Terminal Sacrifice — Mouse Toxicity Study

Group	Adrenal Glands	Epididymides	Prostate	Seminal vesicles	Testes
		Male Animals			
1					
Mean:	0.0055	0.1280	0.0611	0.4396	0.2786
Standard deviation:	0.0007	0.0271	0.0215	0.0604	0.0553
Number of observ. :	(10)	(9)	(10)	(10)	(9)
2					
Mean:		0.1127	0.0561	0.2467+	0.2853
Standard deviation:		0.0147	0.0255	0.1336	0.0396
Number of observ. :		(9)	(10)	(10)	(9)
3					
Mean:	0.0051	0.1329	0.0584	0.4177	0.2412
Standard deviation:	0.0011	0.0156	0.0199	0.1035	0.0282
Number of observ. :	(11)	(9)	(10)	(10)	(9)
4					
Mean:	0.0060	0.1309	0.0738	0.5506	0.2936
Standard deviation:	0.0010	0.0111	0.0249	0.1354	0.0497
Number of observ. :	(10)	(8)	(10)	(10)	(8)

Summary

Both species showed dose-related decreases in rate of body weight gain and loss of body weight at the HD as well as the reduced HD. A maximally tolerated dose was exceeded in both species.

Luteininzing hormone in rats- Given the variability, it is difficult to say that there are any real differences between the groups. However, with all values included in the analysis, the following was seen:

Positive control- mean LH in this group showed a marked increase over all other Groups both ±pulses at weeks 2,4 and 13.

Week 2- there were no differences in mean LH values between nebivolol and Vehicle control except at the HD where there was a 50% decrease in mean LH.

Week 4- there was no consistent pattern but a mild increase in mean LH at 40 Mg/kg which was not seen at week 13.

Week 13- there was a slight decrease in mean LH at LD and MD.

The sponsor then repeated the analysis, removing pulses, or the values ≥2SD from the mean.

The positive control group was still significantly increased compared to all other Groups at weeks 2,4 and 13.

Week 2- the control value LH was greater than that of each of the nebivolol Groups.

Weeks 4, 13- mean control value was greater than that of each nebivolol group.

Luteinizing hormone in mice:

Positive control- mean LH for the finasteride mice was increased only at week 4 With removal of pulses. If all pulses are left in the analysis, the positive control Shows an increase at only 1 point of determination.

If all values are left in the analysis:

Week 2: there is a dose-related decrease in mean LH with increased dose of Nebivolol.

Week 4: mean LH was decreased in the nebivolol animals compared to control

Week 13: some increase in mean LH at MD and HD

Recovery: 3X increase in mean LH at the HD

If the pulses are removed from the analysis:

Weeks 2 and 4: no difference in mean LH values between nebivolol and control

Week 13: LH in MD and HD groups are increased over the positive control.

Rat estradiol data: The variability makes interpretation difficult. Estradiol in the positive control group was increased over all other groups. There was no apparent difference in mean estradiol in the nebivolol-treated groups vs control.

Mouse estradiol: The variability makes interpretation difficult. The positive control showed greater mean values than the other groups at weeks 2 and 4. At other points of determination, there was no difference between the values for the positive control and the other groups. At week 2, there appeared to be a dose-related decrease in estradiol in nebivolol-treated animals vs control. Weeks 4 and 13 there was no discernible pattern. At the recovery measurement, there was an increase in estradiol in MD and HD. There were individual values in the nebivolol LD group exceeding the highest values in the positive control group.

PHA021-005 Amendment of Report for PHA021-005 Endocrine screening study with 12 beta blockers
Study location:

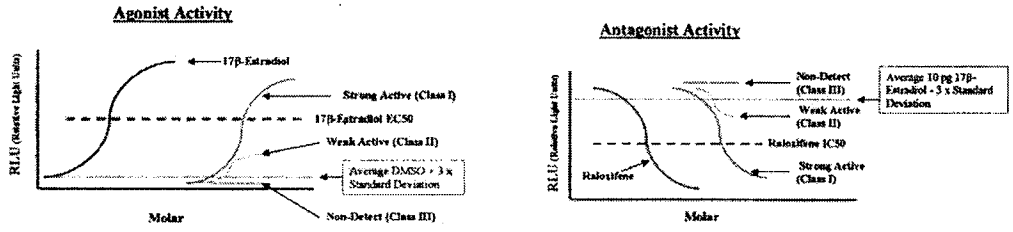
[redacted] has a genetically engineered cell line ([redacted] which contains a stably transfected estrogen inducible reporter responding to chemicals that are estrogen agonists and/or antagonists. The response to these chemicals is induction of the firefly luciferase gene. The samples were analyzed using six 10-fold dilutions.

Samples: metoprolol, atenolol, bisoprolol, propranolol, carvedilol, acebutolol, timolol, sotalol, pindolol, nadolol, betaxolol, nebivolol-HCl

Standards included: a standard curve of β-estradiol (10 points),
Positive controls: methoxychlor, raloxifene, flavone, estradiol
Negative control: DMSO

Results were assessed as non-detectable, weak active, strong active. The definitions are shown below.

- Non-Detect (or Class III) is defined as having no observed activity above background (average of DMSO blanks + 3 x standard deviation of DMSO blanks) for agonist activity or below the 10 pg 17β-estradiol (average 10 pg 17β-estradiol replicates minus 3 x standard deviation of 10 pg 17β-estradiol replicates) for antagonist activity.
- Weak Activity (or Class II) is defined as having observed activity above DMSO background (for agonist) or below the 10 pg 17β-estradiol (for antagonist), but not reaching the EC50 RLU value for 17β-estradiol for agonist activity or not reaching the IC50 RLU value for Tamoxifen for antagonist activity.
- Strong Activity (or Class I) is defined as having observed activity above DMSO background (for agonist) or below the 10 pg 17β-estradiol (for antagonist) and having RLU values equal to or greater than the EC50 RLU value for 17β-estradiol (for agonist activity) or equal to or less than the IC50 RLU value for Raloxifene (for antagonist activity). Please see figures below for clarity.



Pharmacology/Toxicology Consult
To: NDA 21-742 as requested by Division of Cardiovascular & Renal Products (DCRP)
From: Karen Davis-Bruno PhD; Supervisory Pharmacologist; DMEP

Re: Nonclinical endocrinologic effects of Nebivolol
Date: 3/15/06

Material Reviewed: 2-volume briefing document provided by Mylan Bertex Pharmaceticals as well as meeting minutes and pharmacology/toxicology reviews by HFD-110 available electronically in DFS. Reference is made to clinical consults from DRUP (2/4/05 & 5/24/05) and from DMEP by Hylton Joffe (3/6/06).

Administrative background: Nebivolol is a β_1 -adrenergic receptor antagonist indicated for treatment of ——— hypertension at a clinical dose of 2.5-10 mg (10 mg/day or 78 ng h/ml[1] or 6 mg/m^2), although some patients could potentially receive up to 20 mg BID off-label. Nebivolol is currently marketed outside the USA for the management of hypertension and treatment of ischemic heart disease and heart failure. A submitted NDA received an approvable action letter 5/31/05 because of concerns regarding potential adverse endocrinologic and reproductive effects identified in nonclinical studies. These concerns included statistically significant, dose-related increases in benign and malignant Leydig cell tumors in male mice, reductions in adrenal and ovarian weights in long-term rat toxicity studies, dystocia and interference with estrus cyclicity in reproductive toxicity studies. These findings were interpreted to be suggestive of an endocrine disruption signal with nebivolol treatment. The sponsor responded to these concerns in a 2/10/06 submission.

Nonclinical Background: During the review of NDA 21-742 concern was raised about several findings which taken together suggested a signal for endocrine disruption with nebivolol treatment. Evidence of endocrine disruption is based on:

- A 20- month dietary carcinogenicity study in mice given nebivolol at 0, 2.5, 10, 40 mg/kg/day resulted in drug–related Leydig cell tumors (adenomas) with the following incidence: 2/50, 0/50, 1/50, 21/50 in males. A later re-read of the HD group slides indicated 2/50 male mice had Leydig cell carcinomas. The NOEL=10 mg/kg/day (AUC=1450 ng h/ml; 5X MRHD based on body surface area comparisons) for Leydig cell tumors. In rodents this is typically due to an estrogen receptor mechanism. A 3-month mouse toxicity study shows a single male (160 m/k/d) with focal Leydig cell hyperplasia, however lower doses weren't evaluated.
- A 25-month rat carcinogenicity study at 0, 2.5, 10, 40 mg/kg/day by diet did not induce any drug-related tumors. A standard genotoxicity test battery was negative. A recently completed 13-week endocrine evaluation in rats and mice given nebivolol at 40 and 80 mg/kg/day resulted in Leydig cell hyperplasia in association with an increase in serum LH, in the absence of increased estradiol. This suggested that perturbations in estradiol homeostasis were not involved in the pathogenesis of Leydig cell adenomas in mice according to the expert panel. Following a 4-week recovery period the Leydig cell hyperplasia in the 80 mg/kg/day group was still evident in association with a greater increase in LH. At 40 mg/kg/day the Leydig cell hyperplasia showed recovery and the LH response was considered normal.
- Initially the sponsor considered the nonclinical reproductive effects of nebivolol to be attributable to stress or chronic sympathetic stimulation. The rejection of this hypothesis by FDA led the sponsor to a consultation with an expert panel of scientific advisors. They concluded that the reproductive effects in rats (decreased ovarian weight, numbers of corpora lutea, increased atretic follicles, and changes in uterus/vagina and testicular degeneration) were consistent with reduced feed intake and 20% body weight (presumed a decrease in GnRH, estrogen) since the reproductive

[1] 78 ng h/ml is the sponsor's value based on a weighted calculation of human extensive (698 ng h/ml) and poor metabolizers (31 ng h/ml) based on a 93% weight for extensive and 7% weighted for poor metabolizers. It would be more appropriate to derive individual exposure multiples for extensive and poor metabolizers rather than using an arbitrary weighted human average of exposure.

findings only occurred in conjunction with body weight loss. The panel concluded that the decreased pup weight and survival were class effects of beta-blockers. Decreased pup body weight and viability occurred at ≤10 mg/kg/day nebivolol given to pregnant rats during the peripartum period. Maternal toxicity indicated by dystocia at ≥ 5 mg/kg/day was also present. The sponsor concludes that nebivolol has a larger margin of safety compared to other beta adrenergic antagonists. As Dr. Hausner identifies in a series of reviews there has been inconsistencies in the study reports from NDA 21-742, particularly regarding reproductive toxicities.

- In females the sponsor notes a more resting of the repro tract as well as fewer corpora lutea and more atretic follicles
- In males in a 3-month mouse study, Leydig cell hyperplasia (160 mg/k/d), large nucleated tubular cells and testicular atrophy due to delayed maturation were noted. In rats increased gonad weight (unspecified) was reported. In 6-month rat studies decreased gonad weight, testicular degenerative changes with reduced sperm and "possible cellular debris in the epididymis" were noted. A one month dog showed an increase in prostatic weight but no histopath data was provided. The 3-month dog showed increased gonad weight at 2.5, 10 and 40 mg/kg with urolithiasis at LD and prostatitis at MD
- The following is excerpted from Dr. Hausner's review

4) Reproductive toxicology was apparent.
Certain points from the reproductive and developmental toxicology studies are summarized below.

Reviewer's Summary of Findings

Study	Dosages (mg/kg)	Sponsor's statement of results
Seg I	0, 10, 40 and 160 premating, mating, to GD6	40 mg/kg: NOEL for fertility for both sexes according to sponsor, but not conclusive from the data presented.
Seg II	0, 2.5,10 and 40 GD6-GD16	40 mg/kg: Maternal toxicity characterized by decreased food consumption, decreased litter size, increased embryonal resorption, decreased pup weight
Seg III	0, 2.5, 10, 40 GD18-PN21	2.5 mg/kg: Decreased pup birth weight and decreased pup survival 10 mg/kg: Decreased food consumption during lactation as well as decreased pup weight and survival 40 mg/kg: Maternal toxicity characterized by mortality, ptosis, decreased body weight gain + food consumption, increased duration of gestation, decreased nursing behavior. No pups survived in this dosage group
Current Seg III	0, 1.25, 5 and 20	≥1.25 mg/kg decreased pup birth weight and decreased pup survival No surviving pups at the HD. Fertility was decreased in the F1 pups as shown by decreased number of implantations(≥1.25 mg/kg) and decreased number of corpora lutea per female (≥1.25 mg/kg). Maternal toxicity at 20 mg/kg as decreased weight

Study reports indicate cannibalism at ≥10 mg/kg/day and dystocia at doses ≥ 5 mg/kg/day but don't provide specifics on incidence. The sponsor indicates adverse maternal clinical observations at 10 and 40 mg/kg/day. Bad general condition and ptosis were noted in dams at 160 mg/kg/day. Cannibalism was reported in multiples studies. There was no evaluation of estrus. One criticism of the developmental data was that developmental landmark evaluations were performed at a relatively late timepoint compared to the OECD recommended earliest evaluation e.g. eye opening can occur PND 12 but wasn't examined until PND 21 or righting reflex is seen by PND 4 but wasn't examined until PND 21. As nebivolol was administered later in gestation, the dose at which maternal weight gain was affected decreased. The F1

pups showed decreased birth weight and survival to PND 21. This was repeated in two separate studies at doses of 2.5 and 1.25 m/k/d. The adult exposure relative to the MRHD based on body surface area comparison was 3 and 1X respectively. When untreated F1 pups were mated the corpora lutea, number of implantations and live fetuses were decreased from litters of does that received 1.25 and 5 mg/kg/day.

6)Teratogenicity

Summary of significant Seg II Study findings: Affected pups(litter data not available)

N74513 Sprague-Dawley Rats				
Finding	Dose group mg/kg/day			
	0	2.5	10	40
Split center of thoracic vertabrae	41	30	62	100**
Rudimentary sternal bone	1	5	11*	19**
Ureter dilatation	3	2	21**	42***
N74514 Sprague-Dawley rats (same doses)				
Split center of thoracic vertebrae	19	30	31	56**
Rudimentary sternal bone	9	18	20	30**
Ureter dilatation	8	17	16	12
Historical range for split thoracic vertebrae 0-27				
Historical range for rudimentary sternal bone 0-20				
Historical range for dilated ureter 0-29				

*p<0.05, **p<0.01, ***p<0.001

The data from the rabbit studies was not presented in a way that facilitates interpretation. Study N51487 (Seg II in NZW) did not include a tabular summary of incidences. There were 2 control litters, 1 LD litter and 1 MD litter affected with abnormal thoracic vertebrae. At the HD, 4/13 litters were affected by abnormal thoracic vertebrae.

The second rabbit study, N71096, did not report any teratogenic or anomalous findings of significance.

The rat dose of 1.25mg/kg (7.5 mg/m^2) is comparable to 1.2x the human dose of 10 mg (6.2 mg/m^2). A rat dose of 10 mg/kg (60 mg/m^2) is comparable to 9.6x the human dose of 6.2 mg/m^2. At human doses of 20 mg (12mg/m2 for a 60 kg human) and 40 mg (25 mg/m2) as may possibly be used in African Americans, the rat dose of 1.25 mg/kg is equivalent to ~0.6X -0.3Xthe human exposure.

Therefore, the reproductive/developmental effects reported appear at equivalent exposure to the proposed human levels when doses are compared on a mg/m^2 basis.

- Adrenal dysfunction: Nebivolol increased adrenal weight and adrenal cortical hypertrophy in rats but not mice or dogs. Other drugs in the class show increased adrenal weight and hypertrophy (e.g. atenolol, arvedilol) and adrenal hyperplasia (atenolol) in rats. Nebivolol attenuates the corticosterone response to ACTH in rats but had no effect on the human HPA axis as assessed by insulin induced hypoglycemia and ACTH-stimulated glucocorticoid and mineralocorticoid production. Reference is made to Dr. Joffe's clinical review of these studies, suggesting limitations in the sponsor's interpretation of the apparent lack of effect in humans because of the study design. Published literature with various beta-adrenergic receptor antagonists in multiple species given at pharmacologic doses results in adverse synthesis/release of endocrine hormones from testes, ovary, pituitary and adrenal gland as well as perturbations in HPG and HPA axis.

- Consistently enlarged adrenal glands was observed in rodents
- Decreased triglycerides and cholesterol (primarily HDLc in rat) whereas beta agonists are typically associated with increased triglycerides and decreased HDL cholesterol.
- Several studies in rats looked at circulating corticosterone and aldosterone pre and post ACTH stimulation. Animals treated for up to 1 month showed an increase in corticosterone following stimulation but the degree of increase was less than the control. Aldosterone levels with the

MOUSE MODEL SUMMARY TABLE A

	Nebivolol (mg/kg/day)					Finasteride (mg/kg/day)
	0	10	40	80	160[a]	250
	Weeks 1 to 17	Weeks 1 to 13	Weeks 1 to 13	Weeks 6 to 13	Weeks 1 to 3	Weeks 1 to 13
Toxicokinetics (AUC_{0-24})[b]	n/a	3.666	29.674	55.968	71.492	n/a
Mortality[c] Dosing phase	0/120	1/192	3/192	4/146	12/192	2/120
Recovery phase	none	none	none	none	none	none
Clinical Observations						
Dosing phase palpebral ptosis	none	none	25%[f]	45%[e]	>59%[d]	none
Dosing phase paleness	none	none	none	15%[e]	>45%[d]	none
Dosing phase decreased activity	none	none	none	none	5%[d]	none
Recovery phase	none	none	none	p. ptosis	n/a	none
Absolute body weight (grams)						
End of dosing	39.7	38.5	36.8**	34.4**	X	38.0
Recovery phase	40.6	40.4	39.2	39.9[g1]	n/a	40.0
Feed consumption (trend)						
Dosing phase	n/a	no effect	no effect	Slight increase	Marked decrease	Moderate increase

[a] Initial High dose. Actual intake circa 120 mg/kg/day. Dosing was reduced to 80 mg/kg/day after Day 15 and then stopped on Day 20.

