maternal food intake was reduced in all grs including the cont during gd18-20, at 56-64g compared to 140-148g at gd0-6, but no changes during lactation. There was no drug effect on gestating or lactating females re. B.wt or water intake and no effect on gestation duration or nursing behavior. Resorption index was incr dose-dependently in MD&HDf rel to the cont (12.7 and 13.4% vs. 10% in cont). However, the sponsor indicated that these values were within historical data for SD rats at 10.8±1.9 and therefore, may not be drug related. At birth, the no. of live young per gestating female was reduced in all 3 drug grs non-dose dependently with moderate decr in MD (mean±sd: 14.3±2.4, 13.6±2.5, 11.7±5.8, and 12.5±4 in cont, LD, MD, and HD respectively). In addition, the no. of stillborns was elevated in MD&HD rel to the cont at 1.5±3.8 MD, 1.1±2.6 HD vs. 0.3±0.6 in cont. The stillborn rate (stillborn/young born) was: MD 32/290 (11%), HD 26/326 (8%), cont 7/349 (2%). Among the physico-anatomical changes, the startle reflex was

slightly delayed in MD&HD rel to the cont but was earlier in LD.; no other effects. There were no drug effects on organ wts or macroscopic exam.

It is concluded that oral administration of modafinil to female rats caused no maternal tox upto 100mg/kg. There was an incr in RI that was dose-dependent but stated to be within historical data for this strain of rats. The no. of live fetuses per female was reduced in all 3 drug grs but the pattern was non-dose dependent and the stillborns and stillborn rate were incr in MD&HD rel to the cont parameters. Fetal and litter wt were not affected by drug treatment however, growth seemed to be slightly retarded as indicated by the delay in startle response in MD&HD. The maternal NOEL is 100mg/kg whereas, that for embryotoxicity is 20mg/kg.

Seq III peri- and post-natal study in rat with functional and behavioral evaluation/Study conducted in 1995/
 [REDACTED] - PA/GLP/study# DS-95-022

Modafinil was administered by oral gavage to pregnant Sprague-Dawley rats (30f/gr*) at 50, 100, and 200mg/kg/d from gd7 through ppd20. All surviving pregnant females were killed on ppd 21. The cont gr was administered the vehicle. Modafinil was prepared as a suspension in [REDACTED] (white viscous liquid). B.wt at study initiation was 220-271g.

* Blood was collected from 8 mated rats pre-dose on gd6, 5 of these rats were assigned to a postpartum satellite gr for collection of milk and blood (dosing from gd7-11 of lactation). Blood was collected on ppd3&9 from 5 rats pre-dose and pre-administration of oxytocin for collection of milk. Blood was also collected on ppd5&11 at 2hr postdose. Blood was sampled from the jugular or tail vein. Milk was collected pre-dose on ppd3&9 and 2hr postdose on ppd4&10. Oxytocin was injected 5min before milk sampling (see above).

The following parameters were assessed: mortality, signs, B.wt, food intake, mating behavior, gestation period, implantation sites, litter size, pup viability, and nursing behavior. Gross necropsy was done on rats that did not deliver by gd25, any gestating rats that sacrificed moribund or found dead, all surviving females on ppd21, and, all pups on ppd21 not selected for continued observation. Statistical analyses were done on given parameters.

Note that blood levels were not reported in this study.

Also, any functional/developmental/behavioral tests were not done on F1 generation pups because they were performed in the above study# DS-93-017.

Results:

There were no drug related deaths or clinical signs. Mean wt gain was sig reduced in HDf (40%) during the 7-10 gestation period; no difference at later periods. However, this early decr affected the cumulative periods of 7-20 and 0-20 ($p \leq 0.01$ at 12&8% respectively). Mean wt was comparable in HD rel to the cont except a sig decr recorded on gd14&15. During lactation period, a decr in mean wt (3-5% of cont) noted in HD during pp days 2-5 & 7-8 (reaching significant level $p \leq 0.05/0.01$ at pp days 3-4&7-8); no effect on wt gain. Absolute & rel food intake in MD&HD was sig decr during the period 7-10&7-20. The decr was between 4-7% of the cont except during gd7-10 it was 20% in HD relative to cont (this was the period of decr in wt. gain of 40% in HD). No effect on food during lactation period. No deaths occurred in any gr. There were fewer pregnancies in LD rel to the cont (20/25 vs. 25/25 in vehicle), however, this was considered not drug related since it was not seen at the higher doses and dosing was initiated post gd7 which is after implantation. No drug effect on gestation period, duration of delivery per pup per litter, duration of parturition per pup, or on implantation sites. An incr in incidence of stillborn noted in LD ($p \leq 0.01$) and HD (non-sig)(1.4, 4.6, & 3% in cont, LD, & HD respectively). This finding was considered by the sponsor to be drug unrelated since it was not dose-dependent. In the HD satellite gr, the no. of pups found dead or cannibalized was incr ($p \leq 0.01$, at 14.5% vs. 4.4% in cont). Also in this gr, lactation index (no. of live pups on ppd11/live pups on ppd7) was sig ($p \leq 0.01$) decr (84%) rel to the cont (94%). The

sponsor considered these findings non drug related because they were not observed in the main study (more rats than the 5/gr satellite). Milk was absent from stomach on ppd1 of 4/20LD or 80% ($p \leq 0.01$) rel to 1/23 or 12.5% cont. The sponsor indicated that this finding was not dose dependent therefore, not drug related. There were no other drug effects on any of the assessed parameters.

It is concluded that oral administration of modafinil to rats during pregnancy and through lactation day20 was not teratogenic upto 200mg/kg. There was a 40% decr in wt gain of rats dosed 200mg/kg during gestation period 7-10; values were comparable thereafter. This decr affected the cumulative wt during gd7-20&0-20. Mean wts were slightly but sig decr in HD during lactation. Decrease in food intake accompanied the decr in wt. Decrease in food intake noted in MD&HD during gd7-10&7-20. There were no drug effects on the several repro parameters measured in this study except for few sporadic effects. The affected parameters included an increase in incidence of stillborn, lactation index, no. of pups found dead or cannibalized. These findings were small, occurred either in LD or were non-dose-dependent, or noted only in the 5 rats satellite gr and not in the main study. Modafinil therefore, was not embryotoxic upto 200mg/kg. The NOEL for maternal toxicity is 100mg/kg.

SUMMARY AND CONCLUSION(s) FOR THE REPRODUCTIVE STUDIES:

Modafinil effect on reproductive and/or developmental parameters was tested in the following studies:

1. Seg I in rats/doses: 20, 50, 100mg/kg/Non-GLP
2. Seg II in rats/doses: 50, 100, 200mg/kg/d/Non-GLP
3. Seg II in rabbits/doses: 25, 50, and 100mg/kg/Non-GLP
4. Seg III in rats/doses: 20, 50, 100mg/kg/GLP
5. Seg III in rats/doses: 50, 100, 200mg/kg/GLP

The NOEL values are as follows:

Study	End-point	NOEL (mg/kg)
Seg I rat	fertility	100
Seg II rat	maternal tox	200
	teratogenicity	200
	embryotox	200
Seg II rabbit	maternal tox	50
	teratogenicity	100
	embryotox	100
Seg III rat	maternal tox	100
	teratogenicity	100
	embryotox	20
Seg III rat	maternal tox	100
	teratogenicity	200
	embryotox	200

BEST POSSIBLE

Modafinil administered orally to rats and rabbits upto 200mg/kg (this dose is 30x the max anticipated human dose of 400mg/d), was not teratogenic. Male and female fertility in rats was not affected upto 100mg/kg. Embryotox seen as incr resorptions, occurred at 100mg/kg in F0&F1 rats. In absence of maternal toxicity, modafinil in a Seg II rat study incr total no. of resorptions in f dosed 200mg/kg, in addition, there was an incr in litter hydronephrosis and incomp ossification in fetuses from these females.

