

Ovarian hyperplasia may take a number of forms. As the term was undefined in the incidence tables it can only be given consideration in terms of generalities. Many forms of ovarian hyperplasia are associated with age-related loss of ovarian function. But, they can also occur with drug-induced atrophy of the follicular epithelium. (Greaves Histopathology of Preclinical Toxicity Studies, 2<sup>nd</sup> edition. 2000. pages 703-719).

Similarly, there were consistent findings among the male rodents. Giant cells are found in seminiferous tubules under several circumstances but are most frequently associated with tubular atrophy and are considered to be a component of germ cell degeneration. Focal Leydig cell hyperplasia was seen in the 3 month mouse study in combination with large nucleated tubular cells. Greaves notes that Leydig hyperplasia may be seen in perfectly healthy animals but is more commonly associated with atrophic tubules both in rodents and man (p. 657, 2<sup>nd</sup> edition). As all 10 of the HD animals had large nucleated tubular cells, were the combined findings indicative of testicular degeneration?

One can not come to a definitive interpretation of the above data. The lack of reported findings in the dog study also raises questions. Is the seasonal breeding pattern of the dog the reason for lack of stated effects? Were sexually immature animals studied? Were the studies simply underpowered, is there some species specific effect or are all the noted effects simply due to random chance? The data generates the overall impression is that there is a signal to be investigated regarding a possible hormonal effect of nebivolol.

Elizabeth Hausner DVM  
Pharmacology

Al DeFelice, Ph.D.  
Supervisory Pharmacologist

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/s/

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Elizabeth Hausner  
4/11/05 08:59:31 AM  
PHARMACOLOGIST  
Elizabeth Hausner

Albert Defelice  
4/11/05 09:50:56 AM  
PHARMACOLOGIST

## PHARMACOLOGY/TOXICOLOGY REVIEW

### 3.1 INTRODUCTION AND DRUG HISTORY

**NDA number:** 21742

**Review number:** 1

**Sequence number/date/type of submission:** 0/February 24, 2005/BZ, doc type N

**Information to sponsor:** Yes ( ) No ( )

**Sponsor and/or agent:** Bertek

**Manufacturer for drug substance:** Mylan Pharmaceuticals Inc, 781 Chestnut Ridge Road, Morgantown, WV 26505.

**Reviewer name:** Elizabeth Hausner, D.V.M.

**Division name:** Cardio-Renal Drug Products

**HFD #:** 110

**Review completion date:**

#### Drug:

**Generic Name:** Nebivolol Tablets

**Chemical Name** Nebivolol hydrochloride is identified chemically as ( $\pm$ )-  
[2R\*[R\*[R\*(S\*)]]]- $\alpha,\alpha'$ -[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-  
2H-1-benzopyran-2-methanol] hydrochloride

**Code Numbers** R067555

R067138 (d-Nebivolol)

R067145 (l-Nebivolol)

**CAS Registry No.** 152520-56-4

**Trade Name:** To Be Established

The material submitted is from a DMF and is in response to the chemistry reviewer noting that there was an impurity to be qualified. The studies provided by the sponsor are in support of the qualification of an impurity.

## Studies Submitted

**EDMS-PSDB-2876162 Two week repeated dose oral toxicity study in the rat**

**EDMS-PSDB-2892162 In vitro bacterial reverse mutation test with *Salmonella typhimurium***

## Studies Reviewed

**EDMS-PSDB-2876162 Two week repeated dose oral toxicity study in the rat**

**EDMS-PSDB-2892162 In vitro bacterial reverse mutation test with *Salmonella typhimurium***

**EDMS-PSDB-2892162 In vitro bacterial reverse mutation test with *Salmonella typhimurium***

Study location: Global Preclinical Development, Beerse, Belgium

Sponsor: Johnson & Johnson division of Janssen

Study dates: April 15, 2003

GLP: statement included

QA: statement included

Test article: R06755 batch # ZR067555PUB061 for all combinations

R06755 spiked with \_\_\_\_\_ (batch WVLA\_0041\_019\_7) analysis  
2/22/2001

R06755 spiked with \_\_\_\_\_ (GVLO\_0068\_053) **Approval of the analysis was dated 2/8/1993 10 years prior to use in this assay. Re-analysis was dated 1/6/2000, 3 years prior to use in the assay.**

R06755 spiked with \_\_\_\_\_ (GVLO\_0068\_068\_1) **Approval of the material was originally dated 1993. Re-analysis was dated August 2, 1999, 4 years prior to use.**

The vehicle used was DMSO

Positive controls: 2-nitrofluorene, sodium azide, 9-aminoacridine, 2- aminoanthracene, 4-nitroquinoline-N-oxide.

Ames assays were carried out using *Salmonella typhimurium* strains TA1535, TA1537, TA102, TA98 and TA100 ±S9. Each of the 3 combined test articles were tested at 2.5, 7.5, 25, 75, 200, 600 and 1800 µg/plate in the first study and 3.13, 6.25, 12.5, 25, 50, 100 and 200 µg/plate in the second study. The concentrations were determined by the bacteriotoxic effects of decreased revertants, thinning of bacterial background and occurrence of pinpoints at ≥50 µg/plate. Precipitation was seen from ≥ 600 µg/plate in all three test articles.

The positive controls produced appropriate responses. Under the conditions used, none of the combinations of R067555 and impurity caused an increase in revertants.

**EDMS-PSDB-2876162 Two week repeated dose oral toxicity study in the rat**

Study location: Global Preclinical Development, Beerse, Belgium

Study dates: April 29, 2003

Study Number: EDMS-PSDB-2876162

GLP:

QA:

Test articles:

R06755 batch # ZR067555PUB061 for all combinations

R06755 spiked \_\_\_\_\_ (batch WVLA\_0041\_019\_7) analysis  
2/22/2001R06755 spiked \_\_\_\_\_ (GVLO\_0068\_053) **Approval of the analysis  
was dated 2/8/1993 10 years prior to use in this assay. Re-analysis was dated  
1/6/2000, 3 years prior to use in the assay.**R06755 spiked \_\_\_\_\_ (GVLO\_0068\_068\_1) **Approval of the  
material was originally dated 1993. Re-analysis was dated August 2, 1999, 4  
years prior to use.**

Analysis of the impurities in the suspensions was not conducted.

Nebivolol spiked with each impurity at — was compared against neбиволol by itself at doses of either 5 or 40 mg/kg/day ( increased to 60 mg/kg on day 7) for 14 days. Drug was given by oral gavage of an aqueous suspension in 0.5% methylcellulose. A total of 10 rats/sex/dose were used. Parameters measured included clinical signs, body weight, food consumption, clinical pathology and urinalysis. At time of euthanasia gross changes were noted and a standard list of tissues was collected for histopathological analysis.

**Results**

One male in the vehicle group died in week 2 in what the sponsor reports as probably a gavage accident. Clinical signs were noted only for females in the HD groups, both spiked and non-spiked, in the second week of dosing.

**Summary of signs in females**

| Sign/finding | Non-spiked 60 mg/kg | Spiked 60 mg/kg |
|--------------|---------------------|-----------------|
| ptosis       | 5/10 p<0.05         | 8/10 p<0.001    |

There were no effects on male body weight or food consumption apparent in the data.

Body weight was inconsistently decreased in the drug-treated females.

**BODY WEIGHT GAIN**

Mean values per dosage group in g

Experiment: TOX - 5752

2-WEEK REPEATED DOSE ORAL TOXICITY STUDY IN THE RAT  
R067555 - OR/GAV - RAT

| Week/Day | Males       |             |             |              |             | Females     |             |             |             |             |
|----------|-------------|-------------|-------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|
|          | Vehicle     | Low 1:5     | High 1:60   | Low 2:5      | High 2:60   | Vehicle     | Low 1:5     | High 1:60   | Low 2:5     | High 2:60   |
| 1 / 7    | 46<br>(1.9) | 50<br>(1.8) | 45<br>(2.8) | 49<br>(4.0)  | 44<br>(1.8) | 21<br>(2.2) | 21<br>(3.2) | 22<br>(2.5) | 17<br>(3.0) | 20<br>(1.3) |
| 2 / 14   | 90<br>(5.0) | 99<br>(3.2) | 89<br>(4.3) | 100<br>(5.6) | 89<br>(2.8) | 44<br>(2.9) | 43<br>(5.0) | 45<br>(3.4) | 34<br>(5.4) | 40<br>(2.6) |

Significance computed versus the Vehicle group by Mann-Whitney U test (two-tailed): \* p <.05 \*\* p <.01 \*\*\* p <.001  
Standard error is shown between brackets  
Low 2 and High 2 are spiked with each impurity at —

Despite the inconsistent weight effects, food consumption was significantly decreased in the drug-treated females. (3%LD, 10% HD, 8% LD spiked, 14% HD spiked)

**FOOD CONSUMPTION**

Mean values per dosage group in g

Experiment: TOX - 5752

2-WEEK REPEATED DOSE ORAL TOXICITY STUDY IN THE RAT  
R067555 - OR/GAV - RAT

| Week/Day | Males        |              |              |              |              | Females      |              |                 |                |                 |
|----------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------------|----------------|-----------------|
|          | Vehicle      | Low 1:5      | High 1:60    | Low 2:5      | High 2:60    | Vehicle      | Low 1:5      | High 1:60       | Low 2:5        | High 2:60       |
| 1 / 7    | 187<br>(3.6) | 186<br>(3.6) | 179<br>(4.4) | 186<br>(4.2) | 180<br>(3.9) | 130<br>(3.4) | 128<br>(4.7) | 129<br>(3.6)    | 123<br>(3.8)   | 127<br>(3.1)    |
| 2 / 14   | 187<br>(5.3) | 189<br>(4.0) | 181<br>(6.1) | 184<br>(4.9) | 178<br>(4.3) | 133<br>(3.0) | 129<br>(4.4) | 120 **<br>(3.5) | 122 *<br>(3.9) | 115 **<br>(4.9) |
| Total:   | 375          | 375          | 360          | 370          | 358          | 264          | 257          | 250             | 245            | 242 *           |

Significance computed versus the Vehicle group by Mann-Whitney U test (two-tailed): \* p <.05 \*\* p <.01 \*\*\* p <.001  
Standard error is shown between brackets  
Low 2 and High 2 are spiked with each impurity at —

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Hematology: Both males and females in the HD groups (spiked and non-spiked) showed decreases in hemoglobin, hematocrit, reticulocyte (percent and absolute).

| EXPERIMENT : TOX - 5752<br>2-WEEK REPEATED DOSE ORAL TOXICITY STUDY IN THE RAT<br>R067555 - OR/GAV - RAT |                    | HAEMATOTOLOGY<br>Mean values per dosage group<br>Terminal, recorded from day 15 till day 16 |                 |                       |                 |                       |
|--|--------------------|---|-----------------|-----------------------|-----------------|-----------------------|
| Parameter  | Unit               | Dosage Groups (mg / kg)   |                 |                       |                 |                       |
|  |                    | Vehicle   | Low 1<br>5      | High 1<br>60          | Low 2<br>5      | High 2<br>60          |
| White blood cells  | $10^3/\mu\text{l}$ | 12.2<br>(0.6)   | 11.5<br>(0.8)   | 11.8<br>(0.9)         | 10.5<br>(0.9)   | 10.8<br>(0.9)         |
| Red blood cells  | $10^6/\mu\text{l}$ | 8.09<br>(0.13)  | 8.21<br>(0.14)  | 7.86<br>(0.11)        | 8.13<br>(0.12)  | 7.71<br>(0.11)        |
| Haemoglobin  | g/dl               | 16.2<br>(0.2)   | 15.9<br>(0.2)   | 15.5<br>(0.2)<br>*    | 15.9<br>(0.2)   | 15.2<br>(0.2)<br>**   |
| Hematocrit   | %                  | 48.4<br>(0.5)   | 47.8<br>(0.7)   | 47.0<br>(0.4)         | 47.7<br>(0.5)   | 45.9<br>(0.4)<br>**   |
| Mean cell volume   | f                  | 59.9<br>(0.6)   | 58.3<br>(0.6)   | 59.9<br>(0.8)         | 58.7<br>(0.4)   | 59.6<br>(0.5)         |
| Mean cell haemoglobin  | pg                 | 20.0<br>(0.2)   | 19.4<br>(0.2)   | 19.8<br>(0.3)         | 19.5<br>(0.1)   | 19.8<br>(0.2)         |
| Mean cell haemogl. conc.   | g/dl               | 33.4<br>(0.2)   | 33.3<br>(0.2)   | 33.0<br>(0.2)         | 33.3<br>(0.2)   | 33.2<br>(0.1)         |
| Reticulocytes  | %                  | 3.5<br>(0.3)  | 3.2<br>(0.2)    | 2.2<br>(0.1)<br>***   | 3.3<br>(0.2)    | 2.0<br>(0.1)<br>***   |
| Reticulocytes  | $10^3/\mu\text{l}$ | 277.7<br>(23.1)   | 265.3<br>(15.3) | 175.0<br>(7.7)<br>*** | 268.3<br>(13.1) | 157.1<br>(7.4)<br>*** |

Standard Error is shown between brackets if more than 2 animals  
Significance versus Vehicle computed by Mann-Whitney U test (two-tailed): \* P<.05 \*\* P<.01 \*\*\* P<.001

Low 2:5: spiked with each impurity at  
High 2:60: spiked with each impurity at

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| Parameter                | Unit               | Dosage Groups (mg / kg) |                 |                      |                     |                        |
|--------------------------|--------------------|-------------------------|-----------------|----------------------|---------------------|------------------------|
|                          |                    | FEMALES                 |                 |                      |                     |                        |
|                          |                    | Vehicle                 | Low 1<br>5      | High 1<br>60         | Low 2<br>5          | High 2<br>60           |
| White blood cells        | $10^3/\mu\text{l}$ | 9.6<br>(0.8)            | 10.5<br>(0.4)   | 9.9<br>(0.7)         | 11.1<br>(0.8)       | 10.5<br>(0.8)          |
| Red blood cells          | $10^6/\mu\text{l}$ | 7.67<br>(0.12)          | 7.82<br>(0.15)  | 7.66<br>(0.12)       | 8.03<br>(0.10)<br>* | 7.45<br>(0.15)         |
| Haemoglobin              | g/dl               | 15.2<br>(0.1)           | 15.7<br>(0.3)   | 15.0<br>(0.2)        | 15.8<br>(0.2)<br>*  | 14.6<br>(0.2)          |
| Haematocrit              | %                  | 44.7<br>(0.5)           | 45.4<br>(0.8)   | 44.1<br>(0.7)        | 45.8<br>(0.5)       | 42.7<br>(0.7)<br>*     |
| Mean cell volume         | fl                 | 58.4<br>(0.4)           | 58.1<br>(0.6)   | 57.6<br>(0.5)        | 57.1<br>(0.6)       | 57.4<br>(0.5)          |
| Mean cell haemoglobin    | pg                 | 19.9<br>(0.2)           | 20.1<br>(0.3)   | 19.6<br>(0.2)        | 19.6<br>(0.3)       | 19.6<br>(0.2)          |
| Mean cell haemogl. conc. | g/dl               | 34.1<br>(0.2)           | 34.6<br>(0.3)   | 34.0<br>(0.1)        | 34.4<br>(0.2)       | 34.2<br>(0.1)          |
| Reticulocytes            | %                  | 2.4<br>(0.2)            | 2.4<br>(0.2)    | 1.6<br>(0.1)<br>**   | 2.1<br>(0.1)        | 1.6<br>(0.2)<br>**     |
| Reticulocytes            | $10^3/\mu\text{l}$ | 187.9<br>(13.7)         | 189.8<br>(14.3) | 120.7<br>(9.9)<br>** | 167.9<br>(10.8)     | 115.4<br>(11.4)<br>*** |

Standard Error is shown between brackets if more than 2 animals

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

Low 2:5: spiked with each impurity at  
High 2:60: spiked with each impurity at

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Clinical chemistry: In both males and females in the HD groups there were increases in serum potassium. Other changes that were seen in both sexes in the HD groups ( both spiked and non-spiked) were decreases in calcium, total protein, albumin, cholesterol and triglycerides. Both sexes showed increases in inorganic phosphorous.

| Parameter         | Unit   | Dosage Groups (mg / kg) |                    |                     |               |                     |
|-------------------|--------|-------------------------|--------------------|---------------------|---------------|---------------------|
|                   |        | MALES                   |                    |                     |               |                     |
|                   |        | Vehicle                 | Low 1<br>5         | High 1<br>60        | Low 2<br>5    | High 2<br>60        |
| Sodium            | mmol/l | 144<br>(0)              | 144<br>(0)         | 144<br>(0)          | 144<br>(0)    | 144<br>(0)          |
| Potassium         | mmol/l | 4.7<br>(0.1)            | 4.6<br>(0.1)       | 4.9<br>(0.1)<br>*   | 4.6<br>(0.1)  | 4.9<br>(0.1)        |
| Chloride          | mmol/l | 102<br>(1)              | 101<br>(1)         | 102<br>(0)          | 101<br>(0)    | 102<br>(0)          |
| Calcium           | mg/dl  | 11.0<br>(0.1)           | 11.2<br>(0.1)      | 10.9<br>(0.1)       | 11.0<br>(0.0) | 10.7<br>(0.1)<br>** |
| Inorg. phosphorus | mg/dl  | 10.0<br>(0.2)           | 10.6<br>(0.2)<br>* | 10.8<br>(0.2)<br>*  | 10.2<br>(0.2) | 10.7<br>(0.2)<br>** |
| Total protein     | g/dl   | 6.5<br>(0.1)            | 6.5<br>(0.1)       | 5.9<br>(0.1)<br>*** | 6.4<br>(0.1)  | 5.9<br>(0.1)<br>*** |
| Albumin           | g/dl   | 4.6<br>(0.1)            | 4.8<br>(0.0)<br>*  | 4.4<br>(0.1)<br>**  | 4.6<br>(0.1)  | 4.3<br>(0.0)<br>*** |
| Glucose           | mg/dl  | 83<br>(4)               | 81<br>(2)          | 76<br>(3)           | 75<br>(2)     | 74<br>(2)           |
| Cholesterol       | mg/dl  | 57<br>(4)               | 70<br>(4)          | 41<br>(6)<br>*      | 72<br>(6)     | 35<br>(5)<br>**     |
| Triglycerides     | mg/dl  | 66<br>(12)              | 59<br>(4)          | 51<br>(5)           | 63<br>(5)     | 45<br>(7)<br>*      |

Standard Error is shown between brackets if more than 2 animals  
 Significance versus Vehicle computed by Mann-Whitney U test (two-tailed): \* P<.05 \*\* P<.01 \*\*\* P<.001  
 Low 2:5: spiked with each impurity at  
 High 2:60: spiked with each impurity at

