CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-742

PHARMACOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21742
Review number: 2
Sequence number/date/type of submission: 0/November 12, 2004/
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: October22, 2007

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

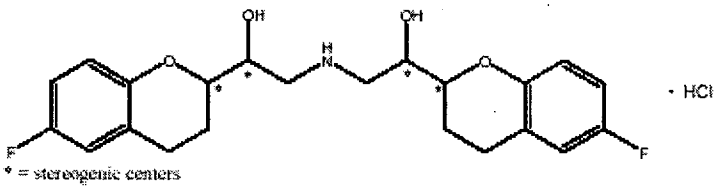
R067138 (d-Nebivolol)

R067145 (l-Nebivolol)

CAS Registry No. 152520-56-4

Trade Name: To Be Established

Figure 3.2-01 Chemical Structure of Nebivolol



* = stereogenic centers

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

Molecular Weight 441.90 g/mol

In the review of the complete response, I requested historical control data for sperm parameters. This review is the consideration of that material, received September 24, 2007.

Text-Table 3.8.1a. Sperm Parameters In The Rat (Terminal Sacrifice). * p<0.05, **p<0.01.

Terminal Sacrifice		Motile Sperm (%)	Progressively Motile Sperm (%)	Cauda Epididymis Sperm Count (millions/g)	Right Testis Sperm Count (millions/g)	Normal Sperm (%)
0 mg/kg/day	Mean	93	46	525	82	96.6
	SD	4	15	121	25	1.3
	n	10	10	10	10	10
100 mg/kg/day (Flutamide)	Mean	86	17**	391	63	79.4**
	SD	8	7	201	31	29
	n	9	9	10	10	10
10 mg/kg/day (Nebivolol)	Mean	94	54	564	92	96.1
	SD	4	17	100	27	1.8
	n	10	10	10	10	10
40 mg/kg/day (Nebivolol)	Mean	83	37	552	89	89.1*
	SD	14	14	111	12	6.3
	n	10	10	10	10	10
80 mg/kg/day (Nebivolol)	Mean	84**	33	485	76	84.4**
	SD	8	13	201	25	11.2
	n	10	10	10	9	10

Motile sperm %: The values presented for flutamide and nebivolol 40 and 80 mg/kg/day are outside of the historical controls for all four strains of rats listed in the historical controls (mean range from 90.2-95.7%).

Progressively motile sperm %: the values for flutamide, MD nebivolol and HD nebivolol are also outside the range of all listed historical controls (mean range 48.1-52.1).

Normal sperm: no values were listed for this.

Some increases in abnormalities were also reported in the complete response studies.

Rats | Terminal Sacrifice | Table 7

Number Animals		Normal Number	%	Decapitate Number	%	Head Abnormal Number	%	Neck Abnormal Number	%	Tail Abnormal Number	%	Mid Tail Blob Number	%
Group 1 – 0 mg/kg/day (Control)													
10	Mean	193.2	96.6	3.4	1.7	2.0	1.0	0.2	0.1	0.4	0.2	0.9	0.5
	SD	2.7	1.3	1.8	0.9	1.7	0.8	0.4	0.2	0.5	0.3	1.4	0.7
Group 2 – 100 mg/kg/day (Flutamide)													
10	Mean	158.5	79.4	16.0	11.3	15.5	7.8	0.7	0.4	3.3	1.7	2.1	1.1
	SD	59.1	29.0	37.1	28.8	24.8	12.4	1.1	0.5	7.7	3.8	2.4	1.2
Group 3 – 10 mg/kg/day (Nebivolol)													
10	Mean	192.1	96.1	4.4	2.2	1.4	0.7	0.1	0.1	0.6	0.3	1.4	0.7
	SD	3.6	1.8	2.0	1.0	1.0	0.5	0.3	0.2	1.1	0.5	1.2	0.6
Group 4 – 40 mg/kg/day (Nebivolol)													
10	Mean	178.3	89.1	8.5	4.2	3.2	1.6	0.9	0.5	1.0	0.5	8.2	4.1
	SD	13.8	6.3	7.3	3.6	1.9	0.9	1.0	0.5	1.6	0.8	11.5	5.8
Group 5 – 160/80 mg/kg/day (Nebivolol)													
10	Mean	169.1	84.4	14.5	7.2	6.3	3.1	2.4	1.2	1.1	0.5	7.8	3.9
	SD	22.3	11.2	12.9	6.5	6.5	3.2	2.3	1.2	1.5	0.8	14.7	7.3

*Significantly different from control mean; p≤0.05.
**Significantly different from control mean; p≤0.01.

Normal number %: the material provided lists the percentage in 4 strains of rats as >96%. Therefore, the flutamide, MD nebivolol and HD nebivolol are outside of the historical range.

Decapitate sperm: listed in the historical controls as <2%. All the nebivolol values are outside the range of historical controls.

Head abnormal: the historical controls are divided into "small", "short", "misshapen", "pinhead", "hook" etc. If one adds them together, the sum of head abnormalities appears to be ≤1% (including "head flat"). Flutamide and HD nebivolol are outside the range of the historical controls.

Tail abnormalities: summation of the different categories is ≤ 0.4. The nebivolol findings are just outside the historical range but could be considered to be at the fringes of historical variability. Mid tail blob was not listed in the historical values.

Mouse Parameters

Text-Table 3.8.2a. Sperm parameters in the mouse (Terminal sacrifice). * p<0.05, **p<0.01.

Terminal Sacrifice		Motile Sperm (%)	Progressively Motile Sperm (%)	Cauda Epididymis Sperm Count (millions/g)	Right Testis Sperm Count (millions/g)	Normal Sperm (%)
0 mg/kg/day	Mean	93	66	1053	251	76.4
	SD	6	19	207	69	13.6
	n	8	8	10	10	10
250 mg/kg/day (Finasteride)	Mean	92	53	776*	220	79.7
	SD	8	25	142	52	12.1
	n	9	9	10	10	10
10 mg/kg/day (Nebivolol)	Mean	85	56	847*	228	81.6
	SD	9	12	261	70	8.7
	n	7	7	10	10	9
40 mg/kg/day (Nebivolol)	Mean	80	55	782*	249	52.7**
	SD	16	26	244	68	21.6
	n	4	4	10	10	10
80 mg/kg/day (Nebivolol)	Mean	69**	17**	584**	215	49.9**
	SD	21	10	360	54	23.6
	n	7	7	10	9	9

Motility and counts: When compared to Control values, statistica

Motile sperm %: only the CD-1 strain of mouse was listed in the historical control data. The range for this parameter was 85-92%. All nebivolol-treated animals were outside the range of historical controls.

Progressively motile sperm %: the range listed for historical animals was 44-51%. The HD nebivolol was outside of this range.

Mice	Terminal Sacrifice	Table 7

Number Animals		Normal Number	%	Decapitate Number	%	Head Abnormal Number	%	Neck Abnormal Number	%	Tail Abnormal Number	%	Mid Tail Blob Number	%
Group 1 – 0 mg/kg/day (Control)													
10	Mean	131.2	76.4	2.5	6.2	5.4	2.7	3.4	1.7	16.3	11.1	6.4	3.2
	SD	67.2	13.6	1.8	12.3	4.0	2.0	3.4	1.7	9.5	4.0	5.0	2.4
Group 2 – 250 mg/kg/day (Finasteride)													
10	Mean	149.4	79.7	3.7	2.0	12.6	6.9	3.1	1.5	17.9	9.4	8.2	4.3
	SD	45.0	12.1	4.1	2.0	18.8	9.1	2.9	1.4	11.5	5.1	7.3	3.4
Group 3 – 10 mg/kg/day (Nebivolol)													
9	Mean	164.2	81.6	3.2	1.6	4.4	2.2	5.2	2.6	18.6	9.2	16.3	8.1
	SD	16.7	8.7	2.5	1.2	3.4	1.7	6.1	3.0	9.7	4.7	7.0	3.4
Group 4 – 40 mg/kg/day (Nebivolol)													
10	Mean	72.7	52.7	7.2	15.3	2.1	1.7	2.5	1.2	35.6	21.6	14.8	12.1
	SD	52.9	21.6	11.1	30.3	2.7	1.6	3.5	1.7	32.9	13.3	14.3	8.4
Group 5 – 160/80 mg/kg/day (Nebivolol)													
9	Mean	88.8	49.9	12.7	8.1	9.3	5.3	3.8	2.6	64.8	36.5	0.0	0.0
	SD	53.9	23.6	5.8	5.4	4.6	2.2	1.4	2.1	49.2	22.2	0.0	0.0

*Significantly different from control mean; p≤0.05.
**Significantly different from control mean; p≤0.01.

% normal : 76-81% for the historical controls. MD and HD nebivolol were outside the range of historical controls.

Decapitate%: ≤3.5% for historical controls. MD and HD were outside this range while the LD animals were at the edge of the range.

From Dr Davis-Bruno's consult on the complete response material:

1. Does the data support a drug-related effect upon spermatogenesis?

Response: Yes, there is a decrease in sperm count and morphology in mice and rats with nebivolol ≥ 40 mg/kg/day in study TOX 021-001. The effect is greater in mice than rats.

From the material submitted there appears to be a drug-related detrimental effect upon sperm count, motility and morphology in both rodent species. According to the re-evaluation of the histopathology from the dog studies, there was no detected effect on canine testes/sperm. As noted in the prior review, there was no statement as to the quality of the slides used for re-evaluation.

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
11/1/2007 07:19:26 AM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
11/1/2007 01:06:27 PM
PHARMACOLOGIST

The sponsor's results are shown below.

Antagonist Activity

ID Number	Client ID Number	Sample Matrix	Sample Test Range	Antagonist Activity Range	Activity Strength	Estrogenic Class
A04927	Acebutolol Raw Dru	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	> $1x10^{-4}M$	Strong	I
A04928	Timolol Raw Drug	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	> $1x10^{-4}M$	Strong	II
A04929	Sotalol Raw Drug	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A05321	Pindolol Raw Drug	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	> $1x10^{-4}M$	Weak	II
A04931	Nadolol Raw Drug	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A04922	Metoprolol Raw Drug	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A04923	Atenolol Raw Drug	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	> $1x10^{-4}M$	Weak	II
A04924	Bisoprolol Raw Drug	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A04925	Propranolol Raw Dru	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A04926	Carvedilol Raw Drug	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	> $1x10^{-5}M$	Strong	I
A04932	Betaxolol	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	> $1x10^{-4}M$	Weak	II
A05334	Nebivolol HCl, Mylan Lot No	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III

Agonist Activity Table

ID Number	Client ID Number	Sample Matrix	Sample Test Range	Agonist Activity Range	Activity Strength	Estrogenic Class
A04927	Acebutolol Raw Drug	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A04928	Timolol Raw Drug #	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A04929	Sotalol Raw Drug #5	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A05321	Pindolol Raw Drug #4	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A04931	Nadolol Raw Drug #	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A04922	Metoprolol Raw Drug #	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A04923	Atenolol Raw Drug	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A04924	Bisoprolol Raw Drug	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	> $1x10^{-5}M$	Weak	II
A04925	Propranolol Raw Dru	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A04926	Carvedilol Raw Drug	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III
A04932	Betaxolol	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	> $1x10^{-5}M$	Weak	II
A05334	Nebivolol HCl, Mylan Lot No	Powder in DMSO	$1x10^{-6}M$ - $1x10^{-3}M$	•	Non-Detect	III

If I am reading the tables correctly, it takes millimolar quantities of the other beta blockers to have any kind of estrogenic effect in this system. Under the conditions of the assay, nebivolol did not any estrogenic activity. This does not address the question of metabolites of nebivolol or the isomers.

Discussion on polyploidy and endoreduplication in the in vitro chromosome aberration test

The sponsor states that the findings of polyploidy in the chromosome aberration assays were fortuitous and within the range of historical controls. They further state that if these findings were real, a signal for aneugenic activity should have been observed. The claim is that there were no positive findings in the mouse lymphoma assay and the mouse micronucleus assay and therefore there was no true polyploidy.

Scientific Advisory Panel Review of Nonclinical and Clinical Research Data and Guidance on Proposed Responses to FDA Regarding Potential Endocrine Effects Associated with Experimental Exposures to Nebivolol

This report is essentially a written restatement of the company's position and points that they have made in past.

1. Page 6 of the report makes the statement:

> Review of the literature revealed that there were no reports of chemicals or drugs that induced Leydig cell hyperplasia or tumors in mice or rats that also caused these effects in humans. To further underline the lack of relevance to humans, the panel noted the absence of any genotoxic effects of nebivolol in a battery of genotoxicity studies. The Panel concludes that

a. The report does not specify what data in the literature is used to support the claim that the drugs causing these tumors in mice do not cause tumors in humans.

b. Genotoxic mechanisms of carcinogenesis are distinct from hormonal mechanisms. The absence of positive genotoxicity is not really pertinent to the discussion.

2. Two further comments in close proximity are also of note:

> carcinogenicity study. The Panel also noted the lack of an effect of nebivolol on basal corticosterone and/or adrenocorticotrophic hormone (ACTH) stimulated corticosterone levels in the mouse following one month administration at high dose levels (20 and 40 mg/kg/day). In the
>
> blockade). However, nebivolol administration to rats (unlike in mice) also blunted the increase of aldosterone and corticosterone levels to ACTH stimulation but did not affect the basal levels of these hormones. The mechanism for this effect of nebivolol on influencing the adrenocortical response to ACTH challenge in the rat is uncertain.

This blunting effect is similar to that of dexamethasone administered to Cushing's patients to blunt the production of ACTH.

3. > The histological and weight changes observed in the female reproductive tract were the result of decreased food intake and reduced body weight gain rather than a direct effect of nebivolol. The inactive ("resting") status of the female reproductive tract of rodents given nebivolol is typical for any chemical causing significant reductions in food intake or body weight gain, and is, therefore, not considered to be a direct effect of nebivolol.

This set of statements is not consistent with my experience as a veterinarian or a toxicologist. This is also contrary to the findings of others. One of the reliable effects of caloric restriction is a delay in the onset of reproductive senescence in females. Males show a feminization of gonadal steroids and a decrease in reproductive function(Leakey, Seng and Allaben "Influence of body weight, diet and stress on aging, survival and pathological endpoints in rodents: implications for toxicity testing and risk assessment." Reg Res Perspec 2004; 4(1):1-29). This will be submitted as one of the questions in the consult request.

4.

pathogenesis of Leydig cell tumors in mice. The lack of estrogen receptor activity in the mechanism of tumor production is further supported by the absence of systemic estrogenic effects in a wide variety of responsive tissues in chronic studies in three species (mouse, rat and dog) and the finding that nebivolol does not bind or have activity with estrogen receptors at therapeutically relevant and/or achievable concentrations.

a. No where in the study reports is there any mention of specific examination of any tissue for estrogenic effects. The original reports were of poor quality and contained statements presented as fact that we were informed were only hypotheses, unsupported by any data.
b. The tubuloalveolar differentiation of mammary tissue in the rat studies is considered a response to a xenoestrogen.
c. The lack of binding to estrogen receptors is also arguable as the sponsor did not examine the metabolites or the enantiomers.

5.

administration were the result of reduced food intake and reduced body weight gain. In females, the decreased ovarian weights and numbers of corpora lutea, increased atretic follicles, and atrophic changes in the uterus and vagina occurred only at doses of nebivolol that caused a significant (≥ 20%) loss of body weight. The tubular atrophy and degeneration of the testes in both rats and mice also was present only at doses of nebivolol that caused a similar body weight loss.

This has been addressed in previous reviews.

6.

Previous statements made with some study reports with regard to potential endocrine findings may have inappropriately raised concern as they often were hypotheses without supporting functional data. This Panel finds no additional evidence that would warrant any further nonclinical or clinical testing and concludes that nebivolol has a well justified and strong risk/benefit ratio.

a. I am unclear how including "hypotheses without supporting functional data" as fact in multiple reports that were signed and submitted to regulatory authorities should increase the level of comfort with the entire pre-clinical safety assessment

b. The last statement in this paragraph is absolutely inappropriate. Did the panel review the current and legacy clinical trials to the same degree as did the Division's Medical Officers and the consulting Medical Officers?

It was also noted that

> Dr. Werner Coussement, the current Global Head Toxicology/Pathology, Global Preclinical Development, for Johnson & Johnson Pharmaceutical Research & Development, was Director of Toxicology for Janssen Pharmaceutica NV at the study site when the general toxicology and reproductive and developmental toxicity studies were conducted. He was in attendance during the August 24-25 panel meeting and provided an initial explanation of statements made in toxicology reports that suggest the reproductive tract as one of the target organs of toxicity. He has provided to Mylan and the Panel, a white paper on the potential endocrinological effects of nebivolol in rodents (Appendix A) that provides more detailed explanation and supporting data for each study that may have attributed endocrine effects to nebivolol exposure. Dr. Coussement's conclusion provides an overall perspective of his white paper:
>
> > "Target organ changes were identified in toxicity studies in rats and mice at 20 and 40 mg/kg body weight of nebivolol and were confined to the adrenals. In the rat, the adrenal changes seem to be linked to a decrease in ACTH-stimulated corticosterone levels. In man, the possible effect of nebivolol on ACTH and corticosterone were examined (BEL-52, BEL-55, and CAN-09). From these investigations, these preclinical findings appear to be of no clinical relevance.

A citation from Dr Coussement's White Paper is shown here:

> In addition to the tumor findings in the mouse and adrenal findings in the rat, some additional changes were noted in the reproductive tract and were also believed to be suggestive of a high dose effect on the endocrine system. In an attempt to describe such findings within the report, often the study director, study toxicologist and/or the study pathologist put forth a hypothesis based on the available information to account for the finding(s) of interest. A listing of example comments has been prepared and is presented in Addendum 2 below along with the comments concerning the presence or absence of data to support the statements. It should be noted that the physiological relevance of many of these changes was very difficult to evaluate and was often confounded by concurrent detrimental changes in animal body weight and health. These statements were not statements of fact, rather they were often statements regarding possibilities for interpretation. For example, the statement that nebivolol has an affect on adrenal steroid biosynthesis or the statement that nebivolol has an affect on steroid metabolism were potential explanations/hypotheses for the apparent reduction in ACTH stimulated levels of aldosterone and corticosterone at 40 mg/kg in rats (106654). These direct statements have no direct mechanistic data (e.g. enzyme studies) to support them.