[b] ng·hr/mL

[c] includes TK animals

[d] after approximately 2 weeks at 160 mg/kg/day

[e] weeks 3 to 13 at 80 mg/kg/day

[f] transitory weeks 8 to 10 at 40 mg/kg/day

[g1] statistics analyses not carried out

n/a = not applicable

X= average -19% weight loss on Study day 13 compared to mean values of this group at the initiation of dosing.

* = $p<0.05$ **= $p< 0.01$

no asterisk = not statistically significant

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On Original

MOUSE MODEL SUMMARY TABLE B

	Nebivolol (mg/kg/day)					Finasteride (mg/kg/day)
	0	10	40	80	160[a]	250
	Weeks 1 to 17	Weeks 1 to 13	Weeks 1 to 13	Weeks 6 to 13	Weeks 1 to 3	Weeks 1 to 13
Hormones (mean, ng/ml.)						
LH week 2[h]	7.172	3.885	3.406	n/a	1.822[#]	6.055
LH week 4[h]	5.547	5.082	3.962	-	-	11.381*
LH week 8[h]	n/a	n/a	n/a	4.672[#]	n/a	n/a
LH week 13[h]	2.787	1.750	3.965*	4.595[#]	n/a	4.506*
LH recovery[h]	3.773	2.790	3.657	11.007[#]	n/a	3.975
LH week 2[t]	0.881	0.979	0.784	n/a	1.235[#]	1.241
LH week 4[t]	1.536	1.403	1.832	-	-	6.717*
LH week 8[t]	n/a	n/a	n/a	2.297[#]	n/a	n/a
LH week 13[t]	0.981	0.749*	2.611*	3.224[#]	n/a	1.787*
LH recovery[t]	2.278	1.078*	2.156	11.007[#]	n/a	1.612
Estradiol[#1] week 2	3.195	5.663	4.159	n/a	2.953	11.087
Estradiol[#1] week 4	3.72	4.093	2.066	-	-	9.601
Estradiol[#1] week 8	n/a	n/a	n/a	1.237	n/a	n/a
Estradiol[#1] week 13	2.733	5.786	1.08	3.735	n/a	2.706
Estradiol[#1] recovery	2.717	2.745	5.582	4.192	n/a	1.998

[a] Initial High dose. Actual intake circa 120 mg/kg/day. Dosing was reduced to 80 mg/kg/day after Day 15 and then stopped on Day 20.

[#] statistics analyses not carried out.

[#1] estradiol statistics analyses were not carried out due to a large percentage of samples below the limit of quantification.

[h] pulses not removed

[t] pulses removed

n/a = not applicable - not collected

*= $p<0.05$, **= $p< 0.01$, no asterisk = not statistically significant

MOUSE MODEL SUMMARY TABLE C

		Nebivolol (mg/kg/day)				Finasteride (mg/kg/day)
		0	10	40	80	250
		Weeks 1 to 17	Weeks 1 to 13	Weeks 1 to 13	Weeks 6 to 13	Weeks 1 to 13
Sperm analyses (means)						
End of dosing	Motility	93	85	80	69**	92
	Prog. Motility	66	56	55	17**	53
	Sperm count	1053	847*	782*	584**	776*
	Spermatid count	251	228	249	215	220
	% normal sperm	76.4	81.6	52.7**	49.9**	79.7
Recovery phase	Motility	76	84	92	96**	84
	Prog. Motility	42	38	39	62	35
	Sperm count	621	657	384	541	542
	Spermatid count	167	172	143	164	176
	% normal sperm	71.7	74.6	65.5	81	77.5
Organ weight (means)						
End of dosing	Adrenals	0.0055	0.0051	0.0060	0.0083**	-
	Epididymides	0.1280	0.1196	0.1309	0.1159	0.1127
	Prostate	0.0611	0.0584	0.0738	0.0936*	0.0561
	Seminal Vesicles	0.4396	0.4177	0.5506	0.4023	0.2467
	Testes	0.2786	0.2171	0.2936	0.2608	0.2853
Recovery phase	Adrenals	0.0052	0.0048	0.0056	0.0073	
	Epididymides	0.1326	0.1352	0.1252	0.1322	0.1371
	Prostate	0.0532	0.0667	0.0556	0.0645	0.0456
	Seminal Vesicles	0.5280	0.4461	0.3742**	0.4539[k]	0.4194
	Testes	0.2669	0.2671	0.2702	0.2712[k]	0.2790
Microscopic Pathology						
Dosing phase		none	none	Leydig cell hyperplasia[l] (13 wks)[M].	Leydig cell hyperplasia[l] (8 & 13 wks).	none
Recovery phase		none	none	Leydig cell hyperplasia[l] recovered	Leydig cell hyperplasia[l] not recovered	none

[k] statistics analyses not applicable

n/a = not applicable

- = not obtained

[l] minimal, diffuse

[M] had no evaluation at 8 weeks

*= $p<0.05$, **= $p< 0.01$, no asterisk = not statistically significant

Mouse: Administration of 160 mg/kg/day nebivolol was associated with increased mortality concurrent with decreased activity, ptosis, >20% weight loss and decreased food consumption. Treatment with 80 m/k/d resulted in ptosis, paleness and 9% decreased body weight and increased (12%) food consumption. Transient ptosis and body weight decreases (<10%) were observed at 40 m/k/d. Statistically significant changes in sperm parameters were observed at all drug treated levels on week 13 consisting of minimal to moderate decreases in caudal epididymal sperm counts (-20, -26, -45% vs. control), decreased motile sperm (25%) at HD and 30-35% decrease in normal sperm at doses ≥40 m/k/d. Sperm effects were reversible following a 4-week recovery. Leydig cell hyperplasia at 80 m/k/d at week 8 & 13 and at 40 m/k/d at week 13 in addition to some apoptosis was observed. LH was significantly increased at ≥40 m/k/d. No increases in estradiol were observed.

RAT MODEL SUMMARY TABLE A

	Nebivolol (mg/kg/day)					Flutamide (mg/kg/day)
	0	10	40	80	160[a]	100
	Weeks 1 to 17	Weeks 1 to 13	Weeks 1 to 13	Weeks 7 to 13	Weeks 1 to 4	Weeks 1 to 13
Toxicokinetics (AUC_{0-24})[b]	n/a	1.136	8.570	33.610	34.673	n/a
Mortality[c] Dosing phase	0/80	0/104	2/104	1/84	6/104	0/80
Recovery phase	none	none	none	none	none	none
Clinical Observations						
Dosing phase palpebral ptosis	none	none	none	30-36%[f]	73-98%[d]	none
Dosing phase paleness	none	none	none	none	sporadic[e]	none
Dosing phase decreased activity	none	none	none	none	sporadic[e]	none
Recovery phase	none	none	none	p. ptosis	n/a	none
Absolute body weight (grams)						
End of dosing	593	565*	537**	458**	x	495**
Recovery phase	622	587*	591	499**	n/a	540**
Feed consumption (trend)						
Dosing phase	n/a	none	none	Moderate decrease	Marked decrease	No effect

[a] Initial High dose. Actual intake circa 120 mg/kg/day. Dosing was reduced to 80 mg/kg/day after Day 20 and then stopped on Day 30.

[b] ng·hr/mL

[c] includes TK animals

[d] after one week at 160 mg/kg/day

[e] after 3 weeks of dosing

[f] after 6 to 7 weeks at 80 mg/kg/day

x average -19% weight loss on Study day 20 when compared to mean values of this group at the initiation of dosing.

n/a = not applicable

- = not obtained

* = $p<0.05$, ** = $p<0.01$. No asterisk = not statistically significant

RAT MODEL SUMMARY TABLE B

	Nebivolol (mg/kg/day)					Flutamide (mg/kg/day)
	0	10	40	80	160[a]	100
	Weeks 1 to 17	Weeks 1 to 13	Weeks 1 to 13	Weeks 6 to 13	Weeks 1 to 3	Weeks 1 to 13
Hormones (mean, ng/mL)						
LH week 2[h]	1.474	1.173	1.198*	n/a	0.667[g]	10.178*
LH week 4[h]	1.095	0.798*	1.793	n/a	0.644[g]	13.010*
LH week 7[h]	n/a	n/a	n/a	0.808[g]	n/a	n/a
LH week 13[h]	0.997	0.677*	0.610*	n/a	n/a	9.443*
LH recovery[h]	0.872	1.096	0.905	1.628[g]	n/a	1.418
LH week 2[l]	1.418	1.002*	0.937*	n/a	0.505[g]	7.969*
LH week 4[l]	1.012	0.684*	0.873	n/a	0.539[g]	13.010*
LH week 7[l]	n/a	n/a	n/a	0.644[g]	n/a	n/a
LH week 13[l]	0.997	0.653*	0.562*	n/a	n/a	8.090*
LH recovery[l]	0.780	0.839	0.905	1.333[g]	n/a	0.983
Estradiol[g1] week 2	8.274	5.674	5.674	n/a	5.627	10.683
Estradiol[g1] week 4	3.914	3.063	4.317	n/a	3.326	11.937
Estradiol[g1] week 7	n/a	n/a	n/a	1.946	n/a	n/a
Estradiol[g1] week 13	2.089	2.723	1.475	n/a	n/a	9.548
Estradiol[g1] recovery	2.747	2.088	4.890	1.557	n/a	1.689

[a] Initial High dose. Actual intake circa 120 mg/kg/day. Dosing was reduced to 80 mg/kg/day after Day 20 and then stopped on Day 30.

[g] Statistic analyses not carried out.

[g1] estradiol statistics analyses were not carried out due to a large percentage of samples below the limit of quantification

[h] pulses not removed

[l] pulses removed

n/a = not applicable

*= p<0.05, **= p< 0.01, no asterisk = not statistically significant

RAT MODEL SUMMARY TABLE C

		Nebivolol (mg/kg/day)				Flutamide (mg/kg/day)
		0	10	40	80	100
		Weeks 1 to 17	Weeks 1 to 13	Weeks 1 to 13	Weeks 6 to 13	Weeks 1 to 13
Sperm analyses (means)						
End of dosing	Motility	93	94	83	84**	86
	Prog. Motility	46	54	37	33	17**
	Sperm count	525	564	552	485	391
	Spermatid count	82	92	89	76	63
	%normal sperm	96.6	96.1	89.1*	84.4**	79.4**
Recovery phase	Motility	85	94	76	52	72
	Prog. Motility	61	55	50	26**	46
	Sperm count	671	613	295**	293**	463
	Spermatid count	80	72	83	59	63
	%normal sperm	94.8	97.4	84.9	53.9**	70.3*
Organ weight (means)						
End of dosing	Adrenals	0.0670	0.0692	0.0868	0.1119**	-
	Epididymides	1.7756	1.7653	1.7293	1.5641	0.9323*
	Prostate	1.6131	1.5953	1.7127	1.6571	0.3287**
	Seminal Vesicles	1.9546	1.9826	2.7728**	3.0099**	0.4053**
	Testes	4.4153	4.6633	4.4398	4.053*	5.0009*
Recovery phase	Adrenals	0.0716	0.0668	0.0072	0.0947**	-
	Epididymides	1.7567	1.7612	1.7292	1.5242	1.3894**
	Prostate	1.1634	1.2521	1.1263	0.6712**	0.7727**
	Seminal Vesicles	2.0501	1.8937	1.9563	1.0723**	1.6013*
	Testes	3.8614	4.2213	4.2201	3.9634	4.5618**
Microscopic Pathology Dosing phase		none	none	Week 13: Spermatid retention, germ cell exfoliation, degeneration of elongating spermatids, distended seminal vesicles & prostatic secretory acini.	Wks. 4&7	All weeks: Diffuse Leydig cell hyperplasia, epididymides ductal atrophy, contracted prostatic acini and epithelial atrophy of the seminal vesicles.
Recovery phase		none	none	recovered	Not recovered: prostate & seminal ves. atrophy.	None.

- = not obtained

*= p<0.05, **= p< 0.01, no asterisk = not statistically significant

Rat: Nebivolol given at 160 m/k/d was associated with increased mortality and concurrent ptosis, >20% body weight loss and decreased food consumption. Administration of 80 m/k/d was associated with ptosis, 9% decrease in body weight and 7-12% decrease in food. Statistically significant sperm changes were observed at ≥40 m/k/d both at 13 weeks and at recovery. At recovery decreased motile sperm cells at 80 m/k/d, >50% decreased decreased epididymal sperm count at ≥40 m/k/d and 40% decreased normal sperm cells at 80 m/k/d. The NOEL for sperm findings was 10 m/k/d. Damaged spermatids present at the end of dosing and released from seminiferous tubules (allowing for transit time and cell cycling for ~3-4-weeks post dose cessation) are likely to contribute to the findings observed during recovery. Increased weight of seminal vesicles at 40 m/k/d by 40% and 54% at 80 m/k/d and increased adrenal weight (32%), decreased (-42%) prostate weight and seminal vesicles (-50%) at 80 m/k/d were observed at recovery. Histopathology was confined to doses ≥40 m/k/d and consisted of spermatid retention, germ cell exfoliation, degenerative spermatid, distended seminal vesicles & prostatic acini and decreased sperm cells

in epididymides as well as mammary differentiation (lobulo-, tubulo-alveolar) at 80 m/k/d. At recovery in the 80 m/k/d group atrophic prostatic acini and contracted seminal vesicles were observed. No histopathology findings were observed at 10 m/k/d. Treatment with nebivolol was not associated with any increase in LH or estradiol.

Summary: An established mechanism has been identified for induction of rodent Leydig cell tumors with a variety of compounds which result in increased LH or Leydig cell responsiveness to LH. The hypothalamic-pituitary-testes axis of rats and humans are similar and compounds that decrease testosterone or estradiol will increase LH levels. Compounds that induce Leydig cell tumors in rats by disruption of the HPT axis pose a theoretical risk to human health. An exception would be GnRH and dopamine agonists because GnRH and DA receptors are not expressed at high levels in human testes. Furthermore, rats generally lack SHBG and exogenous hCG produces Leydig cell hyperplasia in rats and hypertrophy in humans typically. Human Leydig cells are considered less sensitive than rats in their proliferative response to LH. However quantitation of that sensitivity has not been established. Reduced food and decreased body weight gain can result in decreased GnRH secretion by the hypothalamus independent of a glucocorticoid-mediated stress response.

The increased adrenal weight occurred in rats at doses not associated with body weight decreases, thymic atrophy or any increase in glucocorticoid levels and therefore are not likely related to stress, but rather a pharmacologic effect of nebivolol.

Prolonged gestation and dystocia are associated with nebivolol treatment in rats. The expert panel convened by the sponsor concluded that this may be a class effect. Beta blocker pharmacologic effects during gestation result in maternal sedation, increased postimplantation loss (40 mg/kg/day), in utero mortality and decreased stimulation to initiate parturition as proposed by the expert panel. This would result in prolonged gestation, dystocia, increased perinatal mortality and maternal nutrition in the peripartum period. Stillbirths, neonatal deaths and cannibalism of pups are associated with prolonged deliveries. Maternal toxicity is reported at 20 and 40 mg/kg/day in the absence of teratogenicity when treated occurred during organogenesis (but not late fetal period). Decreased pup weight and survival at 1.25 m/k/d occurs in the absence of maternal toxicity.

Pup body weight is likely the most sensitive developmental endpoint measured in the reprotox studies and is adversely affected by nebivolol at doses of 1.25 and 2.5 mg/kg/day. Mean pup weight was reduced during the perinatal period (parturition, lactation). A NOEL for this endpoint has not been established.. The sponsor argues that this is an effect seen with other drugs in the class and within the historical control range for the testing facility. Prolonged gestation, dystocia, cannibalism, stillbirths and postnatal deaths as well as decreased mating and fertility in the second generation occurred at a dose with doses greater than 2.5 mg/kg/day. The maternal behavior may be an influencing factor since the expert panel identifies a NOAEL for fetal body weight reduction at 10 mg/kg/day, a much higher dose than during the peripartum period. The adverse fertility effects of the second generation was considered a general toxicity effect as there were too few surviving pups (only 2/5 surviving pups mated).

- A NOAEL has not been established for decreased pup survival or body weight (in the absence of maternal toxicity)
- Descriptive text suggests a progression of endocrine toxicity:
    - 6-month rat-edematous testes (4/20 @ 160 mg/kg/day) soft testes 2/20, swollen testes 1/20 and small testes 3/20. Reproductive tract of females was described as senescent characterized by fewer corpora lutea, more atretic follicles, decreased uterine glandular development and thinning of the vaginal epithelium
    - 12-month rat-decreased activity of the genital tract noted by degenerative tubules and giant cell sin the testes, low sperm counts and cellular debris in the epididymides at 80 mg/kg/day. Atretic ovarian follicles, decrease of old corpora lutea and reduced glandular development and granulocyte infiltration of the uterus in females given 80 m/k/d.
    - Carci study in mice-decreased absolute and normalized gonad weight in MD, HD females, diffuse atrophy of male mammary glands at HD. Mammary gland stimulation increased in HD females and decreased swelling and cystic presentation in uterus.