These embryo-fetal toxicities may not be of real biological significance because the number of resorptions per female was not affected and the visceral and skeletal effects are relatively small. The NOEL for embryotoxicity in this study is therefore, 200mg/kg. Rabbits dosed modafinil during organogenesis period, showed a small decr in mean wt gain and food intake at 100mg/kg. The NOEL in rabbits was 50mg/kg for maternal tox and 100mg/kg for embryotox and teratogenicity. In a pre- and post-natal Seg III rat study, modafinil was dosed during pregnancy and lactation. There was a dose-dependent incr in RI but stated by the sponsor to be within historical data for this rat strain. Though dose-independent, number of live fetuses per female was decr in all 3 drug grs and the stillborn rate and number of stillborns were incr in females dosed 50&100mg/kg. The only effect on growth was a delay in startle response in the 50&100mg/kg dosed groups. The maternal NOEL is 100mg/kg whereas, that for embryotoxicity is 20mg/kg. In a more recent Seg III study in rats, modafinil was not teratogenic upto 200mg/kg but caused a 40% decr in wt gain in females in this gr during gestation period 7-10; no effect thereafter. This decr however, affected the cummulative wt during gestation periods 7-20&0-20. Mean wt was slightly but sig decr during lactation period. Accompanying the decr in wt was a 7-20% decr in food intake in the 100&200mg/kg dosed grs during gestation periods 7-10&7-20. In contrast to the previous Seg III study where the NOEL for embryotoxicity was 20mg/kg, in this repeate study, modafinil was not embryotoxic upto 200mg/kg; the maternal NOEL was 100mg/kg similar to that in the previous study.

Labelling:

Impairment of Fertility:

DRAFT LABELING

Pregnancy:

DRAFT LABELING

Genetic Toxicology

conducted in 1979 the following assays (DS-93-018) all of which were non-GLP:

- Ames bacterial reverse gene mutation assay Plate incorporation labs conc tested were 10, 100, 1000, and 10,000ug/plate in -/+ S9. An incr in no. of revertants was seen in +S9 in TA98 and TA1538 (the former is a derivative of the latter) when the assay repeated 2x; the findings were negative in -S9. The incr was 2.2-3x negative cont in TA1538 and 2-3x negative cont in TA98. The sponsor concluded that modafinil at 5000 and 10,000ug/plate in +S9 was mutagenic in TA98 and TA1538.

labs repeated Ames test with conc upto 5000ug/plate using the 5 standard strains, each strain tested in 3 plates per conc in -/+ S9 from Aroclor induced rat livers. Modafinil in DMSO was non-toxic upto 5000ug/plate which was the conc used in the main assay. Modafinil was not mutagenic in this assay upto 5000ug/plate in either -/+ S9 including TA98 & TA1538.

A complementary 3rd Ames was done by according to OECD guidelines but still non-GLP in 1982 to check the results found by above: only TA98 & TA1538 were examined in -/+ S9 at 500-10,000ug/plate modafinil. The results were negative and modafinil was found non-mutagenic in these 2 strains.

- A more recent Ames test was done by [REDACTED] 1996 under GLP (study# DS-96-001) and sponsored by Cephalon. This study was done because the other 3 studies were non-GLP and a positive response was seen in strains TA98 and TA1538 in +S9. This study was well done, the appropriate positive controls and their responses were valid and a cytotox assay was done prior to the main assay using TA100 and WP2uvrA (modafinil conc ranged between 5-5000ug/plate and it was not cytotoxic upto 5000ug/plate which is the conc used in the main assay). Modafinil similar to the above 3 assays was dissolved in DMSO so did the positive cont except for 2 (NaAz & MMS) were dissolved in water. The strains tested were TA98, TA100, TA1535, TA1537, and E.coli WP2uvrA. Both the plate incorporation and pre-incubation methods were tested in -/+S9. Conc of modafinil tested in the main assay were 250, 500, 1000, 2500, and 5000ug/plate in -/+S9. The results were clearly negative in -/+S9 in the plate incorporation and pre-incubation assays upto 5000ug/plate modafinil conc, therefore, modafinil is considered non-mutagenic in the Ames bacterial reverse mutation assay.

- In vitro Human lymphocyte assay/GLP [REDACTED] 1986
Cytotox was assessed using modafinil conc at 30, 100, 300, 1000, and 3000ug/ml in DMSO and mitotic index (MI) was determined. The highest conc used in the main assay was 300ug/ml due to limit by solubility as stated by the sponsor. Modafinil conc in the main assay were 30, 100, 300ug/ml in +/-S9 at 1 & 24hr exposure respectively. Modafinil was not clastogenic in human lymphocyte assay upto 300ug/ml conc in -/+ S9.

- In vitro gene mutation of Chinese hamster V79 lung fibroblasts at the HPRT locus/Lafon labs/ followed OECD guidelines but not GLP (in the spirit of GLP as stated by the sponsor)/ Exposure to test sub was 1&3hrs (OECD recommends 3-6hr and sometimes upto 1.5x cell cycle). Modafinil conc was given in mM and upto 10^{-6} mM in +/- S9. The results were negative and modafinil was not mutagenic in this system.

- In vivo bone marrow MN in Chinese hamsters [REDACTED] Non-GLP
Modafinil was orally dosed to 6/gr Chinese hamsters for 5d at 200&1000mg/kg/d and chromosomes checked for abnormalities. This assay failed to meet several of the OECD criteria such as no. of cells used per animal (recommended minimum of 100 here only 50 were used), no. of doses usually one dose and other designs should be justified, no justification was given for dosing for 5days, MI is determined in at least 1000cells/animal in this study only 50 cells were used. A priliiminary tox study showed 2/4 animals died at 2000mg/kg but no deaths at 1000mg/kg dose. The results showed that modafinil was not clastogenic in Chinese hamster bone marrow MN in vivo assay upto 1000mg/kg dose.

As stated, it is the opinion of the reviewer that the above assays were non-GLP and some did not follow OECD giodelines and therefore, firm conclusions can not be made based on the data submitted.

- In vitro forward gene mutation at TK locus in L5178 MLP cells [REDACTED] DS-96-003/1996/GLP.
Modafinil was dissolved in DMSO. Conc were based on cytotox assay and solubility characteristics. The drug remained in sol upto 1500ug/ml but after 4hr treatment period a precipitate was noted at ≥ 1100 ug/ml. Note that the drug did not alter the pH in culture medium. In the dose range finding assay, both untreated and vehicle controls were used, exposure was for 4hr at 37C afterwhich, cells were washed and resuspended in growth medium. Cell count was made after 24hr to measure reduction in cell growth relative to the cont. The appropriate positive controls were used: MMS in -S9 and MCA in +S9. Modafinil in dose range finding was tested between 3.12-1451ug/ml in -/+S9, it was cytotoxic at 1451 with 6.5-11% cytotoxicity relative to negative controls.