Target organ changes were identified in toxicity studies in rats and mice at 20 and 40 mg/kg body weight of nebivolol and were confined to the adrenals. In the rat, the adrenal changes seem to be linked to a decrease in ACTH-stimulated corticosterone levels. In man, the possible effect of nebivolol on ACTH and corticosterone were examined (BEL-52, BEL-55 and CAN-09). From these investigations, these preclinical findings appear to be of no clinical relevance.

Statements made within nonclinical study reports that referred to potential affects on the endocrine system were hypothetical and often did not have directly supporting mechanistic data. As such, these statements should be viewed only as potential explanations of findings and not as direct evidence of a direct affect of nebivolol on the endocrine system. Rather, many findings appear to occur secondary to severe general toxicity.

The clinical trials cited above have been described as inadequate by the medical officers from the Division of Metabolic and Endocrine Drugs. For completeness, included below is the sponsor's summary of various endocrine statements.

Addendum 2: Endocrine Statements in Non-clinical Study Reports

Study	Document Number	Page number	Wording From Report	Data Supporting Statement
Mouse onco	109066	9 of 1270	"This finding in combination with the Leydig cell hyperplasia at the same dosage group is considered to be due to the interference of nebivolol with the steroid metabolism at 40 mg/kg body weight/day, resulting in a disturbance of the hormonal balance. A hormonal imbalance of sex hormones in rodents might result in hyperplastic and/or neoplastic changes in the target organs."	Increased incidence of Leydig cell hyperplasia and Leydig cell adenoma at 40 mg/kg/day No steroid metabolism studies were performed No hormone levels were measured
		10 of 1270	"The findings of Leydig cell hyperplasia and increased incidence of benign Leydig cell tumors in males at 40 mg/kg body weight/day is considered to be due to the interference of nebivolol with the steroid metabolism at 40 mg/kg body weight/day, resulting in a disturbance of the hormonal balance. A hormonal imbalance of sex hormones in rodents might result in hyperplastic and/or neoplastic changes in the target organs."	Increased incidence of Leydig cell hyperplasia and Leydig cell adenoma at 40 mg/kg/day No steroid metabolism studies were performed No hormone levels were measured

		113 of 1270	This effect is estimated to be due to the interference of nebivolol with the steroid metabolism at 40 mg/kg, resulting in a disturbance of the hormonal balance (see also non-neoplastic changes in the male genital tract)."	Increased incidence of Leydig cell adenoma None; no steroid metabolism studies were performed
		114 of 1270	"This finding in combination with the Leydig cell hyperplasia at the same dosage group is considered to be due to the interference of nebivolol with the steroid metabolism at 40 mg/kg body weight/day, resulting in a disturbance of the hormonal balance. A hormonal imbalance of sex hormones in rodents might result in hyperplastic and/or neoplastic changes in the target organs."	Increased incidence of Leydig cell hyperplasia and Leydig cell adenoma at 40 mg/kg/day None; no steroid metabolism studies were performed No hormone levels were measured
Mouse onco	109066	225 of 1270	"In view of the accepted male treatment related effects and the attribution of these to interference with steroid metabolism it seems that some possibility of hormonal disturbance in females should be	Decreased body weight , ↑ mammary gland development, ↓ cystic ovaries, ↓ cystic uteri ↑ incidence of Leydig cell hyperplasia and Leydig cell

Study	Document Number	Page number	Wording From Report	Data Supporting Statement
			considered."	adenoma at 40 mg/kg/day No steroid metabolism studies were performed
3-mon mouse	106653	6 of 220	"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 cholesterol levels.	No steroid metabolism studies were performed No hormone levels were measured

			[illegible]	
Mechanistic mouse study #1	109053	1 of 149	"No consistent changes in basal plasma corticosterone and testosterone levels were observed. At the end of the treatment period, testicular cells of the individual male mice were dispersed and incubated in presence of vehicle, ACTH and HCG. Testosterone production *ex vivo* was not affected by nebivolol, R085547 and R085548 treatment."	Data show that daily oral administration of nebivolol and its enantiomers, d- and l-nebivolol for 1 month did not alter the basal plasma levels of corticosterone or testosterone, No change in ex vivo testosterone production following stimulation with HCG.
Mechanistic mouse study #2	109054	6 of 122 Author	"The physiological relevance of these changes is very difficult to evaluate. Some subtle chronic alteration of the hypothalamic-pituitary-testicular axis by R085547, the d-enantiomer, in mice might have increased the sensitivity of testicular androgen biosynthesis to the gonadotropins and/or of pituitary LH release to LHRH stimulation, amongst other possibilities. No consistent changes in plasma corticosterone were observed with the exception of a statistically significant decrease (-55%) after 40 mg/kg R085548, the l-enantiomer. This is in accordance with the results obtained in rats after 1-month of treatment and in female mice without exogenous stimulation. These data suggest that high doses of R085548, the l-enantiomer, might slightly alter adrenal steroid biosynthesis in rodents."	Plasma LHRH-stimulated testosterone levels increased after the racemate (+33% at 20 mg/kg and +40% at 40 mg/kg) without reaching the level of statistical significance. After 20 and 40 mg/kg d-nebivolol treatment, the testosterone increase was slightly more pronounced and statistically significant (+63 and +70% at 20 and 40 mg/kg), whereas a slight but statistically significant decrease in testosterone was observed after 40 mg/ kg l-nebivolol (-44%)."

Study	Document Number	Page number	Wording From Report	Data Supporting Statement
Mechanistic rat	106654	1 of 330	"These results demonstrate that the morphological and functional alterations of the adrenal gland observed after chronic dosing at 20 and 40 mg/kg body weight of nebivolol in rats are mainly due to R 85548, the l-isomer. However, no marked changes of the basal hormones levels were observed and the alterations in plasma corticosterone and mainly in aldosterone levels were obvious after ACTH stimulation only."	↑ adrenal weights and swollen cells in nebivolol, d- and l-nebivolol treated animals No change in basal corticosterone. ↓ in basal aldosterone in nebivolol 40 mg/kg; ↓ in post ACTH stimulation levels of aldosterone in males and females treated with 40 mg/kg nebivolol and 20 and 40 mg/kg l-nebivolol related females. treated males on day 1 and in nebivolol 20 and 40 mg/kg treated females on days 7 and 26 ↓ in renin in 40 mg/kg nebivolol treated females ↓ in post ACTH stimulation levels of corticosterone at 20 mg/kg in nebivolol and l-nebivolol treated females and at 40 mg/kg in nebivolol treated males and females, and d- and l-nebivolol treated females

Summary And Discussion

— Re-evaluation of testes, epididymides and prostate slides for dogs administered nebivolol for 3,6 or 12 months

Based on the material presented, there is no signal apparent for drug-effects on the tissues re-evaluated. There was no statement in the report assessing the adequacy of the histological methods or assessing the quality of the slides. The methods of collection, fixation, processing, sectioning and staining should produce slides of acceptable quality and this is a critical part of this re-evaluation. The lack of such a vital assessment, pivotal to the remainder of the report reduces the strength of the whole.

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.

Doses of 0, 5, 20, 80 mg/kg. Satellite animals used for tk.

Obvious clinical signs were seen at doses ≥20 mg/kg. Unscheduled mortality was seen in all dose groups including controls. At both day 14 and day 28 euthanasia and organ weight determination, increases were seen in the normalized weight of seminal vesicles, epididymus, and testes. Normalized prostate weight was increased at day 28. These findings are summarized below.

Summary of organ weight changes

	Dose of nebivolol mg/kg			
	0	5	20	80
Day 14 findings				
Normalized epididymus	0.295	0.258	0.292	0.319 (8%)
Normalized seminal vesicle	0.824	0.875	0.937	1.290
Normalized testes	0.699	0.699	0.713	0.790
Day 28 findings				
Normalized epididymus	0.296	0.264	0.325	0.359*
Normalized prostate	0.054	0.062	0.102(+88%)	0.079(+46%)
Normalized seminal vesicle	0.894	0.747	0.994(+11%)	1.125(+26%)
Normalized testes	0.7172			0.763(+6%)

The sponsor did not present any hormonal data in the report but stated that serum LH levels showed a statistically significant increase relative to control values at 6 hours post dose on Day 28. Non-significant increases were noted for the HD at 4 and 8 hours on Day 28 also and at 4,6 and 8 hours on Day 14. A contaminant in one of the assay reagents prevented the generation of meaningful estradiol values. The appendix for the hormonal analysis provided the sponsor's interpretation of results and listed assay results without any indication of the group or timepoint from which the sample was derived. There were no group summary tables. There was also no demonstration of data to support the statement that LH increases correlated with LC hyperplasia. This would have required identifying the specific animal, the LH value, and the histologic findings for the specific animal. No such organized correlation of material was located in the report. After the initial submission of this material, the sponsor was asked to provide datasets for

the hormone data. The sponsor did not provide the data for this study. After seeing the textual statement noted above with no accompanying data, I again specifically requested the datasets for this report.

Descriptive statistics of the datasets show tremendous within-group variability. On Day 14, the mean control LH values were greater than those of the HD drug-treated groups at 5 out of 7 points of determination. On Day 28, the control group's mean LH values were greater than those of the HD group at 4 out of 7 points of determination. I find the sponsor's statements that this study supports the increased LH hypothesis to be unconvincing. The reluctance of the sponsor to provide the data,(indicated by the necessity of asking twice for the datasets) does not promote credulity.

Effect of subcutaneous DHT administration on serum LH levels and Leydig cell proliferation following gavage administration of nebivolol for 28 days to mice

Doses: 0, DHT, Nebivolol 80 mg/kg ± DHT
Overt clinical signs were reported for all groups except the vehicle control group. Nebivolol caused an 11% increase in normalized testicular weight. Adding DHT caused a decrease in normalized testicular weight of 11%.

The serum hormone results were presented graphically without error bars. Analysis of the dataset showed considerable variability within each group. If the sponsor had done the analysis by removing hormonal "pulses" as was done in the 13 week study, there would have been no difference between the groups. Some of the data is summarized below for illustrative purposes. The sponsor states in the Appendix that secretion of hormones is pulsatile, and levels of hormones may vary from 20-50 fold in an individual. This is also the basis of the sponsor's statement that historical controls are meaningless and therefore not provided. Yet in both human and veterinary clinical practice endocrine-related conditions are sometimes diagnosed based upon the comparison of a sample value with a laboratory reference range, something based upon historical controls. It is difficult for me to say that there is a real difference between treatment groups in this study.

parameter	Time point	Vehicle control	Nebivolol 80 mg/kg
LH	Day 28, 4-hour	1.05±1.52 (0.31-8.25)	1.82±2.47 (0.32-9.84)
LH	Day 28, 6-hour	0.876±0.846 (0.15-4.54)	1.77±2.41 (0.28-10.85)
testosterone	Day 28, 4 hour	2.76±5.13 (0.1-20.72)	1.35±2.73 (0-10.27)
testosterone	Day 28, 6 hour	3.38±7.95 (0.12-37.11)	2.26±3.17 (0.02-10.48)

Of the 57 animals given just nebivolol, 44/57 showed Leydig cell hyperplasia. Given the range of LH values, it could be inferred that some animals with LC hyperplasia did not necessarily have high serum levels of LH at the time of determination.

Of the 52 animals given nebivolol+DHT, 0 were reported to have Leydig cell hyperplasia. Increased apoptotic-like bodies in the seminiferous tubules was seen in 39/57 (nebivolol) and 36/52 nebivolol+DHT. The sponsor does not compare measured LH with histologic results.

13 Week endocrine evaluation study in male CD-1 mice and Wistar rats with a 2-week and 1-month interim sacrifice and a 1-month recovery period.
Rat doses: 0, 10, 40, 160/80, flutamide(positive control). HD decreased day 21, 2 week drug-free period.
Mouse doses: 0,10, 40, 160/80 finasteride(positive control). HD decreased day 15, 2 wee drug-free period.

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.

Sperm parameters

	Vehicle control	Nebivolol dose (mg/kg)			Positive control
	0	10	40	160/80	flutamide
Rats at terminal euthanasia					
Motile sperm%	93±4	94±4	83±14	84**±8	86±8
Progressively motile sperm	46±15	54±17	37±14	33±13	17**±7
Right testis sperm count (millions/g)	82±25	92±27	89±12	76±25	63±31
Normal sperm %	96.6±1.3	96.1±1.8	89.1*±6.3	84.4**±11.2	79.4**±29
Rats at recovery euthanasia					
Motile sperm%	85±24	94±5	76±29	52**±30	72±37
Progressively motile sperm	61±27	55±18	50±26	26**±23	46±26
Right testis sperm count (millions/g)	80±23	72±24	83±27	59±32	63±29
Normal sperm %	94.8±5.3	97.4±1.6	84.9±25.4	53.9**±31.9	70.3*±38.3
Mice at terminal euthanasia					finasteride
Motile sperm%	93±6	85±9	80±16	69**±21	92±8
Progressively motile sperm	66±19	56±12	55±26	17**±10	53±22
Cauda epididymus sperm count (millions/g)	1053±207	847*±261	782*±244	584*±360	776*±142
Right testis sperm count (millions/g)	251±69	228±70	249±68	215±54	220±52
Normal sperm %	76.4±13.6	81.6±8.7	52.7**±21.6	49.9**±23.6	79.7±12.1
Mice at recovery euthanasia					
Motile sperm%	76±28	84±18	92±1	96**±2	84±13
Progressively motile sperm	42±30	38±24	39±27	62±19	35±29
Cauda epididymus sperm count (millions/g)	621±253	657±232	384±144	541±149	542±119
Right testis sperm count (millions/g)	167±64	172±57	143±64	164±81	176±57
Normal sperm %	71.7±8.3	74.6±7.6	65.5±24.7	81.0±13.6	77.5±8.1

The breakdown of sperm abnormalities indicates a dose related increase in sperm defects in nebivolol-treated animals.

Summary of sperm defects (mean±sd)

	Vehicle control	Nebivolol dose (mg/kg)			Positive control
	0	10	40	160/80	flutamide
Rats at terminal euthanasia					
Normal number	193.2±2.7	192.1±3.6	178.3±13.8	169.1±22.3	158.5±59.1
Decapitate sperm (%)	1.7±0.9	2.2±1.0	4.2±3.6	7.2±6.5	11.3±29
Mid tail blob(%)	0.5±0.7	0.7±0.6	4.1±5.8	3.9±7.3	1.1±1.2
Rats at recovery euthanasia					
Normal number	189.6±10.5	195.0±2.6	170.7±50.8	109.4±65.0	141.6±77.2
Decapitate sperm (%)	3.2±4.5	1.3±0.9	12.0±25.7	36.4±29.7	24.0±38.7
Midtail blob (%)	0±0	0.2±0.3	0.2±0.3	0.7±0.9	0.5±0.5
Mice at terminal euthanasia					finasteride
Normal number	131.2±67.2	164.2±16.7	72.7±52.9	88.8±53.9	149.4±45.0
Decapitate sperm (%)	6.2±12.3	1.6±1.2	15.3±30.3	8.1±5.4	2.0±2.0
Midtail blob(%)	3.2±2.4	8.1±3.4	12.1±8.4	0±0	4.4±3.4
Tail abnormal (%)	16.3±9.5	18.6±9.7	35.6±32.9	64.8±49.2	17.9±11.5
Mice at recovery euthanasia					
Normal number	134.8±38	149.8±14.8	122.8±56.2	115.3±68.8	149.4±31.5
Decapitate sperm (%)	2.5±2.0	1.4±0.8	8.2±15.4	1.2±1.2	2.2±1.0
Midtail blob(%)	0.5±0.6	5.9±4.7	7.2±3.8	0.3±0.7	4.3±3.6
Tail abnormal (%)	21.5±8.5	18.1±6.4	17.2±10.8	11.3±12.2	13.3±5.6

From the NDA review for Proscar:

Finasteride has no uterotrophic, gonadotropin-inhibiting, progestational, or anti-progestational activity in standard assays. In addition, finasteride produced no significant changes in renal, gastric, respiratory or cardiovascular function in standard assays performed on dogs.

Doses of finasteride as low as 1 mg/kg.po significantly reduced prostate size in beagle dogs treated for 4 or 15 weeks. Administration of Proscar to patients with BPH decreases prostatic DHT levels by 80%, with concomitant 10-fold increases in prostatic testosterone levels. Proscar therapy had no effects on plasma levels of cortisol, prolactin, TSH, thyroxine and estradiol or on plasma lipid profiles (cholesterol, LDL, HDL, triglycerides). Plasma LH, FSH and testosterone levels were increased by 10% in patients receiving Proscar. Treatment with 5 mg/day finasteride for 1 year produced a 20% decrease in prostate volume in man.

Leydig Cell Changes in Response to Elevated Serum LH

Chronic administration of finasteride at doses which produce plasma drug concentrations 10 to 100 times human exposure levels produce Leydig cell changes in rodents. Leydig cell adenomas were observed in mice after 19 months of treatment with 250 mkd finasteride (100 times human exposure). Leydig cell hyperplasia was observed in rats dosed for 1 year with 80 mkd (30 times human exposure) and in mice dosed for 19 months with 25 mkd (10 times human exposure). No Leydig cell changes were observed in the rat carcinogenicity study or in dogs treated with 45 mkd (130 times human exposure) for 1 year. The Leydig cell changes observed in rodents are associated with 200 - 300 % increases in serum LH levels. The elevated LH concentrations in rodents appear to be due to altered hypothalamic-pituitary feedback control of LH secretion. Administration of exogenous DHT to rats treated with high doses of finasteride (250 mkd) prevented elevation of serum LH levels and consequent Leydig cell hyperplasia (14 week study). Chronic finasteride treatment produces only slight (10%), nonsignificant increases in LH concentrations in men. Therefore, the available data suggest that the drug-related alterations in hormonal balance is a rodent-specific effect.

So should we reasonably expect to see changes in these parameters with nebivolol?

The magnitude of the LH change and the margin of exposure was part of the consideration for the safety profile of Proscar. A comparison of AUC and Cmax values for nebivolol follows. The values for the human plasma levels from single and multiple doses of 10 mg are from Elena Mishina, Ph.D., the Biopharmaceutics reviewer for the NDA.