- Blood pressure effects were seen in normotensive rats given >10 m/k/d. Therefore reproductive effects seen at doses less than 10 m/k/d would not be anticipated to have profound alterations in BP. In hypertensive rats the lowest orally effective dose was 10-20 mg/kg.

The sponsor indicates that pregnant rat exposure was much higher than in non-pregnant rats based on changes in route from dietary administration to oral gavage and a consequence of daily adjustment of dose to increased body weight in the pregnant animals. Thus toxicity differences reflect increased exposure and not increased sensitivity. This TK data in gravid and non-gravid animals has not been provided for review. Based on mating performance and fertility, nebivolol did not selectively affect estrus cyclicity in the absence of generally toxicity (doses above 40 mg/kg). The expert panel advises that Nebivolol should not be administered during late pregnancy as with other beta blockers, but concludes an adequate margin of safety for humans is provided given therapeutic doses proposed.

The majority of toxicology studies presented for nebivolol were conducted over 10 years ago by Janssen Research Laboratories to support European marketing. The sponsor suggests that standard analytic designs for exposure at that time did not allow for optimal PK characterization often resulting in underestimation of nonclinical exposure. The current sponsor; Mylan Laboratories therefore conducted bridging studies to the carcinogenicity studies in mice and rats using a 10 mg/kg/day dose for 14 days by dietary admixture in addition to a 13-week dietary study in male mice and rats to look at LH and estradiol levels using 10, 40 and 80 mg/kg/day.

Studies	AUC $_{0\text{-}24h}$ (ng h/ml)			
	10 m/k/d		40 m/k/d	
	M	F	M	F
14-day diet Mylan Mouse	2721	2095		
14-day diet Mylan Rat	592	1914		
13-week diet Mylan Mouse	3993		32,917	
13-week diet Mylan Rat	1529		11,916	
Carc diet Janssen Mouse	1450	442	13,536	
Carc diet Janssen Rat	1486	641	10,680	

Decreased Effect Observed in Offspring	Maternal Animal Dose mg/kg	Animal Exposure mg/m^2	Human Exposure[2]
Fertility	5	30	5X
Wt. gravid uterus	1.25	7.5	1X
Mean litter size	1.25	7.5	1X
Implantations	1.25	7.5	1X
Corpora lutea	1.25	7.5	1X

While other beta antagonists have reported effects on reproduction, there are distinctions with nebivolol that distinguish it:

1. Approved beta blockers have shown >5X margin of safety for adverse nonclinical reproductive effects relative the MRHD
2. Effects on decreased offspring fertility have not been reported for other drugs in the class
3. Dystocia, cannibalism and prolonged parturition have not been reported for other approved beta blockers.
4. Beta blockers have been shown to affect the hypophyseal-gonadal system in various
5. species including humans by affecting levels of some pituitary hormones at different activity profiles. Generally interference has the capacity affect steroidogenesis, androgen production, testosterone release and cellular hypertrophy in rodent Leydig cells, inhibition of cAMP accumulation in rodent Sertoli cells. In various animals interference with beta adrenergic receptors in the ovary leading to production of progesterone and estadiol has been observed. Propranolol can either stimulate or inhibit the release of progesterone depending on the stage of

[2] Based on a human dose of 10 mg or 6 mg/m^2

estrus cycling. However interactions are far from consistent across the class. Likewise inconsistent results have been observed in humans. Atenolol, nadolol and propranol decrease LH and FSH in humans. Other articles propose an association between beta-blockers and sexual dysfunction performance, however there is no consistent correlation between hormone levels and sexual performance. The most consistent response to beta blockers is the stimulatory effect on catecholamine secretion and decreased plasma renin with consequent decreased in angiotensin II and aldosterone. Clinical studies with nebivolol show no evidence of adverse pituitary gonadotrophins, sex hormones or prolactin. Adrenal function and production of glucocorticoids and mineralocoritcoids in basal non-stimulated production is unremarkable. Assessment of the HPA axis appears to be intact based on an ACTH stimulation test and gluco- and mineralo-corticoid production. Hormonal responses differ among beta blockers and appear to be related to dose, duration, species and pharmacological properties (specificity, agonist/antagonist activity) of the individual drug.

Conclusion: There appears to be evidence of some perturbations in endocrine function particularly in reproductive development. Findings identified in the nonclinical data are confounded and inadequately addressed by the inconsistencies apparent in the study reports. The sponsor has not adequately the adrenal and reproductive findings based on the current information. The 13-week male reproductive/endocrine study in mouse and rat attempt to address the Leydig cell tumors. However the inconsistent effects on LH and estradiol further confound interpretation of the data especially since Leydig cell hyperplasia was seen in the 13-week rat study when it hasn't been seen in previous studies. Therefore dismissal of the Leydig cell tumors as being due to LH perturbations and clinically irrelevant cannot be concluded.

By a weight of evidence approach there appears to be an effect on survival and growth of offspring with nebivolol, at exposures in the absence of maternal toxicity. When given during late gestation there are marked adverse effects on parturition for both mothers (dystocia) and offspring survival. This has not been reported for other drugs in this class. The sponsor suggests that this effect is common to beta antagonists because it is related to its pharmacologic activity. However based on the data analysis these repro findings occur at higher exposure multiples with other antagonists in the class. Since effects on blood pressure were seen in normotensive rats given >10 m/k/d, the reproductive effects observed at <5 mg/kg/day would be anticipated to occur in the absence of any significant cardiovascular activity. . The effects on adrenal dysfunction and male reproductive disruption are less clear and further studies would be needed to clarify these signals. The reproductive findings may be addressed by appropriate labeling for nebivolol compared to the general class label, perhaps indicating contraindication during pregnancy; i.e. Pregnancy Category X designation. The adrenal signals occur at relatively high exposures (>100X) in rat (160 mg/kg/day; 960 mg/m^2) relative to therapeutic exposure. The adrenal findings are not present in other species tested (mice, dog). Dr. Joffe's recommendation to address this is by a 3month clinical study to determine if there are perturbations in the adrenal axis in humans would address the clinical relevance of this finding.

In the consult request dated 2/23/06 DCRP posed the following nonclinical questions:

1. In the 13-week study in mice and rats (Appendix 7), in the mouse, there appears to be a significant dose-related decrease in the % normal sperm and sperm count. There was also a significant dose-dependent increase in adrenal and prostate weight and a significant decrease in the weight of the seminal vesicles that did not completely normalize during recovery. Dose-dependent Leydig cell hyperplasia was noted. LH increased significantly and estradiol decreased by Week 13 in the nebivolol 40 mg/kg/day mouse model. Several estradiol measurements were not obtained because they were apparently below the limit of quantification of the assay.
   In your opinion is the assay adequate to appropriately interpret the changes in estradiol seen in this study?
   Response: No
   Also do these study results, rule out a possible endocrine effect of nebivolol?
   Response: No
2. Do you agree that the preclinical reproductive tract observations are not clinically relevant?
   Response: No
3. Do you agree that the reproductive/developmental toxicity profile of nebivolol is consistent with the beta antagonist class?
   Response: No

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/s/

Karen Davis-Bruno
3/22/2006 09:13:05 AM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21742
Review number: 1
Sequence number/date/type of submission: 0/October5, 2005/
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: Bertek
Manufacturer for drug substance: Mylan Pharmaceuticals Inc, 781 Chestnut Ridge Road, Morgantown, WV 26505.

Reviewer name: **Elizabeth Hausner, D.V.M.**
Division name: **Cardio-Renal Drug Products**
HFD #: 110
Review completion date: October 6, 2005

Drug:

Generic Name: Nebivolol Tablets

Chemical Name Nebivolol hydrochloride is identified chemically as (±)-[2R*[R*[R*(S*)]]]-α,α′-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol] hydrochloride

Code Numbers R067555

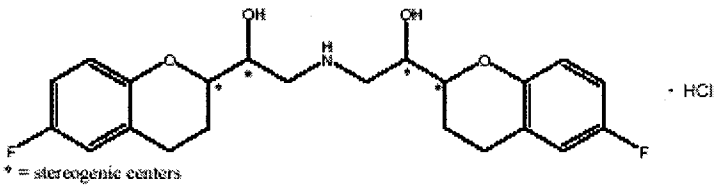
R067138 (d-Nebivolol)

R067145 (l-Nebivolol)

CAS Registry No. 152520-56-4

Trade Name: To Be Established

Figure 3.2-01 Chemical Structure of Nebivolol



Empirical Formula $C_{22}H_{25}F_2NO_4 \cdot HCl$

Molecular Weight 441.90 g/mol

Due to 4 chiral carbons, there are 10 different stereoisomers possible. The drug substance is the racemate of the enantiomeric pair SRRR-nebivolol (d-nebivolol) and RSSS-nebivolol (l-nebivolol).

The sponsor has submitted two protocols to the electronic document room:

10-day dose tolerance study of nebivolol administered by oral gavage to mice (dated July 7, 2005)

Proposed experimental start date of July 21, 2005

28-day toxicity study of nebivolol administered by oral gavage to CD-1 mice with a 14-day interim sacrifice to measure levels of luteinizing hormone and estradiol.(dated July 26, 2005)

Proposed experimental start date of August 18, 2005

Apparently both these studies have started and the in life portions been completed.
No action indicated at this time.

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/s/

Elizabeth Hausner
10/11/2005 03:05:11 PM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
10/12/2005 02:56:05 PM
PHARMACOLOGIST

8/22/05

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21742
Review number: 1
Sequence number/date/type of submission: 000/June22, 2005/BP

Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Bertek
Manufacturer for drug substance: Mylan Pharmaceuticals Inc, 781 Chestnut Ridge Road, Morgantown, WV 26505.

Reviewer name: Elizabeth Hausner, D.V.M.
Division name: Cardio-Renal Drug Products
HFD #: 110
Review completion date: August 22, 2005

Drug:

Generic Name:	Nebivolol Tablets
Chemical Name	Nebivolol hydrochloride is identified chemically as (±)-[2R*[R*[R*(S*)]]]-α,α'-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol] hydrochloride
Code Numbers	R067555
	R067138 (d-Nebivolol)
	R067145 (l-Nebivolol)
CAS Registry No.	152520-56-4
Trade Name:	To Be Established

The purpose of the correspondence is to provide amendments to toxicology protocol TOX021-001. The amendments provide changes to the dosing regimen for the mouse and rat high dose groups due to observed body weight changes.

Mouse Study

In the first two weeks of the current study the mice showed decreased food consumption and corresponding weight loss. By two weeks a "deficit in mean body weight" of 20-25% was reported and 3 animals in the group were premature decedents (unspecified number found dead and unspecified number requiring euthanasia). Some unclear time after study day 14, 2 more mice were found dead and another required euthanasia. After several days (unspecified number) on the reduced dosage nebivolol, there was no obvious difference in the clinical condition of the

mice. The animals were then taken off drug. The 1-month euthanasia and evaluation for this group was postponed.

After 14 days without drug (study day 35), dosing with 80 mg/kg was restarted. Feed intake and mean body weight are reported as stabilized.

The high dose group showed decreased food consumption of the original feed/drug combination. It was estimated that they were actually consuming a dose of 120 mg/kg/day but with no improvement in overt condition. Therefore the dose was further reduced to 80 mg/kg/day.

Rat Study

Rats in the 160 mg/kg/day group showed decreased food consumption and "body weight performance." Approximately 40% of the rats were reported as showing 20% body weight loss. The dose was decreased to 80 mg/kg/day (unclear what study day). The rats in this group continued to show body weight effects. By study day 30, 3 rats had been euthanized in extremis and 1 animal was found dead. Treatment was suspended for 12 days then re-started at the 80 mg/kg/day level.

The proposed changes to dosing have been noted by the Division. Obviously a sufficient number of animals must survive the study in order to have meaningful results. It is noteworthy that these survival problems were not as evident in the original studies.

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/s/

Elizabeth Hausner
8/22/2005 07:42:07 AM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
8/22/2005 02:18:29 PM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21742
Review number: 1
Sequence number/date/type of submission: 000/May 24, 2005/BP
000/May 24, 2005/C
000/May 24, 2005/Response to telephone request
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: Bertek
Manufacturer for drug substance: Mylan Pharmaceuticals Inc, 781 Chestnut Ridge Road, Morgantown, WV 26505.

Reviewer name: **Elizabeth Hausner, D.V.M.**
Division name: **Cardio-Renal Drug Products**
HFD #: 110
Review completion date:

Drug:

Generic Name:	Nebivolol Tablets
Chemical Name	Nebivolol hydrochloride is identified chemically as (±)-[2R*[R*[R*(S*)]]]-α,α′-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol] hydrochloride
Code Numbers	R067555
	R067138 (d-Nebivolol)
	R067145 (l-Nebivolol)
CAS Registry No.	152520-56-4
Trade Name:	To Be Established

The submission May 24, 2005 Doc type N, Mod Type BP is a copy of the protocol "A 13-week endocrine evaluation study in male CD-1 mice and Wistar rats with a 2-week and a 1-month interim sacrifice and a 1-month recovery period.

This is a reiteration of a number of submissions pertaining to the sponsor's plans for the mechanistic study to elucidate pathogenesis of the Leydig cell tumors in mice. Test article and positive control substance will be administered to both rats and mice daily for 13 weeks. In both species there will be

1. toxicokinetic analysis at 2,4,and 13 weeks of treatment (n=4 per group, 6 timepoints)
2. hormone analysis at 2,4 and 13 weeks
3. interim euthanasias at 2,4,13 weeks and end of recovery will include limited postmortem examination (reproductive tract histopathology, n=10 per group per timepoint).

4. sperm analysis (motility, morphology and numbers) will be performed week 13.

The sponsor has constructed a protocol that they feel will provide data to support their contention that the Leydig cell tumors seen in mice are not caused by a mechanism relevant to humans.

Second Article: May 24, 2005 Seq 000/ Doc type N/ Mod type C
With any dataset, multiple interpretations may be made. The sponsor has presented their interpretation of this data. The reviewer is reiterating her interpretation.

This is a response to statements made by myself and Karen Hicks during a May 5, 2005 telecon with the sponsor in which we discussed the variety of phenomena which have raised our concern for an endocrine effect associated with nebivolol.

1. The sponsor cites study N69430 and notes that there was a severe degree of toxicity at the high dose that was probably responsible for the decreased copulatory behavior. It was also stated that the study suggests an absence of endocrine effects based on a lack of reported findings for the 40 mg/kg group, and a median cohabitation interval within one estrus cycle and no effect on the mean number of corpora lutea in any of these groups.

Response: Each group of nebivolol treated females gained less weight than did the control group although the change in the LD group was not statistically significant. Therefore, one could interpret the data to indicate that the decrease in gain is a drug-associated affect that is seen not only with overt toxicity. An alternative conclusion is that no NOAEL was identified for this effect.

As it stands, this report cannot be used to support a lack of endocrine effect. As the HD caused some mortality (3 males died of pneumonia; 1 female died of GI complications, 1 female showed no reported lesions), a lower dose causing less excessive weight loss might have given a better demonstration of any dose response effects. That is, the excessive effects caused by the HD dose may mask effects. In all dose groups, the weight of the gravid uterus was decreased, significantly so in the LD group with no overt toxicity (100.3 control vs 90.9, units not given, LD $p<0.05$), and to a greater extent in the HD group (n=1, 77.2). Decreased weight of the gravid uterus is generally considered an undesirable effect.
There was a slight increase in the average time of cohabitation. If one looks at the individual animal data, the range of cohabitation interval in the control group was 1-7 days. The cohabitation interval in both the LD (data missing for 2 animals) and MD(data missing for 3 animals was 1-14 days. Was this random chance? The report is uninformative regarding effects on cyclicity, information necessary to make the determination of chance versus cause and effect. Nor was there any analysis of sperm parameters. Specific examination of cyclicity is needed in the case of nebivolol to say that an adequate examination was conducted.

It was also asserted in the sponsor's statement that :

> Based on a comparison of dose levels in terms of mg/m2, the 40 mg/kg/day dose level at which no effect on mating was observed is approximately 45 times that proposed for the maximum recommended human dose (10 mg).

First, it can be argued whether or not 40 mg/kg is a no effect level. Second, the effective dose range of nebivolol in humans extends to 40 mg and there is a distinct possibility that nebivolol may be used clinically at this higher dose. For the sake of argument, on a body surface area basis, using 40 mg/kg as a NOAEL, the prospective margin of safety is:

Rat dose: 40 mg/kg x 6 = 240 mg/m2

Human dose 40 mg/ 60 kg patient x 37 = 25 mg/m2

The ratio of doses is now approximately 10 for a 60 kg patient.

2. Observations of reduced fertility in the F1 generation

N92570
"There were no effects on the reproductive capacity of the F1 generation supporting the conclusions that Nebivolol has no effect on fertility."

Response:
The reviewer disagrees with the sponsor's interpretation.
This study used doses of 2.5, 10 and 40 mg/kg/day from day 18 of pregnancy through the 3 week lactation period. Please note that in this study report ptosis was recorded as occurring at 40 mg/kg, something not identified in previous reports. Thus, overt toxicity is appearing at a lower dose than heretofore reported. Two drug-related mortalities out of 5 total mortalities were reported at 40 mg/kg as well as decreased food consumption, increased duration of gestation and decreased nursing behavior.