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| Parameter         | Unit   | Dosage Groups (mg / kg) |                   |                     |                   |                      |
|-------------------|--------|-------------------------|-------------------|---------------------|-------------------|----------------------|
|                   |        | FEMALES                 |                   |                     |                   |                      |
|                   |        | Vehicle                 | Low 1<br>5        | High 1<br>60        | Low 2<br>5        | High 2<br>60         |
| Sodium            | mmol/l | 143<br>(0)              | 142<br>(1)        | 141<br>(1)          | 142<br>(0)        | 142<br>(0)           |
| Potassium         | mmol/l | 4.2<br>(0.1)            | 4.5<br>(0.1)<br>* | 4.9<br>(0.1)<br>*** | 4.6<br>(0.1)<br>* | 4.8<br>(0.1)<br>***  |
| Chloride          | mmol/l | 102<br>(1)              | 103<br>(1)        | 102<br>(0)          | 103<br>(0)        | 102<br>(0)           |
| Calcium           | mg/dl  | 11.0<br>(0.1)           | 11.0<br>(0.1)     | 10.7<br>(0.1)<br>** | 10.9<br>(0.1)     | 10.6<br>(0.0)<br>*** |
| Inorg. phosphorus | mg/dl  | 8.5<br>(0.1)            | 8.5<br>(0.1)      | 9.6<br>(0.2)<br>*** | 8.4<br>(0.1)      | 9.2<br>(0.2)<br>*    |
| Total protein     | g/dl   | 6.5<br>(0.1)            | 6.4<br>(0.1)      | 5.5<br>(0.1)<br>*** | 6.4<br>(0.0)      | 5.3<br>(0.1)<br>***  |
| Albumin           | g/dl   | 4.8<br>(0.1)            | 4.8<br>(0.0)      | 4.1<br>(0.1)<br>*** | 4.7<br>(0.1)      | 3.9<br>(0.1)<br>***  |
| Glucose           | mg/dl  | 80<br>(2)               | 86<br>(2)<br>*    | 78<br>(2)           | 85<br>(2)         | 76<br>(2)            |
| Cholesterol       | mg/dl  | 76<br>(5)               | 76<br>(3)         | 13<br>(2)<br>***    | 84<br>(4)         | 9<br>(2)<br>***      |
| Triglycerides     | mg/dl  | 51<br>(4)               | 58<br>(5)         | 35<br>(4)<br>*      | 53<br>(7)         | 37<br>(3)<br>*       |

Standard Error is shown between brackets if more than 2 animals  
 Significance versus Vehicle computed by Mann-Whitney U test (two-tailed): \* P<.05 \*\* P<.01 \*\*\* P<.001  
 Low 2:5: spiked with each impurity at  
 High 2:60: spiked with each impurity at

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Absolute organ weights increased in both sexes ( in the HD groups) were the spleen, pancreas and adrenals. In the HD females, other organs with increased absolute weight were the lungs, liver and heart.

| Parameter   | Unit | Dosage Groups (mg / kg) |               |                  |                    |                 |
|-------------|------|-------------------------|---------------|------------------|--------------------|-----------------|
|             |      | MALES                   |               |                  |                    |                 |
|             |      | Vehicle                 | Low 1<br>5    | High 1<br>60     | Low 2<br>5         | High 2<br>60    |
| Body weight | g    | 284<br>(5)              | 289<br>(4)    | 283<br>(4)       | 291<br>(6)         | 280<br>(5)      |
| Lungs       | mg   | 1517<br>(61)            | 1546<br>(33)  | 1566<br>(62)     | 1508<br>(61)       | 1493<br>(28)    |
| Spleen      | mg   | 597<br>(29)             | 654<br>(35)   | 666<br>(24)      | 631<br>(27)        | 662<br>(26)     |
| Liver       | mg   | 8748<br>(288)           | 9182<br>(182) | 9080<br>(239)    | 8779<br>(199)      | 9162<br>(221)   |
| Heart       | mg   | 1093<br>(19)            | 1159<br>(29)  | 1187<br>(53)     | 1247<br>(39)<br>** | 1189<br>(45)    |
| Pancreas    | mg   | 1161<br>(78)            | 1204<br>(41)  | 1253<br>(44)     | 1091<br>(57)       | 1270<br>(53)    |
| Kidneys     | mg   | 2214<br>(54)            | 2267<br>(61)  | 2158<br>(55)     | 2282<br>(77)       | 2262<br>(55)    |
| Brain       | mg   | 1981<br>(11)            | 2008<br>(24)  | 1929<br>(26)     | 1954<br>(49)       | 1947<br>(19)    |
| Thymus      | mg   | 571<br>(38)             | 575<br>(38)   | 580<br>(19)      | 591<br>(26)        | 550<br>(52)     |
| Adrenals    | mg   | 55<br>(3)               | 58<br>(3)     | 80<br>(6)<br>*** | 53<br>(3)          | 74<br>(5)<br>** |

Standard Error is shown between brackets if more than 2 animals

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

Low 2:5: spiked with each impurity at

High 2:60: spiked with each impurity a

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| Parameter   | Unit | Dosage Groups (mg / kg) |               |                      |               |                      |
|-------------|------|-------------------------|---------------|----------------------|---------------|----------------------|
|             |      | FEMALES                 |               |                      |               |                      |
|             |      | Vehicle                 | Low 1<br>5    | High 1<br>60         | Low 2<br>5    | High 2<br>60         |
| Body weight | g    | 187<br>(5)              | 188<br>(6)    | 193<br>(5)           | 182<br>(6)    | 188<br>(4)           |
| Lungs       | mg   | 1199<br>(32)            | 1215<br>(48)  | 1367<br>(45)<br>*    | 1179<br>(31)  | 1353<br>(42)<br>**   |
| Spleen      | mg   | 468<br>(26)             | 480<br>(24)   | 565<br>(28)<br>*     | 475<br>(12)   | 560<br>(20)<br>*     |
| Liver       | mg   | 5946<br>(129)           | 6234<br>(307) | 7106<br>(231)<br>*** | 5870<br>(177) | 7116<br>(168)<br>*** |
| Heart       | mg   | 788<br>(24)             | 793<br>(21)   | 836<br>(29)          | 833<br>(26)   | 829<br>(19)          |
| Pancreas    | mg   | 862<br>(28)             | 907<br>(59)   | 1134<br>(64)<br>**   | 805<br>(56)   | 1172<br>(59)<br>***  |
| Kidneys     | mg   | 1502<br>(34)            | 1496<br>(47)  | 1560<br>(53)         | 1463<br>(40)  | 1595<br>(43)         |
| Brain       | mg   | 1883<br>(24)            | 1855<br>(16)  | 1801<br>(20)<br>*    | 1870<br>(23)  | 1850<br>(13)         |
| Thymus      | mg   | 509<br>(36)             | 488<br>(20)   | 520<br>(22)          | 487<br>(31)   | 544<br>(21)          |
| Adrenals    | mg   | 66<br>(3)               | 69<br>(4)     | 113<br>(6)<br>***    | 69<br>(3)     | 128<br>(7)<br>***    |

Standard Error is shown between brackets if more than 2 animals  
 Significance versus Vehicle computed by Mann-Whitney U test (two-tailed): \* P<.05 \*\* P<.01 \*\*\* P<.001  
 Low 2:5: spiked with each impurity at  
 High 2:60: spiked with each impurity a

Normalized lung, liver, spleen, pancreas and adrenal weight were also significantly increased in females. Normalized liver and adrenals were significantly increased in males.

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Gross pathology observations are summarized in the reviewer's table below.

|                   | Dose group |        |          |        |          |
|-------------------|------------|--------|----------|--------|----------|
|                   | veh        | Low1:5 | High1:40 | Low2:5 | High2:40 |
| <b>males</b>      |            |        |          |        |          |
| Adrenals: swollen | 0/10       | 0/10   | 2/10     | 0/10   | 2/10     |
| <b>females</b>    |            |        |          |        |          |
| Adrenals: swollen | 0/10       | 0/10   | 7/10**   | 0/10   | 9/10***  |

|   | Dose group |        |          |        |          |
|---|------------|--------|----------|--------|----------|
|   | veh        | Low1:5 | High1:40 | Low2:5 | High2:40 |
| <b>males</b>  |            |        |          |        |          |
| Adrenal: Swollen zona fasciculate cells, diffuse    | 0          | 0      | 5*       | 0      | 4        |
| Adrenal: Swollen zona reticularis cells, diffuse    | 0          | 0      | 3        | 0      | 4        |
| Adrenal: Vacuolated zona fasciculate cells, diffuse |            |        |          |        |          |
| Lungs: foamy macrophages                            | 1          | 1      | 7*       | 1      | 5        |
| <b>females</b>                                      |            |        |          |        |          |
| Adrenal: small zona glomerulosa                     | 0          | 0      | 9***     | 0      | 10***    |
| Adrenal: swollen zona fasciculate cells, diffuse    | 0          | 0      | 9***     | 0      | 10***    |
| Adrenal: swollen zona reticularis cells, diffuse    | 0          | 0      | 8***     | 0      | 10***    |
| Lungs: foamy macrophages                            | 1          | 1      | 10***    | 0      | 10***    |
|   |            |        |          |        |          |

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Histopathology findings : the sponsor's final point under the "Conclusion" is of special interest and is shown below:

**Conclusion**

When male and female rats were dosed for 14 days with a spiked or non-spiked batch of R067555 up to 60 mg b.e./kg body weight/day, the following histological changes were observed and considered to be drug-related:

- a dose dependent increase of red blood cells in the splenic red pulp was observed in the 5 and 60 mg b.e./kg body weight/day dosed groups, males as well as females;
- a swelling of adrenocortical cells in the zona fasciculata and zona reticularis was observed in the 60 mg b.e./kg body weight/day dosed groups, especially the females. The cells of zona fasciculata and the zona reticularis were swollen in females, sometimes in association with sinusoidal ectasia in the (inner) cortical area and/or in the medulla, sometimes in association with diffuse vacuolation and often associated with a small sized zona glomerulosa;
- a minimal to slight increase in intra-alveolar foamy macrophages was observed in the lung of the 60 mg b.e./kg body weight/day dosed groups. The changes were more prominent in the females than in the males;
- a reduced cyclic activity (more resting appearance) in the female genital tract in the 60 mg b.e./kg body weight/day dosed groups.

The reasoning behind this statement is shown below, taken from the Discussion section:

**Female genital tract** (= Ovaries, oviduct, uterus, cervix, vagina)

No relevant differences between vehicle and 5 mg b.e./kg body weight/day dosed groups. Although some variation in score for the histological changes is due to variation of the cyclic stage between individual animals, the following histological changes in the 60 mg b.e./kg body weight/day dosed groups are retained as treatment-related and are indicative for a reduced cyclic activity (more resting appearance):

- the increased incidence of atrophic follicles with cystic-like appearance and the reduction of basophilic (=recent) corpora lutea in the ovaries;
- the decreased score for vacuolated (=karyorrhectic) epithelium and decreased granulocytic infiltrate in the uterus;
- the increased presence of cellular debris, of granulocytes in the vaginal lumen and in the vaginal wall, and of mucosal mucification.

Findings were comparable in the spiked and non-spiked groups.

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The relevant portions of the sponsor's incidence tables are shown below.

| Organ or Tissue - Observation                   | Dosage group ( mg / kg ) |         |           |         |           |
|---|--------------------------|---------|-----------|---------|-----------|
|   | Vehicle                  | Low 1:5 | High 1:60 | Low 2:5 | High 2:60 |
| <b>Ovaries</b>                                  |                          |         |           |         |           |
| <i>Number examined:</i>                         | 10                       | 10      | 10        | 10      | 10        |
| - atretic follicles                             | 10                       | 10      | 10        | 10      | 10        |
| - atretic follicles with cystic-like appearance | 0                        | 0       | 5 *       | 1       | 7 **      |
| - corpora lutea, basophilic                     | 9                        | 10      | 2 **      | 10      | 0 ***     |
| - corpora lutea, cystic                         | 0                        | 0       | 2         | 0       | 0         |
| - corpora lutea, eosinophilic                   | 10                       | 10      | 10        | 10      | 10        |
| - interstitial tissue                           | 0                        | 0       | 0         | 0       | 1         |
| - luteinized follicles                          | 0                        | 0       | 1         | 0       | 0         |
| - Sertoli-like cells                            | 0                        | 1       | 1         | 1       | 1         |
| - tertiary follicles                            | 10                       | 10      | 10        | 10      | 10        |
| <b>Uterus</b>                                   |                          |         |           |         |           |
| <i>Number examined:</i>                         | 10                       | 10      | 10        | 10      | 10        |
| - dilated lumen (lumina)                        | 5                        | 3       | 4         | 6       | 8         |
| - glandular development                         | 10                       | 10      | 10        | 10      | 10        |
| - granulocytes in lumen                         | 0                        | 0       | 0         | 1       | 0         |
| - high active epithelium                        | 0                        | 2       | 0         | 0       | 1         |
| - infiltrating granulocytes                     | 10                       | 10      | 5 *       | 10      | 4 *       |
| - mitoses (epithelium)                          | 1                        | 1       | 0         | 0       | 0         |
| - vacuolated (karyorrhectic) epithelium         | 6                        | 4       | 2         | 5       | 1         |
| <b>Vagina</b>                                   |                          |         |           |         |           |
| <i>Number examined:</i>                         | 10                       | 10      | 10        | 10      | 10        |
| - cellular debris                               | 0                        | 0       | 2         | 0       | 2         |
| - cornification                                 | 3                        | 4       | 1         | 4       | 3         |
| - desquamation                                  | 3                        | 5       | 3         | 3       | 3         |
| - granulocytes in lumen                         | 0                        | 1       | 2         | 0       | 3         |
| - infiltrating granulocytes                     | 4                        | 3       | 7         | 4       | 5         |
| - mitoses (epithelium)                          | 0                        | 1       | 0         | 1       | 0         |
| - mucified aspect                               | 4                        | 3       | 5         | 3       | 6         |
| - single cell necrosis (epithelium)             | 3                        | 0       | 5         | 1       | 2         |
| - thickness epithelial layer                    | 10                       | 10      | 10        | 10      | 10        |

Significance versus Vehicle computed by the Fisher Exact test (two tailed): \* P < .05 \*\* P < .01 \*\*\* P < .001  
Statistics are only performed if more than 50 % of the animals of the group are examined

There are several troublesome points. Compared to previously submitted reports:

3 month mouse study: 40 mg/kg dose not analyzed, significant effects on ovaries at HD of 160 mg/kg

24 month mouse study: effects seen at 40 mg/kg

6 month rat study: no clear effects on ovaries at 40 mg/kg, significant effects at 160 mg/kg

12 month rat study: significant effects at 80 mg/kg, no clear effects at next dose of 20 mg/kg.

24 month rat study: significant effects on ovary at 40 mg/kg

It is interesting that the sponsor states that there is a drug-related effect on cyclicity seen at 14 days of dosing using a dose that has not produced clear effects after 6 months of dosing.

Overall, the combination of impurities added to nebivolol did not cause any new effects compared to nebivolol alone. Although in some parameters the degree of effect appeared to be of a slightly greater degree than what was seen with nebivolol alone, from the data provided it is not possible to distinguish between a true exacerbation of effect and the variability that may be expected simply from having two separate groups receiving the same dose. There is no discernible signal that this combination of impurities and nebivolol causes any appreciable biological effects.

Elizabeth Hausner DVM  
Pharmacologist

Al DeFelice, Ph.D.  
Supervisory Pharmacologist

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/s/

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3/24/05 07:43:53 AM  
PHARMACOLOGIST  
Elizabeth Hausner

Albert Defelice  
3/24/05 08:29:12 AM  
PHARMACOLOGIST

# PHARMACOLOGY/TOXICOLOGY REVIEW

## 3.1 INTRODUCTION AND DRUG HISTORY

**NDA number:** 21742

**Review number:** 1

**Sequence number/date/type of submission:** 0/January 4, 2005/

**Information to sponsor:** Yes ( ) No ( )

**Sponsor and/or agent:** Bertek

**Manufacturer for drug substance:** Mylan Pharmaceuticals Inc, 781 Chestnut Ridge Road, Morgantown, WV 26505.

**Reviewer name:** Elizabeth Hausner, D.V.M.

**Division name:** Cardio-Renal Drug Products

**HFD #:** 110

**Review completion date:** February 11, 2005

### Drug:

**Generic Name:** Nebivolol Tablets

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

**Code Numbers** R067555

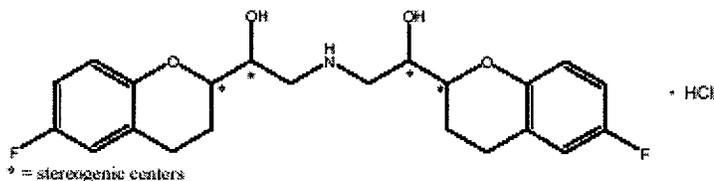
R067138 (d-Nebivolol)

R067145 (l-Nebivolol)

**CAS Registry No.** 152520-56-4

**Trade Name:** To Be Established

**Figure 3.2-01 Chemical Structure of Nebivolol**



**Empirical Formula** C<sub>22</sub>H<sub>25</sub>F<sub>2</sub>NO<sub>4</sub>·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 in this addendum was submitted in response to the Division's repeated requests for data to indicate that the reproductive toxicology findings were not of clinical relevance. The sponsor begins the presentation with 3 citations:

- a) an abstract from a Teratology Society meeting
- b) an abstract from medline
- c) the package insert from Coreg

One of the sponsor's introductory paragraphs is shown below:

Almost all, if not all, reproductive functions including rearing may be affected by degrees of hormonal imbalance. Beta-blockers are associated with embryo lethality (lab animals), low birth weight (humans and lab animals) and suppression of body weight gains of offspring (humans and lab animals) (Tabacova et al. 2002; Bayliss et al. 2000). Mechanisms hypothesized for the effect of beta blockers on reproduction include a reduction of blood pressure resulting in reduced placental and fetal circulation (Tabacova et al. 2002) and direct toxic effects on fetuses (Klug et al 1990). At least one beta blocker, carvedilol, can reduce fertility in rats at dose levels that cause sedation and reduced weight gain (PDR).

The generality of the above statement omits two important points: 1) the approved beta blockers cause embryotoxic/fetotoxic effects at substantial multiples of the maximally recommended human doses 2) no other beta blocker lists decreased fertility of pups as an observed effect. The reviewer's summary of this information from the PDR is shown below.

| Drug        | Effects  | Non-clinical safety margin |
|-------------|--|----------------------------|
| timolol     | No effects on reproduction or fertility  | Up to 125X the MRHD        |
|             | Teratology: no effects up to 40X the MRHD<br>40X MRHD: delayed fetal ossification associated with maternal toxicity ( based on patient body weight of 50 kg)   | 40 x the MRHD              |
| propranolol | Rats: ↓ litter size, ↑ resorptions sites, neonatal deaths  | 10X MRHD                   |
|             | Rabbits: no embryo toxicity, no neonatal toxicity  | 15X MRHD                   |
|             | Intrauterine growth retardation reported in neonates whose mothers received propranolol during pregnancy.<br>Neonates of mothers receiving propranolol at parturition showed bradycardia, hypoglycemia and respiratory depression. |                            |
| nadolol     | Rabbits: embryo/fetotoxicity   | 5-10x MRHD                 |
|             | No teratogenic potential shown   |                            |
|             | Neonates of mothers receiving nadolol at parturition have exhibited bradycardia, hypoglycemia and associated symptoms  |                            |
| Metoprolol  | Rats: ↓ neonatal survival, ↑ post-implantation loss  | 22xMRHD ( 60 kg            |

|            |   |  |
|------------|---|--|
|            | No evidence of teratogenicity or impaired fertility   | patient)   |
| Carvedilol | Rats: ↑ postimplantation loss (associated with maternal toxicity). Decrease in fetal body weight, missing or stunted ribs associated with maternal toxicity.<br>Increased mortality at one week post-partum in neonates from rats treated during last trimester through lactation day 22. | 50X MRHD for teratology<br><br>NOAEL for development was ≥10X MRHD |
|            | Rabbits: ↑ postimplantation loss  | 25X MRHD   |
| Atenolol   | Fertility unaffected  | Up to 100x MRHD  |
|            | Rats: dose-related increases in embryo-fetal absorptions  | ≥25x MHRD (50 kg patient)  |
|            | Rabbits: similar effects to rat not seen.   | Up to 12.5x MRHD   |
|            | Administration starting in the second trimester of pregnancy has been associated with the birth of infants who are small for gestational age.   |  |

Nebivolol produced untoward effects at doses that were not associated with reported or obvious maternal toxicity. Nebivolol is possibly unique among beta blockers in having no margin of safety between reproductive/developmental effects and the therapeutic dosages.