Comparison of plasma levels of drug : mouse to human ratio

parameter	Compared to extensive metabolizers		Compared to poor metabolizers	
28-day toxicity study in mice Tox021-003:				
	d-nebivolol	l-nebivolol	d-nebivolol	l-nebivolol
NOAEL of 5 mg/kg				
AUCng.hr/ml*	668/7.5=89	116/12=9.7	668/105=6.4	116/528=0.22
Cpeak ng/ml	128/1.2=107	33/2.3=14.3	128/6.5=19.7	33/26=1.3
80 mg/kg				
AUCng.hr/ml*	10022/7.5= 1336	6770/12=564	10022/105=95	6770/528=12.8
Cpeak ng/ml	1509/1.2=12575	1032/2.3=449	1509/6.5=232	1032/26=39.7
13 week toxicology study: values from mice in week 13				
160/80 mg/kg				
AUC_{0-24} ng.hr/ml	33,679/7.5=4490	18,214/12=1518	33,679/105=321	18,214/528=35
Cpeak ng/ml	1718/1.2=1432	890/2.3=387	1718/6.5=264	890/26=34
40 mg/kg				
AUC_{0-24} ng.hr/ml	22,229/7.5=2964	10688/12=891	22,229/105=212	10688/105=102
Cpeak ng/ml	1238/1.2=1032	536/2.3=233	1238/6.5=190	536/6.5=82
10 mg/kg NOAEL				
AUC_{0-24} ng.hr/ml	3377/7.5=450	617/12=51	3377/105=3.2	617/105=6
Cpeak ng/ml	195/1.2=163	40/2.3=17	195/6.5=30	40/6.5=6

*$AUC_{0.5-8\ hr}$ for the animal value

Comparison of plasma levels of drug : rat to human ratio

parameter	Compared to extensive metabolizers		Compared to poor metabolizers	
	d-nebivolol	l-nebivolol	d-nebivolol	l-nebivolol
13 week toxicology study: values from rats in week 13				
160/80 mg/kg				
AUC_{0-24} ng.hr/ml	21549/7.5=2873	10692/12=891	21549/105=205	10692/528=20
Cpeak ng/ml	1224/1.2=1020	643/2.3=280	1224/6.5=188	643/26=25
40 mg/kg				
AUC_{0-24} ng.hr/ml	8586/7.5=1145	3330/12=278	8586/105=82	3330/105=32
Cpeak ng/ml	559/1.2=466	194/2.3=84	559/6.5=86	194/6.5=30
10 mg/kg NOAEL				
AUC_{0-24} ng.hr/ml	1194/7.5=159	335/12=28	1194/105=11	335/105=3
Cpeak ng/ml	73/1.2=61	22/2.3=10	73/6.5=11	22/6.5=3

For a patient receiving 10 mg per day, it appears that there is a reasonable margin of safety for extensive metabolizers. The difficulty is in African American patients receiving 20 mg once (possible twice?) a day, especially if they are poor metabolizers.

Is the mechanism rodent specific? There are several difficulties in interpreting the studies provided here.

1. The lack of a robust effect from the positive controls.
2. The intra-group variability of the hormone values.
3. The inconsistent analysis of the hormone values.
4. Very little estradiol data.

At present there is a pre-clinical statistical consult pending and a preclinical consult from the Division of Metabolic and Endocrine Drug Products. A final interpretation of these studies requires the considered opinions of others within OND.

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DEPARTMENT OF HEALTH & HUMAN SERVICES
Food and Drug Administration

Memorandum

Date: 9/21/07
From: Karen Davis-Bruno PhD; Pharmacology Supervisor; DMEP
Subject: Consult Request: Nonclinical endocrine effects
To: NDA 21-742 Nebivolol
Materials reviewed: draft 8/20/07 pharm/tox review NDA 21-742

Reference is made to my March 2006 review of a prior consult request which contains an overview of the nonclinical issues, data presented, conclusions and regulatory recommendations. Nonclinical data indicates perturbations in endocrine function, particularly for reproductive functions with nebivolol treatment. The findings identified in the nonclinical studies are confounded by inconsistencies apparent in the study reports and inadequacies of the study designs. Mylan Bertek has provided mechanistic studies designed to address the Leydig cell tumor findings in male mice. All other reproductive findings, including those observed in female animals are considered secondary to drug induced toxicities by the sponsor. The results of this type of strategy are inadequate in fully explaining the inconsistencies in the data.

Leydig cell tumors: The sponsor designed a 13-week endocrine study in male mice and rats (TOX 021-001) to assess and elucidate two of the most referenced mechanisms of Leydig cell tumorigenesis in mice; 1) stimulation of Leydig cells or 2) increased estrogen. Statistically significant increases in mean LH levels were observed in groups given ≥40 mg/kg/day nebivolol at the 13 week (but not 4 weeks or recovery week 17). Leydig cell hyperplasia was observed concomitantly. Finasteride (250 mg/kg; 5α-reductase inhibitor) was administered as a positive control, it increased LH levels after weeks 4 and 13 of treatment and caused a decrease in epididymis, prostate and seminal vesicle weight without detectable Leydig cell hyperplasia. The sponsor explains the absence of a target tissue response in the positive control as being at the threshold for quantitation. Leydig cell adenomas as well as 2X increases in LH are reported in a 19-month mouse carcinogenicity study with finasteride (see Proscar labeling). Consistent with this hypothesis is the absence of detectable elevations in LH and Leydig cell histopathology in rats. Flutamide (100 mg/kg; anti-androgen at nuclear receptor) given as a positive control produced Leydig cell hyperplasia in all treated animals and is associated with testicular interstitial cell adenomas as well as mammary adnomas/carcinomas and fibroadenomas in male rats in 2-year carcinogenicity studies at doses ≤ 50 mg/kg (see product labeling). One Leydig cell tumor was diagnosed as carcinoma in the 2-year mouse nebivolol study. This lesion was examined and discussed by an additional peer review Pathology Working Group organized by Mylan Bertek. This lesion was described as an expansive mass with some evidence of invasion into the tunica albuginea (capsule) with embolic tumor cells present in the blood vessels external to, but adjacent to the capsule without evidence of cellular anaplasia, increased mitosis or distant metastases. The lesion was considered a large, well differentiated adenoma by the

working group. [redacted] a consultant for Mylan Bertek suggests that focal Leydig cell hyperplasia and Leydig cell adenomas are related findings along a pathology continuum based on size relative to the seminiferous tubules. This criterion was established by the Society of Toxicologic Pathologists. Elevations in estradiol were not observed in any of the studies with male mice. An in vitro estrogen receptor binding assay does not suggest nebivolol binding.

In a 28-day study in male mice (TOX 021-003) nebivolol was administered by oral gavage rather than by diet. At 80 mg/kg, minimal Leydig cell hyperplasia with an increase in serum LH at 4, 6, 8 hours post-dose on day 14 and 28 (statistical significant at 6 h post-dose on day 28 only) was observed. No Leydig cell hyperplasia was observed at 20 mg/kg.

In a 28-day study of nebivolol (NEB-TX-02) administered by oral gavage to male mice increases in serum LH (4, 6 h postdose) and Leydig cell hyperplasia were observed in at day 28. These findings were suppressed by concomitant administration of DHT (dihydrotestosterone) via negative feedback on the hypothalamic-pituitary axis. Taken together this data supports Leydig cell tumors in mice may be mediated by LH surges using the same mechanism as finasteride. However the absence of LH elevations in rats despite testicular and sperm effects remains unexplained by this proposed mechanism.

Reprotoxicity:
Effects on survival and growth of offspring are observed in reproductive developmental assessments with nebivolol at durg exposures in the absence of maternal toxicity. When administered during late gestation, dystocia and decreased survival of progeny are observed which have not been reported for other beta-blockers. The sponsor suggests that these adverse effects are common to the drug class based on the pharmacologic activity. This is inconsistent with the data. Effects on blood pressure were seen in normotensive rats given > 10 mg/kg/d, yet the adverse repro effects were observed at lower doses of < 5 mg/kg/d which are below the established cardiovascular effect of nebivolol in rats. Concomitant toxicity (interpreted as weight loss and decreased food consumption) has been used by Mylan Bertek to explain the adverse sperm and testicular effects.

Adrenal effects: Adrenal adverse effects occur at high exposures; approximately >100X human therapeutic exposure and were present in the rat (160 mg/kg/day) only (not mice or dogs).

Clinical results: Three clinical studies have evaluated basal gonadotropins, sex hormones and prolactin following nebivolol treatment (4 weeks duration; 10 mg/day). The sponsor has provided the results of a 2-month study (NEB-PK-03) evaluating ACTH stimulation, LH, testosterone, and SHBG after 5 and 10 mg/day nebivolol. There were no observed changes in clinical endocrine parameters.

The consult requests a response to the following questions:

1. Do you agree with the sponsor's statements that decreased food consumption and decreased rate of body weight gain are typically associated with senescence or inactivity of the female reproductive tract?

Response: In my experience, decreased ovarian weights, numbers of corpora lutea, increased atretic follicles can be associated with maternal toxicity indicated by significant decreases in maternal body weight gain and food consumption during fertility and early embryonic developmental studies in rats and rabbits. The sponsor suggests that these findings as well as prolonged gestation, dystocia, cannibalism, estrus cyclicity, cohabitation interval, decreased pup weight or survival occurred as a consequence of ≥20% body weight decrement and attributes the testicular atrophy and degeneration in both rats and mice to this response. This is inconsistent with my experience.

2. Does the data support a drug-related effect upon spermatogenesis?

Response: Yes, there is a decrease in sperm count and morphology in mice and rats with nebivolol ≥ 40 mg/kg/day in study TOX 021-001. The effect is greater in mice than rats.

3. Do you agree that report NEB-TX-02 supports the sponsor's hypothesis of a LH hormone mechanism of tumorigenesis?

Response: A similar mechanism has been successfully employed to explain the presence of Leydig cell tumors in mice (but not rats) for finasteride. Study NEB-TX-02 examines the effect of subcutaneous administration of dihydrotestosterone on serum LH levels and Leydig cell proliferation following oral gavage administered nebivolol in male mice for 28 days. Mylan Bertek has proposed that the development of Leydig cell tumors in male mice (but not rats) occurs as a result of increased LH secondary to decreased testosterone in mice. Administration of DHT (2 mg/day) prevented this LH elevation suggesting that the tumors might be mediated by an androgenic rather than an estrogenic mechanism. Evaluation of the a 5α-reductase inhibitor; finasteride in the same study; has been associated with Leydig cell tumors in mice and also produced increases in LH. This study as well as another endocrine study in mice and rats (Tox 021-001) indicates that the LH levels increase in mice but not rats with nebivolol treatment. It is unclear how nebivolol selectively increased LH in male mice but not other species or in females. It is also surprising that similar decreases in estradiol in mice were not observed based on the increase in nebivolol mediated LH increases. However most of the endocrine studies utilized males and not females which may account for this discrepancy. There are issues with the assessments of LH variability in these studies and the estradiol measurements are confounded by a technical problem. In vitro binding data suggests a lack of appreciable estrogen receptor binding for nebivolol. There is precedent for acceptance of this mechanism to explain mouse specific Leydig cell tumors.

4. Do you agree that the mechanism of tumorigenesis appears to be rodent specific?

Response: Leydig cell tumors were not observed in the rat in a lifetime bioassay (2-year carcinogenicity study). Therefore the mechanism of tumorigenesis does not appear to be rodent specific. The presence of these tumors in the mouse at large exposure multiples relative to therapeutic exposure correlates with the observed increased LH levels which are antagonized by DHT is compelling and suggests that the Leydig cell tumors are species specific. This mechanism has been used to explain mouse Leydig cell tumors with finasteride treatment. It is unclear how LH elevations in male mice are regulated differently than in rats, humans or females of other species. Mylan's data suggests that the outcome of a clinical endocrine evaluation following 2-months treatment with nebivolol did not show perturbations in cortisol, estradiol or testosterone. This implies that the Leydig cell tumors have limited clinical relevance.

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/s/

Karen Davis-Bruno
9/21/2007 01:06:24 PM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21742
Review number: 1
Sequence number/date/type of submission: 0/November 12, 2004/
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: September 11, 2007

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

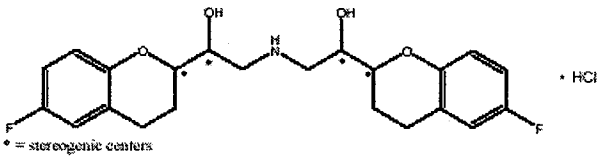
R067138 (d-Nebivolol)

R067145 (l-Nebivolol)

CAS Registry No. 152520-56-4

Trade Name: To Be Established

Figure 3.2-01 Chemical Structure of Nebivolol



* = stereogenic centers

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 material submitted was the sponsor's complete response to the Division's approvable letter.

Material Submitted

- *Method validation of an HPLC-UV assay for nebivolol in [redacted] polysorbate 80 in water*
- *Summary of preclinical evaluations of the potential effects of nebivolol on adrenal function in rodents (third draft, previously submitted)*
- *Re-evaluation of testes, epididymides and prostate slides from dogs administered nebivolol for 3,6 or 12 months*
- *Effect of subcutaneous dihydrotestosterone (DHT) administration on serum luteinizing hormone(LH) levels and Leydig cell proliferation following gavage administration of nebivolol for 28 days to mice*
- *Amendment of report for PHA021-005 Endocrine screening study for 12 beta blockers*
- *PharmSum*
- *Scientific advisory panel review of nonclinical and clinical research data and guidance on proposed responses to FDA regarding potential endocrine effects associated with experimental exposures to nebivolol*
- *13-week endocrine evaluation study in male CD-1 mice and Wistar rats with a 2-week and 1-month interim sacrifice(sic) and a 1-month recovery period*
- *10-day dose tolerance study of nebivolol administered by oral gavage to CD-1 mice*
- *TOX-021- 003 : 28-day study of nebivolol administered by oral gavage to CD-1 mice with a 14-day interim sacrifice(sic) to measure levels of luteinizing hormone and estradiol*
- *Discussion on polyploidy and endoreduplication observed in the in vitro chromosome aberration test with nebivolol.*

Study No. 06-9616 Re-evaluation of testes, epididymides and prostate slides for dogs administered nebivolol for 3,6 or 12 months. December 20, 2006

Study location: [redacted]
QA: "this study reflects the raw data as far as can reasonably be established"
GLP: no statement found

The studies re-evaluated were Janssen protocol numbers 1965, 1591 and 1896, originally conducted at Janssen Pharmaceutica, N.V.

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The re-evaluation of the slides was conducted [redacted]. Each animal was assigned a random animal number to evaluate the slides blindly with respect to treatment group. The original animal number was covered and the slides re-labeled with the newly assigned animal number.

Study 1591: 3 month oral subchronic toxicity study. The report states that on the basis of the histological findings of spermatogenesis, amount of sperm present and secretory expansion of the prostate, the dogs were sexually mature. The pathologist felt that there were no drug-related findings. The one exception noted was described as a subacute, chronic inflammation present in the periurethral tissue and/or within the prostatic tissue. This finding was reported as not present in the control animals and showing a dose-related incidence and severity in nebivolol-treated animals. The finding was dismissed as it was seen in control animals in the longer duration studies.

> no dose relationship. One exception was subacute, chronic inflammatory infiltrate, which was present in a periurethral location or within the prostatic tissue. Although this is a common background finding in Beagle dogs, it was not present in the control dogs of this study and it showed a dose related incidence and severity in Nebivolol treated animals. However, based on the absence of any dose relationship for this finding and its presence in control animals in the 6 month and 12 month studies, where Nebivolol was administered at the same or higher doses for longer periods of time (see below), the finding is considered incidental and unrelated to treatment in this study.

Study 1896: 6 month oral subchronic toxicity. It is reported that all dogs in the study were sexually mature. It is further reported that the microscopic findings were typical of lesions usually identified as background pathology and the findings showed no relationship to dose.

Study 1965: 12 month chronic toxicity. It is again reported that all dogs were sexually mature and that all findings were unrelated to drug-treatment. A subacute to chronic inflammatory process was reported in the prostate of dogs from all treatment groups including controls. Incidence and severity were no longer related to dose, leading the pathologist to the conclusion that the findings in the 3 month study were fortuitous.

Duration of study	Doses used mg/kg				
3 months		0	2.5	10	40
6 months		0	5	20	80
12 months	0	0	2.5	10	40

Something that was conspicuously absent from this report was an evaluation of the quality of the slides themselves. That is, I expected to see a brief statement as to the adequacy of fixation, section thickness, area of tissue suitable for evaluation and staining. While the method of fixation is most critical in studies of 28 days or less, it is still an important issue for studies of any length. The protocol used for generation of the slides and the physical quality of the slides should have been the first consideration. Even a statement to the effect that " the slides were of acceptable diagnostic quality" would have provided some reassurance that the pathologist was not evaluating between fractures in overfixed tissues.

The small sample size in these studies makes it unlikely to see a dose response effect. I have combined findings from different studies into one table.

Dose (mg/kg)	0	2.5	5	10	20	40	80
Total # animals	16	8	4	8	4	8	4
hypospermatogenesis		2	2				
Sperm stasis	1	1		1			1
Focal hypoplasia		2		2		1	1
Epididymus (subacute/chronic inflammation)	2	3	2	1	2	1	
abscess		1					
Prostate: periurethral inflammation Subacute/chronic	8	3	2	4	4	5	1
Prostate: acute inflammation						1	
Increased multinucleate cells						1	
Degenerate tubules+chronic inflammation						1	

Overall I don't see a signal in the material presented.

Appears This Way
On Original

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

Study location: [redacted]
Report number: Tox021-003
Study number: 1187-003
Study dates: initiated July 28, 2005
GLP; statement included
QA: yes
Test article: nebivolol hydrochloride, lot number 4L110, purity [redacted] by chromatographic assay

Three treatment groups (n=420/group) of male Crl:CD1®(Icr) mice were given the test article at doses of 5, 20 and 80 mg/kg/day. One additional group served as the control and received the vehicle [redacted] polysorbate 80 in water. The first 10 surviving animals/group were designated for Day 14 and Day 28 necropsies. Three additional groups of 56 mice/group served as TK animals and received the test article at the same doses as the main study group.

Observations

Signs: twice daily
Body weight, Food consumption: days -1, 7,14,21,28 for main study
Blood for hormone analysis: days 14 and 28 at 0.5, 1,2,3,4,6 and 8 hours post dose (non-fasted)
Necropsies: days 14 and 28 on first 10 animals/group
TK: days 14 and 28 at 0.5, 1,2,3,4,6 and 8 hours after dosing

Results

Exposure to drug

The homogeneity of the drug preparation was within ±4% of target at 5 mg/kg. At 80 mg/kg, the homogeneity was ±11% from target concentration. Average test article concentrations for the formulations were determined for days 1,7 and 14. The mean values of the test article concentrations were reported to be within ±10% of the target.