-S9:

There were 4 assays in -S9, #2 was terminated without mutant selection because there was a shift in cytotox causing too few doses to clone and proper evaluation of the drug; trial 3 was unacceptable because cloning efficiencies were very high and contamination at top dose. The sponsor indicated that in these 2 trials, there was no positive response for mutagenicity (data not provided). Conc tested in -S9 in trial 1 were: 301, 485, 590, 642, 670, & 695ug/ml; in trial 4: 355, 462, 565, 727, 909, 1007, & 1011ug/ml. Other conc were not selected due to excessive toxicity as stated by the sponsor (Day2 cell conc $\leq 3 \times 10^5$ cells/ml). Relative growth (indication of tox) ranged between 3% at 642ug/ml to 87% at 301ug/ml in trial 1 and 9% at 1011ug/ml to 106% at 355ug/ml; note that the highest conc in either trial were not the ones to produce the sig decr in RG but rather the 4th highest conc out of 6 in trial1. It was the lab director's decision to include these conc in the assay although they produced cytotox near the 10% limit, to strengthen the evaluation; it is the opinion of the reviewer that this was the right decision. The positive cont produced the expected positive responses. Modafinil was not mutagenic in -S9 under these conditions.

+S9:

Also 4 trials were attempted but trials 2&3 were not used for the same reasons listed above in -S9. Trial 1, conc were 152, 301, 485, 590, 670, 695ug/ml and those in trial 4: 462, 565, 727, 909, 1007, 1011ug/ml. Cytotox for trial 1 ranged between 43.7% at 152ug/ml to 85% relative growth at 670ug/ml which are not between the 10-20% limit. Mean cloning efficiency of the neg cont was 81% within the acceptable range, positive cont MCA produced the expected positive mutant response. Modafinil at the lowest conc of 152ug/ml/trial 1 caused an incr in mutation frequency that exceeded the minimum criterion for a positive response i.e. 128.1×10^{-6} at 148.2×10^{-6} . However, modafinil at higher conc was negative. In trial 4, cytotox ranged between 13% at 1007ug/ml to 65% relative growth at 727ug/ml. Mean cloning efficiency of the neg cont was 95% demonstrating acceptable level of cloning. The positive cont MCA produced the expected mutagenic response. Modafinil at the lowest conc of 462ug/ml exceeded the minimum criterion for positive response of 62.2×10^{-6} at 62.9×10^{-6} . However, since these positive responses were small, occurred at the lowest conc, and were non dose-dependent, the overall conclusion was that modafinil was not mutagenic at the MLP/TK locus under these experimental conditions.

- Morphological transformation of BALB/3T3 mouse embryo cells. [REDACTED]
 [REDACTED] DS-96-010/1996/GLP.

The objective of this study was to examine the morphological transforming potential of modafinil using BALB/3T3 clone A31-1 mouse embryo cells. Modafinil was dissolved in DMSO; the drug was soluble in treatment medium at final conc of 600ug/ml and lower in the dose-range finding assay, upto 1200&1600ug/ml in main assay. A preliminary tox assay was done at modafinil doses 2, 6, 20, 60, 200, 600, 2000ug/ml with cells exposed to the drug and neg cont for 3days in -S9 and 4hr in +S9 at 36C. Following 10days from start of treatment, cells were scored for colony formation. High dose was selected based on 20% survival. At 2000ug/ml relative cloning efficiency was 6% in +S9 and 0% in -S9. Doses selected in the main assay in -S9/trial1 were 150, 300, 600, 1200ug/ml (data not presented) and in -S9/trial2: 38, 75, 150, 300ug/ml; and in +S9: 400, 800, 1200, 1600ug/ml. MMNG was the positive cont in -S9 and DMN in +S9. The concurrent cytotox assay done with the transformation assay showed marked tox (cell survival) of 63, 37, 9, & 1% at 150, 300, 600, and 1200ug/ml respectively, relative to solv cont. (data not shown). This assay was terminated and a repeat assay at lower conc was done. Relative survival in this assay was 87, 83, 79, 38% at 38, 75, 150, and 300ug/ml respectively, in -S9 and in +S9: 100, 84, 54, and 28% respectively. Modafinil in +/- S9 did not incr the total no. of transformed foci/total dishes or the transformation frequency (# of transformed foci per surviving cell) relative to the neg cont; the positive cont produced the expected positive response. It was concluded that modafinil was negative in the transformation assay in +/- S9 under these experimental conditions.

APPEARS THIS WAY ON ORIGINAL

- In vitro UDS in Fischer rat hepatocytes primary culture [REDACTED] DS-96-016/1990/GLP. The assay was done on freshly isolated non-replicating rat hepatocytes in primary culture. Modafinil dissolved in DMSO. Conc tested in the first cytotox test were 1, 10, 50, 100, 250, 500, 1000ug/ml and those in the 2nd test: 25, 50, 100, 250, and 500ug/ml. Positive cont were 2-AF, the negative cont was pyrene dissolved in DMSO, untreated cultures, and DMSO. Each drug conc was tested in triplicate, following 18-20hr incubation cells were washed and observed under the scope for morphology, attachment, spreading out of cells and any cytotoxicity. This was followed by standard procedures for washing and staining and final mounting on glass slides and autoradiography. Modafinil in the 1st main assay, conc were 50, 100, 250ug/ml, it did not incr the mean nuclear grain count or the net grain count (nuclear - cytoplasmic) and was similar to the neg cont cells (solvent or untreated) also, the % of cells in repair fell within the range of the neg cont. On the other hand, the 2-AF positive cont at the 2 conc tested, produced intense DNA repair as seen from incr in nuclear grain count. It was concluded that modafinil did not induce UDS. Note that data for the cytotox were not provided. In the 2nd trial, cytotox was observed at 500ug/ml with no tox at lower conc. The conc of modafinil tested here were the same as those in trial 1 above (50, 100, 250ug/ml). Modafinil at 250ug/ml showed a large no. of percent of cells in repair (21.7%) above that listed in criteria for a positive result (i.e. 20%). This is the mean of 3 slides at 25, 0, and 40% values. A second reading of all slides in all 3 drug conc was done. The results showed 11.7% percent of cells in repair at 250ug/ml which is below the positive response limit of 20%. Based on the result in this second reading of the slides, the sponsor concluded that the earlier reading is "insignificant" and can be explained by loss in cytoplasmic grains consequent to excessive hypotonic shock. It is the opinion of the reviewer that although the sponsor's explanation is interesting, the reviewer concludes that the result in this 2nd trial was equivocal since it was positive in one and negative in the second reading of the slides. However, because the results were clearly negative in the 1st trial, the overall conclusion is that modafinil did not induce UDS under these conditions in rat hepatocytes.

- In vivo induction of micronuclei in OF-1 mouse bone marrow/DS-96-012/[REDACTED] France/1995/GLP.

The vehicle and negative cont in this assay was 0.5% methyl cellulose. The positive cont was CP dissolved in water. Doses of modafinil tested were 0, 175, 300, and 700mg/kg/d administered by gavage for 2 days to OF-1 mice. A preliminary tox assay was done in 3/sex mice to determine top dose (note that on 3 different occasions the no. of animals varied between 3/sex, 5/sex/ 6/sex). Doses in the preliminary assay were 667 and 1000mg/kg orally dosed once daily for 2days or 2x per day for 2 days. Animals were observed for 48hr for signs and mortality and killed by CO2 inhalation. Hypoactivity, dyspnea, and loss of equilibrium were seen in 2/3m and 1/3f dosed 1000mg/kg, also in this dose, 1m and 1f were found dead 24&48hr post dose respectively. At 667mg/kg, equilibrium loss was seen in 1/3m on d1 and staggering gait on d2. Based on these findings the sponsor selected 700mg/kg as top dose for the cytogenetic assay. In the cytogenetic assay, mice were killed 24hr after the last dose and smears prepared from femur bone marrow. Micronuclei were counted in 2000 PCE per animal, polychromatic (PE) and normochromatic (NE) ratio was calculated by scoring 1000 RBCs (PE+NE). No clinical signs or deaths occurred in mice dose 175 or 350mg/kg but at 700mg/kg, loss of equilibrium was observed in 2/8m and staggering gait in 4/8m and 1/8f. Also, 1/8m in HD was found dead 24hr post the 2nd dose and was replaced. Mean values of MPE for the vehicle cont and positive cont were within the historical data (provided) of that lab. there were no difference in mean MPE or the PE/NE ratio of any of the drug gr to that of the vehicle cont. The PE/NE ratio in the 700mg/kg dose gr was slightly lower than that of the cont at 0.6 ± 0.5 vs. 0.9 ± 0.3 in cont. This was not statistically sig and was caused by 1f out of 5 with high PE value that resulted in a PE/NE ratio for this animal of 1.8 whereas, the ratio for the rest of the mice in this gr ranged between 0.2-0.8 with a mean of 0.5 ± 0.2 . This reduction is considered a support for bone marrow exposure to the drug. It was concluded that modafinil did not induce MN in bone marrow from mice dosed upto 700mg/kg and therefore was not clastogenic.