Interestingly, the report references another report with the statement:

JUSTIFICATION FOR SELECTION OF DOSES
In a previously performed segment III study with nebivolol (Exp.No.1888), doses were 10, 40 and 160 mg/kg. At 10 mg/kg, maternal toxicity, evidenced by dystocia and cannibalism, was associated with a lower number of live pups at birth and a decreased survival.
At 40 mg/kg, decreased body weight gain, dystocia and cannibalism resulted in no surviving pups after 4 days. Dosing at 160 mg/kg revealed a bad general condition with increased mortality, decreased body weight gain, dystocia and cannibalism. As a result, birth weight and survival rate decreased with no survivors after 4 days.
Based on these data, it was decided to reduce the doses in the present study and to select 2.5, 10 and 40 mg/kg body weight/day.

Therefore, there is a repetition of the doses of 10 mg/kg and 40 mg/kg causing adverse effects during pregnancy and parturition.

Decreased food consumption was reported for both the LD and MD groups with pup survival decreased in the MD group. Therefore, no NOAEL for the dams can be identified in this study.

Average food consumption (g)

Dosage group mg/kg body weight /day	prior to dosing d 1 - d 17 (pregnancy)	d 18 - d 21	during dosing d 0 - d 3	d 4 - d 13 (lactation)	d 14 - d 20
0	500.8	135.8	159.3	602.8	500.0
2.5	509.5	137.7	150.2	560.7	484.2
10	531.5	133.5	136.7**	527.8*	436.0**
40	517.7	105.3***	85.7***	254.9***	184.5***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Average body weight (g)

Dosage group mg/kg body weight/day	pregnancy d1	d18	d22	day of delivery	lactation d4	d14	d21
0	201.5	331.0	386.7	299.3	311.0	332.6	321.6
2.5	195.9*	327.1	382.3	296.2	308.6	328.0	323.5
10	201.4	332.0	385.6	306.0	311.7	331.5	324.7
40	200.6	335.2	367.6*	283.1*	280.8**	302.0**	317.1

* $p < 0.05$; ** $p < 0.01$

The average body weight of the LD and MD groups did not differ significantly from the controls, in some cases exceeding control values. Only the 40 mg/kg group showed a significant difference in body weight.

The mean weights of the pups of drug-treated dams were less than the control values with no NOAEL identified. The pups of the LD dams caught up to the control group by the end of the lactation period. Pups of the MD dams did not catch up to the pups of the control dams. This cannot be attributed to overt toxicity based upon body weight effects.

Dosage group mg/kg body weight/ day	Mean body weight (g) of pups at stated age (days) and sex					
	day 0	day 4	day 14		day 21	
	M+F	M+F	M	F	M	F
0	6.7	10.2	28.0	26.3	44.9	41.8
2.5	6.3*	9.5	25.6	24.7	42.4	41.2
10	6.0**	8.5**	23.9*	23.5	38.9	38.3
40	6.1**	-	-	-	-	-

* $p < .05$ ** $p < .01$

There was 100% mortality of the HD pups within 4 days of birth. Malnutrition and dirty aspect of pups was seen in both the LD and MD pups but not in the controls. No NOAEL was identified for these effects. The NOAEL for pup survival was 2.5 mg/kg/day.

Developmental landmarks were also assessed by an insensitive method, thereby invalidating the sponsor's argument that there were no developmental effects. It is customary to record the day that a pup achieves a specific landmark. In this study, the investigators waited days to weeks past the time that one could expect the developmental parameters to be reached and then checked the pups. Some of the problems are summarized in the reviewer's table below.

Developmental milestone	OECD recommended assessment time *	Time of sponsor's actual assessment	
		studyN92570	Study N106655
Vaginal opening	Postnatal (PN) day 30-35	PN Day 42	PN Day 42
Testes descent	PN day 40-45	PN day 42	PN day 42
Surface righting reflex	PN 2-4 days	PN21	PN21
Air righting reflex	PN12-17 days	PN 21	PN 21
Auditory startle	PN10-14 days	PN 21	PN 21
Anogenital distance**	Day 0 of F2 generation	Not done	Not done

*OECD Draft Guidance Document on Reproductive Toxicity Testing and Assessment. November 10, 2004. Available in the OECD website.
** anogenital distance is recommended as an assessment of possible endocrine effects

In the assessment of F1 fertility, the pups of LD dams gained approximately 14% more than the control pups while the pups of MD dams gained approximately 8% more than the control pups during gestation, an unexplained effect. Simple examination of mating and number of corpora lutea is an insensitive method of assessing reproductive effects. No information was given regarding the cohabitation interval, duration of gestation or cyclicity of the females.

N106655: The sponsor states that "...the F1 generation appeared to show decreased fertility...Considering the adverse effects on survival and low body weights at birth and day 4 at the middle dose and the small number of rats mated no reasonable conclusions can be drawn on fertility other than the probability that the overall condition of these rats at 5 mg/kg/day was not conducive to reproduction. There were no significant effects at this dose level on testicular descent and vaginal opening and in the number of corpora lutea which might be expected if an adverse hormonal effect had been induced."

Response:
I agree that the sample size was small but disagree that no conclusions can be drawn and disagree that there were no significant effects on vaginal opening. Some of the same methodological problems that occurred in the first study were repeated in this study.

This study used doses of 1.25, 5 and 20 mg/kg given to the F0 generation 60 days prior to and during mating (males) and 14 days prior to mating, throughout mating and during pregnancy and lactation until weaning of the F1 generation.

Gestation was slightly prolonged in the 5 mg/kg/day group and significantly prolonged in the 20 mg/kg/day group (24.1 days vs 23.1 days for control, $p<0.05$). Dystocia was also reported for the HD group. There was no apparent drug-related effect on body weight gain prior to mating, and only mildly decreased weight gain in the drug-treated groups was seen during gestation. The HD group showed a significant difference in weight gain during the lactation period. Therefore, overt toxicity was seen only in the HD (20 mg/kg) group with significantly decreased food consumption, an unexplained effect.

These tables indicate that the body weight during the precohabitation period was comparable between groups for both males and females.

Dosage group mg/kg body weight/day	Mean weight gain (g) Males (dosed) 1st generation	Females (dosed) 1st generation
0	246.8	58.6
1.25	235.8	55.7
5	244.6	61.2
20	234.3	58.2

As appears from these data, the weight gain during the precohabitation period was comparable between groups.

Body weight was determined individually per pregnant female on a daily basis but recorded on days 1 and 22 of their presumed pregnancy and averaged per group of females in Table 21. Individual data are tabulated in Tables 22, 24, 26 and 28.

Dosage group mg/kg body weight/day	Average body weight (g) day 1	day 22	Mean weight gain (g)
	1st generation dosed		
0	259.1	439.8	180.7
1.25	260.0	433.1	173.1
5	270.9	425.4	154.5
20	258.8	423.3	164.6

Body weight was determined individually per lactating female on the day of delivery and on days 4, 14 and 21 of lactation. Body weight was averaged per group of females in Table 21 and individual data are tabulated in Tables 22, 24, 26 and 28.

Dosage group mg/kg body weight/day	Mean body weight (g) at stated day 1st generation (dosed)			
	birth	4	14	21
0	350.4	358.5	377.8	362.6
1.25	343.4	345.2	368.7	345.8
5	359.9	373.6	380.4	372.9
20	345.4	325.1**	350.2**	356.7

Significance computed by Mann-Whitney U test (two-tailed) ** $p<0.01$

As appears from these data, body weight of dams at birth and during a 3-week lactation period was comparable between the vehicle group and the groups dosed at 1.25 and 5 mg/kg/day. At 20 mg/kg/day, a decreased body weight of dams was noted on day 4 and day 14 of lactation. This effect was related to a decreased food consumption of dams during lactation at this maternally toxic dose level (see A 4.3)

Food consumption was comparable during pre-cohabitation and pregnancy but moderately decreased at 5 mg/kg/day and significantly decreased at 20 mg/kg/day during lactation.

Food consumption of dams during lactation

Food consumption was recorded individually per lactating female during the 3-week lactation period. Food consumption was averaged per group of females in Table 21 and individual data are tabulated in Tables 22, 24, 26 and 28.

Dosage group mg/kg body weight/day	Mean food consumption (g) 1st generation (dosed) days			
	0 - 3	4 - 13	14 - 20	0 - 20
0	176.0	662.1	672.8	1510.9
1.25	154.2	644.5	603.3	1401.9
5	157.5	532.8	509.1	1199.4
20	100.0***	359.6***	276.6***	736.2***

Significance computed by Mann-Whitney U test (two-tailed) *** $p<0.001$

Dystocia was seen in this study at doses ≥5 mg/kg: 0/10 (control), 0/11(LD), 2/9(MD) and 8/12(HD). Note: dystocia has been reported in other studies also in association with nebivolol treatment. The cohabitation interval is open to several interpretations. One is that there were no significant effects. Again, ranges were not presented in the summary tables. Therefore, some data gleaned from the individual animal sheets is presented here.

	Control group	1.25 mg/kg	5 mg/kg	20 mg/kg
Duration of gestation	11rats- 23 days 1 rat-24 days 2 rats –no data	9 rats- 23 days	6rats-23 days 1 rat- 24 days 1 rat 25 days 2 rats-no data 2 rats-not pregnant	5rats-23 days 1 rats-24 days 6 rats-25 days
Cohabitation interval	1-9 days	1-10 days	1-12 days	1-7 days

In the other fertility study conducted with nebivolol, an increase in the range of cohabitation was also seen, without statistically significant prolongation of the mean cohabitation interval, consistent with results here. An alternative interpretation to "no significant effects" is that there is a u-shaped dose response curve, not unusual in endocrine situations.

There was no NOAEL for pup survival or decreased pup weight. While there was no difference in maternal mean weight gains or food consumption at the LD and only minimal effects at the MD, what explanation is there?

Dosage group mg/kg body weight/day	Average weight (g) of fetuses at stated day of age 1st generation (dosed)					
	at birth	day 4	day 14		day 21	
	M + F	M + F	M	F	M	F
0	6.8	10.9	28.9	27.3	49.0	46.0
1.25	6.3*	9.7	28.7	27.1	48.1	44.2
5	6.1*	8.6*	29.1	28.8	49.7	48.8
20	5.5**	5.1	11.9	18.8	38.8	33.3

M: male, F: female

Significance computed by Mann-Whitney U test (two-tailed) * $p<0.05$ ** $p<0.01$

Dosage group mg/kg body weight/day	Number of surviving fetuses/total number of fetuses born (%) at stated day of age		
	day 4	day 14	day 21
0	93.2	92.3	91.5
1.25	88.7	84.2	82.0*
5	85.0	71.3***	71.3***
20	5.4***	5.4***	5.4***

Significance computed by Mann-Whitney U test (two-tailed) * $p < 0.05$, *** $p < 0.001$

We have essentially no information about offspring reaching developmental landmarks due to the insensitive methodology used (see above). It is also not clear when the offspring were assessed for breeding potential :

SECOND GENERATION STUDY PHASE

At the age of ± 3 months, males and females, naturally delivered by the originally dosed mothers, were randomly selected as parents of the next generation. Within each litter one

How old is "±3 months"? In addition to non-informative developmental data, it is not clear if there is a delay in reaching sexual maturity.

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The body weight of the offspring used for mating did not differ significantly during pregnancy although they did eat somewhat less than the control offspring.

Average body weight and body weight gain for the various groups was:

Dosage group mg/kg body weight/day	Average body weight (g) day 1	day 22	Mean weight gain (g)
		2nd generation non-dosed	
0	315.3	473.9	58.2
1.25	301.2	456.1	61.9
5	325.5	470.5	55.0
20	-	-	-

Body weight and body weight gain of dams during pregnancy were comparable between groups.

Dosage group mg/kg body weight/day	Mean food consumption (g) 2nd generation non-dosed
0	724.3
1.25	676.6
5	643.5
20	-

Food consumption of dams during pregnancy was comparable between groups.

I agree with the sponsor's statement that more animals were needed to make an adequate evaluation. Certainly the repeatability of the findings of this study is an important point. The averages of certain parameters presented in the summary table might be more informative with ranges or standard deviations included. Using the individual animal data in the appendices, the following table was compiled by the reviewer.

	Dose mg/kg			
	0	1.25	5	20
Corpora lutea Range Mean	 14-25 (4 rats ≥21CL) 19.3	 13-19 (0 rats ≥21) 16.2	 16 16	No surviving pups to evaluate for breeding potential
Implants Range Mean	 8-18 15	 4-17 13.5	 12-13 12.5	
Gravid uterine weight :range mean	 51.5-132 g 100.5	 39.2-122 93.0	 87.3-92.8 90.1	
	N=11 No data for 1 rat	N=11 No data for 1 rat	N=2 3 not pregnant, no other data	

In summary

1. No NOAEL has been identified for decreased pup weight or decreased pup survival. These effects occurred at doses with no apparent maternal toxicity.
2. It can't be said that there are no effects on reproduction/fertility in general due to
    a. Maternal toxicity was reported at doses of 20 and 40 mg/kg. The dose of 20 mg/kg was not reported to produce toxicity in non-gravid animals in general toxicology studies. The dose of 40 mg/kg caused mild, inconsistent effects in non-gravid animals in general toxicology studies.
    b. Dystocia was reported in multiple studies at doses down to 5 mg/kg/day, with no other maternal toxicity reported.
    c. Cannibalism was reported in multiple studies
    d. Prolonged parturition was reported in multiple studies
    e. There has been no direct examination of female cyclicity
3. It can't be said that there are no effects on development or fertility in the offspring
    a. an insensitive method was used to assess reaching developmental landmarks.
    b. we don't know precisely the age at which the offspring were allowed or able to mate.
    c. There is no reported information on cyclicity or the cohabitation interval

The sponsor has proposed that the reported histopathological effects on the female reproductive tract may be attributed to general weight loss due to the stress of being in a toxicology study. The following material compiled by the reviewer is in response to that assertion.

N106653 3 month mouse study Orally administered through diet. Doses used 0, 10, 40, 160 mg/kg

EXPERIMENT: 1966
Toxicity study
R 67555 - FOOD - MICE - 3 MONTH

| BODY WEIGHT
| Mean values per dosage group in g

	Dosage group (mg / kg)							
	Males				Females			
Week	0	10	40	160	0	10	40	160
0	29	29	29	29	25	25	25	26
1	31	32	31	25 ***	26	26	26	23 ***
2	33	33	33	26 ***	27	27	27	23 ***
3	34	34	34	27 ***	28	28	29	24 ***
4	35	35	35	27 ***	29	27	30	24 ***
5	35	36	36	28 ***	30	29	31	25 ***
6	36	37	37	29 ***	31	30	31	26 ***
7	37	38	38	29 ***	31	30	31	26 ***
8	38	38	38	30 ***	32	30	32	27 ***
9	38	38	39	30 ***	32	30	32	26 ***
10	38	39	39	29 ***	32	31	33	26 ***
11	39	39	39	29 ***	32	31	33	26 ***
12	38	38	39	28 ***	33	31	33	26 ***
13	39	39	40	29 ***	33	32	34	27 ***
14	39	39	40	29 ***	34	33	34	26 ***

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

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	males				Females			
	0	10	40	160	0	10	40	160
Seg neutr	7.5	8.4	5.4	13.1 *	5.6	6.3	8.6	8.6
lympho	91.7	89.6	93.7	85.4 *	92.9	92.3	90.6	90.4
Na mEq/l	149	149	151	155 *	148	149	151	155 *
K mEq/l	6.2	6.2	6.9	7.1	5.2	6.5 *	6.1	6.7 *
Glu mg%	197	188	178	120 **	191	199	168 *	141 **
Chol mg%	129	119	109	22 **	90	106	79	16 **
Trigl mg%	167	149	131	57 **	105	159 *	197 **	66 *
Pancreas mg Mg/100 g	400 989	399 984	408 1005	307 ** 1054	387 1116	347 1021	377 1067	304 *** 1113
Adrenals Mg Mg/100g	5 13	5 12	5 12	10 *** 36 ***	11 32	11 31	10 28	14 ** 52 ***
Gonads Mg Mg/100g	278 693	274 682	285 704	261 905 ***	47 135	50 148	49 140	32 ** 115

Adrenal cortical hypertrophy was reported only at HD. Lymph node atrophy was reported only at the HD and was not prominent.

3 month mouse study: females

	control	LD 10	MD 40	High (160)
Ovaries: Corpora lutea	10/10	ND	ND	5/10**
Ovaries: cystic	4/10			0/10*

*p<0.05, **p<0.01, p<0.001

3 month mouse study: males

	control	LD 10 nd	MD 40 ND	High (160)
Testes		ND	ND	
Focal Leydig cell hyperplasia	0/10			1/10
Large nucleated tubular cells	4/10			10/10**

*p<0.05, **p<0.01, p<0.001

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N76736 Pilot 1 month study in rats: oral administration via the diet. Doses used: 0, βCD, 20, 40 80 mg/kg/day

Persistently decreased body weight gain in HD males and slight, temporary decrease in body weight gain in HD f. Swollen adrenals were found in most HD m and all MD and HD females.