Unscheduled mortality

Unscheduled mortality was seen. The sponsor's summary is shown below.

Unscheduled Fates - Males			
Dose Level (mg/kg/day)	Animal Number	Day of Fate	Type of Fate
0 (Control)	3332	5	Found Dead
	3373	6	Found Dead
5	3743	19	Found Dead
20	3897	10	Found Dead
	4069	3	Found Dead
80	4488	23	Found Dead
	4497	25	Found Dead
	4500	19	Found Dead
	4515	17	Found Dead
	4656	9	Found Dead
	4663	19	Found Dead
	4680	9	Found Dead
	4839*	7	Found Dead
*Toxicokinetic animal			

Signs

5 mg/kg: no treatment related signs reported
20 mg/kg: yellow discoloration of the hair, ptosis starting day 2 and persisting throughout the study
80 mg/kg: decreased activity, yellow discoloration of the hair in ventral and anogenital regions, ptosis
Incidence of signs was dose-related

Body Weight

Body weight of main study animals in grams (mean ±sd)

Study day	0 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
-1	34.9±2.8	34.7±2.7	34.9±2.6	35.0±2.8
7	35.7±2.8	35.2±2.7	35.2±2.5	34.2±2.7(-4%)
14	36.3±2.7	35.9±2.7	35.6±2.5	33.1±2.7(-3%)
21	37.5±2.9	37.6±2.7	36.8±2.6	34.5±2.5(-8%)
28	37.9±3.1	38.2±2.7	36.8±2.8	34.1±2.5(-10%)

(percentage difference from the control group)
Compared to its own baseline, the HD group lost 2.6% body weight. The drop was entirely within the first week. Body weight remained stable thereafter. The MD group gained weight at a lower rate than the other groups.

Food consumption decreased corresponding to the decrease in body weight in the HD group.

Organ Weights

The normalized epididymal weight increased across dose groups as did seminal vesicle and testes' weight. The sponsor's summary is shown below.

Summary of Organ Weight Values - MALE

Table 5 — Day 14

Endpoint	0 mg/kg/day (Control) Mean	SD	N	5 mg/kg/day Mean	SD	N	20 mg/kg/day Mean	SD	N	80 mg/kg/day Mean	SD	N
Body weight g	34.7	2.3	10	34.3	2.3	10	33.9	1.2	10	33.4	2.8	10
Epididymides g	0.103	0.021	10	0.088	0.013	10	0.099	0.013	10	0.105	0.021	10
Epididymides/BWt %	0.2949	0.0496	10	0.2582	0.0383	10	0.2922	0.0306	10	0.3188	0.0827	10
Prostate gl g	0.018	0.007	10	0.023	0.009	10	0.014	0.005	9	0.019	0.007	10
Prostate gl/BWt %	0.0503	0.0189	10	0.0663	0.0246	10	0.0422	0.0145	9	0.0579	0.0209	10
Sem. ves. g	0.286	0.091	10	0.300	0.049	10	0.317	0.080	10	0.434[E]	0.136	10
Sem. ves./BWt %	0.8237	0.2526	10	0.8751	0.1346	10	0.9367	0.2441	10	1.2898[E]	0.3604	10
Testes g	0.243	0.031	10	0.239	0.029	10	0.242	0.054	10	0.263	0.023	10
Testes/BWt %	0.6985	0.0605	10	0.6994	0.0951	10	0.7133	0.1579	10	0.7899	0.0679	10

N - Number of measures used to calculate mean
SD - Standard Deviation
[E]Significantly different from control: ($p<0.01$)

Appears This Way
On Original

Summary of Organ Weight Values - MALE

Table 5

Day 28

Endpoint	0 mg/kg/day (Control) Mean	SD	N	5 mg/kg/day Mean	SD	N	20 mg/kg/day Mean	SD	N	80 mg/kg/day Mean	SD	N
Body weight g	34.5	3.2	10	35.8	3.0	10	34.4	2.5	10	32.0	1.9	10
Epididymides g	0.102	0.012	10	0.094	0.011	10	0.112	0.025	10	0.115	0.026	10
Epididymides/BWt %	0.2958	0.0216	10	0.2637	0.0274	10	0.3253	0.0643	10	0.3591*	0.0701	10
Prostate gl g	0.019	0.014	10	0.022	0.013	10	0.036	0.042	10	0.026	0.018	10
Prostate gl/BWt %	0.0536	0.0342	10	0.0618	0.0328	10	0.1023	0.1178	10	0.0785	0.0518	10
Sem. ves. g	0.308	0.051	10	0.267	0.063	10	0.341	0.071	10	0.357	0.128	10
Sem. ves./BWt %	0.8939	0.1383	10	0.7466	0.1718	10	0.9940	0.2141	10	1.1248	0.4078	10
Testes g	0.247	0.038	10	0.249	0.027	10	0.243	0.033	10	0.243	0.026	10
Testes/BWt %	0.7172	0.0972	10	0.6942	0.0581	10	0.7061	0.0819	10	0.7627	0.0831	10

N - Number of measures used to calculate mean
SD - Standard Deviation
*Significantly different from control; ($p<0.05$)

Hormone analysis

What is shown below is the sum total of hormone results presented in the text of the report:

> Serum luteinizing hormone levels were statistically significantly increased relative to control values at 80 mg/kg/day at 6 hours postdose on Day 28. Although not statistically significant, luteinizing hormone levels were also slightly increased at 80 mg/kg/day at 4 and 8 hours postdose on Day 28, and at 4, 6, and 8 hours postdose on Day 14. The LH AUC $_{0.5-8\ hr}$ was increased on Day 28.

> Due to a contamination in the extraction solvent used to the determination of serum estradiol levels [redacted], a meaningful interpretation of the data pertaining to estradiol levels from the Day 14 and Day 28 intervals could not be determined.

Appendix G, page 404, provides the interpretation of the hormone results by the laboratory who performed the assay analysis. The Background section provides the following information:

> Release of LH in males of virtually all species that have been examined in detail occurs in a pulsatile manner with pulses occurring every 1 to 2 hours. The concentrations of LH from peak to nadir during the pulse may vary more than 10-fold. Due to the expected variability, a large number of animals were incorporated into the design of the study. Each treatment group evaluated 30 animals. The rationale was that testing such a large number of animals would allow for data collection that would have the potential to reveal differences between control and treated animals as well as differences between doses of nebivolol.

> When evaluating an effect of LH on the testis, several different parameters may be considered. Some of these include the mean concentration of LH, the number of pulses of LH, the height of the pulses, the interval between pulses and the basal level of LH between pulses. The mean concentration is actually an integration of the other parameters.

The Appendix containing the description of the analysis listed the assay results without any indication of the group or time point from which the sample was derived. There were no group summary tables. The conclusion of the study report states that:

> The increased incidence of Leydig cell hyperplasia correlated with the increase in serum luteinizing hormone levels at 4,6 and 8 hours post-dose on days 14 and 28 (statistically significant at 6 hours post-dose on Day 28). The LH $AUC_{0.5\text{-}8hr}$ was increased on Day 28.

I would expect to see a table that summarizes individual animal LH levels and histological findings to support the above statement.

Gross changes
No macroscopic changes were reported.

Toxicokinetics

Day 14 values

parameter	5 mg/kg	20 mg/kg	80 mg/kg
Day 14 d,l-nebivolol			
$AUC_{0.5\text{-}8\ hr}$ ng.hr/ml	712.9	5149	14,663
C_{peak} ng/ml	171.2	1143	2498
Day 14 d- nebivolol			
$AUC_{0.5\text{-}8\ hr}$ ng.hr/ml	624.7	3767	8955
C_{peak} ng/ml	135.0	780.3	1464
Day 14 l-nebivolol			
$AUC_{0.5\text{-}8\ hr}$ ng.hr/ml	88.2	1382	5708
C_{peak} ng/ml	36.2	362.8	1034

Day 28 values

parameter	5 mg/kg	20 mg/kg	80 mg/kg
Day 28 d,l-nebivolol			
$AUC_{0.5\text{-}8\ hr}$ ng.hr/ml	784	4684	16791
C_{peak} ng/ml	156	830	2541
Day 28 d- nebivolol			
$AUC_{0.5\text{-}8\ hr}$ ng.hr/ml	668	3448	10022
C_{peak} ng/ml	128	603	1509
Day 28 l-nebivolol			
$AUC_{0.5\text{-}8\ hr}$ ng.hr/ml	116	1236	6770
C_{peak} ng/ml	33	227	1032

Histopathology

Distension of the seminal vesicles was observed in a small number of the mice administered 20 and 80 mg/kg/day nebivolol for 14 days. The incidence had decreased by 28 days of dosing. These changes correlated with an increased weight of seminal vesicles in these dose groups at these time periods. Nebivolol at 80 mg/kg/day produced an increase in incidence of Leydig cell hyperplasia. The text of the report stated that the increased incidence of Leydig cell hyperplasia correlated with observed organ weight differences and

with observed organ weight differences. The increased incidence of Leydig cell hyperplasia correlated with the increase in serum luteinizing hormone levels at 4, 6, and 8 hours postdose on Days 14 and 28 (statistically significant at 6 hours postdose on Day 28). The LH $AUC_{0.5-8\,hr}$ was increased on Day 28. At 20 mg/kg/day, treatment-related effects were

Summary of histological findings at 14-day interim euthanasia

	control	5mg/kg	20 mg/kg	80 mg/kg
Epididymides increased germ cells/cell debris	0	2	0	1
Seminal vesicles: distended	0	0	3	4
Testes: leydig cell hyperplasia	0	0	0	7
Testes: tubular vacuoles	0	0	0	1
Testes: increased germ cell degeneration	0	0	0	1
Testes: tubular dilation	0	0	0	1

Summary of histological findings at 28-day euthanasia

	control	5mg/kg	20 mg/kg	80 mg/kg
Seminal vesicles: distended	0	0	0	2
Testes: leydig cell hyperplasia	3	0	0	7
Testes: atrophic tubules (occasional)	0	1	2	1

Summary

As this report was presented, it is difficult to see this as supporting the sponsor's hypothesis.

1. The lack of interpretable estradiol data due to contamination of the reagents.
2. The lack of numbers (e.g. a summary table) for the LH data in the text of the report.
3. The lack of individual animal data for LH that is labeled as to dose group and time of collection.
4. There are organ weight changes reported to be associated with histopathological changes and specific hormonal changes. The sponsor does not summarize this anywhere on the basis of the individual animals involved and collate the actual numbers of organ weight, serum LH value and histological change.
5. The sponsor does not integrate the findings in this study with the longer duration studies in this species.
6. The sponsor does not integrate the findings in this study with the findings in other species, such as the rat.

July 19, 2007, the sponsor was contacted by email to provide the individual animal data for the LH results for this study and the 13 week study. It was requested that the results be labeled and organized so that they could be independently analyzed.

August 9, 2007

The datasets were received. I analyzed them in Excel and simply did descriptive statistics to determine the variability of the data. Shown below is a summary of the mean ±SD for the luteinizing hormone data as calculated from the sponsor's datasets.

Summary of Luteinizing hormone values (mean ±SD) : Day 14

	G1	G2	G3	G4	
		Day 14 luteinizing hormone means and SD			
Mean	1.58	1.349667	1.138667	1.013667	0.5 hour
Standard Deviation	2.624299	1.542062	1.573232	0.548776	
Mean	5.061034	2.616552	0.602414	0.733448	1 hour
Standard Deviation	5.687875	4.172004	0.343944	0.384673	
Mean	1.77	2.348621	1.754138	1.296207	2 hours
Standard Deviation	2.019524	3.862776	2.410282	3.20638	
Mean	3.166897	1.262414	1.364483	2.262759	3 hours
Standard Deviation	5.00695	1.797651	1.341485	3.440574	
Mean	2.543448	1.364828	1.312069	2.161034	4 hours
Standard Deviation	4.14772	2.298427	2.120112	3.00161	
Mean	0.726897	1.437241	1.22069	1.967241	6 hours
Standard Deviation	0.384699	2.269701	1.48947	3.54603	
Mean	2.778966	1.371724	1.865862	2.856207	8 hours
Standard Deviation	3.287907	1.594334	2.104029	3.134564	

Summary of luteinizing hormone values (mean ±SD): Day 28

	G1	G2	G3	G4	
		Day 28 luteinizing hormones means and SD			
Mean	1.187931	0.808966	0.671379	1.055517	0.5 hour
Standard Deviation	1.009007	0.4081	0.313946	0.63595	
Mean	0.851034	1.229655	1.155172	0.829655	1 hour
Standard Deviation	0.295458	2.076122	1.562195	0.303097	
Mean	3.113793	1.730345	1.83931	1.687586	2 hours
Standard Deviation	3.600495	2.239392	2.950685	2.890716	
Mean	2.357586	1.037586	1.558966	1.870345	3 hours
Standard Deviation	2.415691	0.870618	1.897699	3.124379	
Mean	1.273793	1.808276	1.153448	2.647931	4 hours
Standard Deviation	0.995441	2.547021	0.950847	3.840299	
Mean	1.437931	1.204138	1.383103	3.815517	6 hours
Standard Deviation	2.274753	1.591337	1.561984	5.238789	
Mean	1.490667	1.308	1.64	2.043	8 hours
Standard Deviation	1.502028	1.574478	1.644074	3.588967	

The analysis as described in the Appendix states that the sponsor analyzed the data by first calculating summary statistics for each treatment group by day and hour. An ANOVA was calculated for each day and hour and Dunnett's test was also used. Further analysis was carried out using Tukey's normal scores on the ranked response data "to ensure that extreme values would not have undue impact on results and to stabilize within-group variances."
The Appendix further states

> The general step-down trend test was implemented on the Day 14 data and Day 28 data to check for a dose trend. The general step-down trend test was run with all treatment groups, using the contrast (-1.5 -.5 .5 1.5) for the full dose range, (-1 0 1 0) for the dose range up to Nebivolol mid dose, and (-1 1 0 0) for the dose range up to Nebivolol low dose. An additional trend test was run on just the Nebivolol treatment groups, using the contrast (-1 0 1) for the dose range up to Nebivolol high dose, and (-1 1 0) for the dose range up to Nebivolol mid dose.
>
> Additional analyses were performed on these data. For each group and week, an iterative program was run to identify values greater than mean + 2 SD. A categorical analysis was run, with the larger values identified as pulse and the smaller values identified as baseline. In an additional analysis, the pulse values were deleted from analysis and a series of ANOVAs was run on the remaining data. The analyses were repeated using the normal scores.

Dr Chapman's analysis: ANOVA analysis was supplemented with Wilcoxon scores.

> There was sufficient material available to obtain an accurate value for the concentration of LH in serum from almost every animal in the study. Based on the analyses performed by Mylan statisticians, and those performed by Dr. [redacted], the only significant increase in serum concentrations of LH relative to control animals in this study was at the 6 h time point on Day 28 of treatment in the high dose animals (control = 1.57 ± 0.4 ng/ml and high dose = 4.19 ± 1.01 ng/ml, P<0.05). There was also a tendency for LH concentrations to be elevated at 4 h and 8 h after treatment with the high dosage of nebivolol on day 28. Taken together, these data suggest that drug treatment (high dose only) increased concentrations of LH by 28 d of treatment. Although not statistically different than control, concentrations of LH appeared to be elevated at the same time points (i.e. 4, 6 and 8 h) on d 14 of treatment in the high dose group. Drug concentrations reached near maximum levels within 30 min of administration (see Preclinical Pharmacokinetic Report for TOX 021-003).

Given the number of timepoints and the similarity of values between the treatment groups, is there in fact a true effect on day 28 or is this due to chance? If one looks at the timepoints from earlier in that sampling day, it could be said that the mean control sample values are greater than the drug-treated. A statistical consult regarding the methodology has been requested.

Effect of subcutaneous dihydrotestosterone (DHT) administration on serum luteinizing hormone (LH) levels and Leydig cell proliferation following gavage administration of nebivolol for 28 days to mice

Study number: [redacted]
Report number: NEB-TX-02
Study number: 1281-001
GLP: statement included
QA: yes
Study dates: experiment started July 6, 2006
Test article: nebivolol lot#ZRO67555PUB061, purity [redacted] by chromatography
nebivolol at 80 mg/kg given po in a vehicle of [redacted] polysorbate 80
Dihydrotestosterone in sesame oil given subcutaneously at a dose of 2 mg/day
Bromodeoxyuridine: was prepared in phosphate buffered saline on the days that it was administered

Male Crl:CD1®(Icr) mice, approximately 8-9 weeks of age at the start of dosing were used.

Study Design Summary

Group#	Dose level/route of administration		# of male main study +satellite animals [a,b]
	DHT (mg/day, SC)	Nebivolol (mg/kg/day,PO)	
1	0	0	60+20
2	2	0	60+20
3	0	80	60+20
4	2	80	60+20

SC-subcutaneous, PO-oral gavage

[a] The first 30 main study animals/group began dosing1 day prior to the last 30 main study animals/group. The satellite animals/group began dosing 5 days after the first main study animals per group. Study functions were conducted relative to the respective start dates.

[b] The additional 20 satellite animals/group were used for BrdU labeling and possible future microscopic assessment. Approximately 4 hours after test article administration these animals were given 1 ml intraperitoneal injection of a 1mg/ml BrdU solution once daily for 7 days prior to necropsy.

Signs: days-1, 7,14,21,28
Weight, food consumption: pretest and weekly
Hormone analysis: 4 and 6 hours post-dose Day 28 from cohorts of 30 main study animals who were then necropsied
Necropsy: performed on main study and satellite animals euthanized in extremis or found dead

Results

Drug Exposure

The concentration of the nebivolol dosing preparation was found to be within 10% of the targeted concentration.

Unscheduled Mortality and Clinical Signs

Unscheduled mortality was reported:

0 mg/kg Nebivolol// 2 mg/day DHT : 1 male
80 mg/kg Nebivolol//0 mg/day DHT : 4 males
80 mg/kg Nebivolol//2 mg/day DHT : 16 males died or were euthanized moribund.
Signs associated with these animals included decreased activity, impaired righting reflex and cold to touch.