Dystocia was reported in at least 3 studies, but with incidences clearly reported for only 2 studies. This is summarized below:

#### Reviewer's summary of dystocia incidences

|                                   | Dose mg/kg   |      |      |        |
|-----------------------------------|--|------|------|--------|
|                                   | 0  | 2.5  | 10   | 40     |
| Study N92570 dystocia             | 0/24   | 0/24 | 2/24 | 3/24   |
|                                   | Dose mg/kg   |      |      |        |
|                                   | 0  | 1.25 | 5    | 20     |
| Study N106655 dystocia            | 0/10   | 0/11 | 2/9  | 8/12** |
| cannibalism                       | 0/10   | 0/11 | 0/9  | 4/12   |
| **p<0.01 by two-tailed Chi Square |  |      |      |        |
| N65774                            | Dystocia was reported for all dose groups in the text of the study. The actual incidences to support this statement could not be located in the report. An increase in the number of dead fetuses was attributed to dystocia. Increased cannibalism was used to explain the lack of a dose response in the numbers of dead pups. |      |      |        |

There are throughout the Reproductive and Developmental toxicology studies, textual reports of dystocia, delayed parturition and cannibalism. Incidences are not presented in each study. Maternal toxicity is not clearly obvious in each study. Developmental effects, other than decreased fertility in offspring, remain undescribed due to the insensitive detection method used.

The decreased fertility in offspring of drug-treated dams was reported in N106655. The sponsor has not conducted a more recent study to try to confirm or refute the earlier observations.

Comparison of relative exposure between rats (Study N106655) and humans given Nebivolol

| Effect reported in offspring     | Dose given to dam with effect noted in offspring |                   | Relative to human 10 mg dose(6mg/m <sup>2</sup> ) | Relative to human 40 mg dose(25 mg/m <sup>2</sup> ) |
|----------------------------------|--|-------------------|---|---|
|                                  | Mg/kg  | Mg/m <sup>2</sup> |   |   |
| ↓ fertility                      | 5  | 30                | 5x  | 1.2x  |
| ↓ weight of gravid uterus        | 1.25   | 7.5               | 1.25x   | 0.3X  |
| ↓ mean litter size               | 1.25   | 7.5               | 1.25x   | 0.3X  |
| ↓ implantations, ↓ corpora lutea | 1.25   | 7.5               | 1.25x   | 0.3X  |

The effects listed were reported in the offspring (F1) of treated (F0) dams. A Km of 6 was used to calculate rat doses in mg/m<sup>2</sup>. A Km of 37 was used to convert dosages for a 60 kg human to mg/m<sup>2</sup>.

While other beta adrenergic antagonists have reported non-clinical and clinical effects on reproduction, there are several distinctions that are possibly unique to nebivolol:

1. the approved beta blockers showed in non-clinical studies at least 5X margin of safety with the maximally recommended human doses.
2. effects of decreased fertility in offspring of drug-treated dams has not been reported in approved beta blockers
3. dystocia, cannibalism and prolonged parturition have not been reported for approved beta blockers.

In looking for guidance in the non-clinical characterization of a signal suggesting an endocrine effect, there are several sources of information. These include:

*The OECD Guidelines on Endocrine Disruptors*

Some of the published literature on selective estrogen receptor modulators (SERM) lasofoxifene and raloxifene listed below:

*J. Buelke-Sam et al. The selective estrogen receptor modulator, raloxifene: A segment II/III delivery study in rats. Reproductive Toxicology, Vol 12, No.3, pp.271-288, 1998.*

*D.O. Clarke, et al. The selective estrogen receptor modulator, raloxifene: reproductive assessments following preimplantation exposure in mated females. Reproductive Toxicology, Vol 12, No.3, pp. 247-259, 1998.*

*W.P. Weisenburger, A.R.Hagler, M.S. Tassinari. Pre- and postnatal development studies of lasofoxifene, a selective estrogen receptor modulator (SERM), in Sprague-Dawley rats. Birth Defects Research (Part B)71:171-184(2004).*

*T.R.S. Ozolins and U.Gupta. Embry/fetal toxicity assessment of lasofoxifene, a selective estrogen receptor modulator (SERM), in rats and rabbits. Birth Defects Research (Part B)71:161-170(2004).*

*J.A.Hoyt et al. The selective estrogen receptor modulator,raloxifene: Reproductive assessments in adult male rats. Reproductive Toxicology, Vol. 12, no. 3, pp.223-232, 1998.) morphology.*

There were no significant differences in time to mating, % implantation, live conceptuses per litter, mating index or fertility index. While Leydig cell tumors were reported for male mice treated with raloxifene, other effects on the testes were inconsistent, except for decreased progressive motility of sperm after 28 days of treatment.

*Cappon, G.D., M. Horimoto, M.E. Hurtt. Reproductive toxicity assessment of lasofoxifene, a selective estrogen receptor modulator(SERM), in male rats. Birth Defects research (Part B)71: 142-149 (2004).)*

Male rat body weight was decreased at all doses ( significantly so,  $p \leq 0.01$ , for all drug-treated groups beginning on day 4 and continuing through the end of treatment). Duration of cohabitation was increased at the HD by 0.7 days. The number of males copulating and the number of implantation sites produced per copulation were reduced in the 10 and 100 mg/kg groups. Absolute weights of the seminal vesicles and epididymides were decreased for all groups. However, testes weight and seminal epididymal sperm motility and concentration were not affected by treatment. No microscopic findings were reported for the male reproductive tissues.

Duration of the cohabitation interval was increased only at the highest dose. However, copulation rate was decreased at both 10 and 100 mg/kg and the pregnancy rate showed a dose-related decrease. Implantation sites and viable fetuses were also decreased at the 10 and 100 mg/kg doses.

Nebivolol (N69430) Fertility and Reproduction study.

Doses of 0, 10, 40 and 160 mg/kg administered through the diet. There was decreased body weight gain in all drug-treated groups of males (LD-2%, MD -17%, HD -55% relative to control). In the 173 pages of this report, there was no male organ weight data. There were no gross observations on the male reproductive tract, there was no histopathology data for the male reproductive organs and there was no data for sperm numbers, motility, morphology or kinetics of production. There is no data to conclude either an effect or the lack of a nebivolol-related effect upon the male reproductive system.

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*Hoyt, J.A. et al. The selective estrogen receptor modulator, raloxifene: reproductive assessments following pre-mating exposure in female rats. Repro. Toxicol. Vol 12, No.3, pp.233-245, 1998.*

*Terry, K.K. et al. Reproductive Toxicity Assessment of lasofoxifene, a selective estrogen receptor modulator (SERM), in female rats. Birth Defects Research (Part B) 71:150-160(2004).*

Both references indicated that each drug altered normal female cyclicity reversibly but did not affect fertility per se. Results common to both drugs were increased implantation loss, increased gestation length and reduced litter size. It is interesting to note in the lasofoxifene data that there was less effect apparent at the higher doses than the lower doses.

A textual comment reported that the external appearance of GD21 fetuses resembled that of GD14 fetuses, a severe developmental delay that is beyond being smaller than expected. The text of the paper also noted that 3 animals did not re-establish normal cyclicity after drug administration was terminated. That is, there were permanent effects upon the endocrine axes in these animals. The same paper also referenced the persistence of abnormal cyclicity in rats after treatment with raloxifene (Hoyt et al., 1998). Decreased food consumption may alter cyclicity. A separate food restriction study indicated that a 50% restriction in food consumption and mean decrease in body weight of ~20% compared to controls appear to be needed to achieve the persistent diestrus without pharmacologic means.

#### Nebivolol

N69430 Male and female fertility study in Wistar rats. Doses of 10, 40 and 160 mg/kg were administered through the diet.

As mentioned above for the male fertility, there were few details in this report. There is no information on female cyclicity, histopathology of the reproductive tract, early vs late resorptions or pre- vs post-implantation losses. From the data provided it appears that a dose between 40 and 160 mg/kg might have provided a better description of fertility effects. The median interval to mating was decreased at the HD. Body weight during gestation was decreased in all drug-treated groups compared to control while the weight of the gravid uterus was inconsistently affected. Number of live feti, mean litter size and implantations appeared to fluctuate in parallel. Number of corpora lutea was decreased only in the one surviving high dose female.

The report for study N92570 reported increased duration of gestation (0.8 days greater than control,  $p < 0.01$ ) at the HD of 40 mg/kg. The report for study N106655 indicated increased duration of gestation at the HD of 20 mg/kg: 1 day longer than the control value ( $p < 0.05$ ).

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*Byrd and Francis. The selective estrogen receptor modulator, raloxifene: Segment II studies in rats and rabbits. Reproductive Toxicology, Vol 12, No. 3, pp 261-270, 1998.*

Rats were dosed from GD6-GD17. Other comments from the text of the report included  
Evidence of hemorrhaging from the vagina in a single animal in the 1 mg/kg and  
several animals in the 10 mg/kg group.  
dystocia in one 0.1 mg/kg animal  
The deviations that appeared to be associated with maternal raloxifene in the 1 and 10  
mg/kg groups were wavy ribs and kidney cavitation.

N51171 Seg II Study

Doses of 1.25, 5 and 20 mg given from GD6-GD16. The introduction cites a preliminary study that used doses of 40, 80 and 160mg/kg.

No data was available regarding variations, anomalies and teratogenicity.

N74513SegII Study in Rats

Doses of 2.5, 10 and 40 mg/kg were given from GD6 to GD16 by oral gavage. The introduction of the report cites a preliminary study that used doses of 40, 80 and 160 mg/kg. The details of that preliminary study have not been located.

N74514 Seg II Study in Rats

Doses of 2.5, 10 and 40 mg/kg (repeat of the previous study) were given from GD6 – GD16 by oral gavage.

N51487 Seg II Study in Rabbits

Doses of 1.25, 5 and 20 mg/kg were given orally from GD6 through GD18.  
The data summary for this study is poorly readable. It is reproduced here.

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| Treatment : from day 6 to day 18 after mating |     |               |         |        |        |
|---|-----|---------------|---------|--------|--------|
| Embryotoxicity and teratogenicity in rabbit   |     |               |         |        |        |
|   |     | Dosage groups |         |        |        |
|   |     | R 67555       |         |        |        |
|   |     | Control       | 1.25 mg | 5 mg   | 20 mg  |
| Adult rabbit data                             |     |               |         |        |        |
| No. of dosed females                          |     | 15            | 15      | 15     | 15     |
| No. of dead females                           | (1) | 0/15          | 0/15    | 1/15   | 0/15   |
| No. of pregnant females                       | (1) | 14/15         | 14/15   | 11/15  | 13/15  |
| Weight change of preg. females                | (2) | -357.9        | -192.3  | -188.7 | -296.6 |
| Litter data                                   |     |               |         |        |        |
| Number of living pups / female                | (2) | 9.5           | 6.1 **  | 7.8    | 6.8 ** |
| Number of dead pups / female                  | (2) | 0.2           | 0.2     | 0.6    | 0.6    |
| Mean litter size                              | (2) | 9.7           | 6.2 **  | 7.8    | 6.8 ** |
| Number of resorptions / female                | (2) | 0.8           | 0.5     | 0.6    | 1.2    |
| Number of implantations / female              | (2) | 10.5          | 6.7 **  | 8.4 *  | 8.0 ** |
| Weight of living pups (each sex)              | (2) | 35.9          | 42.0 ** | 39.5   | 39.4   |
| Abnormalities                                 |     | 2/136         | 1/37    | 1/78   | 6/89   |

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Statistics - (1) Chi Square Test      \* p < 0.05  
(2) Mann-Whitney U Test      \*\* p < 0.01  
Date : 08-06-86      \*\*\* p < 0.001  
P. Lasserfs

The incidences of all terata, anomalies and variations were not presented or summarized. There was a textual description of gross observations, all of which were abnormal thoracic vertebrae and bifurcated ribs.

#### Summary

1. There is no data available or at least apparent regarding sperm analysis parameters for nebivolol.
2. There is no data available or at least apparent regarding female cyclicity for nebivolol.
3. There is no data available or apparent regarding any reversibility of effects apparently associated with nebivolol.
4. There is no data available regarding post-natal developmental landmarks after maternal nebivolol treatment.

5. There is no data available regarding pre-natal developmental delays if any.
6. Dystocia, delayed parturition, decreased birth weight of pups, decreased survival of pups have been reported in several studies corresponding to treatment with nebivolol.
7. Detailed descriptions of the delayed parturition (e.g.time in hours) were not apparent in any nebivolol report.
8. Actual incidence of dystocia and cannibalism was not provided for one nebivolol study where the text of the report stated that this occurred.
9. The report for one nebivolol range-finding study with adverse effects has apparently not been submitted.

Although decreased pup weight may prolong the timing of parturition (Leonhardt et al. Perinatal toxicity of ethylene glycol dimethyl ether in the rat. *Reprod Toxicol.* 5:157-162.) disrupted implantation followed by increased gestation length is consistent with estrogen agonist activity as seen with lasofoxifene and raloxifene. The paper by Terry et al. notes that:

Estrogen antagonism and its downstream effects are also likely a mechanism behind CL disruption, increases in pre- and post-implantation losses, and implantation –disruptive events in the oviduct and uterus. Post-ovulatory estrogen and progesterone directly affect nidation and implantation in the rodent...These hormones regulate contractions of the oviduct that influence the rate of transport of ovum to the uterus... On GD3 in the rat, an estrogen surge is required for preparation of the uterine lining for implantation...

Inhibition of ovarian function and disruption of implantation are consistent findings with antiestrogenic activity reported in ER knockout mice affecting disruption of the ER $\alpha$  gene...and for SERMs tamoxifen,...clomiphene,...idoxifene,... and raloxifene.

The raloxifene papers also touch on the subject of endocrine modulation of the immune system. While the raloxifene studies did examine this, and there are published reports relating to this aspect of tamoxifen and diethylstilbesterol, again there is no information generated for nebivolol.

The OECD, lasofoxifene and raloxifene papers are valuable for several reasons. First they show that endocrine modulators may have very subtle effects and may not always follow a classical dose response curve. Second, some effects while present, do not affect overall fertility in the rat. Therefore, alterations in female cyclicity and alterations in sperm motility may exist but simply examining mating and reproduction is likely to disclose a problem in rodents. Third, the papers are very valuable for the level of detail examined and the multiplicity of parameters that can be used to evaluate potential endocrine effects.

A living animal and the physiologic systems contained within that animal have limited ways to respond to chemical/pharmacological injury. Decreased food consumption, decreased implantations, decreased pup survival, dystocia, prolonged parturition and decreased pup weight

are to some extent non-specific signs. However, things that suggest a possible endocrine signal include:

1. leydig cell tumors in mice
2. dystocia
3. prolonged parturition
4. reproductive effects in the offspring of nebivolol-treated dams

The detail is simply not apparent in the nebivolol study reports to be able to come to a conclusion as to the mechanism of toxicity or the potential for clinical relevance.

The sponsor states in the discussion that nebivolol did not affect any of the reproductive or developmental parameters through a mechanism involving hormone imbalance. Unfortunately, the data to support this statement, particularly any exploration of mechanism, is not provided. The reproductive and developmental issues that concern the Division remain unanswered.

E.A. Hausner, D.V.M.  
Pharmacologist

A.F. DeFelice, Ph.D.  
Supervisory Pharmacologist

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Elizabeth Hausner  
2/23/05 10:27:45 AM  
PHARMACOLOGIST  
elizabeth hausner

Albert Defelice  
2/24/05 04:13:31 PM  
PHARMACOLOGIST

NDA21742  
D.V.M.

Reviewer: E.A. Hausner,

*dt/los*

*1*

**NDA 21-742**

**REVIEW AND EVALUATION OF PHARMACOLOGY AND  
TOXICOLOGY DATA**

**Nebivolol**

**Bertek Pharmaceuticals, Inc.  
Morgantown, WV**

**Elizabeth A. Hausner, D.V.M.**

**Division of Cardio-Renal Drug Products (HFD-110)  
Center for drug evaluation and Research**

**November 18, 2004**

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## ***EXECUTIVE SUMMARY***

### **1. Recommendations**

- 1.1 Recommendation on approvability *Approvable depending upon the clinical findings*
- 1.2 Recommendation for nonclinical studies *Characterization of the active metabolites, possible bone marrow toxicity, endocrine disruption, and repolarization effects would have been desirable earlier in the development process. At this stage there may be sufficient clinical data to resolve these issues without conducting further non-clinical testing.*
- 1.3 Recommendations on labeling: See attachment

### **2. Summary of nonclinical findings**

#### 2.1 Brief overview of nonclinical findings

The overall nonclinical package is remarkable for 1) lack of detail in methods and results (particularly histopathology) and 2) scoring systems that negatively impact facility of interpretation.

The toxicology studies were conducted primarily in rats and dogs with a few studies in mice and rabbits. There are few if any reports of clinical signs or behavioral changes in the repeat dose toxicology studies. Throughout the rat and dog studies were effects indicating hemolytic anemia. The rodents showed fairly consistently decreases in HCT, Hb and RBC. While the hematology changes were lacking in the dog, both rat and dog showed increased spleen size, weight and histologic findings of increased rbc in the red pulp. Target organs appear to be lungs, adrenal, heart and reproductive tract.

Clinical chemistry changes consistent across the rodent studies included increased serum potassium, decreased total protein, albumin, cholesterol, triglycerides, phospholipids and inorganic phosphorous. Common organ weight effects in both sexes were increases in lung, liver, spleen, heart and adrenal gland. Other changes reported were decreased pancreas, kidney and "gonad" weight. The histologic changes commonly reported for the lungs were foamy macrophages in the alveolar lumina and inflammatory thickening of the alveolar septae. It is unclear whether the liver is a target organ. There are inconsistent effects on weight in rodents as well as inconsistent effects on liver enzymes. The most consistent indicators of liver functional involvement are the effects noted above of circulating protein levels, cholesterol and triglycerides. The histopathology as presented was mostly non-informative with occasional designations of centrilobular swelling.

There were also indications in both rats and dogs of adrenal effects. Both species showed increases in serum potassium. Rats showed fairly consistent increases in adrenal weight with associated histologic findings of swollen cortical cells, ectasia of sinusoidal spaces and vacuolated zona fasciculata cells, pigmented zona reticularis, small zona glomerulosa and nodular hyperplasia in females. Effects upon the reproductive tract were difficult to discern from the reports as almost no histopathological findings were reported. The primary findings reported were mostly textual comments and organ weight changes. One month of orally administered nebivolol compared to the isomers caused an increase in absolute prostate weight compared to

control: 13%(racemate), 48%(R88548) and 3%(R88547). A three month study in dogs reported no change in prostate weight but did indicate ¼ urolithiasis (2.5 mg/kg) and ¼ prostatitis(10 mg/kg). "Gonad" weight changes appeared in both sexes of dogs but were inconsistent across studies. Histologic effects on the reproductive tract were rarely reported. The few comments that were found were suggestive of a problem. These are summarized below under Section 2.2.

2.1 Pharmacologic activity *A beta adrenergic receptor antagonist with weak  $\alpha 1$  and  $\alpha 2$  adrenergic activity. There are multiple active metabolites. The active metabolites are poorly characterized. According to one published literature report, nebivolol also binds to the estrogen receptor with approximately the same potency as estradiol.*

## 2.2 Nonclinical safety issues relevant to clinical use

### 1. Carcinogenicity

Benign and malignant Leydig cell tumors were present in the males : 2/50 (veh), 0/50 (LD), 1/50 (MD), 21/50 (HD). This was significant by the Exact Method and the Asymptotic method with the p value close to 0. A dose response of Leydig cell hyperplasia was also apparent. The Exec CAC found the Leydig cell tumors in mice to be drug-related.

The HD of 40 mg/kg (239 mg/m<sup>2</sup>) is comparable to 39X a human dose of 10 mg (6.2 mg/m<sup>2</sup>) on a surface area basis. The LD of 2.5 mg/kg is comparable to 2.4x and the MD of 10 mg/kg is comparable to 9.6x the human dose of 10 mg on a surface area basis. The dose successfully achieving decreased systolic pressure in African Americans is 40 mg per day. Using a body surface area comparison, there is only a 5x margin of safety(see Karen Hicks, M.D., Medical Officer's Efficacy Review). It has further been reported that clearance of nebivolol in African American poor metabolizers may be decreased by an average of 30% compared to "average" metabolizers, reducing the margin of safety to nothing.