- In vivo induction of MN in CD-1 mice bone marrow/ [REDACTED] DS-96-002/1996/GLP.

[The sponsor did not explain why a repeat study of the MN was done.] Modafinil was dissolved in Ora-Plus oral suspending vehicle (in the above MN, 0.5% MC was the vehicle). Oral gavage dose range finding study was done at modafinil doses of 176, 315, 561, 876, and 1260mg/kg (3/sex/dose). The doses were administered as a single dose and mice were observed for 3d after dosing for signs and mortality. All mice at 176, 315, and 561mg/kg and all females from 876mg/kg gr were normal without any clinical signs through the observation period of 3d. At 1hr post the 1260mg/kg dose, 1m mouse was hypoactive with dyspnea and squinted eyes, at 2.5hr, all mice in this gr were ataxic and had tremors. By 22hr postdose, all mice in 1260mg/kg were normal except for 1m and 1f that were prostrate and had dyspnea. By 47hr, 1m in 1260mg/kg gr was found dead and at end of observation (70hr) 1f in this gr was found dead. Although males in 876mg/kg gr were stated to have experienced some symptoms, no discussion was provided except that "all males in 876mg/kg were normal at >2.5hr postdose". Based on the findings in the dose range study, doses selected for the cytogenetics were: 339, 696, and 1260mg/kg administered in Ora-Plus vehicle via oral gavage to 5/sex/dose. Animals dosed with the drug and vehicle cont were killed at 24, 48, or 72hr postdose and bone marrow harvested and prepared for cell scoring. The positive cont was CP 80mg/kg and mice killed 24hr postdosing. All neg and positive cont mice were normal postdose. Mice dosed with modafinil were hypoactive immediately postdose. All 1260mg/kg mice were also ataxic 1hr postdose as well as 1m in 696mg/kg gr. At 21hr postdose, all mice in 339mg/kg were hypoactive and those in 696mg/kg were prostrate. In addition to hypoactivity and ataxia in mice dosed 1260mg/kg, at 24hr harvest time, 2m had dyspnea, profound foot splay, red-color lacrimation, and were urine stained as stated by the sponsor. All mice by end of 72hr harvest time were hypoactive with 1m having dyspnea and lacrimation. After 45hr postdose, all mice in 339&696mg/kg were normal. Animals found dead 72hr harvest time included 1m and 4f dosed 1260mg/kg; all surviving mice in 1260mg/kg were hypoactive. Micronucleated polychromatic erythrocytes (MPE), and PE to NE ratio was determined in 1000 PE per animal (OECD recommends at least 2000 PE to be scored per animal). Modafinil did not induce a sig incr in MPE in either sex at any harvest time or any dose except in females dosed 1260mg/kg at 48hr harvest time (0.18 ± 0.05 vs. 0.04 ± 0.02 in cont; $p \leq 0.05$). This was considered by the sponsor to be non-treatment related because the female mean vehicle cont value was low compared to historical data. However, the mean neg cont value was actually high compared to that of historical data: the individual values were 0-1% in the neg cont in the study, and those in historical data ranged from a minimum of 0 to a maximum of 0.24% with a mean of $0.0081 \pm 0.0008\%$ from n=47 mice. Modafinil caused a sig dose-dependent decr in ratio of PE/NE in males dosed 696mg/kg and 1260mg/kg compared with the neg cont at the 72hr harvest time (0.4 ± 0.07 at 696mg/kg and at 1260mg/kg 0.37 ± 0.05 vs. 0.61 ± 0.05 in neg cont).

APPEARS THIS WAY ON ORIGINAL

These values were within historical data (provided for review; n=47 mice from 7/95-12/95) and represent bone marrow toxicity, therefore, of no biological sig (see table below from sponsor). The criteria for a positive response was not met they included: statistically sig dose-dependent incr in MPE, or detection of a reproducible and sig positive response in at least one dose; but ultimate conclusion was based on scientific judgment.

TREATMENT	DOSE	HARVEST TIME (HR)	% MICRONUCLEATED PCE ₁ MEAN OF 1000 PER ANIMAL ± S.E.			RATIO PCE:CE MEAN ± S.E.	
			MALES	FEMALES	TOTAL	MALES	FEMALES
CONTROLS							
VEHICLE	Cis-Plat TM	24 hr	0.10 ± 0.04	0.14 ± 0.06	0.12 ± 0.04	0.70 ± 0.03	0.66 ± 0.03
		48 hr	0.08 ± 0.06	0.04 ± 0.02	0.06 ± 0.03	0.66 ± 0.04	0.65 ± 0.04
		72 hr	0.06 ± 0.04	0.06 ± 0.02	0.06 ± 0.02	0.61 ± 0.03	0.73 ± 0.06
POSITIVE	CP 20.0 mg/kg	24 hr	5.10 ± 0.23*	4.12 ± 0.23*	4.61 ± 0.20*	0.56 ± 0.06	0.66 ± 0.07
TEST ARTICLE	339 mg/kg	24 hr	0.06 ± 0.02	0.14 ± 0.07	0.10 ± 0.04	0.78 ± 0.10	0.57 ± 0.03
		48 hr	0.06 ± 0.02	0.02 ± 0.02	0.04 ± 0.02	0.65 ± 0.10	0.70 ± 0.08
		72 hr	0.12 ± 0.04	0.08 ± 0.04	0.10 ± 0.03	0.66 ± 0.04	0.69 ± 0.07
	606 mg/kg	24 hr	0.06 ± 0.02	0.16 ± 0.07	0.11 ± 0.04	0.65 ± 0.09	0.64 ± 0.07
		48 hr	0.14 ± 0.07	0.06 ± 0.04	0.10 ± 0.04	0.59 ± 0.07	0.63 ± 0.09
		72 hr	0.08 ± 0.04	0.10 ± 0.06	0.09 ± 0.03	0.40 ± 0.07**	0.64 ± 0.07
	1260 mg/kg	24 hr	0.10 ± 0.03	0.10 ± 0.04	0.10 ± 0.03	0.57 ± 0.03	0.66 ± 0.06
		48 hr	0.06 ± 0.02	0.18 ± 0.05*	0.12 ± 0.03	0.55 ± 0.10	0.58 ± 0.04
		72 hr	0.02 ± 0.02	0.10 ± 0.06	0.06 ± 0.04	0.37 ± 0.05**	0.64 ± 0.04

BEST POSSIBLE

* Significantly greater than the corresponding vehicle control, p<0.05.
 ** Significantly lower than the corresponding vehicle control, p<0.05.
 CP = Cyclophosphamide

SUMMARY AND CONCLUSION(s) FOR THE GENETIC TOX STUDIES:

The following assays were conducted, some were GLP others were not:

1. Ames bacterial reverse gene mutation assay (4 assays including pre-incubation).
2. MLP/TK gene mutation assay.
3. Morphological transformation of BALB/3T3 mouse embryo cells.
4. In vitro UDS on rat hepatocytes in primary culture.
5. In vivo bone marrow induction of MN in OF-1 mice.
6. In vivo bone marrow induction of MN in CD-1 mice.