Signs reported

Both Nebivolol groups (±DHT) ptosis, unkempt appearance
DHT alone: lower incidence of ptosis

0 nebivolol// 2 mg/day DHT : 11 mice unkempt
80 mg/kg nebivolol// 0 mg/day DHT : 17 mice unkempt
80 mg/kg nebivolol// 2 mg/day DHT : 26 mice unkempt

Swelling over the dorsum was observed in the majority of control and treated animals of all groups. This was reported to have occurred as early as Day 7 and persisted for the duration of the study. This was considered to be related to the SC injection of the sesame oil.

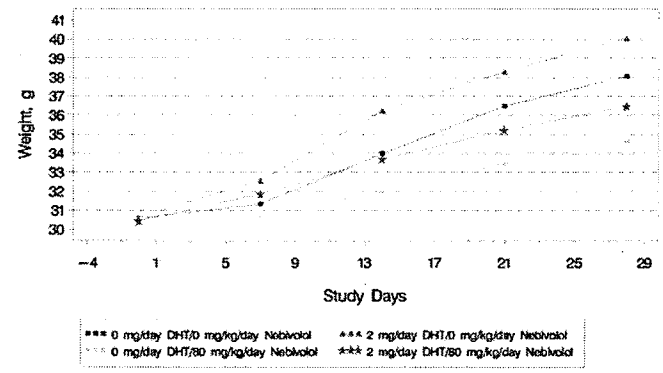
Body Weight and Food Consumption

DHT by itself caused a greater amount of weight gain than was seen in the control groups as might be expected. Nebivolol by itself caused approximately a 50% decreased rate of gain vs the control group. The addition of DHT to nebivolol caused only a partial alleviation of the decreased rate of gain.

While the sponsor provided a summary table, my calculations did not quite match the sponsor's numbers.
Reviewer's calculation of body weight changes

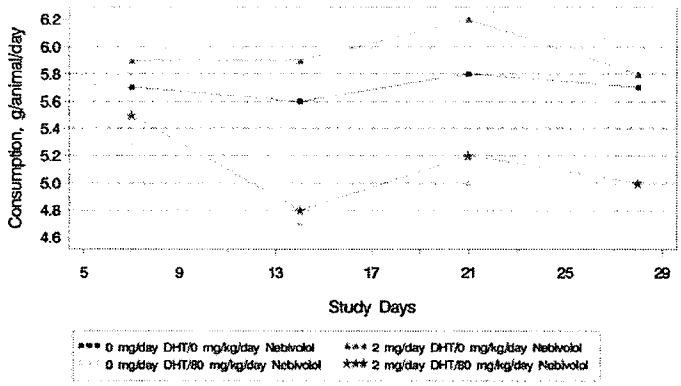
Study day	Control group	2 mg/day DHT 0 mg/kg/d nebiv	0 mg/day DHT 80 m/k/d nebiv	2 mg/day DHT 80 m/k/d nebiv
-1	30.58 ± 1.3	30.65 ± 1.7	30.59 ± 1.4	30.44 ± 1.3
28	38.07 ± 2.5	40.07 ± 2.3	34.63 ± 1.8	36.48 ± 2.0
Difference from day 28-1(mine)	7.49	9.42	4.04	6.0
Sponsor's numbers	7.62	9.65	4.00	5.60

Figure 1 Mean Body Weight Values – MALE



As expected, food consumption was increased in the DHT-treated group. The addition of DHT to the nebivolol group produced a slight increase in food consumption.

Figure 2 Mean Food Consumption Values – MALE



Organ Weights

Nebivolol did not produce a detectable effect on absolute testicular weight but caused an 11% increase in normalized weight. Interestingly, the addition of DHT caused an 11% decrease in normalized weight which was statistically significant compared to the control. In the satellite group, nebivolol caused a 13% increase in normalized weight while the addition of DHT caused only a 6% decrease in normalized weight compared to the control.

Summary of organ weight values (mean±SD) for main study group

endpoint	0 mg/kg DHT 0m/k/d nebiv	2 mg/day DHT 0m/k/d nebivol	0 mg/day DHT 80 m/k/d nebivol	2 mg/day DHT 80 m/k/d nebiv
Body weight (g)	36.7±2.3	38.6±2.1	33.3±1.7	35.0[b]±1.8
Testes(g)	0.255±0.030	0.218±0.024	0.259±0.032	0.216±0.027
Testes/BW %	0.6970±0.086	0.5671±0.068	0.7766±0.093	0.6171[b]±0.076

[b]significantly different from control ($p<0.01$)

Summary of organ weight values (mean±SD) for satellite study group

endpoint	0 mg/kg DHT 0m/k/d nebiv	2 mg/day DHT 0m/k/d nebivol	0 mg/day DHT 80 m/k/d nebivol	2 mg/day DHT 80 m/k/d nebiv
Body weight (g)	38.7±2.7	40.7±1.7	35.5±2.0	35.6[b]±2.2
Testes(g)	0.248±0.035	0.238±0.026	0.257±0.032	0.215[a]±0.029
Testes/BW %	0.6429±0.1014	0.5857±0.061	0.7235±0.077	0.6055±0.083

[b]significantly different from control ($p<0.01$) [a] significantly different from control ($p<0.05$)

Hormone Analysis Results

Something immediately apparent in the sponsor's graphs is the lack of error bars.

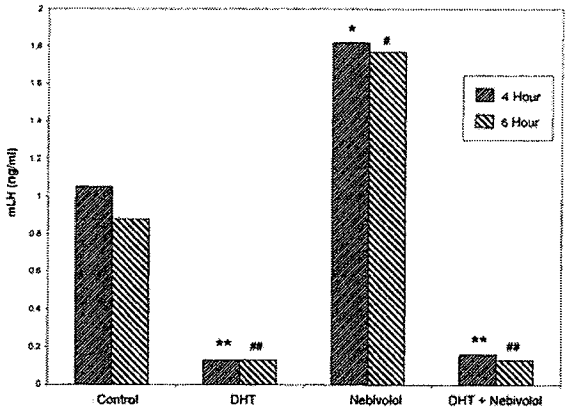


Figure 1. Serum concentrations of LH in mice after administration of vehicle (control), DHT, nebivolol, or DHT + nebivolol for 28 days. Bars with a single * or # differ from control by P<0.01 and P<0.05, respectively. Bars with a double * or # differ from control and from nebivolol alone by P<0.0001.

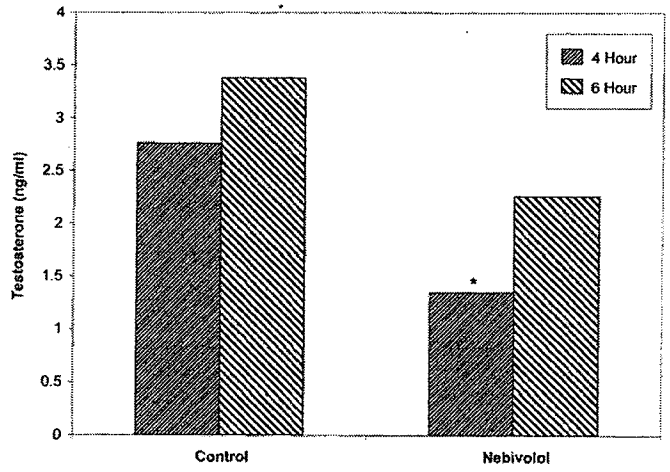


Figure 2. Serum concentrations of testosterone after administration of vehicle or Nebivolol to male mice for 28 days. Bars with a single * differ from control by P<0.0005.

The individual animal data for the LH results was found on page 254. The "ND" found in the tables was defined on page 325 to mean "non-detectable level ≤0.13 ng/ml"). This is noted in the table below. It was also noted that for analysis, LH values below the detection limit of 0.070ng/ml were changed to 0.070. I have included a summary of the values that I calculated. While the numbers do not exactly match the sponsor's, the interpretation is the same.

summary of LH values from p.254 of report

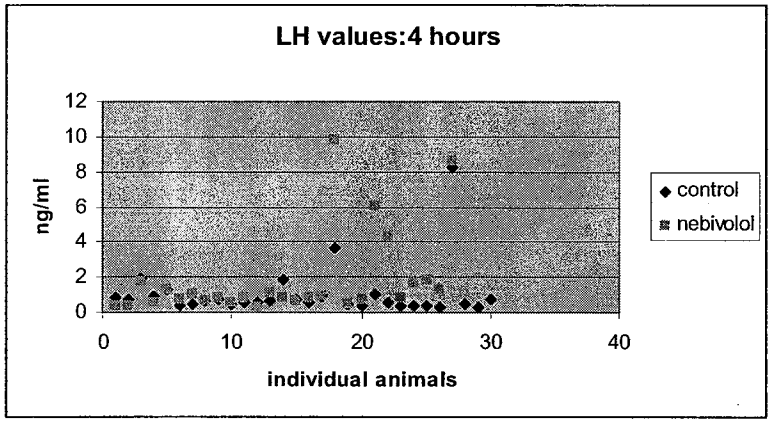
Day 28, 4 hour value			
control	DHT	Nebivolol (3 samples missing from 4 hour determination)	DHT/nebivolol (9 samples missing from 4 hour determination)
1.05	0.18 **(16 o/o 30 samples ND)**	1.82 (n=28)	0.20 **(8 o/o 24 samples ND)**
Day 28, 6 hours value			
0.88	0.19 **(19 o/o 30 samples ND)**	1.77 (n=30)	0.19 **(17 o/o 28 samples ND)**

Summary of LH values (mean ±SD) from dataset as analyzed by the reviewer.

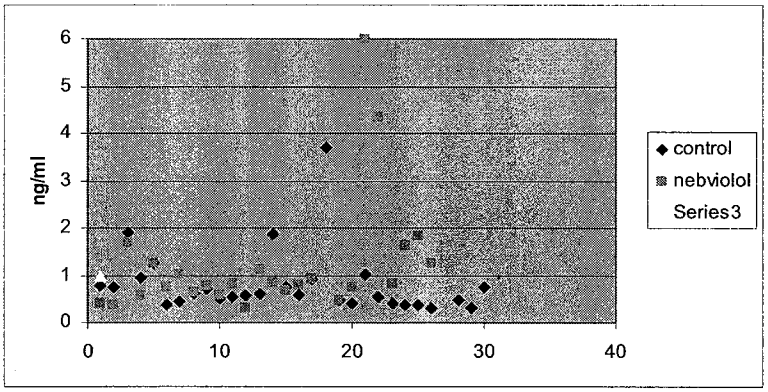
Day 28, 4 hour value			
control	DHT	nebivolol	DNT/nebivolol
1.05±1.52	0.13±0.067	1.82±2.47	0.15±0.083
Range: 0.31-8.25	Range: 0.07-0.26	Range: 0.32-9.84	Range: 0.07-0.38
Day 28, 6 hours value			
0.876±0.846	0.121±0.074	1.77±2.41	0.119±0.066
Range: 0.15-4.54	0.07-0.34	0.28-10.85	0.07-0.3

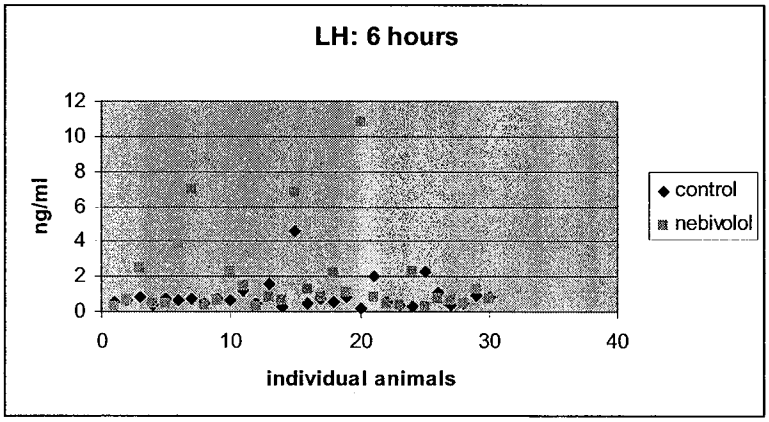
The value 0.07 was used instead of 0 for very low levels (this was the value listed in the dataset)

The graphs below are from my Excel data tables. If the scale was expanded sufficiently, a difference between the groups might be demonstrated.



4 hour values on an expanded scale





Different animals provided the blood for the 4 and 6 hour time points so the internal validation of longitudinal measurements is not available.

I looked to see if there was any obvious pattern between the individual animals with high LH values and the testosterone values for those animals.

Animal number	treatment	LH	testosterone
1003	control	1.89	0.641
1018	control	3.68	6.122
1027	control	8.25	0.7021
1005	control	1.24	5.888
1014	control	1.86	20.719
1021	control	1.01	0.333
3021	nebivolol	9.84	0.086
3024	nebivolol	6.01	Animal not found in testosterone records
3035	nebivolol	4.34	0.025 Found under 6 hour records
3030	nebivolol	8.61	4.853
3008	nebivolol	1.03	0.0985
3016	nebivolol	1.13	0.136
3027	Nebivolol	1.62	0.456
3028	Nebivolol	1.84	6.208
3029	nebivolol	1.27	0.209

6 hour LH and testosterone values

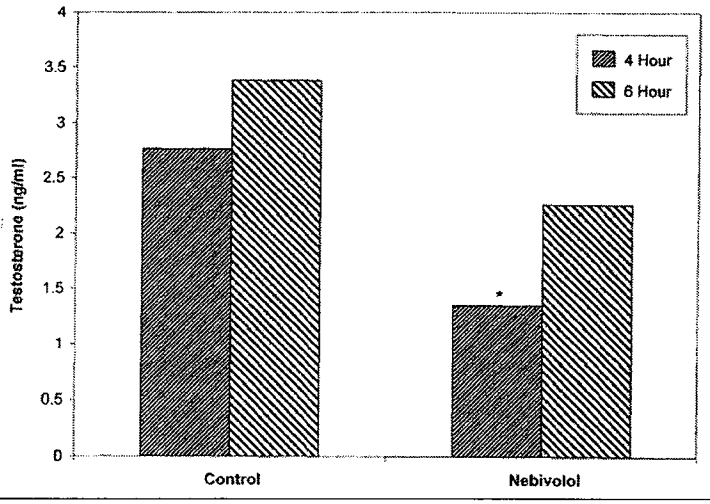
Animal #	treatment	LH	testosterone
1041	control	1.22	0.148
1043	control	1.52	11.308
1045	control	4.54	Not found in dataset
1051	control	2.06	0.234
1055	control	2.31	Not found in dataset
1056	control	1.11	37.226
3036	Nebivolol	3.86	10.483
3037	Nebivolol	6.92	1.36
3040	Nebivolol	2.29	6.467
3041	Nebivolol	1.5	0.296
3045	Nebivolol	6.82	4.804
3046	Nebivolol	1.29	0.271
3048	Nebivolol	2.18	5.716
3049	Nebivolol	1.13	5.605
3050	Nebivolol	10.85	0.172
3054	nebivolol	2.28	9.571
3059	Nebivolol	1.3	2.279

The single animal testosterone values were found starting on page 333 and appear to be the results that the processing lab faxed to the sponsor with minimal explanation. If I misinterpret any results, it can be attributed to the lack of explanatory information provided in this material.

Summary of testosterone values from labs values on page 333 as calculated in Excel: (numbers in parentheses represent the extremes of range). These numbers are consistent with the bar graphs in the body of the report (except that error bars are not shown in the graph).

Day 28, 4 hour value			
control	DHT	nebivolol	DNT/nebivolol
2.76±5.13 (0.1-20.72)		1.35±2.73 (0-10.27)	
Day 28, 6 hours value			
3.38±7.95 (0.12-37.11)		2.26±3.17 (0.02-10.48)	

Treatment of male mice with 80 mg/kg/day of Nebivolol for 28 days decreased serum concentrations of testosterone ($P<0.0005$) when measured at 4 hours after dosing compared to controls. When serum samples collected at 6 hours after dosing were compared to controls, the concentrations of testosterone were lower, although not statistically different ($P>0.25$). These results are depicted in Fig. 2.



Histopathology at 28 days
A summary of the sponsor's findings is shown below.

Histologic finding	control	DHT	nebivolol	DHT+nebivolol
Total number examined	60	60	57	52
Leydig cell hypertrophy/hyperplasia	0	0	44	0
Seminiferous tubules: increased apoptotic-like bodies	1	0	39	36
Leydig cell atrophy	0	60	0	51
Increased germ cell degeneration /sloughing	3	0	0	5
Tubular dilation	0	0	0	6

Toxicokinetics

Summary of Toxicokinetic Parameter Estimates for Nebivolol in Male CD-1 Mice following 13 Weeks tion of Nebivolol HCl in Food at Doses Ranging from 10 to 160mg/kg/day.

Dose Level (mg/kg/day)		10			40			160[1]		
	Group	3			4			5		
Parameter	Week in Study	2	4	13	2	4	13	2	8[1]	13[1]
d-Nebivolol										
$AUC_{0\text{-}24\,hr}$ [(ng•hr)/mL]		2675	3209	3377	16,958	21,577	22,229	38,783	36,859	33,679
C_{peak} (ng/mL)		146	171	195	800	1479	1238	2096	1717	1718
T_{peak} (hr)		20	16	16	24	4	20	16	4	4
C_{ss} (ng/mL)		111	134	141	707	899	926	1616	1536	1403
l-Nebivolol										
$AUC_{0\text{-}24\,hr}$ [(ng•hr)/mL]		500	621	617	7921	9648	10,688	32,709	23,184	18,214
C_{peak} (ng/mL)		35.4	39.9	39.7	376	532	536	1818	1140	890
T_{peak} (hr)		16	4	16	24	24	4	16	4	20
C_{ss} (ng/mL)		20.8	25.9	25.7	330	402	445	1363	966	759
d,l-Nebivolol										

Summary

The sponsor concluded that nebivolol increases the secretion of LH in male mice and that this is likely to be responsible for the increase in Leydig cell tumors. The sponsor further reasoned that the increase in LH was secondary to a decrease in circulating levels of testosterone in mice. Since the increased secretion of LH could be prevented by administration of DHT, the effect was considered to be mediated by an androgenic rather than estrogenic mechanism.

I agree with the sponsor on certain points and would see the study as supporting the sponsor's hypothesis if there was consistency across studies in the analysis of data.
If the hormonal pulses were removed from the analysis as was done in the 13 week study, there would be no difference between the LH values in the control and nebivolol groups. Even though the standard deviation of the LH mean is greater than the SD for the control group, usually an indication of an external effect (such as a drug), given the premise of 1-2 hour pulses of LH, it is difficult to interpret, not knowing the historical variation seen within the performing lab.