### 2. Poorly characterized active metabolites.

### 3. QTc prolongation.

The QTc effect in the animal studies was inconsistently found.

A consistent feature of the QTc evaluation was the lack of detail as to the determination of ECG collection relative to dosing. Also, Bazett's formula appeared to be the only method of correction used even though it was inappropriate given the reported heart rates of the dogs.

In the acute cardiovascular safety study there were no apparent effects on QTc. A 2 week repeat dose study in dogs also showed no QTc effects. A one month oral dosing study showed QTc increased in all groups including controls. One month of intravenous dosing showed a decrease in QTc values. A 3-month oral study showed inconsistent QTc increases from week 4 onward at  $\geq 10$  mg/kg. Using body surface area for comparison, this has a HED of 52 mg/m<sup>2</sup>. A 10 mg dose for a 60 kg patient is equivalent to 6 mg/m<sup>2</sup>. Therefore there is approximately an 8.7X margin between the dose in dogs where the

effect appears and the highest proposed human dose. A HERG assay indicated that nebivolol inhibits the IKr channel with an  $IC_{50} = 3 \times 10^{-7} M$  compared to astemizole,  $IC_{50} = 2 \times 10^{-8} M$  in the same assay.

#### 4 Endocrine disruption

A vague endocrine effect is apparent despite or because of suboptimal reporting. The apparent manifestations of this potential effect were:

- 1). Leydig cell tumors of mice (usually associated with the estrogen receptor)
- 2). weight effects on the reproductive organs of both sexes of animals including mice, rats and dogs
- 3). poorly detailed histologic effects on the reproductive organs of males and females.
- 4). consistent effects upon adrenal glands (enlarged in rodents).
- 5) An equivocal effect was seen in rodents. In rodents, triglycerides were consistently decreased as was total cholesterol, which in the rat is almost entirely HDLc.  $\beta$  adrenergic antagonists typically show some increase in triglycerides and a decrease in HDL cholesterol. Effects upon either the adrenal gland or the liver could produce these effects which would in turn affect cholesterol based steroid hormones.
- 6). The sponsor's statement shown below.

##### Conclusion

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

Secondary to these direct effects on the steroid metabolism are the pulmonary, lymphonodular and splenic changes (namely: the presence of foamy macrophages and thickened septae in the lung, the splenic atrophy and the prominent histiocytes).

The more prominent red blood cells in the splenic red pulp are related to the accumulation of red blood cells. The last is due to the alpha-adrenolytic activity of the test article.

The evidence or reasoning behind this statement was never made apparent. There was little detail given about changes in the reproductive tracts. Some of these comments are shown below.

## N106653/1 3 month study in Swiss mice

Testicular changes were reported as “atrophy due to delayed maturation.” The sponsor’s summary however refers to “The adrenocortical, ovarian and testicular changes in the 160 mg/kg dosed groups are directly related to the interference of the test article with the steroid metabolism.” There is little detail about the referenced changes.

## N92595 1 month toxicity study in rats

The rationale for dosing includes the statement that “Pronounced toxicity was seen after 12 months of dosing with the spleen, adrenals, ...male and female genital tracts as target organs.”

## N64890 6 month oral toxicity in rats

Findings included edematous testes(4/20 @160 mg/kg), soft testes (2/20 ) swollen testes(1/20) and small testes (3/20), Control incidence for each of these findings was 0/20. The reproductive tract of the females was described as looking “more resting,” that is, senescent, as characterized by fewer corpora lutea, more atretic follicles, decreased uterine glandular development and thinning of the vaginal epithelium.

## N79297 12 month study in Wistar rats:

Decreased activity of the genital tract was noted by changes in the testes (degenerated tubuli and presence of giant cells), in the epididymides (low spermatozoa amount and cellular debris) of 80 mg/kg dosed males and in the ovaries (increase of atretic follicles, decrease of old corpora lutea) and in the uterus (reduced glandular development and granulocyte infiltration) of the 80 mg/kg dosed females.

## N109066/1 Carcinogenicity study in Swiss mice

Absolute and normalized gonad weight in the MD and HD females was decreased, significantly so at the HD ( $p < 0.05$  compared to both the vehicle and untreated control groups). Gross results in males included diffuse atrophy of the mammary glands: 6/48(U), 3/49(V), 4/48(LD), 12/48(MD,  $p < 0.05$  vs vehicle) and 12/49(HD,  $p < 0.05$  vs vehicle). Gross necropsy results in the females included mammary gland changes ( decreased number edematous) and uterine changes ( decreased swelling and cystic presentation) that were significantly different from the controls .The sponsor’s table below shows results in the order: untreated, vehicle, LD, MD, HD.

**Females**

|                                      |    |    |    |    |       |
|--------------------------------------|----|----|----|----|-------|
| Lung: congested                      | 0  | 1  | 1  | 1  | 6 *   |
| Uterus: swollen                      | 27 | 17 | 23 | 28 | 8 *** |
| Uterus: swollen, cystic <sup>+</sup> | 20 | 12 | 21 | 21 | 5 **  |

---

Significance computed by Chi-square test (two-tailed):

\* p<0.05 \*\* p <0.01 \*\*\* p<0.001

Mammary gland stimulation in females: 11/50(U), 7/50 (V), 10/50 (LD), 8/50(MD), 22/49(HD), p<0.05 vs untreated and p<0.01 vs vehicle.

N109067 Carcinogenicity study in SPF Wistar rats

Mammary gland stimulation was reported for the males: 11/50(U), 14/50(V), 12/50(LD), 12/49(LD) and 24/50(HD), p<0.01 vs untreated)

Changes reported for the females reported in the order: untreated, vehicle, LD, MD and HD:

|  |    |    |     |      |      |
|--|----|----|-----|------|------|
| Mammary gland: stimulation               | 46 | 48 | 45  | 46   | 37 * |
| tissue mass                              | 19 | 26 | 22  | 30 * | 12   |
| Pituitary gland: tissue mass             | 23 | 23 | 23  | 17   | 8 ** |
| tissue mass,<br>hemorrhagic <sup>+</sup> | 19 | 18 | 19  | 16   | 7 *  |
| Uterus: focus                            | 7  | 8  | 0 * | 5    | 1    |
| focus, swollen +                         | 7  | 8  | 0 * | 5    | 1    |
| swollen                                  | 6  | 2  | 6   | 3    | 0 *  |

---

<sup>+</sup> This observation is already included in the major observation of this tissue

Statistics: Chi-square test (two tailed): \* p<0.05 \*\* p <0.01 \*\*\* p<0.001

X: number of positive animals

By the end of the rat study, the HD males weighed on average 22%(p<0.001) less than the control groups. The HD females weighed on average 28% (p<0.001) less than the control groups. Significant differences in weight gain were apparent in the males from the Week 1 determination through the end of the study. In females, significant differences in weight gain were apparent from the Week 16 determination through the end of the study. A maximally tolerated dose was thus achieved, but the reduction in body weight gain may also have provided a protective effect for the HD animals.

Because of the possibility of body weight effects in the rat study altering the tumor incidence in the HD groups, it was requested that the mammary tumors be statistically re-analyzed omitting the HD group. The re-analysis consisted of a trend test comparing the vehicle control, LD and MD groups but omitting the HD group. Benign (adenomatous) neoplasia was analyzed separately from carcinoma/sarcoma neoplasms. A combination of all mammary tumors was then analyzed also. The untreated control group was compared against the MD group using a pairwise comparison.

The rat study was negative for carcinogenicity when associated tumor types were analyzed separately.

The statistical re-analysis showed:

Summary of female rat tumor re-analysis

| Tumor type  | untreated | vehicle | LD | MD | P value |
|---|-----------|---------|----|----|---------|
| Adenocarcinoma (papillary and acinar)<br>+carcinoma | 1         | 5       | 2  | 1  |         |
| a. trend test with vehicle, no HD group             |           |         |    |    | 0.9375  |
| b. trend test with untreated, no HD                 |           |         |    |    | 0.6082  |
| c. pairwise comparison, untreated and MD<br>group   |           |         |    |    | 0.7097  |
| Combining all other tumors in the mammary<br>gland  | 18        | 25      | 26 | 32 |         |
| a. trend test, vehicle, no HD                       |           |         |    |    | 0.1387  |
| b. trend test, untreated group, no HD               |           |         |    |    | 0.0066  |
| c. pairwise comparison: untreated vs MD             |           |         |    |    | 0.0022  |
| Combining all tumors in the mammary gland           | 19        | 28      | 27 | 32 |         |
| a. trend test, vehicle, no HD                       |           |         |    |    | 0.3045  |
| b. trend test, untreated, no HD                     |           |         |    |    | 0.012   |
| c. pairwise test, untreated vs MD                   |           |         |    |    | 0.0031  |

Several studies were done in rats examining circulating levels of corticosterone and aldosterone pre and post ACTH stimulation. Animals treated with either racemate or one of the enantiomers for up to 1 month showed an increase in corticosterone following stimulation. However, the degree of increase was less than that of the control group, significantly so in most cases. Control and LD aldosterone values were not provided for the racemate. Treatment with R85547 did not affect the day 25 post-stimulation aldosterone results. A dose-dependent statistically significant decrease in day 25 post-stimulation aldosterone values was seen in both males and females treated with R85548. A decrease in plasma renin was shown that is consistent with a beta adrenergic antagonist. This single species, limited examination of the apparent endocrine effects indicates that there is indeed some endocrine effect but provides little in the way of characterization.

## 6. Reproductive and Developmental Toxicity

Reproductive toxicity was apparent in a number of ways.

- 1) Doses that caused little to no reported toxicity in the non-gravid animals used in standard toxicology studies caused decreased food consumption and loss of weight in pregnant animals. Maternal toxicity was reported at doses of  $\geq 10$  mg/kg.
- 2) Study reports contained comments about dystocia and cannibalism occurring in drug-treated dams.

| Finding (Study N 106655) | Dose mg/kg/day |      |     |        |
|--------------------------|----------------|------|-----|--------|
|                          | 0              | 1.25 | 5   | 20     |
| Partus delay             | 0/10           | 0/11 | 0/9 | 7/12*  |
| cannibalism              | 0/10           | 0/11 | 0/9 | 4/12   |
| dystocia                 | 0/10           | 0/11 | 2/9 | 8/12** |

Significance by the two-tailed Chi Square: \*  $p < 0.05$ , \*\*  $p < 0.01$

The test for study number N65774 reported dystocia in all dose groups (10, 40 and 160 mg/kg). However, there was no summary table with precise incidences. The individual animal sheets reported only 1 dystocia (in the 10 mg/kg group). Cannibalism was reported at incidences of 0(Control), 1(LD), 1(MD) and 3(HD). The dose justification portion of the report for N92570 also stated that dystocia had been seen in study N65774 at all doses but with no incidences reported. The sponsor has not been able to clarify this point with specific data. N92750 reported increased duration of gestation at 40 mg/kg, the highest dose for the study. In a 12/23/2004 statement as to the reproductive effects of nebevolo1, the sponsor states that

- 3) In one Seg III study doses  $\geq 2.5$  mg/kg caused decreased pup birth weight and decreased pup survival with no NOAEL identified. This effect was repeated in a separate study where doses  $\geq 1.25$  mg/kg caused decreased pup birth weight and decreased survival with no NOAEL identified.
- 4) fertility in the pups was decreased as evidenced by decreased implantation ( $\geq 1.25$  mg/kg) and decreased numbers of corpora lutea ( $\geq 1.25$  mg/kg). No NOAEL was identified for either effect.
- 5) The developmental landmark data was uninformative due to the insensitive method used. Instead of recording the days of landmarks being achieved, animals were examined on preset days, usually substantial periods of time after the milestones may be expected.

For example:

Eye opening may be achieved PN12, animals not examined for this until PN21

Vaginal opening may be achieved PN28, animals not examined for this until PN42

Righting on surface may be achieved PN4, animals not examined for this until PN21

## 6) Teratogenicity

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

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

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

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

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

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

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

7. Pulmonary effects may be expected from a beta adrenergic antagonist. Bronchoconstriction and decrease in coronary blood flow were not apparent in the cardiovascular/cardiopulmonary safety pharmacology study but were alluded to in several pharmacology studies.

N56072

efficiency of the heart increased. Nebivolol, post-dosing with 0.16mg/kg cumulative administration, induced peripheral vasoconstriction associated with slight decrease in cardiac performance, which led to deterioration of cardiac output and relaxation at higher doses of nebivolol. Nebivolol induced a slight bronchoconstriction and a slight increase in the duration of the PQ-interval of the ECG post-cumulative dosing with the highest dose (0.63mg/kg). These distinct changes or altered responses in cardiac and hemodynamic capacity were not observed with the vehicle control.

N56719

determined in terms of the pressure rate product. Left circumflex coronary blood flow decreased slightly after the 0.04 and 0.16mg/kg doses. The ECG showed prolongation of the QT and PQ interval duration, which was likely to be frequency-related. No changes in behavior were observed.

8. Consistent findings of infiltrates of foamy macrophages in pulmonary alveoli with associated inflammatory changes and increased lung weights in both sexes of rodents

9. In the chromosome aberration assay using cultured human lymphocytes, increased polyploidy was seen at concentrations  $\geq 5\mu\text{g} +\text{S9}$ . Increased endoreduplication was seen at  $\geq 5\mu\text{g/ml} -\text{S9}$  and  $\geq 16\mu\text{g/ml} +\text{S9}$ . The historical control data that was provided did not include ranges for polyploidy and endoreduplication.

10. Possible bone marrow toxicity

N122168 Micronucleus test in mice: single oral dose. Single oral doses of nebivolol in male and female mice showed significant ( $p \leq 0.05-0.001$ ) and dose-related reduction in bone marrow proliferation at the 24 hour sampling time. This dose-related bone marrow toxicity was never referred to again in toxicology reports and has apparently never been characterized. Was the original observation in error? Decreases in HCT, Hb and RBC were seen in the hematology results of most rodent toxicology studies. However, animals did not seem to be dying with non-regenerative anemias. Enlarged spleens with increased RBC in the pulp were reported for most studies, even in situations where hematology changes were not apparent. The points which should have been characterized include:

- a. Did the finding repeat or was the observation in error?
- b. A NOAEL for the bone marrow toxicity
- c. Is the effect reversible, progressive or self-limiting
- d. Are the hematology findings related to the bone marrow effect or a separate phenomenon of hemolytic anemia?

## PHARMACOLOGY/TOXICOLOGY REVIEW

### 3.1 INTRODUCTION AND DRUG HISTORY

**NDA number:** 21742

**Review number:** 1

**Sequence number/date/type of submission:** 0/April 30, 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:**

#### 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)

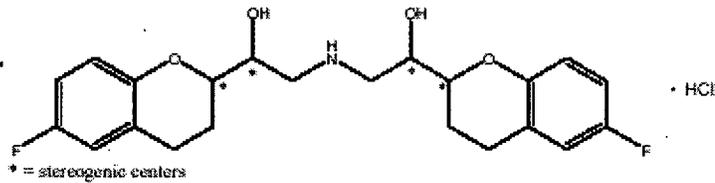
R067145 (l-Nebivolol)

**CAS Registry No.**152520-56-4

**Trade Name:** To Be Established

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Figure 3.2-01 Chemical Structure of Nebivolol



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

**Molecular Weight**      441.90 g/mol

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

**Relevant INDs/NDAs/DMFs:** IND33060

**Drug class:** Beta adrenergic blocker

**Indication:** hypertension

**Clinical formulation:** hypromellose, USP; polysorbate 80, NF; \_\_\_\_\_ lactose monohydrate, NF \_\_\_\_\_; pregelatinized starch, \_\_\_\_\_; microcrystalline cellulose, NF( \_\_\_\_\_); croscarmellose sodium, NF \_\_\_\_\_; FD&C Blue #2 Lake \_\_\_\_\_; D&C Red #27 Lake \_\_\_\_\_; FD&C Yellow #6 Lake \_\_\_\_\_; magnesium stearate/sodium lauryl sulfate, \_\_\_\_\_ and colloidal silicon dioxide NF \_\_\_\_\_.

**Route of administration:** oral

**Proposed use:** management of hypertension

**Disclaimer:** Tabular and graphical information are constructed by the reviewer unless cited otherwise.

**Studies reviewed within this submission:** Many of the studies submitted have already been reviewed in the IND. The drug has had several identifiers which are summarized in the sponsor's table below.

Table 3.5-02 Some Identification Numbers for Nebivolol, *d*-Nebivolol, and *l*-Nebivolol

| Compound  | Identification Numbers                |
|---|---------------------------------------|
| <b>Nebivolol</b><br>Also called in various reports: " <i>d,l</i> -neбиволol", " <i>rac</i> -neбиволol" and " <i>racemic</i> neбиволol". | R67555<br>R067555<br>R65824           |
| <b><i>d</i>-neбиволol</b><br>Also called: " <i>d</i> -enantiomer"   | R67138<br>R0855548<br>R855548<br>SRRR |
| <b><i>l</i>-neбиволol</b><br>Also called: " <i>l</i> -enantiomer"   | R67145<br>R0855547<br>R855547<br>RSSS |

Studies not reviewed within this submission: see Appendix III.

### 3.2 PHARMACOLOGY

**3.2.1 Brief summary:** Nebivolol is a beta blocker as are many of the metabolites. The *d*-isomers of aromatic hydroxyl derivatives were found to have subnanomolar  $K_i$  values for the  $\beta_1$  receptor in vitro. The most potent of these was the 7OH-SRRR isomer which was reported to have ~2x greater binding affinity than does *d*-neбиволol. The N-dealkylated metabolites have lower binding affinity (2000-50,000x) than the parent compound. From a recent receptor binding study (PHA021-001) we know that the racemic mixture has some affinity ( $\geq 300$  nM) for alpha adrenergic receptors present primarily in rat membranes. This does not inform us of agonist or antagonist activity. We do not have information about the active metabolites for other than the  $\beta$  receptors. A peripheral vasoconstrictor effect was reported in some of the pharmacology studies. As these were cumulative dosing studies, it is not clear if the vasoconstriction was due to time (time necessary for the appearance of vasoconstrictive metabolites), or simply a reflex-induced rise in peripheral resistance (no net change in arterial pressure) secondary to decreased blood pressure. The *l*-enantiomer is reported to have vasodilating properties in isolated coronary artery rings and substantially less  $\beta$ -adrenergic receptor antagonist activity. The vasorelaxation produced by the racemate or *l*-enantiomer is reportedly greater in the presence of intact vascular endothelium rather than in denuded vasculature.

A computerized structural similarity consult was requested of Joe Contrera, Ph.D. The report of that structural search is attached as Appendix II. Travoprost showed 86% structural similarity to neбиволol. Travoprost is a synthetic prostaglandin F2 $\alpha$  analogue used to decrease intraocular pressure by increasing uveoscleral outflow. Common side effects of Travoprost are ocular

hyperemia (35-50% of users) and 1-5% of users experience each of the following: angina pectoris, bradycardia, headache, hypercholesterolemia, hyper/hypotension. Nebivolol shared 81% structural similarity with Ezetimibe ( a lipid lowering agent), 80% with l-betaxolol, 79% with oxprenolol and 79% with metoprolol.

Prostaglandins have their own effects on the cardiovascular and endocrine systems. Responses to PGF<sub>2</sub>α vary depending upon the vascular bed and species; however in humans, there is generally no overall effect on blood pressure. Endocrine effects may include stimulation of steroid production by the adrenals, stimulation of insulin release, thyrotropin-like effects on the thyroid and lytic effects on the corpus luteum.