Modafinil was negative in all of these assays in +/- S9 under these experimental conditions.
 Appendix I Carcinogenicity Studies and CAC Recommendations

APPEARS THIS WAY ON ORIGINAL

Amendment# 009
Carcinogenicity Studies

IND# [REDACTED]
Drug: Modafinil Tab/CEP1538
Sponsor: Cephalon Inc.
West Chester, PA
Category: α 1 Agonist
Indication: Narcolepsy
Sub Date: Jan 24 1994
Stamp Date: Jan 26 1994
Rec Date: Jan 27 1994
Rev Date: July 19 1996
Reviewer: Aisar Atrakchi, Ph.D. AA
Team Leader: Glenna Fitzgerald, Ph.D.
Related IND/NDAs: none

Cephalon Inc., submitted final reports for two carcinogenicity studies:

1. Potential tumorigenic effects of long-term, 78 week, dietary administration of Modafinil to mice/report# DS-92-012.
2. 104 week oncogenicity study by the oral route (dietary admixture) in Sprague-Dawley rats with a 52-wk toxicity study/report# DS-92-013.

1. Mouse study# DS-92-012/Lab: [REDACTED] completion date: Apr 1992/GLP statement signed and dated 5/1983.

Lot# CRL 40476

Species/strain/age/wt.: mouse/ICO:OF1/6wks old/32g males and 22g females.

Route/Dose/Duration/no. per dose per sex: oral dietary admix/6, 30, 60mg/kg/50/sex/dose/controls received untreated diet and consisted of 2 groups, T1 (65/sex) and T2 (50/sex). The first 15 mice of T1 and treated groups were used for PK data analysis.

Parameters measured:

mortality, clinical signs (daily), B.wt&food intake(weekly 1st 13wks then every 4wks over 7d period till end of study), ophthalmology (indirect ophthalmoscope wks 53-55 and slit lamp wks 56&59), hematology (wk52&78; orbital sinus; differential WBC only in cont and HD), no clinical chemistry was done, organ wt, macroscopic exam, and histopath in cont and HD only and, liver, any gross lesions, and masses from LD&MD (only the liver from the satellite gr was preserved in 10% formalin and embedded in parapiast); no histopath in the satellite grs.

Results:

Doses were selected based on the results of a 13-wk study (#HIFT 702580). The drug was mixed with the diet and content analyzed frequently for homogeneity and chemical stability. The drug was administered daily for 7 d/wk for at least 77 wk and at the most 78 wks. Blood was collected on wks 4, 52, and 78 of treatment.

APPEARS THIS WAY ON ORIGINAL

Mortality: There was no difference in mortality rate or time of death between any gr (Table below from sponsor). Main cause of death in all grs including the cont was malignant lymphomas and/or myeloid leukemia.

After the 78-week treatment period, the distribution of mortality was as follows:

Dose levels (mg/kg/day)	0 (T1)	0 (T2)	6	30	60
Males					
Sacrificed prematurely	20/50	15/50	20/50	13/50	17/50
Found dead	18/50	15/50	11/50	17/50	10/50
Total	38/50	30/50	31/50	30/50	27/50
%	76	60	62	60	54
Females					
Sacrificed prematurely	19/50	24/50	17/50	25/50	23/50
Found dead	12/50	6/50	13/50	7/50	12/50
Total	31/50	30/50	30/50	32/50	35/50
%	62	60	60	64	70

Clinical signs: no drug related findings. Signs common to old mice occurred in all grs around wk60 of the study (piloerection, hypoactivity, dyspnea, swollen abdomen etc). Piloerection was seen at a slightly higher number in MD&HDf (52&46% vs. 36&42% in the 2 cont grs). Signs found in drug grs but not in cont included immobilized hind limbs, lacrimation, soiled urogenital area, prostration, hair loss (these signs were not dose dependent and occurred in 1-4 mice of 50 total per gr).

B.wt and Food Intake: no drug related findings (see figures from sponsor).

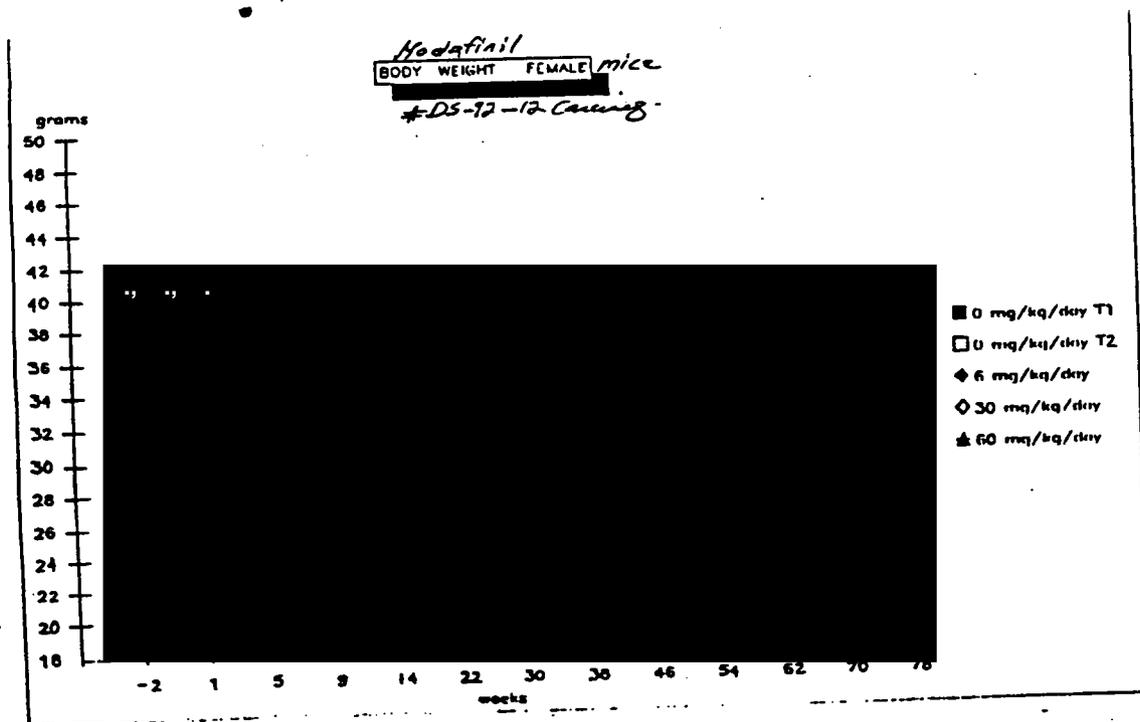
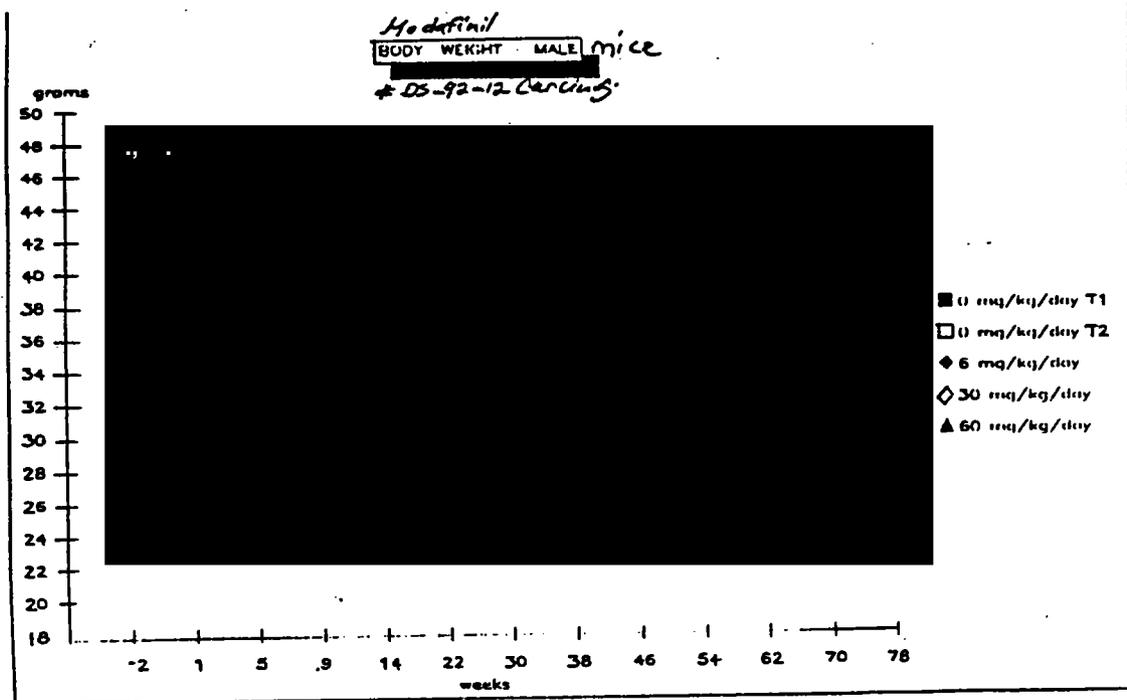
Ophthalmology: no drug related findings. The following was noted only in drug grs or at a slightly higher incidence in drug grs vs. the cont: unilateral corneal opacity (12/38 each in MD&HDm vs. 3/47&8/43 in the 2 cont; no difference in females), corneal neovascularization (13/38&11/38 MD&HDm vs. 1/47&2/43 in 2 cont m; no difference in females).