A 13-week endocrine evaluation study in male CD-1 mice and Wistar rats with a 2-week and 1-month interim sacrifice and a 1-month recovery period.

Study location: [redacted]
Study number: Tox021-001
HLS number: 04-2875
GLP: statement included
QA: yes
Study dates: initiated Feb17, 2005 for mice (dosing started March 31, 2005)
March 15, 2005 for rats (dosing started April 19, 2005)
Test article: the micronized test article was used in the belief that this would approximate the exposure achieved with the original material in βcyclodextrin.
Lot number 4L110, purity [redacted]

Positive controls: finasteride: 5α reductase inhibitor that inhibits the synthesis of DHT, [redacted] pure
Flutamide: has produced LCT in rats through a LH mechanism, [redacted] pure
Both positive controls were given in a vehicle of 0.5% methylcellulose

Mice: CD-1 COBS Swiss Albino [Crl:CD-1®(ICR)BR]
Approximately 8 weeks of age at receipt

Rats: SPF Wistar, Approximately 16 weeks at receipt
Male Wistar rats (80/group) and CD-1 mice (120/group) were fed daily with 0, 10, 40 or 160 mg/kg/day nebivolol for up to 13 weeks. An additional 80 male rats were gavaged daily with 100 mg/kg/day flutamide and an additional 120 male mice were gavaged with 250 mg/kg/day finasteride as positive controls. Adverse effects in both species necessitated decreasing the HD to 80 mg/kg beginning day 15 for the mice and day 21 for the rats. Despite lowering the dose, it was necessary to give both species a drug-free period of approximately 2 weeks, resuming treatment day 35 for the mice and day 42 for the rats.

At the end of weeks 2,4 and 13, 20 rats/group and 30 mice/group were euthanized and necropsied. The remaining mice and rats were given a 4 week drug-free recovery period, then euthanized and necropsied.

Sponsor's summary of study design for mice

Group	Denomination	Daily Doses*			Total	
		Dose (mg/kg)	Route	Conc. (mg/mL)	Tox	TK
1	Control (standard diet)	0	NA	NA	120	ND
2	Finasteride (Positive Control)[a]	250	Gavage (5 mL/kg)	50	120	ND
3	Nebivolol (Low Dose)	10	Dietary	variable	120	72
4	Nebivolol (Mid Dose)	40	Dietary	variable	120	72
5	Nebivolol (High Dose)	160/80[b]	Dietary	variable	120	72
6	Predose (Control for hormone assays)	0	NA	NA	30[c]	ND

Study design for mice continued

Group	Denomination	Number of Animals						
		Necropsy/Hormone Evaluations (Toxicity Animals)[c,d]				Microscopic Pathology[e]		Sperm Analysis[f]
		Week 2	Week 4	Term Week 13	Rec Week 17	PME/ MP	In situ	Weeks 13 and 17
1	Control (standard diet)	30	30	30	30	10	20	10
2	Finasteride (Positive Control)[g]	30	29	30	29	10	20	10
3	Nebivolol (Low Dose)	30	30	30	29	10	20	10
4	Nebivolol (Mid Dose)	30	30	29	29	10	20	10
5	Nebivolol (High Dose)	30	29[i]	30	20	10	20	10
6	Predose (Control for hormone assays)	NA	NA	NA	NA	NA	NA	NA

Sponsor's summary of study design for rats

Group	Denomination	Daily Doses[a]			Totals		TK[b]
		Dose (mg/kg)	Route	Conc. (mg/mL)	Tox	TK	Weeks 2, 4 and 13
1	Control (standard diet)	0	NA	NA	80	ND	NA
2	Flutamide (Positive Control)[i]	100	Gavage (5 mL/kg)	20	80	ND	NA
3	Nebivolol (Low Dose)	10	Dietary	variable	80	24	24
4	Nebivolol (Mid Dose)	40	Dietary	variable	80	24	24
5	Nebivolol (High Dose)	160/80[j]	Dietary	variable	80	24	24
6	Predose (Control for hormone assays)	0	NA	NA	20[c]	ND	NA

Group	Denomination	Dose (mg/kg)	Number of Animals						
			Necropsy/Hormone Evaluations (Toxicity Animals)[c,d]				Microscopic Pathology[e]		Sperm Analysis[f]
			Week 2	Week 4	Term Week 13[k]	Rec Week 17[k]	PME/ MP	In situ	Weeks 13 and 17
1	Control (standard diet)	0	20	20	20	20	10	10	10
2	Flutamide (Positive Control)[i]	100	20	20	20	20	10	10	10
3	Nebivolol (Low Dose)	10	20	20	20	20	10	10	10
4	Nebivolol (Mid Dose)	40	20	20	20	20	10	10	10
5	Nebivolol (High Dose)	160/80[j]	20	20	20	20	10	10	10
6	Predose (Control for hormone assays)	0	NA	NA	NA	NA	NA	NA	NA

The sponsor had contacted us before initiating the study with regard to a reversed light cycle for sampling purposes.
Observations included
Signs: daily
Body weight: twice pretest and weekly during treatment
Feed consumption: weekly

Hormone analysis: the sponsor states in one of the appendices (p.1019) that release of LH occurs in a pulsatile manner with pulses occurring every 1-2 hours. Furthermore, it is stated that concentrations of LH may vary more than 10-fold during the pulse. The sponsor uses this as a rationale for analyzing the LH data after removing the pulses. At a meeting prior to submission of the final reports, the Division requested that the data also be presented with the pulses included.

The interassay precision for estradiol for both species was listed as 6.4-23.9%. The interassay precision for the LH assay was listed as 3.8-9.6% CV. The sponsor makes an interesting statement about historical controls:

> A brief comment is in order concerning the historical reference ranges for our facility. It is our opinion that historical control ranges for reproductive hormones are virtually meaningless. This is due to the fact that hormones are secreted in a pulsatile manner and their concentrations may vary as much as 20-50 fold in an individual animal depending upon the time relative to the beginning or ending of a pulse when the sample was collected. Likewise, there are numerous parameters that can impact the mean concentrations of reproductive hormones within a species/strain of mice or rats. These include: time of day when the samples were collected, age of the animal, the relative stress the animal was experiencing immediately prior to the sample being collected, and the time samples were collected relative to feeding. These parameters have not been systematically examined in rats or mice but almost assuredly will have dramatic impacts on the serum concentrations of reproductive hormones. The reason these evaluations have not been made in mice and rats is that they would require collecting multiple samples from individual animals at frequent intervals for an extended time (i.e. 15-min intervals for 8 h). This requires a Herculean effort that is beyond the technical capabilities of almost all investigators. Moreover, as indicated above, the reference standard is the only parameter used in our assays that changes with time. For LH, the reference standard is prepared by purification of hormone from rat and mouse pituitary glands. It obviously will require thousands of pituitary glands to prepare a sufficient amount of reference standards for LH that is then used by many different laboratories around the world. The NIH has contracted with ———————— to prepare these standards. Due to the limited amounts of the standards that can be prepared at any one time, new standards are produced quite often. Over the years, the ability to prepare highly purified LH has improved. As such, the material that we are using as reference standard has changed over the years. As the purity of the reference standard has increased, the value that is reported for any given serum sample will decrease. Therefore, for LH it is not possible to compare absolute amounts of hormone reported over time even in the same samples from the same laboratory (i.e. baseline values with a previous standard may have been 5 ng/ml for LH, but measuring the same samples with all the same reagents and the new more highly purified reference preparation, the baseline value may be 0.7 ng/ml).

TK: samples were collected after 2,4,(8 for group 5 mice) and 13 weeks of treatment. Blood was collected over the period of one complete light cycle at 4 hour intervals (0,4,8,12,16 and 20 hours). 4 animals were sampled per time point. This was a terminal collection for mice.
4 TK rats contributed one sample per timepoint, 6 timepoints, 3 occasions during the study.

Necropsy: performed on animals from groups 1-4 up to 30/species per group after the animals had been treated for 2,4, or 13 weeks or following 13 weeks treatment and 4 weeks drug-free recovery. Limited macroscopic examinations were performed on up to 10 animals/species/group at each euthanasia. Testes, epididymides, prostate, seminal vesicles and mammary glands were examined for the presence of macroscopic abnormalities, collected and preserved.

At 13 week euthanasia, the left testis and epididymis were collected for histopathology. The right testis and epididymis were used for sperm analysis.

Organ weights were determined for up to 10 animals/species/group.
The sponsor's summary of tissue processing is shown below.

TABLE I

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY
adrenal glands[a]	X	X	
epididymides	X	X	X
mammary gland (inguinal)		X	X
prostate gland	X	X	X
seminal vesicles	X	X	X
testes	X	X	X
remaining organs (in situ)		X	

[a]Adrenal glands from 10 animals/group/species (randomly selected and excluding positive control groups) at termination and recovery only.

Results

Exposure: The sponsor's analysis of feed mixtures for nebivolol indicates that the concentrations achieved were within ±10% of the target values except for 2 occasions in the rat preparations (12-14 % variation).

Unscheduled mortality was reported. Deaths were almost exclusively in the nebivolol groups. The sponsor's summary table is shown.

Text Table 3.3. Mortality: The table includes the toxicokinetic subgroups. The dose level for the High dose groups is depicted in superscript. The total number of animals in each group is shown in parenthesis. The flutamide (Flu.) and finasteride (Fin.) groups are depicted in the first column.

		Rats					Mice			
		Nebivolol (mg/kg/day)					Nebivolol (mg/kg/day)			
Week	**Flu.**	**0**	**10**	**40**	**160/80**	**Fin.**	**0**	**10**	**40**	**160/80**
1	0	0	0	0	0^{160}	0	0	0	0	0^{160}
2	0	0	0	0	1^{160}	0	0	0	0	1^{160}
3	0	0	0	1	0^{160}	0	0	0	1	11^{80}
4	0	0	0	0	1^{80}	1	0	1	1	$0^{holiday}$
5	0	0	0	1	$4^{holiday}$	0	0	0	0	$0^{holiday}$
6	0	0	0	0	$0^{holiday}$	1	0	0	0	0
7	0	0	0	0	1^{80}	0	0	0	1	0
8	0	0	0	0	0^{80}	0	0	0	0	1^{80}
15	0	0	0	0	0^{80}	0	0	0	0	2^{80}
16	0	0	0	0	0^{80}	0	0	0	0	1^{80}
Total	0 (80)	0 (80)	0 (104)	2 (104)	7 (104)	2 (120)	0 (120)	1 (192)	3 (192)	16 (192)

The majority of nebivolol deaths were in weeks 2-5 and in both species were associated with weight loss and decreased food consumption. Given the effect on the animals, dosing for the rats and mice was first decreased to 80 mg/kg/day, suspended for "approximately" 2 weeks and re-started at 80 mg/kg//day.

Deaths were again seen at 80 mg/kg: 1/84 rats and 4/146 mice.
40 mg/kg nebivolol: 2/ 104 rats died. 3/192 mice died
10 mg/kg nebivolol: 1/192 mice died

During the time when food consumption was decreased, the animals were getting approximately -36% (Day 6), -24%(day 13) and -40%(day 20) less than the targeted dose of nebivolol. Otherwise, mean test article intake was within ±15% of target.

Signs:

Nebivolol: 160 mg/kg 73-98% of the rats showed ptosis and decreased activity
80 mg/kg 30-36% (11/36 and 13/36 rats) showed ptosis
No clinical signs were reported for 10 mg/kg or 40 mg/kg nebivolol.
Flutamide: no signs reported

Rat Body Weights

Nebivolol caused a dose-related decrease in body weight gain. The highest dose caused a loss of body weight. Removing the drug caused a recovery of body weight.

Figure 1: Body weights (rat model)

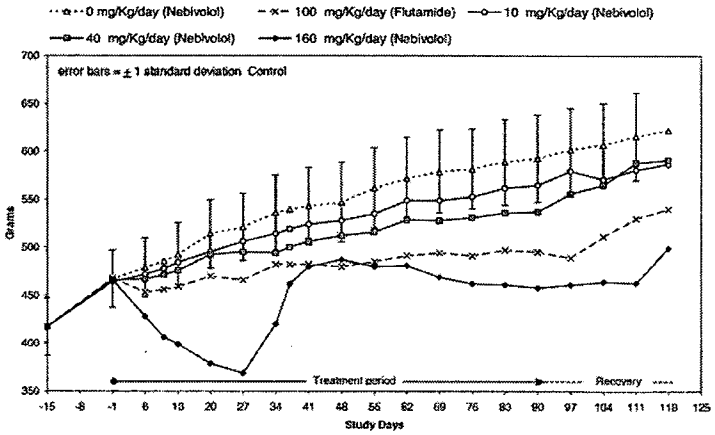
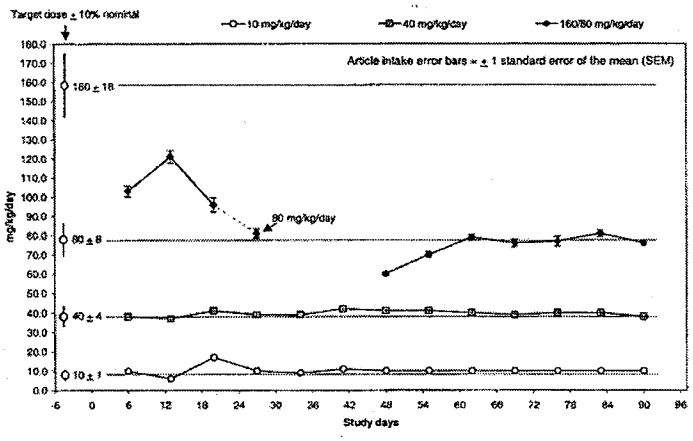


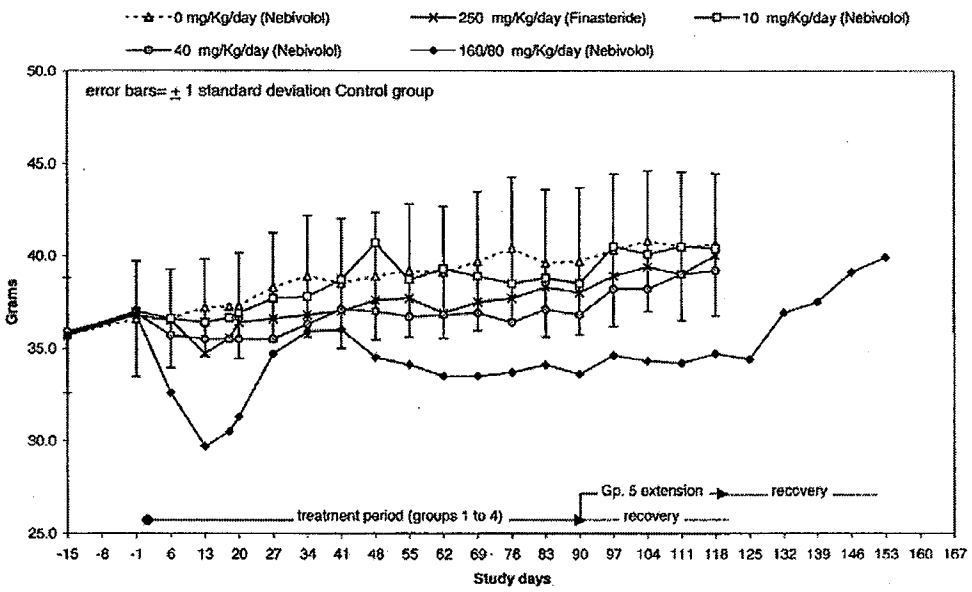
Figure 3: Test article intake (rat model)



Mouse Body Weights

As noted in the rats, there appears to be a dose-related decrease in body weight gain with increased dose of nebivolol. The mice receiving the highest dose showed a loss of body weight. Removal of the drug and implementation of a lower dose caused recovery of body weight.

Figure 2: Body weight (mouse model)



LH levels in rats

Text Table 3.7a. Rat LH prior to the removal of hormone pulses.

		Study Weeks				
		2	4	7	13	Recovery
0 mg/kg/day	mean	1.474	1.095		0.997	0.872
	s.e.m.	0.109	0.084	n/a	0.076	0.104
	n	20	20		20	20
Flutamide 100 mg/kg/day	mean	10.178*	13.010*		9.443*	1.418*
	s.e.m.	1.396	1.394	n/a	0.533	0.213
	n	20	20		20	20
Nebivolol 10 mg/kg/day	mean	1.173	0.798		0.677*	1.096
	s.e.m.	0.108	0.078	n/a	0.043	0.201
	n	20	20		20	20
Nebivolol 40 mg/kg/day	mean	1.198*	1.793*		0.610*	0.905
	s.e.m.	0.220	0.839	n/a	0.062	0.077
	n	20	20		19	18
Nebivolol 160/80 mg/kg/day[a,b]	mean	0.667	0.644	0.808		1.628
	s.e.m.	0.107	0.098	0.107	n/a	0.259
	n	20	19	19		17

[a] Due to reduced feed consumption, the achieved test article intake at 160 mg/kg/day was -24 to -50% of the target dose level during the first 4 weeks. After a 2 weeks drug holiday the rats were re-started at 80 mg/kg/day. A terminal sacrifice for this group occurred after 7 weeks of continued dosing at 80 mg/kg/day (which coincided with the 13 weeks terminal sacrifice of the other groups), and it was followed by a recovery sacrifice 4 weeks after the last day of dosing..

[b] Statistic analyses were not carried out for the high dose group.

* $p<0.05$ s.e.m. = standard error of the mean

Text Table 3.7b. Rat LH After The Removal Of Hormone Pulses.