ıfluent of toxicology

:RIMENT: 2183
city study
'555 - FOOD - RAT - 1 MONTH

| BODY WEIGHT
| Mean values per dosage group in g

	Dosage group (mg / 100g food)									
	Males					Females				
leek	0	0	20	40	80	0	0	20	40	80
0	161	161	161	161	161	130	130	131	130	130
1	207	206	206	202	190	147	151	153	149	142
2	259	261	256	251	233	167	168	176	168	163
3	297	297	289	288	267	181	186	192	185	180
4	331	326	312	318	284 *	196	195	206	195	190

nificance computed by Mann-Whitney U test (two tailed) - * P < .05 ** P < .01 *** P < .001

males

parameter	0	veh	20	40	80
neutr	12.5	10.8	7.2	11.2	12.8
lymph	80.5	83.2	86.6	84.8	85.0
Na mEq/l	142	142	141	141	143
K mEq/l	5.1	4.9	5.5 ɑɑ	5.5 ɑ	5.7 ɑɑ
Gluc mg%	169	186 *	176	171 ɑ	170
Chol mg%	68	62	57	56 *	37 * ɑɑ
Trig mg%	132	126	102	121	96

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Females

parameter	0	veh	20	40	80
Neutr	7.4	10.0	9.0	14.2*	13.2
Na mEq/l	144	143	145	145	144
K mEq/l	4.3	4.3	4.4	4.9	5.4**@@
Gluc mg%	159	163	180*	155	153
Chol mg%	79	76	68	45*@	24**@@
Trig mg%	59	60	82	66	76

Summary of organ weight changes for males

	Dose mg/kg/day				
	0	veh	20	40	80
Adrenals mg	62	66	65	77	104*@@
Mg/kg	188	207	214	247	374*@@
Gonads mg	2575	2968	3097	3065	2993
Mg/kg	8049	9331	10212	9873	10814
Pancreas mg	1189	1200	1130	1332	1234
Mg/kg	3645	3743	3718	4294	4442

Summary of organ weight changes for females

	Dose mg/kg/day				
	0	veh	20	40	80
Adrenals mg	86	89	90	119**	168**@@
Mg/kg	453	451	444	614*@	886**@@
Gonads mg	148	156	171	142	153
Mg/kg	773	793	843	734	807
Pancreas mg	927	957	1080*	1203*	1171*
Mg/kg	4907	4818	5292	6198*	6178@

Histopathology results were not found.

The adrenal weight effects were unequivocal both in the mice (3 months of dosing at 160 mg/kg) and in the rats at 40 to 80 mg/kg as shown in the studies above.

N92595 D-enantiomer tested in a month rat toxicity: orally administered
Doses used: R85547 veh, 10, 40 mg/kg/day
R67555 veh, 20, 80 mg/kg/day

	males					Females				
Week	veh	85547 10	85547 40	67555 20	67555 80	veh	85547 10	85547 40	67555 20	67555 80
0	160	160	160	160	160	143	143	142	142	142
1	220	218	215	220	204**	177	173	176	172	166*
2	282	278	277	285	260*	203	201	205	198	190
4	365	362	359	363	327*	244	231	239	231	217**

Water consumption was decreased with drug, significantly so at 80 mg/kg.

Hematology/clinical chemistry

	Males					Females				
Week	veh	85547 10	85547 40	67555 20	67555 80	veh	85547 10	85547 40	67555 20	67555 80
Neutro	13.4	11.0	9.8	11.3	15.4	13.2	6.4	8.5	8.5	11.2
Lympho	81.7	84.7	87.1	85.0	80.5	82.1	89.5	87.8	88.6	85.9
Na mEq/l	145	144	144	144	143**	141	141	141	142	142
K mEq/l	4.7	5.1	5.1	5.0	5.8***	4.3	4.7	4.7	4.7	5.4***
Glu mg%	177	176	168	172	164	168	167	167	168	154*
Chol Mg%	72	78	73	64	29***	83	78	76	67***	20***
Trig Mg%	155	150	151	177	127	91	85	101	107	127*

Summary of organ weight changes : males

	Dose and drug				
	veh	R85547 10	R85547 40	R67555 20	R67555 80
Pancreas mg Mg/kg	1308 3598	1255 3468	1176 3273	1216 3360	1347 4099*
Adrenal mg Mg/kg	56 154	58 160	58 162	60 166	98*** 300***
Gonad mg Mg/kg	3089 8496	3005 8332	3191 8925	3175 8749	3040 9304**

Summary of organ weight changes : females

	Dose and drug				
	veh	R85547 10	R85547 40	R67555 20	R67555 80
Pancreas mg	1075	970	1002	974	1252*
Mg/kg	4309	4166	4159	4200	5657***
Adrenal mg	75	71	77	77	164***
Mg/kg	304	305	323	329	737***
Gonad mg	157	159	162	158	157
Mg/kg	633	682	677	678	710

No lymphoid atrophy was reported.

Summary of histologic changes reported for the female reproductive tract (scoring system)

	veh	R85547 10	R85547 40	R67555 20	R67555 80
Ovaries					
Developing 3° follicles	0.10	Not done	0.10	Not done	0.20
Focal atrophy	0.80		1.00		1.30
Old CL	2.20		2.60		2.10
Recent CL	1.40		1.20		0.70
3° follicles	1.20		1.40		1.20
Uterus					
Dilated lumen	0.80	Not done	0.30	Not done	0.60
Epithelial granulocytes	0.50		0.70		0.30
Glandular development	1.70		1.60		1.40
Subepithelial granulocyte	1.20		1.50		0.70
Vagina					
Cornification	0.70	Not done	0.90	Not done	0.80
Inflamm cells in epithel	0.90		0.80		0.80
Mucification	1.10		0.40		0.60
Thickness of epith layer	3.40		3.30		3.50

N92594 l-enantiomer vs parent drug. 1 month study in rats, oral administration

Doses used : veh, R85548 10 and 40 mg/kg/day
R67555 20 and 80 mg/kg/day

Summary of weight changes

	Males					Females				
Week	veh	R85548		R67555		veh	R85548		R67555	
		10	40	20	80		10	40	20	80
0	143	143	143	143	142	133	133	133	133	133
1	200	204	191	198	180**	160	163	158	164	149*
2	266	270	256	262	239**	188	196	181	195	167*
4	342	350	327*	342	313**	223	232	215	233	204*

Summary of clinical chemistry/hematology/organ weights

	Males					Females				
Week	veh	R85548		R67555		veh	R85548		R67555	
		10	40	20	80		10	40	20	80
Neutr	14.1	13.9	14.1	10.9	17.4	9.6	6.6	13.3	5.9	11.4
Lymph	81.5	81.0	82.3	84.4	77.9	85.5	90.3	84.5	89.7*	85.0
Na Meq/l	144	144	143*	143	143	143	144	143	143	143
K Meq/l	4.8	4.8	5.7***	5.0	5.6***	4.4	4.6	5.2***	4.8**	5.6***
Glu Mg%	168	165	162	164	156*	164	160	148	162	149
Chol Mg%	81	72	34***	72	32***	80	71	23***	71*	22***
Trig Mg%	146	138	131	179	120	87	76	83	103	91

Summary of organ weight changes in males

	Males				
Week	veh	R85548		R67555	
		10	40	20	80
Pancreas mg	1238	1307	1356	1226	1375
Mg/kg	3646	3778	4162*	3590	4450*
Adrenals mg	54	54	77***	56	78***
Mg/kg	158	157	236***	163	251***
Gonads mg	2955	2982	3029	2951	2900
Mg/kg	8692	8571	9306	8647	9334

Summary of organ weight changes in females

	Females				
Week	veh	R85548		R67555	
		10	40	20	80
Pancreas mg	1091	1062	1119	1046	1233
Mg/kg	4929	4595	5147	4529	6059**
Adrenals mg	69	74	129***	80*	141***
Mg/kg	310	322	597***	346	695***
Gonads mg	147	162*	154	167	140
Mg/kg	662	702	711	722	683

Summary of histologic findings in the reproductive tract of females (scoring system)

	females				
Week	veh	R85548		R67555	
		10	40	20	80
Ovaries					
Atretic follicles	1.10		1.30		1.70
Cysts	0.10		0.20		0.20
Old CL	2.80		2.60		2.70
Recent CL	1.10		1.00		0.70
2° follicle	1.20		1.20		1.20
3° follicle	1.90		1.80		1.50
Uterus					
Dilated lumen	0.80		0.40		0.40
Glandular development	1.20		1.30		1.10
WBC infiltration	1.50		1.00		1.10
Vacuolated epithelium	0.10		0.00		0.00
Vagina					
Cornification	0.70		1.10		0.50
Desquamation	0.10		0.30		0.20
Epithelial thickness	3.40		4.00		3.30
Leucocytes in epithelium	0.80		0.60		1.10
mucification	0.30		1.00		0.50

N88268 1 month in Wistar rats. Intravenous administration
Doses used: 0, vehicle, 0.31, 1.25 and 5 mg/kg/day

Summary of body weight effects

	Males dose (mg/kg)					Females dose (mg/kg)				
Week	0	Veh	0.31	1.25	5	0	veh	0.31	1.25	5
0	221	222	223	223	225	173	173	174	173	173
1	279	278	282	280	280	196	203	198	197	194
2	334	329	337	330	333	216	218	216	218	215
4	400	395	405	399	393	245	249	238	247	244

Summary of hematology/clinical chemistry

	Males dose (mg/kg)					Females dose (mg/kg)				
	0	Veh	0.31	1.25	5	0	veh	0.31	1.25	5
Neutr	11.8	9.0	7.4	7.4	11.6	7.8	6.8	10.4	8.4	5.8
Lymph	82.0	83.8	86.2	89.0	83.6	89.8	87.8	84.6	87.4	89.0
Na mEq/l	144	143	144	144	144	143	142	142	143	142
K mEq/l	4.8	5.1	5.2	4.9	5.3	4.4	4.6	4.6	4.6	5.0*
Gluc Mg%	170	159	165	162	164	177	173	177	174	168
Chol Mg%	78	85	82	66@@	64@	82	84	91	77	78
Trig Mg%	153	146	246	207	133	92	85	114	105	92

Summary of organ weigh changes

	dose (mg/kg)				
	0	veh	0.31	1.25	5
Males					
Adrenals mg	61	58	66	63	68
Mg/kg	153	145	163	158	172
Females					
Adrenals mg	74	73	75	76	73
Mg/kg	295	295	310	304	301
Gonads mg	157	168	148@	166	141@@
Mg/kg	629	682	614	667	578@@

No histological results for the reproductive tract were provided.

N54353 3 month rat study with oral administration via the diet. Doses of 0, 10, 40 and 160 mg/kg/day were used.

Body weight effects were seen only at the HD of both sexes.

	Dosage group (mg / 100g food)							
	Males				Females			
Week	0	10	40	160	0	10	40	160
0	132	132	132	132	101	101	101	101
1	190	191	190	183 *	141	140	140	135
2	252	254	251	240 *	167	170	171	164
3	307	307	306	289 **	185	191	191	185
4	345	348	347	327 *	204	208	209	202
5	378	379	379	354 **	218	224	224	216
6	406	408	407	381 **	231	236	236	228
7	425	427	426	398 **	237	245	244	233
8	443	446	443	415 **	247	254	253	242
9	464	467	464	433 **	257	262	261	249
10	475	479	480	447 **	264	271	271	257
11	489	490	491	456 ***	266	275	274	262
12	491	496	497	460 **	265	271	276	264
13	501	506	506	467 **	272	285 *	281	267

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

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Mild neutrophilia was apparent in the HD groups of both sexes and a minimal decrease in lymphocytes in the female HD group. While serum sodium levels were unaffected, there was an increase in potassium levels, consistent with other studies.

	males				Females			
	0	10	40	160	0	10	40	160
Segmented Neutrophils	14.7	13.5	13.5	17.5	13.7	13.6	17.8	20.2 **
lymphocyte	81.4	82.6	81.3	79.2	83.0	82.4	77.9	76.7 *
Sodium mEq/l	150	149	150	150	148	147	148	147
Potassium mEq/l	4.9	4.7	4.9 *	5.1 ***	4.5	4.6	4.8 *	4.8 ***
Glucose mg%	173	180	177	164	163	169	169	153
Cholesterol mg%	90	89	86	64 ***	95	86	84 *	63 ***
Triglycerides mg%	145	145	153	117	86	95	107	113 *
Adrenal weight mg Mg/kg	58 117	59 118	60 121	66 * 143 **	80 301	83 296	82 299	108 *** 418 ***
Gonads mg Mg/kg	3574 7188	3629 7216	3636 7283	3541 7653 **	173 648	166 591	165 603	162 623

No adrenal hypertrophy or related findings were noted in the histopathology findings. But such has been seen in rats, at 40-80mg/Kg as was noted above, and down to 10-40 as reported below. There were no histopathology findings in the males that seemed relevant to the reproductive tract. However, in the females, with analysis of only the control and HD groups, there were reported findings of decreased mammary glandular development, decreased old and recent corpora lutea and increased atretic follicles. There was also a decrease in apparent uterine activity.

Histologic findings	0	LD	MD	160 mg/kg
Mammary gland				
- glandular development	0.26	-	-	0.16
Ovary				
- atretic follicles	0.84	-	-	1.16
- corpora lutea	2.58	-	-	2.53
- cyst(s)	0.00	-	-	0.05
- recent corpora lutea	0.21	-	-	0.16
- tertiary follicles	1.16	-	-	1.16
Uterus				
- dilated lumen	0.58	-	-	0.95
- glandular development	1.84	-	-	1.58
- high epithelium with mitoses	0.26	-	-	0.21
- inflammatory cells (epithelial)	0.21	-	-	0.21
- vacuolation (epithelium)	0.32	-	-	0.21
Vagina				
- cornification	0.68	-	-	0.68
Vagina				
- inflammatory cells (epithelial)	0.42	-	-	0.26
- mucification (epithelium)	0.21	-	-	0.47
- necrotic cells (epithelium)	0.16	-	-	0.11
- thickness of the epithelium	3.53	-	-	3.74

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N64890 6 month rat study, oral administration through the diet. Dose of 0, 10, 40 and 160 mg/kg/day were used.

Three HD females died, 2 of which deaths were reported as drug related. Body weight gain was decreased in the HD groups of both sexes and in the MD males.

	Dosage group (mg / kg)							
	Males				Females			
Week	0	10	40	160	0	10	40	160
0	177	177	177	177	149	149	149	149
1	222	231 *	227	212 **	175	173	176	158 ***
2	283	289	284	267 ***	197	194	200	191
3	330	336	328	307 ***	214	212	217	210
4	365	370	363	336 ***	228	226	231	218 *
5	394	400	390	359 ***	239	235	242	229 *
6	419	427	415	377 ***	247	243	251	231 *
7	437	445	434	387 ***	253	249	257	239 *
8	460	466	451	401 ***	264	259	267	249 *
9	469	477	461	402 ***	264	260	269	248 **
10	483	492	474	410 ***	271	270	277	258 *
11	494	500	482	411 ***	275	272	279	252 **
12	507	513	490 *	404 ***	281	279	286	246 ***
16	534	537	507 **	373 ***	300	294	299	242 ***
20	555	557	529 *	407 ***	306	299	306	257 ***
21	560	563	536 *	414 ***	308	299	307	263 ***
22	564	565	540 *	417 ***	312	305	315	266 ***
23	570	572	545 *	421 ***	313	306	314	262 ***
24	570	569	544 *	408 ***	318	311	317	256 ***
25	579	579	554 *	420 ***	322	314	322	261 ***
26	565	570	547	410 ***	320	313	322	263 ***

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

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	Males dose (mg/kg/day)				Females (mg/kg/day)			
	0	10	40	160	0	10	40	160
Seg neutrophils	17.2	13.7	12.9 *	25.8 **	15.3	15.6	17.1	25.3 ***
Lymphocytes	76.0	81.3 *	80.2 *	70.5	78.8	79.7	78.4	71.1 **
Sodium mEq/l	145	145	144	145	143	144	144	145 *
Potassium mEq/l	5.0	5.1	5.3 ***	5.8 ***	4.3	4.6	5.0 ***	5.4 ***
Glucose mg%	151	157	148	131 **	145	152	145	118 ***
Cholesterol mg%	104	101	84 **	33 ***	105	101	76 ***	17 ***
Triglycerides mg%	135	124	109	42 ***	103	125	140	45 ***
Adrenals mg	60	62	65	93 ***	81	89 *	109 ***	183 ***
Mg/kg	106	109	119 *	234 ***	253	289 **	348 ***	725 ***
Gonads mg	3654	3691	3782	2978 ***	177	170	175	139 ***
Mg/kg	6477	6507	6965 *	7394 *	562	554	557	543

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Histopathology was reported in the scoring format and was later supplied at the reviewer's request in standard incidence table summary.