The question of selectivity for the β<sub>1</sub> receptor arises as well as the contributions of the two isomers. One of the sponsor's receptor binding profiles is included in Appendix I. In human β<sub>1</sub> and β<sub>2</sub> receptors expressed in *E. coli*, the l-nebivolol showed a K<sub>i</sub> of 55 nM for the Huβ<sub>1</sub>AR and 87 nM for the Huβ<sub>2</sub>AR. The d-nebivolol had K<sub>i</sub> values of 0.21(Huβ<sub>1</sub>AR) nM and 2.50 (Huβ<sub>2</sub>AR) nM. Using the data from this assay system, the d-isomer therefore has ~260x more affinity for the β<sub>1</sub> receptor than does the l-isomer. The selectivity of nebivolol has been questioned in the published literature. A study by van de Water et al. (1988, *J. Cardiovasc. Pharmacol.* 11,552-563) showed a 293-fold β<sub>1</sub> selectivity when dissociation constants were determined by Schild plot analysis of β-adrenergic effects using guinea pig right atria(β<sub>1</sub>) and trachea (β<sub>2</sub>). A study by Pauwels et al (1988, *Mol Pharmacol*, 34:843-851) used rabbit lung ( β<sub>1</sub>-) and rat lung (β<sub>2</sub>-). The study system showed approximately 48-55 fold selectivity for β<sub>1</sub>. A paper by Maack et al (2001, *British J Pharmacol*; 132, 1817-1826) referenced a 1991 study by Pauwels (*Biochem Pharmacol*42, 1683-1689) using CHO cells transfected with HuβARs showed a 10 fold β<sub>1</sub> selectivity of nebivolol as assessed by radioligand binding. The study by Maack et al, used human ventricular myocardium and showed only a 2-4fold selectivity for β<sub>1</sub>(essentially non-selective). A comparator of bisoprolol was processed in the same system and showed approximately 16- 19 fold β<sub>1</sub> selectivity. The in vitro receptor screen reported in study PHA021-001 showed approximately a 40 fold selectivity for β<sub>1</sub> over β<sub>2</sub> using recombinant human receptors expressed in a variety of cell lines. In this study, the selectivity appears similar to carvedilol.

There is also a question as to the vasodilating properties. The sponsor claims that this is due to endogenous production of nitric oxide (NO). The toxicology studies did not include any information to support this such as signs of reddening, flushing, signs of altered thermal regulation, or hypotension, even though substantial multiples of human doses were used in some studies. Also, increased NO production is generally a transient event that may be triggered through a variety of stimuli such as shear stress and a number of different receptor-ligand

interactions. A very interesting perspective on nebivolol's vasodilatory properties comes from the laboratory of Nobel laureate L.J. Ignarro, Ph.D. Dr Ignarro states that:

Previous studies from our laboratory revealed that the endothelium-dependent vasodilator response to nebivolol involves endogenous generation of both NO and cyclic GMP in vascular smooth muscle cells. Moreover, in no experimental circumstances tested did we find any evidence that nebivolol per se generates NO or higher oxides of nitrogen either by enzymatic, chemical or photolytic means.<sup>1</sup> However, the molecular mechanism by which nebivolol induced the generation of nitric oxide remains unclear. Therefore, based on the striking structural and chemical similarities between nebivolol and 17 $\beta$ -estradiol, we hypothesized that the NO-dependent vasodilating properties attributed to nebivolol might be partially mediated through its interaction with the membrane ER and consequent generation of NO. *Reference: J Cardiovasc Pharmacol. Volume 43, Number 5, May 2004. : Estrogen receptor-mediated vascular responsiveness to nebivolol: a novel endothelium-related mechanism of therapeutic vasorelaxation.*

This consideration is very interesting in light of an estrogen receptor-mediated effect possibly manifested as the mouse Leydig cell tumors. This reference examined the parent drug, leaving questions as to possible effects of the many metabolites. There are a number of papers in the peer-reviewed literature showing a mechanistic connection between the estrogen receptor and NO-mediation of vasodilation. This is not meant to be a definitive review of the various receptor ligand combinations associated with NO production, therefore only a few articles will be cited here:

M. Page Haynes, et al. :*Membrane estrogen receptor engagement activates endothelial nitric oxide synthase via the PI3-kinase-Akt pathway in human endothelial cells.* Circ Res. 2000;87:677-682.

M.E.Mendelsohn. *Nongenomic, estrogen receptor-mediated activation of endothelial nitric oxide synthase: How does it work? What does it mean?* Circ Res. 2000;87: 956-960

T. Simoncini, et al. *Novel non-transcriptional mechanisms for estrogen receptor signaling in the cardiovascular system interaction of estrogen receptor  $\alpha$  with phosphatidylinositol 3-OH kinase.* Steroids 67(2002) 935-939.

The vasodilating properties are also interesting in light of the structural similarities to a PGF2 $\alpha$  analogue.

During the review process, one of the medical officers raised the question whether there was any information in the non-clinical material that would indicate a connection between nebivolol and myocardial ischemia and/or cardiovascular collapse. Looking through the pharmacology studies, this reviewer found several studies conducted in dogs where the reports contained statements that

might be considered pertinent to the M.O.'s question. The data in these studies was not presented in the optimum manner for analysis therefore the sponsor's words are quoted below:

N43984: Instrumented dogs. At lower doses the drug causes vasodilation and at higher doses causes vasoconstriction.

range. From 0.04 mg.kg<sup>-1</sup> on the substance induces peripheral vasoconstriction as indicated by the significant and long lasting increase in systemic vascular resistance (median peak effect of 38 %). The effect is more pronounced on the systemic circulation because pulmonary vascular resistance only increases significantly following the administration of 0.16 mg.kg<sup>-1</sup> i.v. of the compound. The vasoconstricting effect of R 65 824 is associated with a slight decrease in cardiac performance starting after injection of 0.04 mg.kg<sup>-1</sup> intravenously. This is indicated by the significant decrease in LV dp/dt max, LV dp/dt max/P, Aov max, Ao dv/dt max, and in stroke volume (median peak effect of 18 %) in the absence of significant changes in heart rate and left ventricular end-diastolic pressure. Systolic aortic blood pressure and heart rate do not change following the administration of the various doses of the compound. Consequently pressure rate product doesn't change. This indicates that the oxygen demand of the heart does not change after administration of the compound in the dose range tested in the anaesthetized dog.

N45119: anesthetized instrumented dogs in a comparison of nebivolol's enantiomers to propranolol. "...in contrast to propranolol R65824 and R65825 acutely induce peripheral vasodilation ...(0.0025 – 0.16 mg/kg dose range)."

N56719: conscious instrumented dogs. "Left circumflex coronary blood flow tends to decrease slightly after injection of 0.04 and 0.16 mg/kg." QT prolongation was noted starting after injection with 0.04 mg/kg. Data was not available to calculate QTc. Presentation of data was graphical and of a nature that made interpretation difficult.

N56072 : Cardiac and Hemodynamic effects of cumulative intravenous injections of nebivolol in closed-chest anesthetized mongrel dogs

Starting at 0.16 mg.kg<sup>-1</sup> i.v. the compound induces peripheral vasoconstriction associated with a slight decrease in cardiac performance. As a consequence cardiac output and the relaxation of the heart deteriorates in this higher dose range of R67555.

The changes described must be attributed to the compound because the solvent does not induce distinct changes in the various cardiac and haemodynamic variables.

Deterioration of cardiac output was reported. The bronchoconstriction was deduced by the increase in the ventilatory pressure needed for the volume controlled ventilator to maintain

ventilation volume. The sponsor then stated that

**PQ-interval in the highest dose range. The rate corrected QT-interval increases slightly throughout the experimental period.**

The data for this study was presented as line graphs on a scale that made interpretation and/or verification of the results difficult.

The secondary activity of the many active metabolites is not characterized.

***N74969  $\beta$ -adrenergic receptor binding affinity of possible nebivolol metabolites measured using cloned human  $\beta$ 1- and  $\beta$ 2- adrenergic receptors expressed in Escherichia coli. 1990.***

d- and l-nebivolol and their hydroxylated and N-dealkylated derivatives (racemic mixtures and separated isomers) were tested for inhibition of  $^{125}\text{I}$  Iodocyanpindolol binding ( $^{125}\text{ICYP}$ ) to human  $\beta$ 1 and human  $\beta$ 2-adrenergic receptors (Hu $\beta$ AR) expressed in E.coli. d-Nebivolol shows a 12x higher affinity for for Hu $\beta$ 1AR than for Hu $\beta$ AR2 and was 250x more potent than l-nebivolol for  $\beta$ 1 binding.

The d-isomers of aromatic hydroxyl derivatives were found to have sub-nanomolar  $K_i$  values for the  $\beta$ 1 receptor. The most potent of these was the 7OH-SRRR isomer which had apparently 2x higher binding affinity than d-nebivolol. The metabolites obtained through N-dealkylation have significantly less (2000-500000x) affinity for the  $\beta$ 1 and  $\beta$ 2 receptors.

Overall, the binding profile is very similar to that of Carvedilol ( which was used as a comparator in one assay) with less affinity than Carvedilol for the non- $\beta$  adrenergic receptors. The sponsor's summary of binding profile is attached as Appendix I and II.

***N46826 In vitro receptor binding and amine uptake inhibition profile of R65824 (January 1986).***

Selective radioligand binding assays to measure  $\beta$ 1 and  $\beta$ 2 adrenergic receptor sites in rabbit and rat lungs respectively are described. The in vitro binding of nebivolol was compared to reference compounds . Cell membrane preparations from tissue homogenates were incubated with tritiated ligand. Specific receptor binding was distinguished by cold competition methods.

To measure inhibition of neurotransmitter uptake,  $^3\text{H}$ -amines were incubated with a crude mitochondrial fraction, containing synaptosomes, obtained from different brain areas, suspended in Krebs medium. After "short" incubations, the tritiated amines taken up in the synaptosomal fraction were separated from the free radioactivity by filtration methods. Specific uptake of  $^3\text{H}$ -amine was defined by incubation in the presence or absence of known inhibitors of the corresponding amine-uptake system.

The technique also used in this study to measure dissociation time was described.

Nebivolol bound to the  $\beta_1$  receptor with high affinity ( $K_i=1.0\text{nM}$ ) and with approximately 47X lower affinity to the  $\beta_2$  receptor. Time of dissociation also differed.

Time of dissociation from  $\beta_1$  receptors:  $t_{1/2} > 90$  minutes (no dissociation noted during the 90 minute observation period).

Time of dissociation from  $\beta_2$  receptors:  $t_{1/2}=36\pm 12$  minutes

The sponsor describes the results as the binding affinity for  $\beta_1$  was 7 and 380 times greater than that of comparator compounds betaxolol and atenolol with a greater  $\beta_1/\beta_2$  selectivity ratio.

Nebivolol weakly inhibited neurotransmitter uptake in brain synaptosomes ( $\mu\text{molar}$  concentrations).

| receptor site:<br>tissue:<br>$^3\text{H}$ -ligand<br>$K_D$ -value of the $^3\text{H}$ -<br>ligand | $\beta_1$ -adrenergic<br>rabbit lung<br>$^3\text{H}$ -dihydroalprenolol<br>0.9 nM | $\beta_2$ -adrenergic<br>rat lung<br>$^3\text{H}$ -dihydroalprenolol<br>0.2 nM | $\frac{K_1 \beta_2}{K_1 \beta_1}$ |
|---|---|--|-----------------------------------|
| R 65 824 $\text{pIC}_{50}$ (M)*   | $8.7 \pm 0.2$ (3)   | $6.55 \pm 0.07$ (2)  |                                   |
| $K_1$ (nM)  | 1.0   | 47   | 47                                |
| betaxolol $K_1$ (nM)  | 7.1   | 67   | 9.4                               |
| atenolol $K_1$ (nM)   | 380   | 2970   | 7.8                               |
| ICI 118 551 $K_1$ (nM)  | 34  | 1.3  | 0.038                             |
| 1-propranolol $K_1$ (nM)  | 0.9   | 0.27   | 0.3                               |

\* mean value  $\pm$  S.D. (number of independently performed experiments in duplicate)

Table 3: Potency of R 65 824 to inhibit neurotransmitter uptake in synaptosomes.

| neurotransmitter<br>tissue                               | $^3\text{H}$ -noradrenaline<br>rat hypothalamus | $^3\text{H}$ -serotonin<br>rat cortex | $^3\text{H}$ -dopamine<br>rat striatum |
|--|---|---------------------------------------|--|
| R 65 824 $\text{pIC}_{50}$ (M)*<br>$\text{IC}_{50}$ (nM) | $6.25 \pm 0.07$ (2)<br>562                      | 6.55<br>280                           | 6.35<br>450                            |

\* mean value  $\pm$  S.D., number of independently performed experiments in duplicate

*PHA021-001 In vitro receptor binding study for neurotransmitter, steroidal, ion channel, uptake and growth factor related targets. (May 2003)*

This study was previously reviewed. Atenolol ( $\beta_1$  blocker), nebivolol (non-selective  $\beta$  blocker) and carvedilol (non-selective  $\beta$  blocker) were tested at concentrations of  $1 \times 10^{-9}$ ,  $1 \times 10^{-8}$ ,  $1 \times 10^{-7}$ ,  $1 \times 10^{-6}$  and  $1 \times 10^{-5}$  M in 50 receptor binding assays. Atenolol showed little affinity for any receptor other than the beta adrenergic receptors. Both carvedilol and nebivolol showed affinity for alpha adrenergic,  $\beta$  adrenergic, dopamine and serotonin receptors. Carvedilol showed a greater affinity than nebivolol for the various receptors. None of the 3 compounds showed an affinity for the estrogen, glucocorticoid, progesterone, testosterone, ATP-sensitive K channel, non-selective corticotrophin releasing factor, oxytocin or thyrotropin releasing hormone receptors. Among the receptors tested, nebivolol showed a similar binding profile to carvedilol. The binding affinities for nebivolol showed higher  $K_i$  values compared to carvedilol. Data was not presented for the metabolites.

**N49477 In vitro pharmacologic profile of R65824, a potent and selective  $\beta_1$  adrenergic antagonist.** The effect of d,l-nebivolol was determined in vitro on isolated cardiovascular, gastrointestinal and respiratory preparations.

$\beta_1$ - adrenergic receptors: Drug inhibition of increases in heart rate caused by increasing concentrations of isoprenaline was determined in guinea pig right atria after 30, 60 and 120 minutes. The drug's inhibitory effect was further compared to other known  $\beta$ -adrenergic antagonists by determination of "A-10" values in the same model system. "A-10" values for nebivolol for antagonism of isoprenaline-induced relaxation were determined on dog coronary arteries.

$\beta_2$ - adrenergic receptors: Inhibition of isoprenaline-induced relaxation was determined in guinea pig trachea. Isoprenaline was used at a concentration of  $4.7 \times 10^{-7}$  M. The inhibitory effect of nebivolol on relaxations induced with cumulatively increasing concentrations of isoprenaline was compared to that of other  $\beta$ -adrenergic antagonists in guinea pig trachea. A-10 values for antagonism of salbutamol-induced relaxations, inhibition of isoprenaline-induced relaxation in the presence of  $3.4 \times 10^{-6}$  M phentolamine were determined in canine saphenous veins.

A-adrenergic receptors: Antagonism of noradrenaline-induced contractions by nebivolol was determined in rat "caudal" arteries and rabbit spleen. The effect of nebivolol on cumulative dose-response curves to noradrenaline was determined on rabbit femoral arteries.

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#### 2.1.2.2. Contractions elicited by adrenergic nerve stimulation

The effect of R 65 824 on contractile responses to adrenergic nerve stimulation was studied in femoral arteries of the rabbit mounted for isometric tension measurement.

#### 2.1.3. Dopamine

Inhibition by R 65 824 of relaxations of the guinea-pig stomach, evoked with dopamine, was determined.

#### 2.2. Histamine antagonism

Histamine antagonism by R 65 824 was determined in the guinea-pig ileum ( $H_1$ -receptor) made to contract with cumulatively increasing concentrations of histamine and in the guinea-pig right atrium ( $H_2$ -receptor) activated with linearly increasing concentrations of histamine.

#### 2.3. 5-Hydroxytryptamine (5-HT)-antagonism

Antagonism of contractions induced with a submaximal concentration of 5-HT was determined in the caudal artery of the rat ( $S_2$ -receptor), the ileum of the guinea pig (M-receptor) and the fundus of the rat (D-receptor).

#### 2.4. Calcium antagonism

The effect of R 65 824 was determined on rat caudal arteries kept in depolarizing solution and made to contract with cumulatively increasing concentrations of  $CaCl_2$ . The effect of R 65 824 on depolarized ear arteries of the rabbit (high  $K^+$ -ion concentration and 1.8 mM of  $CaCl_2$ ) was also determined.

To evaluate the effect of R 65 824 on  $Ca^{++}$ -induced positive inotropic responses of the heart, papillary muscles of the cat were electrically stimulated and cumulatively increasing amounts of  $CaCl_2$  were added before and after the administration of the substance.

#### 2.5. Cholinergic mechanisms

The effect of R 65 824 on muscarinic receptors was determined on the duodenum of the rabbit made to contract with a submaximal concentration of acetylcholine, and on guinea-pig ileum made to contract with cumulatively increasing concentrations of methacholine. The effect of R 65 824 on cholinergic nerve stimulation was investigated on the ileum of the guinea pig made to contract with submaximal coaxial electric stimulation. The effect of R 65 824 on nicotinic receptors was determined in the ileum of the guinea pig made to contract with a submaximal concentration of nicotine.

#### 2.6. Interaction with peptides

The effect of R 65 824 on contractions of the ileum of the guinea pig induced with a submaximal concentration of angiotensin II or bradykinin was determined.

### 2.7. Intrinsic mechanisms

The effect of R 65 824 on inotropy was determined on electrically stimulated papillary muscle of the cat and on spontaneously beating atria of the guinea pig. On the latter preparation also the effect on chronotropy was determined. Changes of myogenic activity upon administration of R 65 824 were determined in the portal vein of the rat and the duodenum of the rabbit.

Results: A<sub>10</sub> values were defined as the concentration of antagonist inducing a 10-fold shift to the right of the agonist dose-response curve. When the maximal response to the agonist was depressed by more than 50%, A<sub>h</sub> values (concentration of the antagonist reducing the maximal response to 50% of the control response) are given. Selectivity between β<sub>1</sub> and β<sub>2</sub> adrenergic effects was defined as the ratio between A<sub>10</sub> values for antagonism of isoprenaline-induced responses on the guinea pig right atrium and the guinea pig trachea or the canine saphenous vein.

Table 1. Inhibitory effect of R 65 824 on adrenergic responses

| Tissue                         | Agonist       | Receptor       | Incubation time | ED <sub>50</sub> (M)     |
|--------------------------------|---------------|----------------|-----------------|--------------------------|
| right atrium of the guinea pig | isoprenaline  | β <sub>1</sub> | 30 min          | 2.2 × 10 <sup>-8</sup>   |
| right atrium of the guinea pig | isoprenaline  | β <sub>1</sub> | 60 min          | 7.9 × 10 <sup>-10</sup>  |
| right atrium of the guinea pig | isoprenaline  | β <sub>1</sub> | 120 min         | 7.9 × 10 <sup>-10</sup>  |
| trachea of the guinea pig      | isoprenaline  | β <sub>2</sub> | 30 min          | 2.2 × 10 <sup>-5</sup>   |
| caudal artery of the rat       | noradrenaline | α <sub>1</sub> | 5 min           | 1.4 × 10 <sup>-6</sup>   |
| spleen of the rabbit           | noradrenaline | α <sub>1</sub> | 3 min           | > 2.5 × 10 <sup>-5</sup> |

R65824 was reported to inhibit the isoprenaline-induced increases in heart rate at the concentrations shown at the left.