		Study Weeks				
		2	4	7	13	Recovery
0 mg/kg/day	mean	1.418	1.012		0.997	0.780
	s.e.m.	0.099	0.069	n/a	0.076	0.050
	n	19	18		20	19
Flutamide 100 mg/kg/day	mean	7.969*	13.010*		8.090*	0.983
	s.e.m.	0.529	1.394	n/a	0.194	0.082
	n	17	20		14	15
Nebivolol 10 mg/kg/day	mean	1.002*	0.684*		0.653*	0.839
	s.e.m.	0.060	0.052	n/a	0.038	0.040
	n	17	17		19	17
Nebivolol 40 mg/kg/day	mean	0.937*	0.873		0.562*	0.905
	s.e.m.	0.067	0.055	n/a	0.042	0.077
	n	18	17		18	18
Nebivolol 160/80 mg/kg/day[a,b]	mean	0.505	0.539	0.644		1.333
	s.e.m.	0.056	0.073	0.068	n/a	0.164
	n	17	17	16		15

[a] Due to reduced feed consumption, the achieved test article intake at 160 mg/kg/day was -24 to -50% of the target dose level during the first 4 weeks. After a 2 weeks drug holiday the rats were re-started at 80 mg/kg/day. A terminal sacrifice for this group occurred after 7 weeks of continued dosing at 80 mg/kg/day (which coincided with the 13 weeks terminal sacrifice of the other groups), and it was followed by a recovery sacrifice 4 weeks after the last day of dosing..

[b] Statistic analyses were not carried out for the high dose group.

* $p<0.05$ s.e.m. = standard error of the mean

Values greater than 2 SD from the mean were removed. If this was applied to the mouse study would there be any difference in LH levels?

Mouse LH

Text Table 3.7d. Mouse LH After The Removal Of Hormone Pulses.

		Study Weeks				
		2	4	8	13	Recovery
0 mg/kg/day	mean	0.881	1.536		0.981	2.278
	s.e.m.	0.102	0.199	n/a	0.071	0.283
	n	15	19		18	25
Finasteride 250 mg/kg/day	mean	1.241	6.717*		1.787*	1.612
	s.e.m.	0.217	1.174	n/a	0.196	0.190
	n	20	23		21	22
Nebivolol 10 mg/kg/day	mean	0.979	1.403		0.749	1.078*
	s.e.m.	0.105	0.160	n/a	0.038	0.063
	n	17	20		18	18
Nebivolol 40 mg/kg/day	mean	0.784	1.832		2.611*	2.156*
	s.e.m.	0.034	0.176	n/a	0.237	0.264
	n	14	23		25	23
Nebivolol 160/80 mg/kg/day[a,b]	mean	1.235		2.297	3.224	11.007
	s.e.m.	0.138	n/a	0.221	0.216	2.192
	n	26		23	25	20

[a] Due to reduced feed consumption, the achieved test article intake for the mice at 160 mg/kg/day was -21% of the target dose during the first 2 weeks. The mice were given a 2 week drug holyday starting on Day 21, therefore the 4-weeks interim sacrifice for the mice in this group was not carried out. Treatment was re-started at 80 mg/kg/day on Day 35. The new high dose group had an interim sacrifice after 8 weeks of continued dosing at 80 mg/kg/day (coinciding with the 13 weeks terminal sacrifice of the other groups), this was followed by a terminal sacrifice at 13 weeks of continued dosing at 80 mg/kg/day (one week after the recovery sacrifice of the other groups), and a recovery sacrifice 4 weeks after the last day of dosing at 80 mg/kg/day.

[b] Statistic analyses were not carried out for the high dose group.

* $p<0.05$ s.e.m. = standard error of the mean

Text Table 3.7e. Mouse LH Prior To The Removal Of Hormone Pulses.

		Study Weeks				
		2	4	8	13	Recovery
0 mg/kg/day	mean	7.172	5.547		2.787	3.773
	s.e.m.	1.727	1.357	n/a	0.596	0.743
	n	30	30		30	30
Finasteride 250 mg/kg/day	mean	6.055	11.381*		4.506	3.975
	s.e.m.	1.834	2.070	n/a	1.106	1.224
	n	30	29		30	29
Nebivolol 10 mg/kg/day	mean	3.885	5.082		1.750	2.790
	s.e.m.	0.836	1.128	n/a	0.275	0.674
	n	29	30		30	29
Nebivolol 40 mg/kg/day	mean	3.405	3.962		3.965*	3.657
	s.e.m.	0.867	0.878	n/a	0.846	0.679
	n	30	30		29	29
Nebivolol 160/80 mg/kg/day[a,b]	mean	1.822		4.672	4.595	11.007
	s.e.m.	0.348	n/a	1.259	0.613	2.192
	n	30		29	30	20

[a] Due to reduced feed consumption, the achieved test article intake for the mice at 160 mg/kg/day was -21% of the target dose during the first 2 weeks. The mice were given a 2 week drug holyday starting on Day 21, therefore the 4-weeks interim sacrifice for the mice in this group was not carried out. Treatment was re-started at 80 mg/kg/day on Day 35. The new high dose group had an interim sacrifice after 8 weeks of continued dosing at 80 mg/kg/day (coinciding with the 13 weeks terminal sacrifice of the other groups), this was followed by a terminal sacrifice at 13 weeks of continued dosing at 80 mg/kg/day (one week after the recovery sacrifice of the other groups), and a recovery sacrifice 4 weeks after the last day of dosing at 80 mg/kg/day.

[b] Statistic analyses were not carried out for the high dose group.

* $p<0.05$ s.e.m. = standard error of the mean

The values for LH are lower in the nebivolol-treated mice than the LH values in the control mice at all time points except for week 13 and the HD group at the receovery evaluation. Given the size of the standard deviations, is there a real difference at week 13?

If the numbers from weeks 2 and 4 are compared to the values from the 28 day study, the numbers here are higher. The sponsor does not discuss this difference.

How can the appearance of Leydig cell hyperplasia at 8 weeks with no increase in LH be explained? See histopath summary below.

If all pulses are left in the analysis, the mean LH for the nebivolol-treated animals is not greater than the control value except after 4 weeks of drug-free recovery. The sponsor did not address this point. Even the positive control shows an increase over control at only 1 point of determination.

Estradiol

Text Table 3.7e. Rat Estradiol[a]

		Study Weeks				
		2	4	7	13	Termination
0 mg/kg/day	mean	8.274	3.914		2.089	2.747
	s.e.m.	3.399	0.539	n/a	0.176	0.194
	n	11	5		15	19
Flutamide 100 mg/kg/day	mean	10.683	11.937		9.548	1.689
	s.e.m.	0.989	0.556	n/a	0.916	0.136
	n	18	19		20	14
Nebivolol 10 mg/kg/day	mean	5.674	3.063		2.723	2.088
	s.e.m.	1.534	0.460	n/a	0.200	0.371
	n	7	4		13	9
Nebivolol 40 mg/kg/day	mean	5.674	4.317		1.475	4.890
	s.e.m.	0.579	1.195	n/a	0.078	2.998
	n	9	7		4	10
Nebivolol 160/80 mg/kg/day[b]	mean	5.627	3.326	1.946		1.557
	s.e.m.	1.800	0.297	0.233	n/a	0.055
	n	7	8	7		3

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

[b] Due to reduced feed consumption, the achieved test article intake for the rats at 160 mg/kg/day was -24 to -50% of the target dose level during the first 4 weeks. After 2 weeks drug holiday the rats were re-started at 80 mg/kg/day on day 42, and had a terminal sacrifice after 7 weeks of continued dosing at 80 mg/kg/day, followed by a 4 weeks recovery.

p<0.05 s.e.m. = standard error of the mean

Text Table 3.7f. Mouse Estradiol[a].

		Study Weeks				
		2	4	8	13	Termination
0 mg/kg/day	mean	3.195	3.720		2.733	2.717
	s.e.m.	0.412	0.316	n/a	0.684	1.020
	n	19	23		12	10
Finasteride 250 mg/kg/day	mean	11.087	9.601		2.706	1.998
	s.e.m.	1.024	0.771	n/a	0.546	0.246
	n	22	22		12	11
Nebivolol 10 mg/kg/day	mean	5.663	4.093		5.786	2.745
	s.e.m.	0.917	0.323	n/a	3.179	0.956
	n	15	20		10	4
Nebivolol 40 mg/kg/day	mean	4.159	2.066		1.080	5.582
	s.e.m.	0.862	0.225	n/a	--	1.894
	n	18	16		1	5
Nebivolol 160/80 mg/kg/day[b]	mean	2.953		1.237	3.735	4.192
	s.e.m.	0.497	n/a	0.235	0.832	0.335
	n	15		12	8	14

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

[b] Due to reduced feed consumption, the achieved test article intake for the mice at 160 mg/kg/day was -21% of the target dose during the first 2 weeks. The mice were given a drug holyday starting on Day 21. The 4-weeks interim sacrifice for the mice in this group was not carried out. Treatment was re-started at 80 mg/kg/day on Day 35. The new high dose group had an interim sacrifice after 8 weeks of continued dosing at 80 mg/kg/day, this was followed by a terminal sacrifice at 13 weeks of continued dosing at 80 mg/kg/day, and a recovery sacrifice 4 weeks after the last dose.

p<0.05 s.e.m. = standard error of the mean

This is the only estradiol data we have for the series of studies.

If we use the same reasoning that was applied in the 28-day study for the LH levels, it would be said that mean estradiol levels in the nebivolol groups were elevated above those of the control group at weeks 2 (LD, MD), 4(LD) and 13(LD) and at termination (MD and HD).

The table of descriptive statistics in the appendix (p.272 of the 2nd volume) is difficult to read. It is reproduced below.
Are the values interpretable with so many missing and the size of the variability?

Summary of estradiol values in mice

group	week	n	N missing	mean±sd	range
Pre-dose control	0	13	7	3.34±0.47	1.48-7.31
	2	19	6	3.19±0.41	1.23-8.60
	4	23	1	3.72±0.32	1.06-6.83
	13	12	13	2.73±0.68	0.59-9.21
	17	10	16	2.72±1.02	0.37-8.61
Positive control	2	22	0	11.09±1.02	2.46-25.20
	4	22	0	9.60±0.77	3.65-15.50
	13	12	12	2.71±0.55	0.76-7.03
	17	11	16	1.99±0.25	0.92-3.68
Nebivolol low dose	2	15	1	5.66±0.92	1.84-12.12
	4	20	0	4.09±0.32	1.21-7.63
	13	10	14	5.79±3.18	0.42-34.03
	17	4	21	2.75±0.96	1.51-5.54
Nebivolol mid dose	2	18	0	4.16±0.86	1.32-18.30
	4	16	3	2.07±0.23	0.81-4.31
	13	1	20	1.08	1.08
	17	5	20	5.58±1.89	2.41-11.59
Nebivolol high dose	2	15	1	2.95±0.50	1.13-8.28
	8	12	10	1.24±0.23	0.44-2.76
	13	8	19	3.74±0.83	1.45-7.66
	17	14	6	4.19±0.33	2.18-6.21

I have the same question about the variability and interpretability that has surfaced in the other studies.

Summary of estradiol values in rats

group	week	n	N missing	mean±sd	range
Pre-dose control	0	7	13	2.42±0.36	1.50-4.26
	2	11	9	8.27±3.39	1.56-32.07
	4	5	15	3.91±0.54	2.64-5.43
	13	15	5	2.09±0.18	1.04-3.25
	17	19	1	2.75±0.19	1.22-4.06
Positive control	2	18	3	10.68±0.99	6.23-24.84
	4	19	1	11.94±0.56	6.26-15.79
	13	20	0	9.55±0.92	2.89-16.73
	17	14	6	1.69±0.14	0.98-2.49
Nebivolol low dose	2	7	13	5.67±1.53	1.72-13.87
	4	4	16	3.06±0.46	2.31-4.38
	13	13	7	2.73±0.20	1.05-3.83
	17	9	11	2.09±0.37	1.31-4.79
Nebivolol mid dose	2	9	11	5.67±0.58	3.09-7.91
	4	7	13	4.32±1.20	1.90-11.17
	13	4	15	1.48±0.08	1.33-1.63
	17	10	8	4.89±3.00	1.05-31.78
Nebivolol high dose	2	7	13	5.63±1.80	2.29-16.25
	4	8	12	3.33±0.30	2.12-4.36
	7	7	12	1.95±0.23	1.23-3.00
	11	3	14	1.56±0.05	1.45-1.63

Text-Table 3.8.1a. Sperm Parameters in The Rat (Terminal Sacrifice). * p<0.05, **p<0.01.

Terminal Sacrifice		Motile Sperm (%)	Progressively Motile Sperm (%)	Cauda Epididymis Sperm Count (millions/g)	Right Testis Sperm Count (millions/g)	Normal Sperm (%)
0 mg/kg/day	Mean	93	46	525	82	96.6
	SD	4	15	121	25	1.3
	n	10	10	10	10	10
100 mg/kg/day (Flutamide)	Mean	86	17**	391	63	79.4**
	SD	8	7	201	31	29
	n	9	9	10	10	10
10 mg/kg/day (Nebivolol)	Mean	94	54	564	92	96.1
	SD	4	17	100	27	1.8
	n	10	10	10	10	10
40 mg/kg/day (Nebivolol)	Mean	83	37	552	89	89.1*
	SD	14	14	111	12	6.3
	n	10	10	10	10	10
80 mg/kg/day (Nebivolol)	Mean	84**	33	485	76	84.4**
	SD	8	13	201	25	11.2
	n	10	10	10	9	10

There appears to be a slight decrease in the number of normal sperm , motility, and progressive motility.

Recovery Sacrifice

Text-Table 3.8.1b. Sperm Parameters in the Rat (Recovery Sacrifice). * p<0.05, **p<0.01.

Recovery Sacrifice		Motile Sperm (%)	Progressively Motile Sperm (%)	Cauda Epididymis Sperm Count (millions/g)	Right Testis Sperm Count (millions/g)	Normal Sperm (%)
0 mg/kg/day	Mean	85	61	671	80	94.8
	SD	24	27	128	23	5.3
	n	10	10	10	10	10
100 mg/kg/day (Flutamide)	Mean	72	46	463	63	70.3*
	SD	37	26	273	29	38.3
	n	10	10	10	10	10
10 mg/kg/day (Nebivolol)	Mean	94	55	613	72	97.4
	SD	5	18	196	24	1.6
	n	10	10	10	10	10
40 mg/kg/day (Nebivolol)	Mean	76	50	295**	83	84.9
	SD	29	26	167	27	25.4
	n	10	10	10	10	10
80 mg/kg/day (Nebivolol)	Mean	52**	26**	293**	59	53.9**
	SD	30	23	167	32	31.9
	n	9	9	10	10	10

The breakdown of sperm abnormalities indicates a dose-related increase in defects in sperm from nebivolol-treated animals.

Rats | Terminal Sacrifice | Table 7

Number Animals		Normal Number	%	Decapitate Number	%	Head Abnormal Number	%	Neck Abnormal Number	%	Tail Abnormal Number	%	Mid Tail Blob Number	%
Group 1 – 0 mg/kg/day (Control)													
10	Mean	193.2	96.6	3.4	1.7	2.0	1.0	0.2	0.1	0.4	0.2	0.9	0.5
	SD	2.7	1.3	1.8	0.9	1.7	0.8	0.4	0.2	0.5	0.3	1.4	0.7
Group 2 – 100 mg/kg/day (Flutamide)													
10	Mean	158.5	79.4	18.0	11.3	15.5	7.8	0.7	0.4	3.3	1.7	2.1	1.1
	SD	59.1	29.0	37.1	28.8	24.8	12.4	1.1	0.5	7.7	3.8	2.4	1.2
Group 3 – 10 mg/kg/day (Nebivolol)													
10	Mean	192.1	96.1	4.4	2.2	1.4	0.7	0.1	0.1	0.6	0.3	1.4	0.7
	SD	3.6	1.8	2.0	1.0	1.0	0.5	0.3	0.2	1.1	0.5	1.2	0.6
Group 4 – 40 mg/kg/day (Nebivolol)													
10	Mean	178.3	89.1	8.5	4.2	3.2	1.6	0.9	0.5	1.0	0.5	8.2	4.1
	SD	13.8	6.3	7.3	3.6	1.9	0.9	1.0	0.5	1.6	0.8	11.5	5.8
Group 5 – 160/80 mg/kg/day (Nebivolol)													
10	Mean	169.1	84.4	14.5	7.2	6.3	3.1	2.4	1.2	1.1	0.5	7.8	3.9
	SD	22.3	11.2	12.9	6.5	6.5	3.2	2.3	1.2	1.5	0.8	14.7	7.3

*Significantly different from control mean; p≤0.05.
**Significantly different from control mean; p≤0.01.

There was some persistence of these changes into the recovery phase.

Rats | Recovery Sacrifice | Table 7

Number Animals		Normal Number	%	Decapitate Number	%	Head Abnormal Number	%	Neck Abnormal Number	%	Tail Abnormal Number	%	Mid Tail Blob Number	%
Group 1 – 0 mg/kg/day (Control)													
10	Mean	189.6	94.8	6.3	3.2	3.2	1.6	0.3	0.2	0.7	0.4	0.0	0.0
	SD	10.5	5.3	8.9	4.5	2.9	1.4	0.9	0.5	0.7	0.3	0.0	0.0
Group 2 – 250 mg/kg/day (Finasteride)													
10	Mean	141.6	70.3	48.1	24.0	8.8	4.4	0.9	0.4	1.8	0.9	1.0	0.5
	SD	77.2	38.3	77.4	38.7	8.4	4.2	1.4	0.7	1.7	0.8	1.1	0.5
Group 3 – 10 mg/kg/day (Nebivolol)													
10	Mean	195.0	97.4	2.6	1.3	2.0	1.0	0.1	0.1	0.2	0.1	0.4	0.2
	SD	2.6	1.6	1.8	0.9	2.3	1.1	0.3	0.2	0.4	0.2	0.7	0.3
Group 4 – 40 mg/kg/day (Nebivolol)													
10	Mean	170.7	84.9	24.1	12.0	4.7	2.3	0.3	0.2	0.9	0.4	0.4	0.2
	SD	50.8	25.4	51.4	25.7	4.0	2.0	0.5	0.2	1.3	0.6	0.5	0.3
Group 5 – 160/80 mg/kg/day (Nebivolol)													
10	Mean	109.4	53.9	73.9	36.4	15.6	7.6	3.2	1.6	2.5	1.2	1.4	0.7
	SD	65.0	31.9	60.6	29.7	13.4	6.5	2.9	1.4	2.5	1.2	1.8	0.9

*Significantly different from control mean; p≤0.05.
**Significantly different from control mean; p≤0.01.

Text-Table 3.8.2a. Sperm parameters in the mouse (Terminal sacrifice). * p<0.05, **p<0.01.