	0	10	40	160 mg/kg
Mammary gland				
- atrophy	0.00	-	-	0.30
- autolysis	0.00	-	-	0.15
- glandular development	1.05	-	-	1.20
Ovary				
- atretic follicles	0.65	0.80	0.75	0.95 *
- autolysis	0.00	0.00	0.00	0.20
- clear interstitial tissue	0.20	0.10	0.10	0.00
- corpora lutea	2.80	2.65	2.55	1.55 ***
- cystic	0.15	0.05	0.05	0.00
- hemorrhagic follicle	0.00	0.05	0.00	0.00
- tertiary follicles	1.05	1.10	1.00	1.05
Uterus				
- autolysis	0.00	0.00	0.00	0.20
- development	2.65	2.60	2.85	2.10 **
- dilated lumen	1.45	1.05	1.20	0.70 *
- infiltrating granulocytes	0.10	0.00	0.00	0.00
Vagina				
- autolysis	0.00	0.00	0.00	0.33
- cornified epithelium	0.63	0.30	0.35	0.33
- epithelial cell necrosis	0.00	0.05	0.10	0.00
- inflammatory cells (epithelium)	0.26	0.40	0.45	0.61
- mucified epithelium	0.26	0.15	0.20	0.39
- thickness of epithelium	3.68	3.30	3.20	2.89

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

From the review of the histology addendum is the following table:

Rat 6 month study: female

	control	LD 10	MD 40	High (160)
Ovaries: atretic follicles	13/20	16/20	15/20	19/20*
Ovaries: corpora lutea	20/20	19/20	19/20	15/20***
Ovaries: cystic	3/20	1/20	1/20	0/20
Vagina: cornified epithelium	8/19	6/20	5/20	4/18

*p<0.05 vs control , ***p<0.001

By comparison with the histology score table, it may be seen how much detail may be obscured by the scoring system. It may also be seen that In this study, adrenal hypertrophy/plasia is now evident down to 10-40 mg/Kg levels, without weight loss and without the clinical chemistry changes that are considered part of the stress reposnse. The effects upon serum cholesterol and serum potassium seem more indicative of a specific modulation of adrenal metabolism than of stress.

N79297 12 month rat study with drug administered through the diet. Doses used were untreated control, vehicle, 5, 20 and 80 mg/kg.

	Dosage group (mg / kg)									
	Males					Females				
Week	Control	Placebo	5	20	80	Control	Placebo	5	20	80
0	156	155	156	156	156	133	133	133	133	133
1	204	206	204	203	196	157	156	154	158	148 *
2	257	260	257	255	241 **	173	171	168	174	166
3	293	297	291	291	274 **	190	187	184	188	180 *
4	322	328	322	322	299 ***	204	200	196	201	192 **
5	346	351	348	346	317 ***	215	211	206	211	202 **
6	369	376	371	371	336 ***	228	225	218	221	211 **
7	388	396	388	388	349 ***	234	230	226	229	214 **
8	407	416	405	407	361 ***	242	236	231	235	222 **
9	424	434	424	425	374 ***	249	244	238	243	227 ***
10	441	452	440	440	383 ***	258	254	249	250	234 ***
11	451	465	451	451	388 ***	262	258	255	254	231 ***
12	464	475	463	462	396 ***	268	261	260	258	240 ***
13	466	481	464	462	397 ***	268	264	260	261	242 ***
14	474	489	473	467	399 ***	271	264	262	262	239 ***
15	474	490	473	476	400 ***	271	266	267	264	242 ***
16	488	504	485	488	400 ***	274	268	268	267	241 ***
17	492	505	480	489	411 ***	279	273	273	270	248 ***
18	489	506	483	489	409 ***	279	273	272	267	243 ***
19	497	514	491	501	418 ***	283	280	279	272	248 ***
20	506	521	498	505	416 ***	287	277	279	274	249 ***
21	508	525	503	508	421 ***	290	281	284	277	252 ***
22	506	524	504	509	419 ***	292	288	286	275	251 ***
23	509	523	501	509	413 ***	292	285	286	278	245 ***
24	516	533	515	520	422 ***	297	291	292	284	248 ***
25	514	533	505	513	417 ***	294	290	289	283	251 ***
26	520	539	510	521	416 ***	297	292	292	282	248 ***

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

	Dosage group (mg / kg)									
	Males					Females				
Week	Control	Placebo	5	20	80	Control	Placebo	5	20	80
27	526	547	520	530	421 ***	300	296	295	284	250 ***
28	533	550	523	534	423 ***	302	298	297	288	251 ***
29	538	556	527	533	431 ***	308	304	304	292	256 ***
30	543	559	530	538	432 ***	310	306	308	294	257 ***
31	547	561	534	542	431 ***	309	303	303	292	254 ***
32	549	563	540	546	432 ***	313	305	307	296	255 ***
33	542	562	537	542	427 ***	312	309	308	294	255 ***
34	555	569	545	547	430 ***	315	311	312	295 *	255 ***
35	555	568	542	545	426 ***	318	312	310	296 *	251 ***
36	560	569	544	548	423 ***	321	315	313	297 *	251 ***
37	559	575	547	552	436 ***	318	314	319	295 *	258 ***
38	552	562	537	541	422 ***	316	309	315	292 *	248 ***
39	559	574	548	549	429 ***	319	313	318	296 *	251 ***
40	563	578	548	552	430 ***	322	321	323	302	255 ***
41	570	585	563	561	443 ***	328	324	330	305	260 ***
42	575	589	564	562	438 ***	332	330	337	310	260 ***
43	579	594	569	566	438 ***	334	330	335	311	261 ***
44	579	594	571	565	437 ***	339	331	338	316	260 ***
45	577	601	566	569	435 ***	341	343	332	316	261 ***
46	582	601	574	575	438 ***	341	342	337	321	259 ***
47	579	599	569	575	430 ***	341	343	338	320	257 ***
48	585	599	572	574	421 ***	340	340	339	317	252 ***
49	591	608	581	582	432 ***	346	345	347	323	261 ***
50	591	606	581	577	432 ***	347	351	352	324	262 ***
51	587	599	572	575	434 ***	348	343	345	316 *	253 ***
52	590	608	575	574	433 ***	347	345	346	319	252 ***

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

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Clinical chemistry and hematology

	males				
Wk 26 Seg neutr	14.4	12.9	14.2	15.1	22.1 *
Wk 26 lympho	79.7	81.2	78.7	80.4	73.7
Wk 39 Seg neutr	17.7	18.4	27.0 *	22.8	34.4 ***
Wk 39 lymph	75.3	74.4	65.7 *	72.3	63.1 **
Wk52 Seg neutr	21.3	21.0	25.0	23.4	31.1 ***
Wk52 lympho	71.5	72.4	68.2	68.9	66.2 *
WK 26 Na	146	147	146	146	148 ***
Wk39 Na	145	147 *	146	146	147 *
Wk52 Na	146	145	147	145 *	146
WK 26 K	5.2	5.2	5.2	5.3	5.6 *
Wk39 K	5.2	5.3	5.3	5.6 **	5.4
Wk52 K	4.7	4.6	4.8	5.1 **	5.3 ***
WK 26 Glucose	109	115	112	105	106
Wk39 Glucose	116	115	113	108	111
Wk52 Glucose	143	146	143	136	127 *
WK 26 Cholester	122	107	101	104	48 ***
Wk39 Cholester	145	113	118	137	55 ***
Wk52 Cholester	172	158	156	154	42 ***
WK 26 Triglycer	232	170 *	213	179	98 ***
Wk39 Triglycer	239	164	218	212	119 **
Wk52 triglycer	196	209	220	243	113 ***

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	females				
Wk 26 Seg neutr	12.8	12.4	13.8	12.5	19.1 *
Wk 26 lympho	84.1	82.4	80.1	83.0	78.3
Wk 39 Seg neutr	19.1	14.0	16.2 *	21.1	28.1 *
Wk 39 lymph	75.2	81.5	79.5	74.9	67.7 *
Wk52 Seg neutr	22.1	16.4 *	19.6	24.4	36.5 ***
Wk52 lymph	72.3	77.8 *	75.2	71.5	59.2 ***
WK 26 Na	147	146	147	146	147
Wk39 Na	144	145	145	145	145
Wk52 Na	142	143	143	143	143
WK 26 K	4.3	4.7 *	4.8 *	4.9 **	5.7 ***
Wk39 K	4.5	4.3	5.1 **	5.0 *	5.3 ***
Wk52 K	4.0	3.9	4.1	4.4 **	5.3 ***
WK 26 Glucose	117	118	114	116	118
Wk39 Glucose	126	127	120	117	125
Wk52 Glucose	138	142	140	133	117 ***
WK 26 Cholester	121	107	92	72 **	18 ***
Wk39 Cholester	213	126	119	84 **	21 ***
Wk52 Cholester	228	157	130	79 ***	25 ***
WK 26 Triglycer	228	161	197	172	50 ***
Wk39 Triglycer	310	184	262	357	102 ***
Wk52 triglycer	807	350 *	445	426	108 ***

Males: organ weight changes					
	0 mg/kg	vehicle	5mg/kg	20 mg/kg	80 mg/kg
Adrenals	57	61	61	60	102 ***
mg	98	101	106	104	236 ***
Mg/kg					
Gonads mg	3676	3804	3615	3742	2857 ***
	6290	6399	6302	6547	6650
Mg/kg					
Females: organ weight changes					
Adrenal mg	76	79	80	106 ***	369 ***
Mg/kg	217	230	229	332 ***	1500 ***
Gonads mg	181	171	177	163	147 **
Mg/kg	517	499	506	515	585 *

Adrenal cortical cells were described as swollen but not hyperplastic.
From the histology addendum review:

Rat Oral 12 month study: males

	0 mg/kg	vehicle	5 mg/kg	20 mg/kg	80mg/kg
	m	m	m	m	m
Epididymides: cellular debris in ductules	0/20	0/20	1/20	0/20	12/20***
Epidiymides: Low spermatozoa count	0/20	0/20	1/20	0/20	8/20**
Testes: degenerated tubuli	0/20	0/20	1/20	0/20	9/20***
Testes: giant cells in tubuli	0/20	0/20	0/20	0/20	5/20*

*significantly different from control $p<0.05$, **$p<0.01$, $p<0.001$

Rat Oral 12 month study: females

	0 mg/kg	vehicle	5 mg/kg	20 mg/kg	80mg/kg
Ovaries: atretic follicles	12/20	9/20	9/20	11/20	17/19**
Ovaries: old corpora lutea	12/20	10/20	12/20	15/20	3/19*
Ovaries: recent corpora lutea	13/20	12/20	7/20	12/20	6/19
Uterus: Infiltrating granulocytes	4/20	8/20	8/20	3/20	0/19*
Vagina: cornification	11/19	13/20	7/20	4/20*	8/19
Vagina: mucification	5/19	6/20	5/20	6/20	8/19

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1 month dog study:
Doses 80 mg/kg R67555 40 mg/kg R85548, 40 mg/kg R85547

histology limited to spleen ..

GOM: Gonads m	g	13.0	13.0	13.5	13.0
	g / 10 kg	13.3	13.1	12.3	11.6
GOF: Gonads f	g	0.641	0.408	0.621	0.703
	g / 10 kg	0.670	0.505	0.696	0.803
HYP: Hypophysis	g	0.044	0.052	0.063	0.045
	g / 10 kg	0.045	0.058	0.062	0.045
PRS: Prostate	g	3.15	3.55	4.65	3.25
	g / 10 kg	3.19	3.61	4.08	2.89

N88712 One month intravenous administration in dogs. Doses used 0, 0.31, 1.25 and 5 mg/kg.

No change in clinical chemistry/hematology. No organ weight effects.

N54352 3 month repeat dose study in dogs, oral administration. Doses used 0, 2.5, 10 and 40 mg/kg/day

Decreased rate of weight gain was seen in the HD animals.

	Dosage group (mg / kg)			
Week	Control	2.5	10	40
1	0.3	0.2	0.2	0.2
2	0.6	0.5	0.5	0.3
3	0.9	0.8	0.8	0.6
4	1.3	0.9	1.2	0.7
5	1.7	1.3	1.4	1.1 *
6	1.4	1.3	1.4	1.0
7	2.0	1.6	1.8	1.4
8	2.0	1.6	1.7	1.4
9	2.0	1.7	2.0	1.4
10	2.3	1.9	2.1	1.6
11	2.4	2.1	2.1	1.6
12	2.5	2.1	2.2	1.8
13	2.4	2.1	2.3	1.7

Hematology: no effects on lymphocytes or neutrophils
Clinical chemistry: no effect on sodium, glucose, cholesterol, triglycerides

EXPERIMENT: 1591
Toxicity study
R 67555 - OR - DOG - 3 MONTH

! POT: Potassium mEq/l
! Mean values recorded at stated week and dose

Week	Date	Dosage group (mg / kg) Control	2.5	10	40
-2	4/ 3/86	5.3	5.7 *	5.6	5.6
0	18/ 3/86	5.3	5.3	5.1	5.4
3	3/ 4/86	5.2	5.3	5.7 *	5.7 **
4	15/ 4/86	5.1	5.2	5.3	5.4
8	13/ 5/86	5.1	5.2	5.3	5.4 **
12	10/ 6/86	5.4	5.1	5.3	5.5

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

N64891 6 month dog study: oral administration, doses used were 0, 5, 20 and 80 mg/kg
No effect on body weight.
No effect on neutrophils, lymphocytes, potassium, glucose, cholesterol, triglycerides
No effect on organ weights

Ovary				
- atretic follicles	1.25	1.00	1.00	1.25
- developed tertiary follicles	0.25	0.00	0.00	0.00
- old corpora lutea	1.00	0.00	0.50	0.75
- recent corpora lutea	0.00	1.50	0.00	0.50
- tertiary follicles	1.00	1.00	1.00	1.00
Pituitary gland				
Testis				
- aspermatogenic tubuli	0.00	0.00	0.00	0.25

N79298 12 month oral study in dogs Doses used: 0,βCD, 2.5, 10 and 40 mg/kg/day

No effect on body weight, neutrophils, lymphocytes, sodium, glucose, inconsistent triglyceride effects. No effect on adrenal weight. No lymphoid effects, repro effects not discernible

Potassium

	Dosage group (mg / kg)				
Week	Control	Placebo	2.5	10	40
-2	5.3	5.3	5.2	5.2	5.5
0	5.1	5.2	5.2	5.1	5.4
2	5.2	5.1	5.0	5.1	5.6 @
5	5.4	5.1	5.0	5.2	5.5 @@
8	5.3	5.2	5.0	5.0	5.3
12	5.2	5.0	5.0	5.1	5.4 @
17	4.8	4.7	4.6	4.9	5.2 @
20	5.2	5.1	5.0	5.2	5.7 * @
24	5.2	4.9	4.9	5.0	5.4
28	5.2	5.1	5.1	5.1	5.3
32	5.4	5.3	5.1	5.1	5.5
36	5.3	5.2	4.7 *** @@	5.0	5.7 * @
40	5.4	5.1 *	5.0 **	5.0 **	5.5 * @@
44	5.2	5.1	4.7 *	4.8 *	5.5
48	5.1	5.0	4.8	4.9	5.5 * @@
52	4.9	4.7	4.7	4.8	5.2 @

Significance computed by Mann-Whitney U test (two tailed)
Control versus all groups : * P < .05 ** P < .01 *** P < .001
Placebo versus other groups : @ P < .05 @@ P < .01 @@@ P < .001

EXPERIMENT: 1965
Toxicity study
R 67555 - OR - DOG - 12 MONTH

| CHO: Cholesterol mg%
| Mean values recorded at stated week and dose

Week	Dosage group (mg / kg) Control	Placebo	2.5	10	40
-2	161	158	147	153	146
0	171	164	153	159	142 * @
2	145	151	152	158	133 @
5	168	160	148	152	135 ** @
8	153	146	142	151	133 *
12	144	141	133	144	132
17	164	139	134	148	130 *
20	167	135	131 *	147	124 *
24	162	141	135	148	130
28	151	145	145	160	133 *
32	148	144	140	146	126 * @
36	151	150	152	156	141
40	160	165	144	162	145
44	164	151	140	147	138
48	165	143	127 *	139	131 *
52	159	153	147	155	140 *

Significance computed by Mann-Whitney U test (two tailed)
Control versus all groups : * P < .05 ** P < .01 *** P < .001
Placebo versus other groups : @ P < .05 @@ P < .01 @@@ P < .001

Gonads male mg	19.0	17.5	17.8	17.5	16.0
Mg/kg	13.6	13.1	12.3	13.3	11.3
Gonads female mg	0.901	1.124	0.735	0.614 *	1.313
Mg/kg	0.805	1.032	0.677	0.531	1.057

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N45717 Pilot 1 month repeat dose study in dogs Doses used 0, 2.5, 10 mg/kg/day
No effect on neutrophils or lymphocytes, potassium, glucose, cholesterol. Minimal histopath reported

N79298 12 month oral study in dogs Doses used: 0,βCD, 2.5, 10 and 40 mg/kg/day
No effect on body weight, neutrophils, lymphocytes, sodium, glucose, inconsistent triglyceride effects. No effect on adrenal weight. No lymphoid effects, repro effects not discernible

Overview of studies

3 month mouse study: Significant weight loss effects were seen at the HD only. The clinical chemistry and hematology changes associated with stress were not present in the data. Serum potassium was significantly increased in both sexes while serum cholesterol and glucose were decreased. Adrenal weight was significantly increased in both sexes at the HD. Gonadal weight was significantly decreased in both sexes at the HD. No histology data was presented for LD or MD groups. However, there was a significant decrease in corpora lutea at the HD in females, Leydig cell hyperplasia and large nucleated tubular cells in the HD males.

N76736 Pilot 1 month study in rats. There were no significant body weight effects at any dose in either sex. There was a dose-related statistically significant increase in potassium. Serum cholesterol was significantly decreased. Adrenal weight was significantly increased in both males and females. No histopathology results were provided so this data cannot be adequately assessed.