Table 2. Inhibitory effect of various β-adrenergic antagonists on β<sub>1</sub>- and β<sub>2</sub>-adrenergic responses.<sup>a</sup>

| Antagonist        | Guinea-pig right atrium (β <sub>1</sub> ) A <sub>10</sub> -value (M) | Guinea-pig trachea (β <sub>2</sub> ) A <sub>10</sub> -value (M) | Ratio trachea/atrium (β <sub>2</sub> /β <sub>1</sub> ) |
|-------------------|--|---|--|
| R 65 824          | 5.2 × 10 <sup>-8</sup>   | 1.5 × 10 <sup>-5</sup>  | 288  |
| R 65 824 (60 min) | 1.2 × 10 <sup>-8</sup>   | 1.4 × 10 <sup>-5</sup>  | 1,166  |
| R 65 825          | 1.8 × 10 <sup>-7</sup>   | > 2.5 × 10 <sup>-5</sup>  | > 143  |
| metoprolol        | 2.3 × 10 <sup>-7</sup>   | 5.9 × 10 <sup>-6</sup>  | 25   |
| atenolol          | 1.2 × 10 <sup>-6</sup>   | 1.8 × 10 <sup>-5</sup>  | 15   |
| practolol         | 2.7 × 10 <sup>-6</sup>   | > 9.3 × 10 <sup>-6</sup>  | > 3.5  |
| bucindolol        | 1.1 × 10 <sup>-8</sup>   | 4.2 × 10 <sup>-8</sup>  | 3.5  |
| pindolol          | 7.0 × 10 <sup>-9</sup>   | 1.1 × 10 <sup>-8</sup>  | 1.9  |
| propranolol       | 2.2 × 10 <sup>-8</sup>   | 4.2 × 10 <sup>-8</sup>  | 1.9  |
| bunolol           | 4.7 × 10 <sup>-8</sup>   | 7.9 × 10 <sup>-9</sup>  | 0.17   |

The A<sub>10</sub> values for the comparator compounds are shown below. Nebivolol seems to be similar to bucindolol, propranolol and bunolol this assay system.

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<sup>a</sup>incubation time with the antagonist 30 minutes except when indicated otherwise

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Nebivolol inhibited salbutamol-induced relaxations in the canine saphenous vein higher concentration was used to inhibit the of isoprenaline.

Table 1. Inhibitory effect of R 65 824 on B<sub>1</sub>- and B<sub>2</sub>-adrenergic relaxations in canine blood vessels (A<sub>10</sub>-values in molar; incubation time of 30 minutes)

| Tissue agonist  | A <sub>10</sub>        | Ratio B <sub>1</sub> /B <sub>2</sub> |
|---|------------------------|--------------------------------------|
| B <sub>1</sub> : canine coronary artery<br>isoprenaline   | 7.9 x 10 <sup>-8</sup> | -                                    |
| B <sub>2</sub> : canine saphenous vein<br>salbutamol  | 1.0 x 10 <sup>-6</sup> | 12.7                                 |
| B <sub>2</sub> : canine saphenous vein<br>isoprenaline in presence of<br>phenolamine (3.4 x 10 <sup>-6</sup> M) | 2.0 x 10 <sup>-6</sup> | 25.4                                 |

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NDA #21-742

Table 3. Inhibitory effect of R 65 824 on isolated tissues

| Tissue                         | Agonist              | Receptor/<br>mechanism    | Incubation<br>time | ED <sub>50</sub> or A <sub>h</sub><br>or A <sub>h</sub> in molar |
|--------------------------------|----------------------|---------------------------|--------------------|--|
| ileum of the guinea pig        | histamine            | H <sub>1</sub>            | 5 min              | A <sub>h</sub> 5.2 x 10 <sup>-6</sup>                            |
| right atrium of the guinea pig | histamine            | H <sub>2</sub>            | 30 min             | ED <sub>50</sub> > 2.5 x 10 <sup>-5</sup>                        |
| ileum of the guinea pig        | serotonin            | M                         | 3 min              | ED <sub>50</sub> 5.4 x 10 <sup>-6</sup>                          |
| fundus of the rat              | serotonin            | D                         | 3 min              | ED <sub>50</sub> > 2.5 x 10 <sup>-5</sup>                        |
| caudal artery of the rat       | serotonin            | S <sub>2</sub>            | 5 min              | ED <sub>50</sub> 2.5 x 10 <sup>-6</sup>                          |
| caudal artery of the rat       | Ca <sup>++</sup>     |                           | 5 min              | ED <sub>50</sub> 1.8 x 10 <sup>-6</sup>                          |
| ear artery of the rabbit       | K <sup>+</sup>       |                           | 30 sec             | ED <sub>50</sub> - 0.16 mg (total amount)                        |
| papillary muscle of the cat    | Ca <sup>++</sup>     |                           | 30 min             | ED <sub>50</sub> 2.2 x 10 <sup>-5</sup>                          |
| ileum of the guinea pig        | methacholine         | muscarinic                | 5 min              | A <sub>h</sub> - 3.1 x 10 <sup>-6</sup>                          |
| duodenum of the rabbit         | acetylcholine        | muscarinic                | 3 min              | ED <sub>50</sub> > 2.5 x 10 <sup>-5</sup>                        |
| ileum of the guinea pig        | nicotine             | nicotinic                 | 3 min              | ED <sub>50</sub> 1.8 x 10 <sup>-6</sup>                          |
| ileum of the guinea pig        | electric stimulation | nerve stimulation         | 15 min             | ED <sub>50</sub> 7.8 x 10 <sup>-7</sup>                          |
| ileum of the guinea pig        | angiotensin II       | AT II                     | 3 min              | ED <sub>50</sub> 3.1 x 10 <sup>-6</sup>                          |
| ileum of the guinea pig        | bradykinin           | BK                        | 3 min              | ED <sub>50</sub> 3.1 x 10 <sup>-6</sup>                          |
| papillary muscle of the cat    | electric stimulation | inotropy                  | 30 min             | ED <sub>50</sub> - 2.5 x 10 <sup>-5</sup>                        |
| right atrium of the guinea pig |                      | heart rate                | 30 min             | starting from 1.5 x 10 <sup>-6</sup>                             |
| right atrium of the guinea pig |                      | contractile force         | 30 min             | starting from 1.5 x 10 <sup>-6</sup>                             |
| portal vein of the rat         |                      | amplitude myogenic contr. | 60 min             | ED <sub>50</sub> 6.9 x 10 <sup>-6</sup>                          |
| duodenum of the rabbit         |                      | tone                      | 3 min              | starting from 6.2 x 10 <sup>-6</sup>                             |
| duodenum of the rabbit         |                      | contractile force         | 3 min              | starting from 2.5 x 10 <sup>-5</sup>                             |

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### **3.2.2 Primary pharmacodynamics**

Mechanism of action: Beta adrenergic receptor blocker.

Drug activity related to proposed indication: Beta adrenergic antagonism decreases blood pressure by several mechanisms including decreased myocardial contractility, decreased cardiac output and a reduction in renin secretion with secondary decrease in angiotensin II (AII) production.

***N45729 Beta adrenoceptor blocking properties of R65824 (0.31 mg.kg<sup>-1</sup> orally) in conscious dogs.1985***

Seven female Labradors of varying and unspecified age were surgically instrumented. R65824 was dissolved in polypropylene glycol (20%). Isoprenaline was dissolved in dH<sub>2</sub>O. The dosing schedule for isoprenaline and nebivolol was not described in words, but a diagram is shown which seems to indicate that repeat injections of isoprenaline were given at -60, -30, 0, 30, 60, 90, 120, 150, 180, 210 and 240 minutes. Isoprenaline was given at a dose of 0.5 µg/kg iv 60 minutes before a single oral dose of nebivolol was given at time 0. Parameters studied included heart rate, PQ, QRS, QT, QTc, diastolic aortic blood pressure, systolic aortic blood pressure, LVdP/dt max and LV dP/dt max/P.

Presentation of the results leaves something to be desired. Most of the data is presented graphically. Heart rate was detailed from the point of oral administration of nebivolol. We do not see values for a stable baseline or the response to isoproterenol.

The variability of the results indicates no change in the parameters measured although the median values measured suggest trends. However, even this interpretation cannot be made with confidence since the trends occur over the duration of the study and there is no untreated or vehicle control to show the effects of time on the measured parameters. There is only 1 dose and no comparator either of other doses or a control group. A dose response effect can't be demonstrated. For that matter, a stable baseline can't be demonstrated. While this reviewer considers the study uninformative, the sponsor sees a long-acting inhibition of isoprenaline-induced effects with a negative inotropic effect evident in a decreased LV dP/dt max and LVdP/dt max/P ratio.

### **3.2.3 Secondary pharmacodynamics**

### **3.2.4 Safety pharmacology**

#### **Overall Summary**

The sponsor has presented evaluations of cardiovascular, electrophysiology, overt neurological and renal effects of nebivolol.

Electrophysiology: Studies using canine Purkinje fibers and auricular muscles of guinea pigs had poorly presented results. The sponsor's conclusion was that nebivolol affected sodium ion movement similar to a Class I antiarrhythmic. This was supported in a separate study examining the local anesthetic properties of nebivolol compared to lidocaine, propranolol and atenolol.

Aqueous and HPβCD preparations of nebivolol had lower EC<sub>50</sub> values than lidocaine: 0.57 and 1.14, respectively, vs 11.0 (aqueous lidocaine) and 2.36 (cyclodextrin lidocaine). Atenolol and propranolol showed similar EC<sub>50</sub> values to nebivolol.

A recent study using whole cell voltage clamp techniques with CHO cells stably expressing HERG channels compared d,l-propranolol, d,l-nebivolol and astemizole. Nebivolol was intermediate in potency between propranolol and astemizole for mean percent HERG current reduction as summarized in the reviewer's table below.

#### Summary of HERG channel current reduction

| concentration               | Mean %current reduction |               |            |
|-----------------------------|-------------------------|---------------|------------|
|                             | D,l propranolol         | D,l nebivolol | astemizole |
| $1 \times 10^{-7} \text{M}$ | 5.4±2.6                 | 21.7±5.2      |            |
| $3 \times 10^{-7} \text{M}$ | 11.4±5.3                | 52.3±2.9      |            |
| $3 \times 10^{-6} \text{M}$ | 26.0±6.8                | 86.5±6.5      |            |
| $3 \times 10^{-9} \text{M}$ |                         |               | 11.6±1.6   |
| $1 \times 10^{-8} \text{M}$ |                         |               | 36.8±0.9   |
| $3 \times 10^{-8} \text{M}$ |                         |               | 75.9±4.9   |

Therefore, it may be seen that nebivolol has some ability to block I<sub>Kr</sub> channels.

Several in vivo electrophysiology studies used anesthetized guinea pigs and conscious instrumented dogs. The data showed changes expected from the pharmacology. That is, decreased heart rate, decreased blood pressure, slight increase in PQ interval and decreases in LVdp/dt max. There were no effects apparent on QTc in these acute dosing studies. It is of interest to note that cardiovascular effects persisted for as long as 12 hours (RC847748) after a single dose of 10 mg/kg. This is consistent with the sponsor's information that the hydroxylated metabolites have beta-adrenergic antagonist activity.

The one study that examined respiratory parameters did not indicate significant respiratory effects.

The renal function study in anesthetized dogs used atenolol as a comparator. Both nebivolol and atenolol caused an increase in mean renal arterial pressure (as did vehicle) although atenolol caused a slightly greater effect. Vehicle, atenolol and nebivolol all caused decreases in renal blood flow, although nebivolol caused the least change from baseline. Creatinine clearance decreased in the vehicle group and increased after treatment with both drugs while urinary volume followed the same pattern. In the data as presented, there did not appear to be a significant difference between atenolol and nebivolol in this test system.

A recent neurological evaluation used doses of 200, 400 and 800 mg/kg, toxicologic rather than pharmacologic doses. Except for abnormal licking, reported at all doses, the signs reported could be explained by exaggerated pharmacology. There was no data presented to support that sedation, decreased vocalization, piloerection, exertional tremors, decreased urination and defecation were secondary to hypotension rather than due to primary neurologic effects.

Cardiovascular effects:**RCC847748*****Effects on cardiovascular and respiratory function in the telemetered dog. August, 2003***

Nebivolol in 20% hydroxypropyl- $\beta$ -cyclodextrin was given in single oral doses of 2.5, 10 and 40 mg/kg to 4 conscious telemetry instrumented beagles. The animals were given 4 oral doses using a sequential design. A period of 6 treatment free days was allowed between each treatment. Before dosing and at intervals after dosing, systolic, diastolic and mean blood pressures, heart rate, electrocardiograms, respiratory parameters, locomotor activity and clinical signs were measured.

pharmacology, toxicology and kinetics. In a previously conducted chronic toxicity study in beagle dogs (repeated dosage for 6 months) (Verstraeten A et al, 1989) R067555 was administered orally (in gelatin capsules) to beagle dogs at 5, 20 and 80 mg/kg body weight/day. Heart rate and E.C.G. measurements revealed that heart rates were comparable between control and dosed groups. No relevant effects on E.C.G. were observed at 5 and 20 mg/kg. At 80 mg/kg, a tendency to a prolonged PQ-interval was observed. In another chronic toxicity study (12-Month Toxicity Study in Beagle Dogs) (Verstraeten A et al, 1991) R067555 was

During acclimatization, a recording was made of blood pressure, heart rate, locomotor activity and ECG for each dog to confirm viability of the parameters to be recorded. The data were retained in the raw data archive but not reported. Before dosing and at intervals after each dosing, systolic, diastolic and mean blood pressures, heart rate, ECGs, respiratory parameters, locomotor activity and clinical signs were measured. A previous study in dogs showed that:

weight/day. After dosing at 10 mg/kg/day, a tendency to a decreased heart rate with a slight PQ interval prolongation was noted. Dosing at 40 mg/kg resulted in a PQ and QT prolongation associated with a decreased heart rate. In a previously conducted

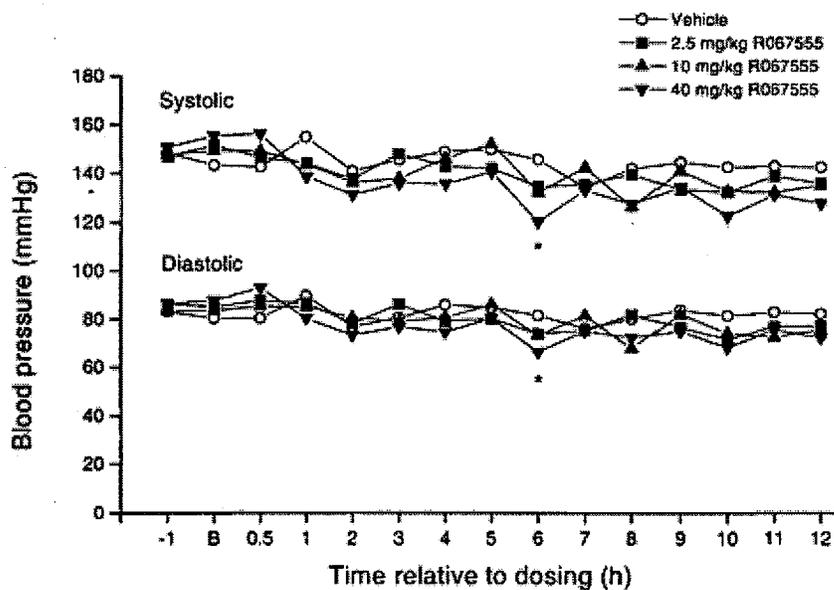
The doses in the present study were based upon these preceding studies.

In this study, ECGs and cardiovascular parameters as listed above were recorded before dosing and for at least 12 hours after dosing. Respiratory parameters of rate, tidal volume and minute

volume were also recorded for 5 minutes before dosing and for 5 minutes immediately after completing the 1,2 and 4 hour ECG. Venous blood was collected Pre-dose, 0.5, 1, 2 and 4 hours after dose for determination of plasma level exposure.

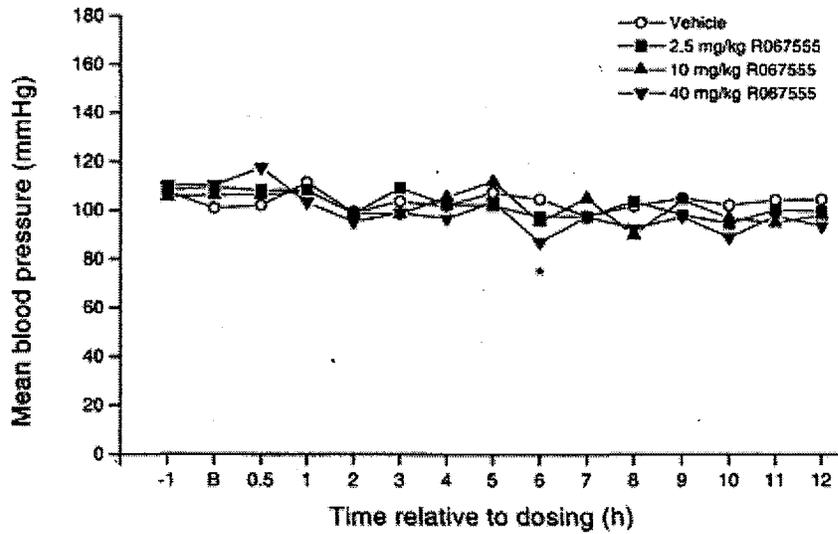
Results

Blood pressure: a decrease in both systolic and diastolic pressure was seen at 6 hours in all drug treated groups. Statistical analysis was Dunnett's test based on pooled variance, significance at 5% or 1% level.

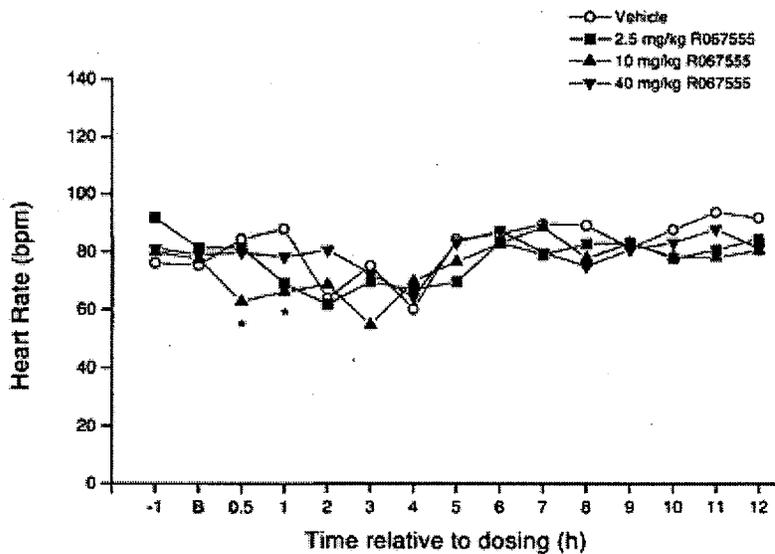


Mean blood pressure was also shown to decrease at 6 hours after dosing.

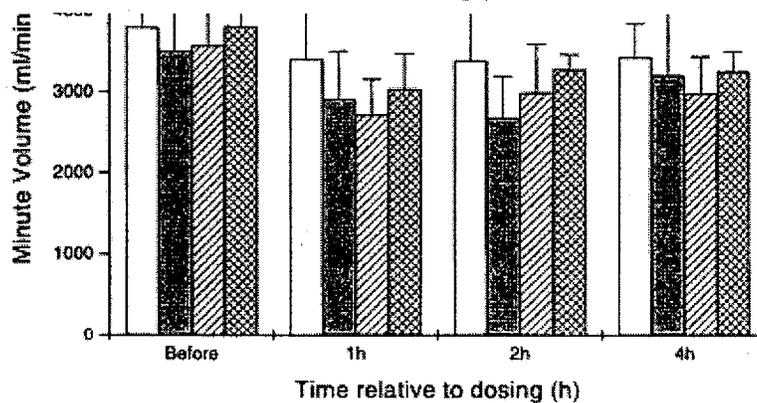
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A slight decrease in heart rate was seen at 0.5 and 1 hour after dosing and was seen again in all drug-treated groups compared to control at 10-12 hours after dosing.