Hematology: no drug effect. The few findings that reached statistical sig were non dose dependent, minimal, and were common findings in aged mice: MCHC decr in males in LD (5%) and HD (3%) not sig in MD.

Organ wts: the absol and relative wt of the liver was sig and dose dependently incr in male mice in the 2 high doses compared with one of the cont grs (10-29%) and in all 3 female grs (absol wt: 30-54% of cont; rel wt: 13, 20, 40%). Mean liver wt was also sig higher in drug grs compared with the 2nd cont gr (rel wt 23&44% in MD&HDf higher than the cont). Though not reaching sig level, the absol and rel wts of the ovaries were incr in HD compared with both cont but the values were highly variable among all grs. The absol and rel wt of the spleen was incr in male and female mice (non dose dependently and not sig) but decr from cont2 in male mice.

APPEARS THIS WAY ON ORIGINAL

BEST POSSIBLE



Gross and Histopath: no gross findings except for enlarged liver mainly in HD mice. Non-neoplastic lesions included **liver hypertrophy** found in 22/50HDm and 8/50HDf relative to 0/100 in both cont1&2, and, extramedullary hematopoiesis (dose dependent in f at 25, 28, 37 of 50mice each in LD, MD, & HD vs. 23/50 in cont1 and similar findings relative to cont2). **Kidney** chronic interstitial nephritis, chronic pyelitis were observed in HD and MDm at incidences higher than these in the cont. In MD&HDm, **seminal tubular atrophy** (4/31MD and 5/50HD rel to cont1 but no difference rel to cont2) and **inhibition of spermatogenesis** in 4/31MD and 5/50HD vs. 5/100 in both conts, were noted. There was a non dose-dependent decr in no. of **corpora lutea** in all 3f grs relative to cont1 (46-62% few corp.lutea vs. 36% cont1/this percent represents the few no. of corp.lutea divided by the total no. examined), mastocytosis in 5/49 HDf vs. 1/50cont1 but no difference from cont2. All other lesions were found at comparable incidence in drug grs and cont. **There were no sig drug related or sig neoplastic findings in any drug gr vs. the cont (no difference with respect to no. of mice with neoplasia, no. of mice with more than 1 primary tumor, or latency of tumor appearance rel to the cont).** Tumor data were analyzed for trend using the method of Peto et al., 1980. The following table from the sponsor presents incidence of malignant lymphoma and myeloid leukemia which were present primarily in the spleen, thymus, lymph nodes, liver, bone marrow, and kidneys.

APPEARS THIS WAY ON ORIGINAL

Number of animals showed malignant lymphoma and myeloid leukaemia:

Lesions	Control T1		Control T2		A 6 mg/kg/day		B 30 mg/kg/day		C 60 mg/kg/day	
	M	F	M	F	M	F	M	F	M	F
Lymphoblastic malignant lymphoma	10	7	5	8	5	7	7	8	3	5
Lymphocytic malignant lymphoma	1	2	1	1	-	2	-	-	1	3
Heterogenous malignant lymphoma	9	(15)	10	(15)	12	(16)	11	(18)	8	(19)
Plasmocytic malignant lymphoma	-	1	2	-	1	2	1	1	-	2
Myeloblastic leukaemia	3	-	3	-	-	1	1	-	3	1
Granulocytic leukaemia	-	2	1	2	3	2	2	1	1	1

APPEARS THIS WAY ON ORIGINAL

PK: in the protocol, modafinil plasma levels were to be measured on wks 4, 52, and 78. However, due to technical problems during transportation of samples (from the [redacted] lab to the sponsor), data were determined only during wk4. Also, because of the small sample volume and low concentration, the levels of the metabolite were not measured. Detection limit of the [redacted] was 0.02ug/ml. Similar to the rat study, the animals were not fasted prior to sampling perhaps accounting for the variability in levels, and the analytical techniques were not validated.

Modafinil:

Dose (mg/kg)	Plasma levels (ng/ml) week 4
--------------	------------------------------

6	102±20(23±0.0)
30	197±47(59±18)
60	402±143(205±92)

Values are means±s.e.m for males and () for females; n=5/sex/dose.

From these data it is concluded that modafinil plasma levels incr with dose but the incr was non linear.

Summary and Conclusion(s):

Dietary administration of modafinil to OF1 Swiss mice at 6, 30, 60mg/kg, did not cause any drug-related deaths. There were no drug related clinical signs, no effect on hematology and ophthalmology except for a modest incr in incidence of unilateral corneal opacity in male mice(12/38MD&HDm vs. 3/47&8/43 in the 2 cont; no difference in females), and corneal neovascularization (13/38&11/38MD&HDm vs. 1/47&2/43 in 2 cont m; no difference in females). Modafinil produced a sig and dose-dependent incr in absol and relative wt of the **liver** in male mice in the 2 high doses compared with one of the cont grs. (10-29%) and in all 3 female grs (absol wt: 30-54% of cont; rel wt: 13-40%). Mean liver wt was also sig higher in drug grs compared with the 2nd cont gr. The absol and rel wt of the **spleen** was incr in male and female mice (non dose dependently and not sig) but a decr from the cont2 in male mice. There was no gross findings except for **enlarged liver** mainly in HD mice. Non-neoplastic lesions included **liver hypertrophy** found in 22/50HDm and 8/50HDf and extramedullary hematopoiesis (dose dependent in f at 25, 28, 37 of 50mice each in LD, MD, & HD vs. 23/50 in cont1). Some **kidney** effects such as congestion, chronic interstitial nephritis, chronic pyelitis observed in HD and MD. In MD&HDm, **testicular degeneration (seminal tubule)** (4/31MD and 5/50HD) and **inhibition of spermatogenesis** in 4/31MD and 5/50HD vs. 5/100 both cont were noted. There was a non dose-dependent decr in no. of **corpora lutea** in all 3f grs relative to cont1 (46-62% of cont)(23/37, 19/41, 26/49 vs. 41/100 both cont), mastocytosis in 5/49 HDf vs. 1/50cont1 (6/100 both cont), and spindle cell proliferation in HDf (28/49 vs. 16/50 cont1 or 46/100 both cont). All other lesions were found at comparable incidence in drug grs and cont. **There were no sig drug related or sig neoplastic findings in any drug gr vs. the cont.** Due to technical problems during transportation of samples, plasma levels were measured only during wk4. However, the data were highly variable, sample volume small, and the conc low, these obstacles led to inability to measure modafinil metabolites. Mean plasma levels ranged between 102-402ng/ml, values incr non-linearly with dose. **The NOEL for this study is 6mg/kg. The validity of this study is questionable since no sig drug related toxic findings or any neoplastic lesions were observed.**