N92595 D-enantiomer tested in a 1 month rat toxicity study. R85547 (d-enantiomer) tested at 10 and 40 mg/kg/day, R67555 (nebivolol) tested at 20 and 80 mg/kg/day versus the vehicle. Statistically significant decreases in body weight were seen only at the 80 mg/kg dose of R67555. Serum potassium was increased in all drug treated groups, significantly so at R67555-80 mg/kg in females. Serum glucose and cholesterol were significantly decreased in this group also. Only cholesterol was significantly affected in the corresponding male group. Adrenal weight was significantly increased in both sexes of the R67555 80 mg/kg group. Very limited histologic assessment was reported and a scoring system was used that makes interpretation somewhat difficult. In the ovaries, focal atrophy was seen in increased amounts compared to the vehicle in both the R67555-80 mg/kg/ and the R85547 40 mg/kg group and recent CL were in decreased amounts. In these same two groups the number of animals with a dilated uterine lumen and glandular development were decreased while cornification of the vagina was increased and mucification decreased. These findings are suggestive of effects on cyclicity. Note that the R85547 group showed no effects indicative of "stress."

N92594 L-enantiomer tested in a 1 month rat toxicity study. R85548 (l-enantiomer) tested at 10 and 40 mg/kg/day, R67555 (nebivolol) tested at 20 and 80 mg/kg/day versus the vehicle.

A significant effect on body weight was seen at the R67555-80mg/kg dose. Serum potassium was significantly increased in the R85548-40 and both the R67555 dose groups. Serum cholesterol was significantly decreased in the same groups while glucose was non-significantly decreased. Adrenal weight was significantly increased in the same groups in females and in the R85548-40 mg/kg and the R67555-80 mg/kg group. The number of atretic follicles was increased in the R85548-40 and R67555-80 groups as were cysts. In these groups, the numbers of recent follicles, tertiary follicles and old corpora lutea were decreased compared to the vehicle. In the uterus, there was a decrease in dilated lumen in the two reported treatment groups as well as decreased glandular development and decreased leucocyte infiltration. Again, the R85548 group was one with no weight or apparent stress effects.

N88268: 1 month rat study. Doses of 0, vehicle, 0.31, 1.25 and 5 mg/kg/day were given intravenously

Drug administration had no effects on body weight. Clinical chemistry showed increased serum potassium in the drug treated groups of both sexes with a significant increase in the HD females. Cholesterol was significantly decreased in the MD and HD males. Adrenal weight was not affected in this study in either sex. Gonad weight was significantly decreased in the LD and HD female groups. Histological results for the reproductive tract were not provided.

N54353: 3 month rat study with oral administration. Doses of 0, 10, 40 and 160 mg/kg/day were used. Body weight decreases were seen at the HD of both sexes. Mild neutrophilia was apparent in the HD groups of both sexes and a minimal decrease in lymphocytes in the female HD group. Serum potassium levels were significantly increased in the MD and HD groups of both sexes. Serum glucose was minimally decreased in the HD groups while cholesterol was significantly decreased in HDm and MD and HDf. Adrenal weight was significantly increased in the HD groups of both sexes. There was a mild decrease in gonad weight in the HD groups. No adrenal hypertrophy or related findings were described in the histopathology results.In the females, only the control and HD groups were analyzed. An increase in atretic follicles, a decrease in corpora lutea, decreased uterine glandular development, decreased inflammatory cells in the vagina and increased mucification of the vagina were reported using the scoring system.

N64890 6 month rat study. Doses of 0, 10, 40 and 160 mg/kg/day were administered orally. Bodyweight gain was decreased in the HD of both sexes and the MD males. Neutrophilia was seen in the HD groups with a mild decrease in lymphocytes. Serum potassium showed dose related increases in both sexes. Serum glucose was significantly decreased in the HD of both sexes while cholesterol was significantly decreased in the MD and HD of both sexes. A significant increase in adrenal weight was seen in the MD and HD males and in all drug-treated female groups. Gonad weight was significantly decreased in the HD of both sexes.

EDMS-PSDB-2876162 Two week repeated dose oral toxicity study in the rat for qualification of an impurity. This is a report that was obtained separately from the DMF and has been reviewed as a separate report that is available in DFS (file name: impurity_addendum.doc) so it will be only briefly discussed here. The high dose rats were given 40 mg/kg for the first week and 60 mg/kg for the second week. One group received nebivolol alone and the other group received

nebivolol plus impurity. At 60 mg/kg there was no effect on body weight gain with nebivolol alone. Nebivolol + impurity showed 9.5% less weight gain than the control group. Serum K+ was significantly increased in all female drug-treated groups. Serum cholesterol was significantly decreased in the HD±impurity group. Adrenal weight was increased in both sexes at the HD±impurity. Both of the female HD groups (±impurity) showed changed in the reproductive tract. While these changes may correlate to adrenal changes, the correlation to "stress" does not seem to be supported.

Summary

As with any experimental data, multiple interpretations may be assigned to that information. The sponsor has proposed that instead of an endocrine effect of nebivolol the reported histological effects are due to "stress" as evidenced by weight loss. However, the written description of this interpretation also states that nebivolol has a pharmacological effect upon the adrenal gland and therefore weight loss is not a reliable indicator of stress. It has also been stated that the findings are "far more consistent with a generalized inactivity of the reproductive cycle than they are of stimulation." It is not a desirable attribute for an anti-hypertensive drug to send the reproductive tract into a state of generalized inactivity.

It is worthwhile at this point to present certain definitions from the OECD Draft Guidance Document on Reproductive Toxicity Testing and Assessment (November10, 2004). The draft guidance defines adverse effects on reproductive ability or capacity to include the following (Section I, part 10):

- Alteration of onset of puberty
- Reproductive cycle normality
- Fertility
- Parturition
- Gamete production and transport
- Premature reproductive senescence
- Modifications in other functions that are dependent on the integrity of the reproductive systems

Developmental toxicity was defined to include (Section I, part 11):

- Any effect which interferes with normal development of the conceptus, either Before or after birth, and resulting from exposure of either parents prior To conception, or exposure of the developing offspring during prenatal Development or post-natally, to the time of sexual maturation. The major Manifestations of developmental toxicity include:
  - Death of the developing organism
  - Structural abnormality
  - Altered growth
  - Functional deficiency

(part 31) A change in offspring body weight is a sensitive indicator of developmental toxicity.

The reviewer reiterates again several points.

1. There have been statements in several study reports that the reproductive tract is one of the target organs of toxicity. One of the statements is quoted here:

From N106653 3 month mouse study:

Conclusion

The adrenocortical, ovarian and testicular changes in the 160 mg/kg dosed groups are directly related to interference of the test article with the steroid metabolism. This interference results in a hormonal imbalance as evidenced by the organ weight changes of the adrenals and the ovaries, by the swelling of the adrenals and by the severely decreased serum

The data supporting this statement has not been made available to the Division. Sponsor has speculated that steroid metabolism is disrupted , but whether levels of pregnenolone or any of its ultimate estrogenic, androgenic or corticoid derivatives is affected remains unexplored.

2. The sponsor conducted several studies investigating a possible effect of nebivolol upon the adrenal gland. The results indicate that nebivolol interferes with the ability of the adrenal to respond to ACTH. This would be a potential explanation for the consistent effect of increased adrenal weight seen in multiple studies in several species. However, the examination of this was limited in scope. No mechanistic data has been generated or presented to explore the relevance or lack of relevance to humans.
3. There is poor support for the sponsor's hypothesis of stress. A typical stress response includes components of altered hematology, clinical chemistry and histopathology. In the early stages of "stress" the most typical feature is a neutrophilia. With time, the neutrophilia may abate, but a lymphopenia is left as well as increased serum cholesterol, increased serum glucose and possibly increased serum triglycerides. Furthermore, lymphoid atrophy is also part of the histopathology findings. Muscle wasting, particularly the abdominal muscles, resulting in a pot-bellied appearance, hairloss or poor pelage quality are grossly obvious features and may also influence the clinical chemistry findings. These features were lacking in essentially every study for nebivolol. In addition, serum cholesterol and glucose, when affected, were *decreased* rather than showing an increase. What was also interesting was that serum potassium showed fairly consistent increases. The overall picture is suggestive of a specific adrenal modulation

ADR: Adrenals	g	1.023	1.170	1.046	1.006
	g / 10 kg	0.925	1.092	0.967	0.975
TYR: Thyroids	g	0.930	0.869	0.884	0.924
	g / 10 kg	0.814	0.786	0.810	0.874
GOM: Gonads m	g	14.8	16.3	15.5	16.8
	g / 10 kg	11.2	14.1	13.3	13.6
GOF: Gonads f	g	0.631	0.619	0.736	0.765
	g / 10 kg	0.664	0.616	0.717	0.846
HYP: Hypophysis	g	0.052	0.047	0.041 *	0.050
	g / 10 kg	0.048	0.044	0.039	0.049
PRS: Prostate	g	5.50	6.00	6.25	4.50
	g / 10 kg	4.00	5.23	5.13	3.65

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Organ or tissue - observation	Dosage group (mg / kg)			
	Control	2.5	10	40
Ovary				
- mature tertiary follicles	0.00	0.00	0.00	0.50
- old corpus luteum	0.25	0.00	0.00	0.00
- tertiary follicles	1.25	1.25	1.00	1.00
Pituitary gland				
- cystic	0.50	0.25	0.29	0.63
- prominent dark blue cells	0.00	0.13	0.00	0.00
Uterus				
- glandular development	1.50	1.75	1.75	2.00
- inflammatory cells (epithelium)	0.25	0.00	0.00	0.00
Vagina				
- cornification of the epithelium	0.25	0.00	0.00	1.00
Vagina				
- inflammatory cells (epithelium)	0.50	1.50	1.75	1.00
- necrotic cells (epithelium)	0.75	1.25	0.50	0.50
- thickness of the epithelium	3.25	3.00	2.50	3.50

rather than the vague, general designation of "stress" , sometimes referred to as chronic sympathetic stimulation.

4. If one looks at each of the following points in isolation:
    a. the histologic changes in the female reproductive tract,
    b. the clinical chemistry and histopathology changes indicating adrenal involvement,
    c. the reproductive toxicology studies,
    d. the carcinogenicity studies
    e. and the various statements throughout the toxicology reports stating that the reproductive tract is a target organ of toxicity

    then one could possibly dismiss each point in isolation as a spurious finding. When the information is integrated, considering the biology of the whole animal, it becomes more plausible to say that there is some perturbation of metabolism. Possibly the observed results are secondary to some adrenal effect. The overall conclusion that this reviewer makes is that there is a signal here for endocrine or metabolic disruption, perhaps reduced levels of sex and adrenal cortex steroids, , provoking release of trophic hormones (LH and ACTH). Whether or not this is in anyway relevant to humans is unknown at this time. The data to make this determination has not been presented.

There is also a distinct "Alice in Wonderland" feel to this situation. October 22, 2002, Drs Defelice, Hausner and Williams held a telecon with the sponsor expressing our concerns over the apparent endocrine signal. At that time our stated concerns included the fact that the female reproductive tracts appeared to be more quiescent and that there seemed to be adrenal involvement. We also specifically asked about the effects, if any, of nebivolol and its metabolites on glucocorticoids and mineralocorticoids. Before us now is the sponsor's reply to our concerns that essentially does nothing more than restate that nebivolol has some undefined effect upon the adrenal gland and that the female reproductive tract seems less active. Curiously, the sponsor seems to consider the adrenal gland in isolation, ignoring that there is histology from multiple studies indicating changes in the zona glomerulosa (involved in mineralocorticoid metabolism and K+ retention) and the zona reticularis (which produces several sex steroids) and that the adrenal in general is involved in cholesterol metabolism, the nucleus for steroid hormones. There seems to be a disconnect in associating the adrenal involvement with the apparent reproductive changes. The concern over the potential relevance of these findings to humans is still unaddressed.

Information to sponsor:

The material provided in the May 24, 2005 communication do not provide data to dispel the concerns over a possible endocrine effect of nebivolol.

Regarding the possible effects on fertility, reproduction and developmental effects, the following points are still unaddressed:

1. No NOAEL has been identified for decreased pup weight or decreased pup survival. This also occurred at doses with no apparent maternal toxicity.
2. It can't be said that there are no effects on reproduction/fertility in general due to

a. Maternal toxicity was reported at doses of 20 and 40 mg/kg. The dose of 20 mg/kg was not reported to produce toxicity in non-gravid animals in general toxicology studies. The dose of 40 mg/kg caused mild, inconsistent effects in non-gravid animals in general toxicology studies – where the consequences of hormonal imbalance may not be so evident.
b. Dystocia was reported in multiple studies at doses down to 5 mg/kg/day, with no other maternal toxicity reported.
c. Cannibalism was reported in multiple studies
d. Prolonged parturition was reported in multiple studies
e. There has been no direct examination of female cyclicity

3. It can't be said that there are no effects on development or fertility in the offspring
   a. an insensitive method was used to assess reaching developmental landmarks. An adequate assessment evaluates when a milestone or landmark was reached, not if a landmark was reached. In the developmental studies, landmarks were assessed substantial periods of time after they should have been achieved, thereby measuring if, not when.

Developmental milestone	OECD recommended assessment time *	Time of sponsor's actual assessment	
		Study N92570	Study N106655
Vaginal opening	Postnatal (PN) day 30-35	PN Day 42	PN Day 42
Testes descent	PN day 40-45	PN day 42	PN day 42
Surface righting reflex	PN 2-4 days	PN21	PN21
Air righting reflex	PN12-17 days	PN 21	PN 21
Auditory startle	PN10-14 days	PN 21	PN 21
Anogenital distance**	Day 0 of F2 generation	Not done	Not done

*OECD Draft Guidance Document on Reproductive Toxicity Testing and Assessment. November 10, 2004. Available in the OECD website.
** anogenital distance is recommended as an assessment of possible endocrine effects

   b. we don't know precisely the age at which the offspring were allowed or able to mate. The stated time in the reports is "±3 months."
   c. There is no reported information on cyclicity or the cohabitation interval of the mated offspring
   d. The lack of information as to the age of mating, cyclicity, cohabitation interval and the insensitive developmental data do not provide information as to whether or not the drug-exposed pups reach sexual maturity at the same time as the control pups.

Regarding the signal for an endocrine effect:

As with any experimental data, multiple interpretations may be assigned to a given dataset. It has been proposed that instead of an endocrine effect of nebivolol the reported histological effects are due to "stress" as evidenced by weight loss. However, the written description of this interpretation also states that nebivolol has a pharmacological effect upon the adrenal

gland and therefore weight loss is not a reliable indicator of stress. It has also been stated that the findings are "far more consistent with a generalized inactivity of the reproductive cycle than they are of stimulation." Inactivity of the reproductive organs is not a more desirable attribute for an anti-hypertensive drug than is stimulation.

It is worthwhile at this point to present certain definitions from the OECD Draft Guidance Document on Reproductive Toxicity Testing and Assessment (November10, 2004). The draft guidance defines adverse effects on reproductive ability or capacity to include the following (Section I, part 10):

Alteration of onset of puberty
Reproductive cycle normality
Fertility
Parturition
Gamete production and transport
Premature reproductive senescence
Modifications in other functions that are dependent on the integrity of the reproductive systems

Developmental toxicity was defined to include (Section I, part 11):

Any effect which interferes with normal development of the conceptus, either Before or after birth, and resulting from exposure of either parents prior To conception, or exposure of the developing offspring during prenatal Development or post-natally, to the time of sexual maturation. The major Manifestations of developmental toxicity include:

Death of the developing organism
Structural abnormality
Altered growth
Functional deficiency

(part 31) A change in offspring body weight is a sensitive indicator of developmental toxicity.

Several points are again reiterated:

1. There have been statements in several study reports that the reproductive tract is one of the target organs of toxicity. One of the statements is quoted here:

From N106653 3 month mouse study:

Conclusion

The adrenocortical, ovarian and testicular changes in the 160 mg/kg dosed groups are directly related to interference of the test article with the steroid metabolism. This interference results in a hormonal imbalance as evidenced by the organ weight changes of the adrenals and the ovaries, by the swelling of the adrenals and by the severely decreased serum

The data supporting this statement has not been made available to the Division.

2.The original sponsor conducted several studies investigating a possible effect of nebivolol upon the adrenal gland. The results indicate that nebivolol alters the ability of the adrenal to respond to ACTH. However, the examination of this was limited in scope. No mechanistic data has been generated or presented to explore the relevance or lack of relevance to humans.
3. There is poor support for the hypothesis of stress. A typical stress response includes components of altered hematology, clinical chemistry and histopathology. In the early stages of "stress" the most typical feature is a neutrophilia. With time, the neutrophilia may abate, but a lymphopenia is left as well as increased serum cholesterol, increased serum glucose and possibly increased serum triglycerides. Furthermore, lymphoid atrophy may be part of the histopathology findings. Muscle wasting, particularly the abdominal muscles, resulting in a pot-bellied appearance, hairloss or poor pelage quality are grossly obvious features and may also influence the clinical chemistry findings. These features were lacking in essentially every study for nebivolol. In addition, serum cholesterol and glucose, when affected, were *decreased* rather than showing an increase. What was also interesting was that serum potassium showed fairly consistent increases. The overall picture is suggestive of a specific adrenal modulation rather than the general designation of "stress", sometimes referred to as chronic sympathetic stimulation.
4. If one looks at each of the following points in isolation:
    a. the histologic changes in the female reproductive tract,
    b. the clinical chemistry and histopathology changes indicating adrenal involvement,
    c. the reproductive toxicology studies,
    d. the carcinogenicity studies
    e. and the various statements throughout the toxicology reports stating that the reproductive tract is a target organ of toxicity

    then one could possibly dismiss each point in isolation as a spurious finding. When the information is integrated, considering the biology of the whole animal, it becomes more plausible to say that there is some perturbation of metabolism. Possibly the observed results are secondary to some adrenal effect. The overall conclusion that this reviewer makes is that there is a signal here for endocrine or metabolic disruption. Whether or not this is in anyway relevant to humans is unknown at this time. The data to make this determination has not been presented.