There did not seem to be any apparent effect on respiratory rate or tidal volume. There did seem to be a decrease in minute volume at 1 and 2 hours after dosing.



An increase in PQ interval was seen in all dose groups from approximately 30 minutes to ~18 hours after dosing.

There were no significant effects upon respiratory rate although there was a non-significant decrease in tidal volume.

Tidal volume: Reviewer's calculation of percent change from before dosing

| Time relative to dosing | vehicle | 2.5 mg/kg | 10 mg/kg | 40 mg/kg |
|-------------------------|---------|-----------|----------|----------|
| Before                  | 160.40  | 171.26    | 157.68   | 165.08   |
| 1 hour                  | 0       | -7%       | -6%      | -8%      |
| 2 hours                 | -1%     | -12%      | +5%      | 0        |
| 4 hours                 | 0       | -9%       | 0        | 0        |

Minute volume: Reviewer's calculation of absolute change and percent change from before dosing

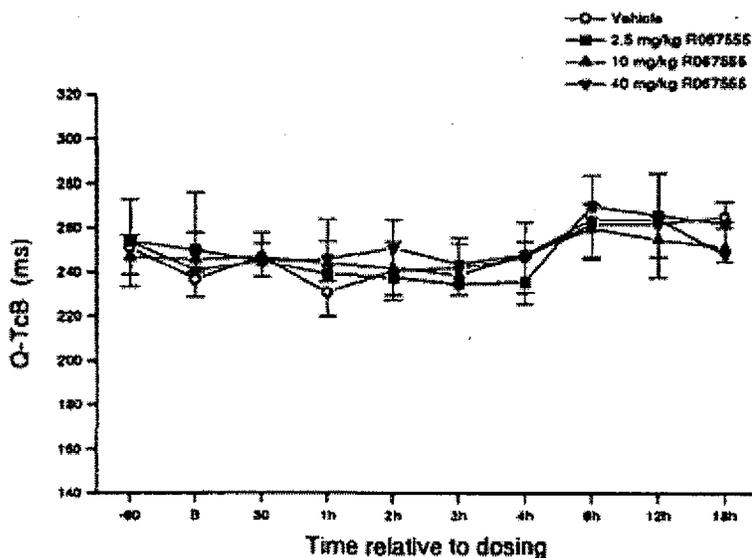
| Time relative to dosing | vehicle   | 2.5 mg/kg | 10 mg/kg  | 40 mg/kg  |
|-------------------------|-----------|-----------|-----------|-----------|
| Before                  | 3803.2    | 3512.6    | 3574.1    | 3815.6    |
| 1 hour                  | -393/-10% | -598/-17% | -856/-24% | -783/-21% |
| 2 hours                 | -413/-11% | -834/-24% | -585/-16% | -530/-14% |
| 4 hours                 | -373/-10% | -307/-9%  | -596/-17% | -565/-15% |

P-Q Interval (ms)  
MALES

|                         |         | VEHICLE | 2.5 MG/KG<br>R067555 | 10 MG/KG<br>R067555 | 40 MG/KG<br>R067555 |
|-------------------------|---------|---------|----------------------|---------------------|---------------------|
| TIME RELATIVE TO DOSING |         |         |                      |                     |                     |
| -60 min                 | MEAN    | 93      | 93                   | 91                  | 90                  |
|                         | ST.DEV. | 15      | 15                   | 14                  | 14                  |
|                         | N       | 4       | 4                    | 4                   | 4                   |
| Before                  | MEAN    | 94      | 91                   | 93                  | 92                  |
|                         | ST.DEV. | 12      | 14                   | 12                  | 17                  |
|                         | N       | 4       | 4                    | 4                   | 4                   |
| 30 min                  | MEAN    | 92      | 103                  | 103                 | 105                 |
|                         | ST.DEV. | 12      | 10                   | 7                   | 8                   |
|                         | N       | 4       | 4                    | 4                   | 4                   |
| 1 hour                  | MEAN    | 95      | 106                  | 105                 | 106                 |
|                         | ST.DEV. | 13      | 8                    | 7                   | 6                   |
|                         | N       | 4       | 4                    | 4                   | 4                   |
| 2 hours                 | MEAN    | 94      | 109 *                | 107                 | 111 *               |
|                         | ST.DEV. | 10      | 7                    | 10                  | 2                   |
|                         | N       | 4       | 4                    | 4                   | 4                   |
| 3 hours                 | MEAN    | 93      | 106                  | 108 *               | 108 *               |
|                         | ST.DEV. | 14      | 4                    | 4                   | 4                   |
|                         | N       | 4       | 4                    | 4                   | 4                   |
| 4 hours                 | MEAN    | 94      | 106                  | 108                 | 107                 |
|                         | ST.DEV. | 12      | 7                    | 9                   | 6                   |
|                         | N       | 4       | 4                    | 4                   | 4                   |
| 6 hours                 | MEAN    | 86      | 101                  | 104 *               | 102                 |
|                         | ST.DEV. | 14      | 5                    | 6                   | 8                   |
|                         | N       | 4       | 4                    | 4                   | 4                   |
| 12 hours                | MEAN    | 90      | 98                   | 104 *               | 105 **              |
|                         | ST.DEV. | 4       | 3                    | 8                   | 7                   |
|                         | N       | 4       | 4                    | 4                   | 4                   |
| 18 hours                | MEAN    | 91      | 100                  | 103                 | 103                 |
|                         | ST.DEV. | 13      | 10                   | 6                   | 6                   |
|                         | N       | 4       | 4                    | 4                   | 4                   |

**ELECTROCARDIOGRAMS SUMMARY**  
**Q-T Interval (ms)**  
**MALES**

|                                |         | VEHICLE | 2.5 MG/KG<br>R067555 | 10 MG/KG<br>R067555 | 40 MG/KG<br>R067555 |
|--------------------------------|---------|---------|----------------------|---------------------|---------------------|
| <b>TIME RELATIVE TO DOSING</b> |         |         |                      |                     |                     |
| -----                          |         |         |                      |                     |                     |
| -60 min                        | MEAN    | 226     | 218                  | 229                 | 227                 |
|                                | ST.DEV. | 4       | 15                   | 9                   | 8                   |
|                                | N       | 4       | 4                    | 4                   | 4                   |
| Before                         | MEAN    | 236     | 223                  | 229                 | 228                 |
|                                | ST.DEV. | 12      | 13                   | 13                  | 12                  |
|                                | N       | 4       | 4                    | 4                   | 4                   |
| 30 min                         | MEAN    | 227     | 221                  | 239                 | 220                 |
|                                | ST.DEV. | 6       | 20                   | 5                   | 8                   |
|                                | N       | 4       | 4                    | 4                   | 4                   |
| 1 hour                         | MEAN    | 224     | 224                  | 228                 | 222                 |
|                                | ST.DEV. | 11      | 9                    | 6                   | 10                  |
|                                | N       | 4       | 4                    | 4                   | 4                   |
| 2 hours                        | MEAN    | 235     | 228                  | 237                 | 229                 |
|                                | ST.DEV. | 9       | 2                    | 11                  | 6                   |
|                                | N       | 4       | 4                    | 4                   | 4                   |
| 3 hours                        | MEAN    | 226     | 220                  | 235                 | 230                 |
|                                | ST.DEV. | 9       | 8                    | 4                   | 11                  |
|                                | N       | 4       | 4                    | 4                   | 4                   |
| 4 hours                        | MEAN    | 234     | 231                  | 229                 | 233                 |
|                                | ST.DEV. | 11      | 7                    | 8                   | 6                   |
|                                | N       | 4       | 4                    | 4                   | 4                   |
| 6 hours                        | MEAN    | 228     | 225                  | 230                 | 228                 |
|                                | ST.DEV. | 12      | 13                   | 16                  | 5                   |
|                                | N       | 4       | 4                    | 4                   | 4                   |
| 12 hours                       | MEAN    | 224     | 224                  | 225                 | 233                 |
|                                | ST.DEV. | 9       | 11                   | 13                  | 8                   |
|                                | N       | 4       | 4                    | 4                   | 4                   |
| 18 hours                       | MEAN    | 196     | 216                  | 225 *               | 225 *               |
|                                | ST.DEV. | 15      | 14                   | 17                  | 7                   |
|                                | N       | 4       | 4                    | 4                   | 4                   |



The graph of QTc shows an increase in all groups from 6 to 18 hours.

The uncorrected QT values showed a significant increase at 18 hours in the MD and HD groups.

No clinical signs were reported for the study. However, at 30 minutes after receiving the HD of 40 mg/kg, all four of the dogs vomited a yellow liquid or white mucous or foam. At 1 hour 1 dog vomited again.

When the blood pressure data was expressed as percent change from pre-dose values, the values at 40 mg/kg were significantly lower starting at 1 hour after dose and continuing through 12 hours (the last recorded point of determination) after dosing. The decrease in blood pressure was seen to a lesser degree at 2.5 and 10 mg/kg. At all doses tested, small but statistically significant increases in PQ interval were seen and persisted to 12 hours, the last point of determination, after a single oral dose. The PQ interval may be increased by drugs which slow atrial and AV nodal conductance and therefore this effect may be related to the mechanism of action of nebivolol.

The sponsor summarizes the results as follows:

No effects on the morphology of the electrocardiogram or on P wave amplitude, P duration or QRS Interval were measured at any dose tested. At 18h after administration of 10 and 40 mg/kg R067555, the QT interval was seen to be significantly higher ( $p < 0.05$ ) than at 18h after vehicle control treatment. However, the absolute values at this time point were actually lower than before dosing and the statistical finding is therefore thought to be due to the fact that a fall in QT interval was seen at this time after vehicle control treatment. The corrected QT interval, both according to van de Water and Bazett, demonstrated that R067555 had no effect in the conscious dog under the conditions of the test.

While there was no apparent effect on QTc under the conditions of this acute dosing study, this does not negate the effects of the chronic toxicology studies. Something lacking in the analysis of these studies is plasma and tissue levels of parent drug and metabolites.

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*N56637 Electrophysiological data of R067555*1. Methods

Conventional microelectrode recordings of transmembrane action potentials were used on the following preparations: Purkinje fibers and trabecular preparations from dog hearts and auricular muscles of the guinea-pig. The preparations were spontaneously active or could be stimulated at a basal rate of 60 min. The normal tyrode solution contained in mM: NaCl 136.9, KCl 5.4, CaCl<sub>2</sub> 1.8, MgCl<sub>2</sub> 1.04, NaHCO<sub>3</sub> 11.9, NaH<sub>2</sub>PO<sub>4</sub> 0.42, glucose 5.55. The pH was 7.4 and the temperature 33° C. The tyrode solution was saturated with 95 % O<sub>2</sub> and 5 % CO<sub>2</sub>. Spontaneous activity in Purkinje fibers was investigated at a reduced K concentration of 2.7 mM.

Depending on the type of cells the following variables were measured: resting membrane potential, action potential amplitude and duration, maximum rate of depolarization (by electrical differentiation of the upstroke), conduction time (between two intracellular electrodes), effective refractory period and recovery time (defined as the period needed for the rate of rise of an extrasystolic action potential to recover its full amplitude). The refractory period and recovery time were measured by interpolating an extra stimulus of twice the threshold at variable intervals in the regular series of stimuli every sixth beat. In spontaneous active guinea-pig auricles and dog Purkinje fibers the change in frequency and in rate of diastolic depolarization were measured. Control values were determined after a period in which the measured variables had stabilized. Then the preparations were exposed for 30 minutes to a tyrode solution containing either 0.08 or 0.31 or 2.5 mg/L neбиволол.

Thereafter, the preparations were allowed to recover as long as necessary in free tyrode. Changes in the values of the determined variable after administration of neбиволол compared with the values before compound administration were evaluated for statistical significance by applying Wilcoxon's matched pairs signed rank test (two-tailed probability). The same procedure was applied to the changes in the values determined after recovery in free tyrode.

Concentrations of 0.08, 0.31 or 2.5 mg/l neбиволол were tested. Parameters were measured after 30 or 60 minutes of exposure to the test article.

The results were presented as box plots, with poor organization that makes interpretation difficult. The values for concentrations and time points were presented in isolation. That is, data for 0.08 mg/l after 30 minutes was presented as a graph discrete and separate from data for 0.31 mg/l at 30 minutes but in comparison to the different tissue types. A table of values would greatly facilitate the review process. The sponsor's summary statement is shown below.

However, at 0.31 and 2.5 mg/L tyrode nebivolol behaved typically as the compounds belonging to the Class I antiarrhythmics. It affected most of the sodium related parameters in Purkinje fibers.

These results strongly suggest that at these relative high doses nebivolol may possess some local anaesthetic (membrane stabilizing) properties.

***N170545-2 effects of d,lpropranolol (R009035) and of d,lneбиволol(R067555) on the membrane K<sup>+</sup> current I<sub>kr</sub> in HERG-transfected CHO cells: a comparison with effects on human  $\beta$ 1 adrenergic receptors. 2002***

Three concentrations of d,l propranolol and d,l nebivolol ( $10^{-7}$ ,  $3 \times 10^{-7}$  and  $3 \times 10^{-6}$ M) were compared with 3 concentrations of astemizole ( $3 \times 10^{-9}$ ,  $10^{-8}$  and  $3 \times 10^{-8}$ M). CHO cells stably expressing the HERG potassium channel were used. All 3 drugs were dissolved in DMSO then further diluted with the cell bath solution. Voltage clamp mode was used to record whole cell currents. Potassium concentration of the solutions was not detailed.

The sponsor's results are summarized in the reviewer's table below.

| concentration        | Mean %current reduction |               |            |
|----------------------|-------------------------|---------------|------------|
|                      | D,l propranolol         | D,l nebivolol | astemizole |
| $1 \times 10^{-7}$ M | 5.4±2.6                 | 21.7±5.2      |            |
| $3 \times 10^{-7}$ M | 11.4±5.3                | 52.3±2.9      |            |
| $3 \times 10^{-6}$ M | 26.0±6.8                | 86.5±6.5      |            |
| $3 \times 10^{-9}$ M |                         |               | 11.6±1.6   |
| $1 \times 10^{-8}$ M |                         |               | 36.8±0.9   |
| $3 \times 10^{-8}$ M |                         |               | 75.9±4.9   |

While nebivolol shows a greater potency for inhibition of the HERG current than d,l-propranolol, it shows much less potency than astemizole in this test system.

***N170233(CPF645): Effects of dl-neбиволol and dl-propranolol on cardio-hemodynamic and electrophysiological parameters in anesthetized guinea pigs January 2003***

dl-neбиволol or dl-propranolol was given intravenously in increasing doses to instrumented, anesthetized female Duncan-Hartley guinea pigs. After 30 minutes of stabilization, 10 minutes of baseline values were recorded. The animals were then dosed with dl-neбиволol, dl-propranolol or the vehicle. Doses used were 0.16, 0.32, 0.64, 1.25, 2.5 and 5 mg/kg, n=7 for each series. The doses were given at 15 minute intervals to the same animal. The total dose received was thus 9.87 mg/kg i.v. in 757 minutes. After each injection the catheter was flushed with saline. ECG parameters and cardiac function were determined before the first iv injection and at 5 minutes

after each iv injection of solvent or drug. In those animals receiving either propranolol or nebivolol, an arterial blood sample was taken at 5 minutes after the intravenous injection of the highest dose. The results of the plasma samples were not complete at the time of the writing of the report.

A decrease in heart rate was apparent with both nebivolol and propranolol starting at ~35 minutes after dosing and reaching a maximum at 80 minutes, the last point of determination. Nebivolol reached a maximum decrease in heart rate of 11% vs baseline and propranolol a maximum decrease of 24% vs baseline. Both drugs showed an increase in PQ interval of 12% for propranolol (80 minutes after dosing) and 3% for nebivolol (35 minutes after dosing). Bazett's formula was used to calculate the corrected QT interval. However, this was not the optimum formula given the high baseline heart rates. Under the conditions of the study, the changes apparent in the ECG parameters are those that would be expected with a beta- adrenergic antagonist.

Effects of solvent on heart rate (HR), mean arterial blood pressure (BPm) and on ECG parameters in anaesthetized guinea pigs (data are expressed in actual values).

| Parameters            | Solvent i.v.; n=7 |                  |                  |                  |                  |                  |                  |
|-----------------------|-------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                       | 0 min             | 5 min            | 20 min           | 35 min           | 50 min           | 65 min           | 80 min           |
|                       | Baseline          | 0.16 mg/kg       | 0.32 mg/kg       | 0.64 mg/kg       | 1.25 mg/kg       | 2.5 mg/kg        | 5 mg/kg          |
| HR<br>(/min)          | 218<br>(205/238)  | 223<br>(204/239) | 225<br>(204/230) | 226<br>(206/232) | 228<br>(207/239) | 234<br>(208/243) | 232<br>(213/251) |
| BPm<br>(kPa)          | 4.5<br>(3.9/5.8)  | 4.7<br>(4/8.6)   | 4.9<br>(3.8/6.8) | 5.3<br>(4.3/7.5) | 5.5<br>(4.2/7.6) | 5.7<br>(4.2/7.6) | 5.8<br>(4.6/8.2) |
| PQ-interval<br>(ms)   | 58<br>(56/63)     | 60<br>(55/68)    | 60<br>(55/64)    | 63<br>(58/64)    | 63<br>(58/64)    | 63<br>(56/69)    | 62<br>(57/63)    |
| QRS-duration<br>(ms)  | 43<br>(39/50)     | 44<br>(39/46)    | 44<br>(39/48)    | 44<br>(39/46)    | 44<br>(40/48)    | 43<br>(37/49)    | 44<br>(35/49)    |
| QT-interval<br>(ms)   | 190<br>(180/197)  | 194<br>(181/196) | 191<br>(185/199) | 189<br>(181/199) | 190<br>(179/201) | 185<br>(173/199) | 183<br>(164/201) |
| QTcB-interval<br>(ms) | 370<br>(350/376)  | 365<br>(360/379) | 366<br>(361/382) | 367<br>(351/374) | 369<br>(351/380) | 362<br>(344/378) | 363<br>(335/380) |

Values are median (min/max).

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Effects of dl-nebivolol on heart rate (HR), mean arterial blood pressure (BPm) and on ECG parameters in anaesthetized guinea pigs (data are expressed in actual values).

| Parameters         | dl-nebivolol i.v.; n=7 |                  |                  |                  |                  |                  |                  |
|--------------------|------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                    | 0 min                  | 5 min            | 20 min           | 35 min           | 50 min           | 65 min           | 80 min           |
|                    | Baseline               | 0.16 mg/kg       | 0.32 mg/kg       | 0.64 mg/kg       | 1.25 mg/kg       | 2.5 mg/kg        | 5 mg/kg          |
| HR (1/min)         | 230<br>(200/260)       | 228<br>(198/261) | 225<br>(201/251) | 218<br>(203/241) | 216<br>(212/242) | 211<br>(203/245) | 204<br>(194/242) |
| BPm (kPa)          | 5.3<br>(4.2/7.5)       | 6.0<br>(4.8/8.7) | 5.9<br>(5.3/8.2) | 6.1<br>(5/8.8)   | 6.6<br>(5.3/9)   | 6.5<br>(5.4/8.2) | 5.9<br>(4.2/7.7) |
| PQ-interval (ms)   | 58<br>(54/62)          | 58<br>(56/60)    | 59<br>(56/62)    | 62<br>(58/63)    | 61<br>(58/65)    | 61<br>(59/64)    | 60<br>(57/64)    |
| QRS-duration (ms)  | 44<br>(39/47)          | 44<br>(41/51)    | 45<br>(41/49)    | 46<br>(42/48)    | 45<br>(41/46)    | 45<br>(41/45)    | 43<br>(40/47)    |
| QT-interval (ms)   | 180<br>(162/206)       | 180<br>(167/204) | 182<br>(175/205) | 185<br>(175/202) | 186<br>(176/200) | 186<br>(163/200) | 189<br>(165/205) |
| QTcB-interval (ms) | 361<br>(331/375)       | 356<br>(335/370) | 358<br>(346/375) | 353<br>(347/371) | 354<br>(343/376) | 350<br>(329/373) | 349<br>(331/378) |

Values are median (min/max).