APPEARS THIS WAY ON ORIGINAL

Dietary carcinogenicity study in rats for 104wks with interim sacrifice at 52wks/study# 311/501/completion date: Jun 26 1992/initiation date: Nov 23 1988/Lab: ██████████ GLP.

Batch# 5/2171

Species/Strain/Age/wt: SD rats/5-6wks old/113-207g at beginning of study.

Dose/No. per dose/Duration: 6, 30, 60mg/kg/d*/50/sex/dose/2 control grs 50/sex/gr (grs1&5)/104wks study duration.

* dose selection was based on the following: the 6mg/kg is similar to the clinical dose of 3-5mg/kg/d and the 30&60mg/kg/d were based on 3mo oral dietary admixture study in rats (# 702579). At doses ≥ 75 mg/kg liver wt was incr, and serum protein incr and serum creatinine decr. At the highest dose of 150mg/kg level of BUN incr and liver and kidney wts incr. No other changes.

Plasma drug levels were determined on wks13, 26, 52, and 104 of study; blood was collected from unfasted 10/sex/gr. Blood samples were sent to the sponsor ██████████ for analysis.

Note that animals were fasted for 16hr prior to blood collection for hematology, clinical chemistry, urine sampling, and time of terminal sacrifice.

The following parameters were evaluated:

Clinical signs (weekly), mortality, B.wt (prior to study, once weekly the 1st 28wks, then every 4wks thereafter), food intake (once weekly for the 1st 28wks then every 4wks thereafter), ophthalmology (direct and indirect; pre-test in 20/sex/gr and in 20/sex wks 52 & 104 in the 2 cont grs and HD), clinical chem (from 10/sex/gr on wk 104), hematology (10/sex/gr wks 52&104; bone marrow smears also done), urinalysis, organ wts, gross and histopath (all rats for macroscopic exam; liver, lungs, and kidneys for all rats; all rats from the 2conts and HD for histopath, and all 3 drug grs for rats found dead or killed moribund).

Historical data were also provided for comparison in addition to the concurrent controls.

Results:

Mortality: the incidence was slightly higher in HDm vs. the combined conts and other male drug grs (27/50 HDm vs. 15&19 of 50 each in the 2cont). Most of these deaths occurred during the last 6mo. The cause of death was **severe chronic progressive nephropathy which was considered drug related.**

Clinical Signs: no drug related clinical signs in any gr.

B. wt and Food Intake: there was a small but statistically sig decr in mean wt of HDm (6.5% of cont) on wk80 and mean wt gain was also decr (47% of cont) in HDm on wks 52-80. Mean wt gain was also reduced in MD&HDf (26% of cont in each gr) on wks 52-80. However, **by the end of the study, mean wts and wt gains were comparable between all drug grs and the cont.** There was no drug related effect on food intake. (See figures from sponsor).

Ophthalmology: no findings on wk52 exam. On wk104, bilateral corneal dystrophy and keratitis was noted in 5/20HDm vs. 3/20 cont m. This finding did not appear to be drug related and the sponsor indicated that this and other minor changes were common results in aging rat. [only cont and HD were evaluated).

Hematology: no drug related findings. There were very small changes in HDm rats in Hb, PCV, and RBC at end of study. None of the effects reached statistical sig. In LDm one animal had very high values of WBCs that led to incr mean total WBC in this gr. This rat was found later to have leukaemic lymphoma that contributed to the elevated WBC count. There were no changes in bone marrow smears.

Clinical Chemistry: no drug related findings. A small but statistically sig incr in Ca, BUN, and chol were noted in HDm compared with the 2 cont grs combined.

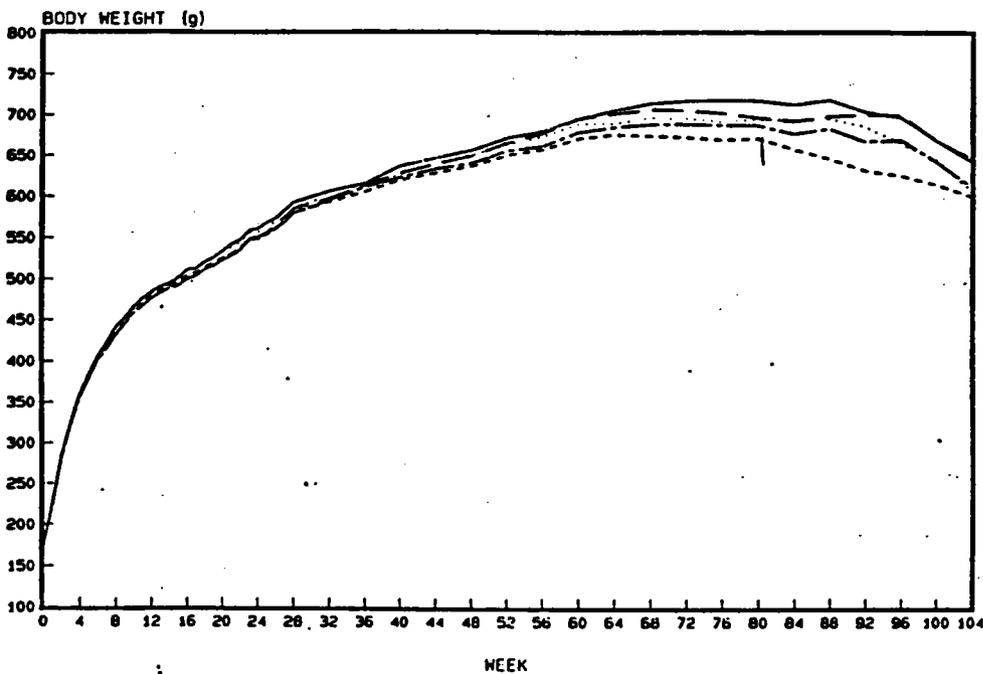
Urinalysis: no findings.