We would also like to remind you that on October 22, 2002, Drs Defelice, Hausner and Williams held a telecon with several Mylan participants expressing our concerns over the apparent endocrine signal. At that time our stated concerns included the fact that there seemed to be some effect upon the female reproductive tract and that there was apparent adrenal involvement. We also specifically asked about the effects, if any, of nebivolol and its metabolites on glucocorticoids and mineralocorticoids. Data has not yet been presented to support the contention that this signal is clinically unimportant. What is before us now is a restatement that nebivolol has some undefined effect upon the adrenal gland and that the female reproductive tract seems less active. The concern over the potential relevance of these findings to humans is still unaddressed.

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/s/

Elizabeth Hausner
7/6/05 07:40:47 AM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
7/6/05 04:53:48 PM
PHARMACOLOGIST

.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21742
Review number: 1
Sequence number/date/type of submission: 0/March15, 2005/BP, doc type N
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: Bertek
Manufacturer for drug substance: Mylan Pharmaceuticals Inc, 781 Chestnut Ridge Road, Morgantown, WV 26505.

Reviewer name: Elizabeth Hausner, D.V.M.
Division name: Cardio-Renal Drug Products
HFD #: 110
Review completion date:

Drug:

Generic Name:	Nebivolol Tablets
Chemical Name	Nebivolol hydrochloride is identified chemically as (±)-[2R*[R*[R*(S*)]]]-α,α′-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol] hydrochloride
Code Numbers	R067555
	R067138 (d-Nebivolol)
	R067145 (l-Nebivolol)
CAS Registry No.	152520-56-4
Trade Name:	To Be Established

In the late summer of 2004, the Division again asked the sponsor to provide evidence that the Leydig cell tumors in mice were not relevant to humans. The sponsor stated an intention of searching the patient use records. The information thus provided was not useful. I disagree with the sponsor's statement on page 1 of Attachment 1 that the "…Agency requested that Bertek conduct a toxicology study in rats and mice to evaluate whether repeated administration of nebivolol is associated with changes in luteinizing hormone (LH) and estradiol levels in mice and rats." The Division asked for a *mechanistic study* to provide support for the sponsor's assertion that nebivolol did not pose a cancer risk to humans. It was the sponsor's decision what mechanistic hypothesis was to be the center of the study.

The current submission was provided to let the Division know of a change made to the protocol. The sponsor proposes that given in the diet, nebivolol will reach peak plasma levels in the middle of the dark period when the animals are eating. The sponsor further feels that the proposed use of a reversed light cycle is necessary to minimize variation in hormone levels between group caused by the pusatile nature of the release of LH as well as the influence of circadian levels.

The sponsor asks the question :

> It is the belief of Mylan Bertek and the identified consultants that the importance of sampling nebivolol and finasteride animals at Cmax outweighs the potential disadvantages of modifying light-dark cycles. Does the Division concur with our changes to the light/dark cycle and sampling times?

Response: Yes.

Information to sponsor: Dr Defelice has already conveyed this information to the sponsor via an email exchange. A copy of those emails are included herein.

From: Defelice, Albert F
Sent: Saturday, March 05, 2005 12:20 PM
To: 'kelly.tate@mylanlabs.com'; Hausner, Elizabeth A; Defelice, Albert F
Cc: Hicks, Karen; Robb, Melissa; 'Andrea.Miller@mylanlabs.com'; Stockbridge, Norman L; Lemtouni, Salma
Subject: RE: NDA 21-742, Toxicology Protocol
Please be advised that our concurrence is neither necessary nor necessarily prudent. You/your consultants are most aware of the PK of your compound and the dynamics of the hormone assays. Having said that,I fully concur with your changes which sound reasonable and appropriate *prima facie*. I have not yet had the chance to discuss with Drs Hausner/Hicks/Lemtouni/Stockbridge: If you do not hear from any of us by COB 3/7/05, proceed expeditiously.
I do not recall the results of estrogen receptor (species?) binding assays....any idea of whether they might be occupied at plasma nebivalol levels afforded at 40 mg/Kg in the mouse?
-----Original Message-----
From: kelly.tate@mylanlabs.com [mailto:kelly.tate@mylanlabs.com]
Sent: Friday, March 04, 2005 4:29 PM
To: elizabeth.hausner@fda.hhs.gov; albert.defelice@fda.hhs.gov
Cc: karen.hicks@fda.hhs.gov; melissa.robb@fda.hhs.gov; Andrea.Miller@mylanlabs.com
Subject: NDA 21-742, Toxicology Protocol

Drs DeFelice and Hausner,

Please review the attached Microsoft Word document which summarizes and provides the rationale behind a proposed protocol amendment to the ongoing 13 week endocrine evaluation in male mice and rats (TOX 021-001). We would appreciate your quick review and your concurrence so that we may make the change to the light/dark cycle as soon as possible.

Kelly Tate
Dir, Regulatory Affairs
Mylan Bertek Pharmaceuticals Inc.
Phone: 304 599 2595 ext 6580
fax: 304 285 6407
email: kelly.tate@mylanlabs.com

No further action is required at this time.

Elizabeth Hausner, D.V.M.
Pharmacologist

Al DeFelice, Ph.D.
Supervisory Pharmacologist

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Elizabeth Hausner
4/11/05 11:02:35 AM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
4/11/05 11:22:05 AM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21742
Review number: 1
Sequence number/date/type of submission: 0/February 8, 2005/BP, doc type N
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: Bertek
Manufacturer for drug substance: Mylan Pharmaceuticals Inc, 781 Chestnut Ridge Road, Morgantown, WV 26505.

Reviewer name: **Elizabeth Hausner, D.V.M.**
Division name: **Cardio-Renal Drug Products**
HFD #: 110
Review completion date:

Drug:

Generic Name:	Nebivolol Tablets
Chemical Name	Nebivolol hydrochloride is identified chemically as (±)-[2R*[R*[R*(S*)]]]-α,α'-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol] hydrochloride
Code Numbers	R067555
	R067138 (d-Nebivolol)
	R067145 (l-Nebivolol)
CAS Registry No.	152520-56-4
Trade Name:	To Be Established

The material in this submission was submitted in response to a request from the Division. The toxicology reports did not contain incidence tables of histopathological findings. What was originally presented was 1) group mean histo scores, 2)scores per individual animals and 3) the individual animal sheets. The sponsor was asked to provide summary tables of the incidences of the histological findings. The purpose of this was to clarify various references throughout the reports that indicated that the reproductive tract was one of the target organs of toxicity.

The 3 month mouse study showed that one HD m was reported as having hyperplasia in the Leydig cells. All of the HD animals were reported to have large, nucleated tubular cells, a term that was not defined.
A limitation of the 3 month mouse study is that the LD and MD groups were not analyzed to determine any kind of dose response relationships.

3 month mouse study: males

	control	LD 10 nd	MD 40 ND	High (160)
Testes		ND	ND	
Focal Leydig cell hyperplasia	0/10			1/10
Large nucleated tubular cells	4/10			10/10**

*p<0.05, **p<0.01, p<0.001

Non-carcinogenic endocrine findings from the mouse carcinogenicity study are summarized below. Statistically significant increases in the incidences of mammary gland atrophy and endocrine hyperplasia (an undefined term) were seen in the HD males. Significant decreases were reported in the incidences of amyloidosis and mineralization.

Mouse Carcinogenicity Study : males

	0 mg/kg	vehicle	2.5 mg/kg	10 mg/kg	40mg/kg
	m	m	m	m	m
Epididymus: benign LCT	0/50	0/50	0/50	1/50	0/50
LCT	1/50	2/50	0/50	1/50	21/50***@@@
Mammary gland: Diffuse atrophy	6/48	3/49	4/48	12/48	12/49@
Testis: amyloidosis	15/50	16/50	13/50	6/50*@	2/50**@@@
Testis: Endocrine hyperplasia	5/50	6/50	4/50	4/50	14/50*
Testis: mineralization	15/50	16/50	14/50	10/50	3/50**@@

** p<0.01, *** p<0.001 vs control group @ p<0.05, @@ p<0.01 @@@ p<0.001 vs vehicle group

In the 6 month rat study, testicular and epididymal degeneration were reported in the HD group with a concurrent low sperm count.

Rat 6 month study: males

	control	LD 10	MD 40	High (160)
Epididymides: cellular debris in ductules	0/20	0/20	0/20	4/20*
Epididymides:low spermatozoa count	0/20	0/20	0/20	5/20*
Testes: degenerated tubules	1/20	0/20	0/20	7/20*

*p<0.05 vs control , ***p<0.001

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The results of the 6-month rat study were repeated in the 12 month rat study at half the dose used in the shorter study.

Rat Oral 12 month study: males

	0 mg/kg	vehicle	5 mg/kg	20 mg/kg	80mg/kg
	m	m	m	m	m
Epididymides: cellular debris in ductules	0/20	0/20	1/20	0/20	12/20***
Epidiymides: Low spermatozoa count	0/20	0/20	1/20	0/20	8/20**
Testes: degenerated tubuli	0/20	0/20	1/20	0/20	9/20***
Testes: giant cells in tubuli	0/20	0/20	0/20	0/20	5/20*

*significantly different from control $p<0.05$, **$p<0.01$, $p<0.001$

Similar results of testicular and epididymal degeneration were not reported in the carcinogenicity study. However, prostatic atrophy showed an increased incidence at the HD while focal hyperplasia showed a decrease at the MD and HD. Testicular vasculopathy, also undefined, showed a statistically significant decrease at the MD and HD.

Rat carcinogenicity study: males

	control	veh	2.5 mg/kg	10 mg/kg	40 mg/kg
Prostate: diffuse atrophy	5/50	4/49	4/50	7/49	12/50
Prostate: focal hyperplasia	9/50	7/49	10/50	2/49	1*/50
Testis: mineralization	9/50	12/50	13/50	15/49	17/50
Testis:vasculopathy	15/50	14/50	7/50	3/49**@@	1/50**@@@

*$p<0.05$, **$p<0.01$, ***$p<0.001$ vs control group, @$p<0.05$, @@$p<0.01$, @@@$p<0.001$ vs vehicle

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In the 3 month mouse study, the number of mice with corpora lutea present was decreased by half compared to the controls. The incidence of cystic ovaries was significantly decreased.

3 month mouse study: females

	control	LD 10	MD 40	High (160)
Ovaries: Corpora lutea	10/10	ND	ND	5/10**
Ovaries: cystic	4/10			0/10*

*p<0.05, **p<0.01, p<0.001

In the carcinogenicity study, the incidence of cystic ovaries was again decreased. A finding of "corpora lutea hyperplasia", undefined, also showed a decrease compared to control at MD and HD. The uterine findings indicated decreased glandular development, increased incidence of atrophy and decreased incidence of cystic uteri. The mammary glands showed significantly increased incidence of glandular development and a non-significant increase in glands with secretion present.

Cyclicity of the females was not directly or specifically mentioned. The histopathology associated with the vagina may or may not be indicative of cyclicity. That is, when vaginal cytology is performed, an increased presence of leucocytes is generally associated with either proestrus or diestrus. The actual stage of the cycle is not determined by the presence of leucocytes alone. Granulocytes may also indicate an infectious process. However, the information necessary to distinguish between estrus cycle and infection was not provided.

Mouse Carcinogenicity Study: females

	0 mg/kg	vehicle	2.5 mg/kg	10 mg/kg	40mg/kg
	f	f	f	f	f
Mammary gland: glandular development	11/50	7/50	10/50	8/50	22/49$^{*@@}$
Mammary gland: secretion present	1/50	1/50	2/50	4/50	4/49
Ovary: corpora lutea hyperplasia	5/50	4/50	5/50	3/50	2/49
Ovary cystic	41/50	32/50	37/50	38/50	25/49**
Uterus: cystic	37/50	24/50*	30/50	30/49	16/49***
Uterus: diffuse atrophy	2/50	4/50	2/50	0/49	7/49
Uterus: glandular devel	30/50	19/50*	23/50	27/49	7/49$^{***@}$
Uterus inflammatory cell infiltration	0/50	0/50	1/50	1/49	4/49
Vagina individual cell necrosis	13/50	17/49	17/49	14/47	7/45
Vagina infiltrating granulocytes	18/50	19/49	19/49	17/47	9/45

**p<0.01, ***p<0.001 vs control group $^{@}$p<0.05, $^{@@}$p<0.01 $^{@@@}$p<0.001 vs vehicle group

The rat study also showed a decrease in corpora lutea, an increase in atretic (aged) follicles and a decrease in cystic ovaries. Vaginal cytology that is primarily cornified epithelium is associated with estrus. Again, there is insufficient information provided to say that there is a difference in the mean cyclicity associated with drug treatment.

Rat 6 month study: female

	control	LD 10	MD 40	High (160)
Ovaries: atretic follicles	13/20	16/20	15/20	19/20*
Ovaries: corpora lutea	20/20	19/20	19/20	15/20***
Ovaries: cystic	3/20	1/20	1/20	0/20
Vagina: cornified epithelium	8/19	6/20	5/20	4/18

*p<0.05 vs control , ***p<0.001

The results seen in the 12 month study are consistent with those from the mice and the shorter duration rat studies. There was a decrease in corpora lutea and an increase in atretic follicles. Again, there is insufficient information to be able to comment about cyclicity vs vaginitis and metritis or mucometra.

Rat Oral 12 month study: females

	0 mg/kg	vehicle	5 mg/kg	20 mg/kg	80mg/kg
Ovaries: atretic follicles	12/20	9/20	9/20	11/20	17/19**
Ovaries: old corpora lutea	12/20	10/20	12/20	15/20	3/19*
Ovaries: recent corpora lutea	13/20	12/20	7/20	12/20	6/19
Uterus: Infiltrating granulocytes	4/20	8/20	8/20	3/20	0/19*
Vagina: cornification	11/19	13/20	7/20	4/20*	8/19
Vagina: mucification	5/19	6/20	5/20	6/20	8/19

The decreased incidence of cystic uteri was again seen, consistent with previous studies. The findings of "prominent sex cords" was undefined.

Rat carcinogenicity study: females

	control	veh	2.5 mg/kg	10 mg/kg	40 mg/kg
Mammary gld: Adenoma,fibroadenoma,adenofibroma	18/50	25/50	26/50	32/50**	15/50
Ovary :prominent sex cords	25/50	26/50	21/50	26/50	13*/50
Uterus:cystic	17/50	14/50	16/50	11/50	6/50

*p<0.05, **p<0.01, ***p<0.001 vs control group, $^{@}$p<0.05, $^{@@}$p<0.01, $^{@@@}$p<0.001 vs vehicle

3 month study in Beagles

	Dose mg/kg			
	0	2.5	10	40
No significant findings				

6 month study in Beagles

	Dose mg/kg							
	0		2.5		10		40	
	m	f	m	f	m	f	m	f
Prostate: chronic inflammation	1/4		4/4		4/4		3/4	
Prostate: chronic inflamm (urethra)	1/4		1/4		3/4		2/4	
Testis: aspermatogenic	0/4		0/4		0/4		1/4	

12 month study in Beagles

	Dose mg/kg							
	0		2.5		10		40	
	m	f	m	f	m	f	m	f
No significant findings reported								

Several findings were consistent in the female rodents: decreased corpora lutea, increased atretic follicles, increased uterine atrophy, and increased mammary stimulation. There was an isolated report of corpora lutea hyperplasia. The findings are not simple to interpret. The first question is whether all findings are simply random. If not random events, what are the possible mechanisms of action and what is the relevance to humans.

There are several considerations for the decreased number of corpora lutea and the increased number of atretic follicles.
It should be noted that oocytes themselves are lacking in receptors for gonadotrophins; follicular cells link hypothalamic activity to follicle and oocyte development. The activity of the corpus luteum is incompletely understood. LH, which is released in largest amounts in mid-cycle, causes the luteinization of granulosa and theca cells. Simultaneously, aromatization of androgens to estrogens is decreased and the quantity of progesterone increased. In the rat, prolactin also has significant luteotrophic and luteolytic properties. Prolactin appears to regulate the luteal receptor for LH. Prolactin may also be responsible for the lysis of mature corpora lutea during the estrous cycle, pregnancy and lactation because specific inhibitors of prolactin release can prevent luteolysis.

Drug-induced destruction of oocytes may manifest primarily by atresia rather than through necrosis and inflammation. Drugs that damage primordial or primary follicles may produce a delayed, but permanent, decrease in fertility. Ovarian atrophy as a result of drug treatment may appear as an absence of corpora lutea with no generalized atrophy as in the case of 17β-estradiol, diethylstilbesterol or tamoxifen or clomiphene.