Terminal Sacrifice		Motile Sperm (%)	Progressively Motile Sperm (%)	Cauda Epididymis Sperm Count (millions/g)	Right Testis Sperm Count (millions/g)	Normal Sperm (%)
0 mg/kg/day	Mean	93	66	1053	251	76.4
	SD	6	19	207	69	13.6
	n	8	8	10	10	10
250 mg/kg/day (Finasteride)	Mean	92	53	776*	220	79.7
	SD	8	25	142	52	12.1
	n	9	9	10	10	10
10 mg/kg/day (Nebivolol)	Mean	85	56	847*	228	81.6
	SD	9	12	261	70	8.7
	n	7	7	10	10	9
40 mg/kg/day (Nebivolol)	Mean	80	55	782*	249	52.7**
	SD	16	26	244	68	21.6
	n	4	4	10	10	10
80 mg/kg/day (Nebivolol)	Mean	69**	17**	584**	215	49.9**
	SD	21	10	360	54	23.6
	n	7	7	10	9	9

Motility and counts: When compared to Control values, statistics

As in the rats, there appeared to be slight decreases in normal sperm, motile sperm and progressively motile sperm.

The sponsor dismissed all spermatic changes as being within the range of historical controls.

Something of interest is the number of samples used for the determination of each of the parameters. The nebivolol groups frequently have lower n values than the vehicle and positive control groups. In the individual animal data, animals were marked "E= excluded due to low number analyzed."

Text-Table 3.8.2b. Sperm parameters in the mouse (Recovery sacrifice). * p<0.05, **p<0.01.

Recovery Sacrifice		Motile Sperm (%)	Progressively Motile sperm (%)	Cauda Epididymis Sperm Count (millions/g)	Testis Sperm Count (millions/g)	Normal Sperm (%)
0 mg/kg/day	Mean	76	42	621	167	71.7
	SD	28	30	253	64	8.3
	n	8	8	10	10	10
250 mg/kg/day (Finasteride)	Mean	84	35	542	176	77.5
	SD	13	29	119	57	8.1
	n	10	10	10	9	10
10 mg/kg/day (Nebivolol)	Mean	84	38	657	172	74.6
	SD	18	24	232	57	7.6
	n	9	9	8	9	9
40 mg/kg/day (Nebivolol)	Mean	92	39	384	143	65.5
	SD	1	27	144	64	24.7
	n	9	9	10	10	10
80 mg/kg/day (Nebivolol)	Mean	96**	62	541	164	81.0
	SD	2	19	149	81	13.6
	n	7	7	10	10	10

Defects in sperm were reported in the mice also.

Mice	Terminal Sacrifice	Table 7

Number Animals		Normal Number	%	Decapitate Number	%	Head Abnormal Number	%	Neck Abnormal Number	%	Tail Abnormal Number	%	Mid Tail Blob Number	%
Group 1 – 0 mg/kg/day (Control)													
10	Mean	131.2	76.4	2.5	6.2	5.4	2.7	3.4	1.7	16.3	11.1	6.4	3.2
	SD	67.2	13.6	1.8	12.3	4.0	2.0	3.4	1.7	9.5	4.0	5.0	2.4
Group 2 – 250 mg/kg/day (Finasteride)													
10	Mean	149.4	79.7	3.7	2.0	12.6	6.9	3.1	1.5	17.9	9.4	8.2	4.3
	SD	45.0	12.1	4.1	2.0	18.8	9.1	2.9	1.4	11.5	5.1	7.3	3.4
Group 3 – 10 mg/kg/day (Nebivolol)													
9	Mean	164.2	81.6	3.2	1.6	4.4	2.2	5.2	2.6	18.6	9.2	16.3	8.1
	SD	16.7	8.7	2.5	1.2	3.4	1.7	6.1	3.0	9.7	4.7	7.0	3.4
Group 4 – 40 mg/kg/day (Nebivolol)													
10	Mean	72.7	52.7	7.2	15.3	2.1	1.7	2.5	1.2	35.6	21.6	14.8	12.1
	SD	52.9	21.6	11.1	30.3	2.7	1.6	3.5	1.7	32.9	13.3	14.3	8.4
Group 5 – 160/80 mg/kg/day (Nebivolol)													
9	Mean	88.8	49.9	12.7	8.1	9.3	5.3	3.8	2.6	64.8	36.5	0.0	0.0
	SD	53.9	23.6	5.8	5.4	4.6	2.2	1.4	2.1	49.2	22.2	0.0	0.0

*Significantly different from control mean; p≤0.05.
**Significantly different from control mean; p≤0.01.
If no asterisks, no statistically significant differences from control mean.

Mice	Recovery Sacrifice	Table 7

Number Animals		Normal Number	%	Decapitate Number	%	Head Abnormal Number	%	Neck Abnormal Number	%	Tail Abnormal Number	%	Mid Tail Blob Number	%
Group 1 – 0 mg/kg/day (Control)													
10	Mean	134.8	71.7	3.9	2.5	5.6	2.8	4.0	2.1	39.9	21.5	0.8	0.5
	SD	38.0	8.3	2.4	2.0	4.8	2.4	4.7	2.2	18.9	8.5	0.8	0.6
Group 2 – 250 mg/kg/day (Finasteride)													
10	Mean	149.4	77.5	4.2	2.2	9.6	4.8	5.3	2.7	25.6	13.3	11.8	5.9
	SD	31.5	8.1	2.3	1.0	6.3	3.0	4.7	2.3	12.5	5.6	10.0	4.7
Group 3 – 10 mg/kg/day (Nebivolol)													
9	Mean	149.8	74.6	2.8	1.4	8.4	4.2	3.6	1.8	36.3	18.1	14.6	7.2
	SD	14.8	7.6	1.6	0.8	3.7	1.9	2.9	1.5	12.9	6.4	7.7	3.8
Group 4 – 40 mg/kg/day (Nebivolol)													
10	Mean	122.8	65.5	4.4	8.2	5.9	8.4	2.9	6.5	33.7	17.2	0.7	0.3
	SD	56.2	24.7	4.0	15.4	4.4	14.8	2.4	15.3	22.7	10.8	1.3	0.7
Group 5 – 160/80 mg/kg/day (Nebivolol)													
10	Mean	115.3	61.0	2.3	1.2	7.2	4.0	2.9	1.6	21.1	11.3	7.6	4.3
	SD	66.8	13.6	2.4	1.2	6.3	3.4	1.9	1.0	24.7	12.2	6.5	3.6

Note: finasteride causes LC hyperplasia in rats. Nebivolol apparently did not.

Text Table 3.10.2a. Histopathology findings in the rat. The table depicts the incidence and severity of testicular and epididymal findings following 13 weeks of dosing (7 weeks of continuous dosing at 80 mg/kg/day).

	Control	Flutamide	Nebivolol		
mg/kg/day	0	100	10	40	160/80
No. rats examined	10	10	10	10	10
Testes					
Spermatid retention	0	0	0	3	6
Exfoliation of germ cells	0	0	0	1	7
Degeneration of elongating spermatids	0	0	0	1	6
Leydig cell hyperplasia	0	10	0	0	0
Epididymis					
Increased sloughed germ cells/cell debris	0	0	0	2	7
Reduced sperm content	0	0	1	1	5

There were also findings in the mammary gland of the male mice. Males given 160/80 mg/kg nebivolol showed a differentiation from a lobuloalveolar to a tubuloalveolar structure. This was also seen in 7/10 flutamide-treated rats at 13 weeks. A minimal differentiation was noted in 2 control rats at 13 weeks. The sponsor notes that:

also present in 2 control rats. This is a relatively common age related change in the rat but the incidence and severity of the finding was increased in the Nebivolol and flutamide treated rats.

Incidences in nebivolol-treated rats:

2 weeks: 8/10 rats
4 weeks: 7/10 rats
13 weeks(7 weeks at 80 mg/kg): 4/10

This is also characteristic of a response to a xenoestrogen(Tox Path 35:199-207, 2007).

The textual summary for the rat noted that disturbance in spermatogenesis were present in the testes of rats dosed with 40 mg/kg/day for 13 weeks and at 160/80 mg/kg/day following 4 and 13 weeks. The incidence and severity of the findings was described as dose and time related. The changes were described as minimal to moderate spermatid retention (stage 19 spermatids), minimal to slight germ cell exfoliation and minimal to slight degeneration of elongating spermatids. This was reflected in the decreased sperm count in the epididymides of affected animals. The flutamide-treated animals showed diffuse interstitial cell hyperplasia of the testes, ductal atrophy of the epididymides, reduced secretion and contracted acini of the prostate, and contraction and epithelial atrophy of the seminal vesicles at 2,4 and 13 weeks of dosing. Flutamide did not have an apparent effect on spermatogenesis.

13 weeks of dosing. Flutamide had no effects on testicular spermatogenesis. These findings correlate with the changes seen in organ weights of these tissues. Although there were no detectable histopathological effects on testicular spermatogenesis, there was a slight reduction in testicular spermatid head count (not statistically significant) and significant reductions in the % of motile sperm in the epididymis. These findings are consistent with the anti-androgenic mechanism of flutamide, which causes decreased efficiency of testicular spermatogenesis and disturbed maturation of epididymal sperm.

Comment [EAH1]: This strikes me as inconsistent. Is there some interpretation that I'm missing?

This is not necessarily consistent with previous work showing that flutamide given to rats causes a decrease in the number of leptotene spermatocytes and round spermatids (Greaves "The male genital tract" Chapter X in **Histopathology of Preclinical Toxicity Studies**, 2nd edition. 2000)

Other histopathology findings for the rat

	Animals affected				
Dose group	ctls	Flutami	Nebivo 10 m/k	Nebivo 40m/k	Nebivo 80m/k
Week 2					
Prostate acute/subacute inflammation	0	4/10	0	0	1/9
Prostate distended acini/epithelial thinning	0	0	1	1	9
Testes: tubular degeneration and atrophy	0	0	0	1	0
Mammary: tubuloalveolar differentiation	0	1	0	0	8/10
Seminal vesicles: contracted/epithelial atrophy	0	10	1	0	0
Seminal vesicles: distension/epithelial atrophy	0	1	0	7	10
Testes: Leydig cell hyperplasia	0	10	0	0	0
Week 4					
Epididymides: increased sloughed germ cells	0	0	0	0	4
Prostate: reduced secretion, contracted acini	0	10/10	0	0	2/10
Prostate: acute/subacute inflammation	0	2/10	3/10	0	2/10
Prostate distended acini/epithelial thinning	1	0	2	1	9
Seminal vesicles: contracted, epithelial atrophy	1	10	0	0	1
Seminal vesicles: Distension/ epithelial thinning	0	0	0	7	9
Testes: tubular degeneration and atrophy	0	0	1	1	1
Testes: spermatid retention	0	0	0	0	4
Testes: germ cell expoliation	0	0	0	0	3
Testes: leydig cell hyperplasia	0	10	0	0	0
Mammary: tubuloalveolar differentiation	0	1	0	0	7
Terminal (week 13)					
Epididymides: reduced sperm	0	0	1	1	5
Epididymides: increased sloughed germ cells	0	0	0	2	7
Prostate: acute/subacute inflammation	2	0	4	4	6
Prostate: distended acini/ epithelial thinning	4	0	3	10	9
Seminal vesicles: contracted/epithelial atrophy	0	10	0	0	0
Seminal vesicles: distension/epithelial thinning	0	0	0	3	8
Testes: tubular degeneration/atrophy	0	0	1	1	2
Testes: spermatid retention	0	0	0	3	6
Testes: elongating spermatid retention	0	0	0	1	6
Testes: germ cell exfoliation	0	0	0	1	7
Testes: tubular vacuoles	0	0	1	1	1
Testes: Leydig cell hyperplasia	0	10	0	0	0
Mammary: tubuloalveolar differentiation	2	7	1	2	4
recovery					
Epididymides: reduced sperm	1	0	0	0	3
Epididymides: increased sloughed germ cells	1	0	0	0	5
Mammary gland: tubuloalveolar differentiation	0	2	1	1	5
Seminal vesicles: contracted/epithelial atrophy	0	0	0	0	10
Testes : occasional atrophic/degenerate tubule	1	1	3	0	3

Testes : Leydig cell hyperplasia	0	0	0	0	0

Mouse histopathology

It is of interest to note that the vehicle control and positive control showed the same level of LC hyperplasia and that nebivolol produced a higher incidence of the finding than did the positive control. Also, LC hyperplasia was still found in the HD group at the end of the 4 week recovery period but was not found in the positive control animals. The sponsor addresses this lack of findings:

> Finasteride was used as a positive control for this study because it is known to produce Leydig cell tumors in mice following chronic administration and to produce increased serum LH levels when administered over a 13-week period (Prahlada, et al 1994). In the current study, Finasteride produced increased LH levels similar to those reported in the literature, but Leydig cell hyperplasia was not detected by microscopic examination. It is likely that an increase in Leydig cell numbers did occur, but was below the threshold needed to be detectable by qualitative microscopy.

Text Table 3.10.2b. Histopathology findings in the mouse. The table depicts the incidence and severity of testicular and epididymal findings following 13 weeks of dosing (7 weeks of continuous dosing at 80 mg/kg/day).

	Control	Finasteride	Nebivolol		
mg/kg/day	0	250	10	40	160/80
No. mice examined	10	10	10	10	10
Testes					
Leydig cell hyperplasia	2	2	0	9	8
Increased germ cell apoptosis	0	0	0	2	0
Epididymis					
Increased sloughed germ cells/cell debris	0	0	0	2	1

Additional mouse findings

	Animals affected				
Dose group	ctls	2	3	4	5
Week 2					
Prostate: subacute/chronic inflammation	0	0	0	0	1/9
Seminal vesicles: distension	0	0	0	4/10	10/10
Leydig cell hyperplasia	0	0	0	2	0
Week 4					
Testes: occasional degenerate/atrophic tubules	0	5	2	2	
Week 8					
Epididymides: increased sloughed germ cells					3/9
Leydig cell hyperplasia					9/9
Terminal (week 13)					
Epididymides: increased vacuolation/apoptosis	0	0	1/9	1/9	1/10
Epididymides: increased sloughed germ cells	0	0	0	2/10	1/10
Prostate: subacute/chronic inflammation	0	0	0	1/10	0
Leydig cell hyperplasia	2	2	0	9	8
Testes: elongating spermatid depletion	0	0	0	1	0
recovery					
Leydig cell hyperplasia	0	0	0	0	7/10

Organ weights

Rats:
Week 2: absolute weight of the epidiymides, prostate and seminal vesicles was reduced in the positive control animals.

Interim Sacrifice - Week 2 — Rat Toxicity Study

Group	Epididymides	Prostate Seminal vesicles		Testes
		Male	Animals	
1				
Mean:	1.5604	1.0156	2.0589	4.3741
Standard deviation:	0.1639	0.1356	0.1405	0.5468
Number of observ. :	(10)	(10)	(10)	(10)
2				
Mean:	1.2194*	0.4926+	0.7533$	4.2722
Standard deviation:	0.2235	0.1446	0.1252	0.8644
Number of observ. :	(10)	(10)	(10)	(10)

Week 4: absolute weight of epididymides, prostate and seminal vesicles were decreased in the positive control animals.

Interim Sacrifice - Week 4 — Rat Toxicity Study

Group	Epididymides	Prostate Seminal vesicles		Testes
		Male	Animals	
1				
Mean:	1.5756	0.9975	2.0461	4.3626
Standard deviation:	0.1168	0.2466	0.2466	0.4333
Number of observ. :	(10)	(10)	(10)	(10)
2				
Mean:	0.9830+	0.2928+	0.4055$	4.4202
Standard deviation:	0.0749	0.1168	0.0867	0.2887
Number of observ. :	(10)	(10)	(10)	(10)

Week 13: dose-related increase in absolute weight of adrenal glands and seminal vesicles in nebivolol-treated animals

Terminal Sacrifice — Rat Toxicity Study

Group	Adrenal Glands	Epididymides	Seminal vesicles	Prostate
		Male	Animals	
1				
Mean:	0.0670	1.7756	1.6131	1.9546
Standard deviation:	0.0089	0.2640	0.6107	0.3398
Number of observ. :	(10)	(10)	(10)	(10)
2				
Mean:		0.9323+	0.3287$	0.4053$
Standard deviation:		0.1192	0.1533	0.0932
Number of observ. :		(10)	(10)	(10)
3				
Mean:	0.0692	1.7653	1.5953	1.9826
Standard deviation:	0.0090	0.2456	0.5135	0.2851
Number of observ. :	(10)	(10)	(10)	(10)
4				
Mean:	0.0868%	1.7293	1.7127	2.7728$
Standard deviation:	0.0211	0.2022	0.5463	0.4910
Number of observ. :	(10)	(9)	(10)	(10)
5				
Mean:	0.1135$	1.5813	1.7251	3.0427$
Standard deviation:	0.0283	0.1836	0.3169	0.5843
Number of observ. :	(10)	(9)	(10)	(10)

Recovery euthanasia: flutamide animals showed epididymides, prostate and seminal vesicle weights below those of the controls. Testes weight was increased.

Adrenal gland weight was increased in the HD nebivolol animals. Epididymides, prostate and seminal vesicle weight were decreased at the HD.

Mice:

Week 2: positive control mice showed a decrease in the absolute weight of epididymides, prostate and seminal vesicles. HD nebivolol showed a decrease in prostate weight.

Interim Sacrifice - Week 2 — Mouse Toxicity Study

Group	Epididymides	Prostate Seminal vesicles	
		Male	Animal
1			
Mean:	0.1372	0.0532	0.3596
Standard deviation:	0.0256	0.0165	0.0883
Number of observ. :	(10)	(10)	(10)
2			
Mean:	0.1100%	0.0499	0.2178*
Standard deviation:	0.0156	0.0180	0.0709
Number of observ. :	(10)	(10)	(10)
3			
Mean:	0.1485	0.0638	0.3631
Standard deviation:	0.0245	0.0230	0.0993
Number of observ. :	(10)	(10)	(10)
4			
Mean:	0.1284	0.0619	0.5388+
Standard deviation:	0.0170	0.0201	0.1543
Number of observ. :	(10)	(10)	(10)
5			
Mean:	0.1577	0.0415	0.4677
Standard deviation:	0.1460	0.0200	0.0923
Number of observ. :	(10)	(10)	(10)

Week 4: positive control animals showed a decrease in epididymides and seminal vesicles. HD animals showed an increase in epididymides.

Week 13: HD nebivolol animals showed an increase in adrenal gland and prostate weight. Epididymides weight decreased.

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