Modafinil

Rat Carcinog. # DS-92-013

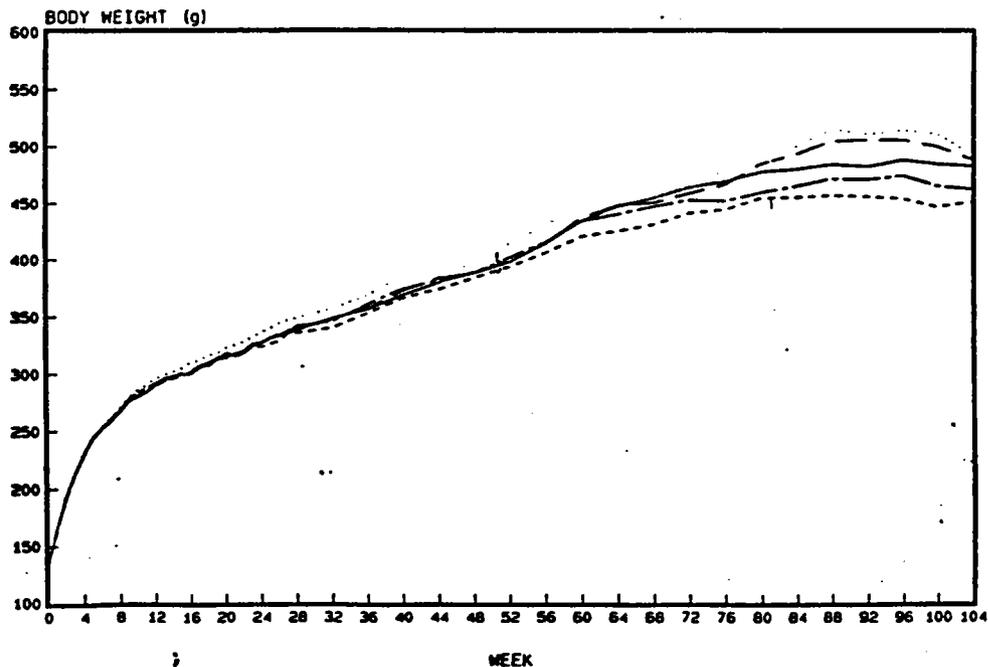
STUDY NUMBER 311/501 - FIGURE 3 -

Cont GROUP 1 GROUP MEAN BODY WEIGHT - MALES - Cont GROUP 5
GROUP 2 GROUP 3 GROUP 4



STUDY NUMBER 311/501 - FIGURE 4 -

Cont GROUP 1 GROUP MEAN BODY WEIGHT - FEMALES - Cont. GROUP 5
GROUP 2 GROUP 3 GROUP 4



Gross and Histopath: no drug-related macroscopic findings in any gr except in kidneys. HDm that were killed moribund or found dead, their kidneys had irregular surface, pale, and cysts were present. The incidence for the kidneys was as follows:

irregular surface	13/23LDm, 9/18MDm, 21/27HDm, 13/35 both conts (23/50LDm, 25/50MDm, 38/50HDm, 40/100both cont)
pale	7/23LDm, 8/18MDm, 13/27HDm, 3/35both conts (8/50LDm, 8/50MDm, 16/50HDm, 6/100both cont)
cyst	5/18MDm, 9/27HDm, 3/35both cont (11/50MDm, 17/50HDm, 17/100both cont)

() is the incidence in all animals (terminal sacrifice + moribund and found dead animals)

The only histopath finding considered by the sponsor to be drug related, was **chronic progressive nephrosis (CPN) the severity of which incr with dose**. The incidence was as follows:

CPN	22/23LDm, 16/18MDm, 26/27HDm, 31/35both conts (47/50LDm, 48/50MDm, 49HDm, 96/100both conts)
mineralization	3/27HDm, 2/35both conts [none in LD&MDm] (3/50HDm, 2/100both conts)
periarteritis nodosa (PAN)*	2/18MDm, 4/27HDm, 2/35both conts (6/50MDm, 5/50HDm, 8/100both conts)

() is the incidence in all animals (terminal sacrifice + moribund and found-dead animals)
The incidence in females was comparable to the controls.

* PAN was found in other organs including the testes with the following incidence: 31/100cont, 19/50, 20/50, and 37/50 in LD, MD, and HD respectively.

In the testes from rats that killed moribund or found dead, there was degeneration/atrophy (14/23LDm, 12/18MDm, 22/27HDm, 12/35both conts) and PAN (11/23LDm, 9/18MDm, 24/27HDm, 9/35both conts). The testicular atrophy/degeneration was considered **secondary to PAN which is usually noted with CPN** (Gray DE, 1986, CPN in rat, monographs of lab animals). The corresponding incidence for all male rats at end of study was: for degeneration/atrophy 21/50LD, 24/50, 30/50, 32/100 (conts) respectively, and for periarteritis nodosa: 19/50, 20/50, 37/50, 31/100 respectively.

CPN is a common age-related finding in the rat (Anver MR & Cohen BV, 1979; The lab rat; Burek JD, 1978, Pathology of aging rat). The sponsor considered this finding to be drug related stating that some cpds are known to affect the incidence and/or severity of CPN with long term exposure (Peter CP et al., 1986, Spontaneous nephropathies in the rat, Tox Path 14:91-100).

Tumor incidence was comparable between cont and drug groups and tumors were spontaneous and related to aging rats.

PK:

The sponsor indicated that non-fasted rat samples had the main peaks contaminated with endogenous peaks in the control, relative to fasted rats. The sponsor also indicated that such problems were accounted for by evaluating the linearity of the control samples, by considering the range in control plasma, and limit of quantification. The sponsor did not validate the quantitative techniques because of the small no. of samples to be quantified and the small blood volume collected from each rat. Detection

limit was 0.02ug/ml. From the tables below, it can be seen that the incr in conc was non-linear with dose and generally, it seems that steady state was not reached since the conc kept incr with time.

Modafinil:

Dose (mg/kg)	Plasma levels (ng/ml)		
	week 13	week 52	week 104
6	11±4.3(30±7.7)	0.0(35±8)	0.0(0.0)
30	24±6.6(40±10)	57±10(77±36)	171±33(168±43)
60	44±6.5(88±10.7)	109±13(180±53)	97±32(90±38)

Acid Metabolite:

Dose (mg/kg)	Plasma levels (ng/ml)		
	week 13	week 52	week 104
6	3±3(35±13)	4±4(6.7±4.5)	0.0(0.0)
30	123±36(142±40)	19±13(46±15)	40±22(32±17)
60	212±38(296±49)	61±19(143±43)	59±27(69±30)

all values are means±sem for males and () for females, n=10 rats/sex/dose.

Blood was collected between 8-9 a.m.; the elimination half life of modafinil in the rat is short, 1-3hr compared with 10-12hr in humans. There was large variability in values that the sponsor contributed to the timing of blood collection. Plasma levels incr non-linearly with dose. The metabolite was detectable on wk52 in LD at which time the parent could not be detected. The plasma levels of the metabolite in MD&HD on wk13 were markedly higher than the parent but lower during wks 52 and 104. This is in agreement with results obtained in the PK studies where the levels of parent decreased upon repeat dosing compared with levels after single dose. This indicated that modafinil induced its own metabolism with repeat dosing.

Summary and Conclusions:

Dietary administration of modafinil to SD rats for 104 wks at 6,30, 60mg/kg, did not cause clinical signs. The mortality was slightly higher in HDm contributed to chronic progressive nephrosis. There was no drug effect on hematology, ophthalmology, clinical chem, or urinalysis. Mean wt and wt gain were slightly but sig decr in HDm (6.5% and 47% of cont respectively) on wk80. Mean wt gain was also reduced in MD&HDf (26%) on wks 52-80. However, **by the end of the study, mean wts and wt gains were comparable between all drug grs and the cont.** There was no drug related effect on food intake. There were no marked drug related effect on macroscopic findings and **there was no difference in tumor incidence, type, or distribution.** The only effect noted in histopath was CPN in male rats and testes degeneration/atrophy. The sponsor indicated that this finding was drug related, however, this lesion is commonly found in aging rats specifically males and from the incidence in male controls, it may be concluded that this finding is not drug related.

It is the opinion of the reviewer, that this rat carcinogenicity study similar to the mouse study discussed above, is not valid because the doses selected produced minimal drug related toxicity in all of the measured parameters including tumor findings.