Effects of dl-propranolol on heart rate (HR), mean arterial blood pressure (BPm) and on ECG parameters in anaesthetized guinea pigs (data are expressed in actual values).

| Parameters         | dl-propranolol i.v.; n=7 |                  |                  |                  |                  |                  |                  |
|--------------------|--------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                    | 0 min                    | 5 min            | 20 min           | 35 min           | 50 min           | 65 min           | 80 min           |
|                    | Baseline                 | 0.16 mg/kg       | 0.32 mg/kg       | 0.64 mg/kg       | 1.25 mg/kg       | 2.5 mg/kg        | 5 mg/kg          |
| HR (1/min)         | 241<br>(227/274)         | 228<br>(212/262) | 226<br>(208/274) | 224<br>(205/262) | 213<br>(204/257) | 195<br>(185/250) | 183<br>(153/224) |
| BPm (kPa)          | 4.5<br>(3.7/5.3)         | 5.2<br>(3.8/6.3) | 5.2<br>(3.8/5.9) | 4.9<br>(3.3/5.8) | 4.8<br>(3.4/5.4) | 4.9<br>(2.8/6)   | 4.9<br>(3/5.8)   |
| PQ-interval (ms)   | 60<br>(54/61)            | 60<br>(55/62)    | 61<br>(56/66)    | 63<br>(57/68)    | 61<br>(59/66)    | 65<br>(59/70)    | 67<br>(65/72)    |
| QRS-duration (ms)  | 42<br>(41/45)            | 44<br>(40/46)    | 45<br>(39/50)    | 44<br>(40/49)    | 43<br>(37/51)    | 43<br>(35/50)    | 45<br>(41/59)    |
| QT-interval (ms)   | 181<br>(162/196)         | 194<br>(168/215) | 187<br>(163/204) | 188<br>(170/211) | 193<br>(170/209) | 197<br>(173/215) | 214<br>(182/224) |
| QTcB-interval (ms) | 363<br>(346/381)         | 378<br>(339/405) | 364<br>(346/382) | 363<br>(346/388) | 370<br>(349/387) | 367<br>(340/387) | 365<br>(352/383) |

Values are median (min/max).

The sponsor reported that neither the solvent nor nebivolol caused any changes in ECG waveforms. After administration of 2.5 mg/kg i.v. of dl-propranolol, atrio-ventricular (AV) blocks type II and type III were observed in 1 animal and an intraventricular bundle branch block

(BBB) was reported for another animal. These 2 animals died after administration of 5 mg/kg i.v. of the compound.

Effects of dl-nebivolol, expressed as changes in percent, relative to the pre-medication values, on heart rate (HR), mean arterial blood pressure (BPM) and on ECG parameters in anaesthetized guinea pigs.

| Parameters    | dl-nebivolol i.v.; n=7 |               |              |               |              |                |
|---------------|------------------------|---------------|--------------|---------------|--------------|----------------|
|               | 5 min                  | 20 min        | 35 min       | 50 min        | 65 min       | 80 min         |
|               | 0.16 mg/kg             | 0.32 mg/kg    | 0.64 mg/kg   | 1.25 mg/kg    | 2.5 mg/kg    | 5 mg/kg        |
| HR            | -1<br>(-3/1)           | -3<br>(-7/0)  | -5<br>(-8/2) | -7<br>(-9/6)  | -8<br>(-9/2) | -11<br>(-15/0) |
| BPM           | 15<br>(-16/21)         | 19<br>(-7/26) | 17<br>(5/38) | 26<br>(-1/55) | 21<br>(3/38) | 13<br>(-28/40) |
| PQ-interval   | 0<br>(-3/4)            | 0<br>(-2/7)   | 4<br>(0/15)  | 5<br>(0/13)   | 5<br>(-2/11) | 3<br>(-3/11)   |
| QRS-duration  | 2<br>(0/9)             | 4<br>(0/7)    | 2<br>(0/10)  | 2<br>(-2/5)   | 2<br>(-4/5)  | 2<br>(-9/7)    |
| QT-interval   | -1<br>(-2/3)           | 2<br>(-2/8)   | 2<br>(-2/9)  | 3<br>(-3/9)   | 1<br>(-6/6)  | 6<br>(-5/9)    |
| QTcB-interval | -1<br>(-1/1)           | 0<br>(-3/5)   | 0<br>(-3/5)  | 0<br>(-5/4)   | -2<br>(-9/1) | -1<br>(-8/2)   |

Values are median (min/max).

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Effects of dl-propranolol, expressed as changes in percent, relative to the pre-medication values, on heart rate (HR), mean arterial blood pressure (BPM) and on ECG parameters in anaesthetized guinea pigs.

| Parameters    | dl-propranolol i.v.; n=7 |               |                |                |                 |                  |
|---------------|--------------------------|---------------|----------------|----------------|-----------------|------------------|
|               | 5 min                    | 20 min        | 35 min         | 50 min         | 65 min          | 80 min           |
|               | 0.16 mg/kg               | 0.32 mg/kg    | 0.64 mg/kg     | 1.25 mg/kg     | 2.5 mg/kg       | 5 mg/kg          |
| HR            | -4<br>(-9/0)             | -7<br>(-13/0) | -8<br>(-14/-2) | -9<br>(-15/-6) | -15<br>(-23/-9) | -19<br>(-33/-18) |
| BPM           | 16<br>(-4/51)            | 6<br>(-9/57)  | 7<br>(-8/49)   | 11<br>(-13/19) | 13<br>(-15/43)  | 5<br>(-19/29)    |
| PQ-interval   | 3<br>(-8/7)              | 5<br>(-8/13)  | 7<br>(3/13)    | 7<br>(0/10)    | 8<br>(-2/18)    | 18<br>(10/24)    |
| QRS-duration  | 2<br>(-2/7)              | 7<br>(-5/12)  | 0<br>(-4/9)    | 5<br>(-10/13)  | 0<br>(-15/17)   | 0<br>(-7/31)     |
| QT-interval   | 4<br>(-1/11)             | 4<br>(-4/8)   | 5<br>(-3/8)    | 7<br>(-4/10)   | 9<br>(1/16)     | 15<br>(12/18)    |
| QTcB-interval | 2<br>(-3/7)              | 1<br>(-9/1)   | 2<br>(-4/3)    | 2<br>(-7/4)    | 2<br>(-9/4)     | 1<br>(-5/5)      |

Values are median (min./max).

***NI84620 (CPF897) (Report date March 2003): Effects of d,l-nebivolol and d,l-propranolol on cardiovascular and behavioural parameters in instrumented awake dogs.***

Healthy trained and chronically instrumented female Beagle dogs of varying age and ranging in body weight from 8.2 to 14.0 kg, were used for recording of the following cardiovascular parameters: heart rate, diastolic and systolic blood pressure, pressure rate product, LV dp/dt max, LV dp/dt max/pd, LV dp/dt min, cardiac output, stroke volume, systemic vascular resistance and the ECG parameters PQ-, QRS-, QT-, QTcBazett- (QTcB), QTcFridericia- (QTcF) and QTcVan de Water- (QTcVdW) interval duration and QT-dispersion.

Instrumented, awake dogs were used with external ECG electrodes for the measurement of a 7-lead ECG. During the last 18 hours prior to the experiments, the dogs had no access to food. Water was available *ad libitum*. At the beginning of each experiment, control values of the various parameters were recorded for at least 30 min. Thereafter, (dl)-nebivolol 10 mg/kg (n=7), (dl)-propranolol 10 mg/kg (n=7), or the corresponding volume of the solvent (n=7) was administered orally by gavage. The various hemodynamic parameters were recorded continuously for 4 hours thereafter.

Before (time -15 and 0 min) and 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225 and 240 min after administration of the compounds or the solvent, the

results of the automatically calculated ECG intervals were visually validated, adjusted if necessary, and reported.

Arterial blood samples were taken 15, 30, 60, 120 and 240 min after administration of the compounds and were collected with EDTA. (dl)-Nebivolol and (dl)-propranolol were dissolved at a concentration of 10 mg/ml in a solution containing hydroxypropyl-beta-cyclodextrin 20% and water. A similar solution without compound, was also prepared and used as solvent (pH=5.7).

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**Table 4a: Effects of solvent of (dl)-nebivolol and (dl)-propranolol in awake, chronically instrumented dogs (data are expressed as median changes versus pre-medication in percentage; n=7).**

| Parameters            | 15 min       | 30 min        | 45 min        | 60 min       | 75 min        | 90 min        | 105 min      | 120 min       | 135 min      | 150 min      | 165 min       | 180 min      | 195 min       | 210 min       | 225 min       | 240 min      |              |
|-----------------------|--------------|---------------|---------------|--------------|---------------|---------------|--------------|---------------|--------------|--------------|---------------|--------------|---------------|---------------|---------------|--------------|--------------|
| HR (b/min)            | -1<br>-10/5  | -13<br>-26/17 | -10<br>-33/19 | 10<br>-28/51 | -2<br>-23/48  | -12<br>-26/51 | 0<br>-23/36  | -2<br>-28/46  | 1<br>-21/48  | 5<br>-33/73  | 2<br>-27/49   | 0<br>-32/51  | -9<br>-37/21  | -6<br>-35/25  | -6<br>-37/51  | -2<br>-38/35 |              |
| AoP's (kPa)           | -5<br>-8/4   | -1<br>-9/4    | -5<br>-16/3   | -5<br>-18/2  | -8<br>-20/1   | -8<br>-26/2   | -1<br>-8/4   | -5<br>-9/9    | -6<br>-9/16  | -1<br>-18/16 | -5<br>-16/16  | -2<br>-22/20 | -2<br>-15/15  | -3<br>-19/7   | -2<br>-12/9   | -6<br>-19/7  | -7<br>-9/7   |
| AoPd (kPa)            | -4<br>-12/1  | -7<br>-12/-2  | -10<br>-20/-2 | -3<br>-16/0  | -14<br>-21/-1 | -13<br>-32/2  | -5<br>-13/8  | -7<br>-16/11  | -4<br>-16/22 | -8<br>-14/21 | -11<br>-20/19 | -3<br>-23/27 | -10<br>-19/15 | -9<br>-21/17  | -11<br>-23/19 | -5<br>-21/11 | -5<br>-21/11 |
| PRP (kPa.b/min)       | -2<br>-15/4  | -15<br>-30/22 | -17<br>-39/16 | 0<br>-33/59  | -19<br>-28/54 | -13<br>-39/18 | -7<br>-28/28 | -4<br>-31/52  | -4<br>-25/44 | -6<br>-35/82 | -6<br>-32/35  | -7<br>-35/61 | -7<br>-43/37  | -16<br>-43/36 | -2<br>-47/43  | -2<br>-44/27 | -2<br>-44/27 |
| LV dp/dtmax (kPa/s)   | -3<br>-6/2   | -6<br>-11/2   | -8<br>-16/2   | -3<br>-12/11 | -6<br>-16/11  | -7<br>-18/4   | -4<br>-14/4  | -2<br>-17/9   | -1<br>-13/12 | 0<br>-17/21  | -2<br>-17/13  | -2<br>-17/19 | -5<br>-20/12  | -5<br>-20/13  | -4<br>-24/14  | -5<br>-26/6  | -5<br>-26/6  |
| LV dp/dtmax/ped (1/s) | 0<br>-2/4    | -3<br>-10/2   | -7<br>-10/13  | -2<br>-11/6  | -4<br>-13/8   | 4<br>-16/6    | -4<br>-12/0  | 0<br>-11/3    | -2<br>-8/0   | 0<br>-9/10   | 2<br>-9/9     | 0<br>-7/6    | 2<br>-11/11   | 0<br>-8/8     | -4<br>-9/6    | -4<br>-15/6  | -4<br>-15/6  |
| LV dp/dtmin (kPa/s)   | -4<br>-5/6   | -5<br>-9/2    | -5<br>-8/0    | -6<br>-8/6   | -5<br>-16/0   | -5<br>-24/2   | -3<br>-9/4   | -2<br>-12/4   | -6<br>-12/13 | 2<br>-15/19  | 0<br>-14/17   | 2<br>-19/22  | 3<br>-19/17   | 3<br>-21/13   | 3<br>-22/12   | 2<br>-18/10  | 2<br>-18/10  |
| CO (l/min)            | -1<br>-6/6   | -9<br>-27/14  | -12<br>-33/21 | -3<br>-27/38 | -2<br>-22/39  | -8<br>-27/28  | -3<br>-23/32 | 0<br>-28/38   | 10<br>-21/36 | 15<br>-34/47 | 4<br>-26/36   | 14<br>-33/41 | -8<br>-38/20  | -4<br>-38/25  | 6<br>-40/30   | -5<br>-42/24 | -5<br>-42/24 |
| SV (ml)               | 0<br>-5/5    | 0<br>-4/5     | 0<br>-4/5     | 0<br>-12/6   | -3<br>-7/6    | 0<br>-7/5     | 0<br>-5/5    | 0<br>-7/35    | 0<br>-14/35  | -2<br>-11/32 | -2<br>-7/29   | -3<br>-7/32  | -3<br>-8/32   | -5<br>-7/32   | -6<br>-13/34  | -6<br>-13/34 | -6<br>-13/34 |
| SVR (kPa/min)         | -2<br>-12/10 | 5<br>-20/24   | 2<br>-28/29   | -4<br>-29/21 | -10<br>-30/26 | -8<br>-30/33  | -4<br>-30/26 | -12<br>-33/31 | -8<br>-33/21 | -8<br>-39/41 | -4<br>-35/24  | -2<br>-35/40 | -2<br>-24/39  | -2<br>-30/36  | 0<br>-27/33   | 0<br>-27/33  | 0<br>-27/33  |

Values are median (min/max).

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**Table 4b: Effects of solvent of (dL)-nebivolol and (dL)-propranolol in awake, chronically instrumented dogs (data are expressed as median changes versus pre-medication in percentage; n=7).**

| Parameters  | 15 min      | 30 min      | 45 min       | 60 min       | 75 min       | 90 min      | 105 min       | 120 min      | 135 min     | 150 min     | 165 min     | 180 min        | 195 min       | 210 min      | 225 min       | 240 min      |
|-------------|-------------|-------------|--------------|--------------|--------------|-------------|---------------|--------------|-------------|-------------|-------------|----------------|---------------|--------------|---------------|--------------|
| PQ (ms)     | -2<br>-8/9  | -1<br>-9/7  | -1<br>-10/14 | 0<br>-7/7    | -1<br>-9/5   | 0<br>-8/8   | 2<br>-12/9    | -2<br>-11/5  | -5<br>-11/3 | -2<br>-8/5  | 2<br>-8/7   | 0<br>-4/8      | 2<br>-10/16   | 0<br>-4/5    | 2<br>-7/8     | 5<br>-5/21   |
| QRS (ms)    | 0<br>-4/7   | 2<br>-4/8   | 0<br>-4/9    | 2<br>-9/7    | 0<br>-6/9    | 4<br>-6/11  | 0<br>-7/7     | 4<br>-4/6    | 6<br>2/6    | 2<br>-8/13  | 0<br>-9/7   | 2<br>-7/7      | 4<br>-13/20   | 0<br>-7/13   | 2<br>-11/13   | 6<br>-6/9    |
| QT (ms)     | 1<br>-3/4   | 3<br>-4/6   | 5<br>-2/11   | 2<br>-5/8    | 5<br>-6/8    | 8<br>-4/9   | 7<br>-5/13    | 1<br>-2/13   | 0<br>-6/9   | 2<br>-5/10  | 4<br>-4/9   | 5<br>-5/11     | 6<br>-3/14    | 5<br>-1/11   | 7<br>-7/13    | 4<br>-3/16   |
| QTcB (ms)   | 2<br>-3/5   | -1<br>-5/6  | 4<br>-9/9    | 5<br>-4/7    | 5<br>-5/11   | 0<br>-3/15  | 1<br>-5/14    | 3<br>-7/16   | 3<br>-3/7   | 9<br>-1/15  | 4<br>-5/12  | 3<br>-4/15     | -3<br>-10/21  | 3<br>-13/18  | 1<br>-9/17    | 2<br>-7/15   |
| QTcF (ms)   | 1<br>-1/4   | 1<br>-2/5   | 3<br>-4/10   | 2<br>-1/12   | 3<br>-2/9    | 2<br>-2/13  | 4<br>-1/14    | 2<br>-2/13   | 4<br>-3/9   | 5<br>2/13   | 3<br>-3/11  | 2<br>-1/14     | 1<br>-7/19    | 4<br>-10/16  | -1<br>-4/16   | 3<br>-3/15   |
| QTcVDW (ms) | 2<br>0/3    | 1<br>-2/4   | 1<br>-3/8    | 2<br>-1/7    | 2<br>0/7     | 2<br>-2/10  | 2<br>-1/11    | 2<br>-3/10   | 4<br>-3/6   | 3<br>2/10   | 2<br>-2/9   | 1<br>-1/11     | 1<br>-5/15    | 3<br>-7/12   | 0<br>-2/13    | 2<br>-1/13   |
| QTd (ms)    | 0<br>-29/29 | 0<br>-65/75 | 17<br>-44/88 | 11<br>-28/76 | 24<br>-48/88 | 0<br>-67/50 | -25<br>-52/82 | -8<br>-53/12 | 0<br>-65/4  | 6<br>-41/38 | 0<br>-47/71 | -24<br>-56/100 | -24<br>-60/24 | 18<br>-42/50 | -12<br>-33/75 | 25<br>-52/75 |

Values are median (min/max).

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**Table 5a: Effects of (dl)-nebivolol (10 mg/kg orally) in awake, chronically instrumented dogs (data are expressed as median changes versus pre-medication in percentage; n=7).**

| Parameters           | 15 min        | 30 min         | 45 min         | 60 min         | 75 min         | 90 min        | 105 min        | 120 min        | 135 min       | 150 min       | 165 min       | 180 min        | 195 min       | 210 min       | 225 min       | 240 min       |
|----------------------|---------------|----------------|----------------|----------------|----------------|---------------|----------------|----------------|---------------|---------------|---------------|----------------|---------------|---------------|---------------|---------------|
| HR (b/min)           | 0<br>-18/20   | -5<br>-19/13   | -8<br>-2/8     | -9<br>-26/8    | -15<br>-23/0   | -9<br>-23/-3  | -10<br>-26/0   | -9<br>-27/-1   | -13<br>-30/-5 | -14<br>-29/-3 | -13<br>-27/-4 | -14<br>-32/12  | -14<br>-34/15 | -7<br>-26/13  | -5<br>-24/5   | -8<br>-37/11  |
| AoPs (kPa)           | 2<br>-5/4     | 1<br>-10/16    | 1<br>-9/13     | -2<br>-15/9    | -6<br>-20/15   | -8<br>-15/3   | -7<br>-16/2    | -6<br>-10/2    | -7<br>-17/0   | -6<br>-16/1   | -5<br>-15/0   | -5<br>-17/3    | -4<br>-19/9   | -1<br>-18/8   | -3<br>-14/16  | -7<br>-17/6   |
| AoPd (kPa)           | 6<br>4/9      | 4<br>-6/20     | 9<br>0/23      | 3<br>-12/9     | -1<br>-18/23   | -6<br>-19/10  | -9<br>-19/10   | -3<br>-12/9    | -3<br>-20/5   | -7<br>-18/6   | -7<br>-15/6   | -10<br>-14/11  | -1<br>-25/11  | 4<br>-14/20   | 1<br>-8/30    | -2<br>-23/18  |
| PRP (kPa.b/min)      | -2<br>-14/25  | -7<br>-22/13   | -9<br>-16/10   | -14<br>-29/1   | -15<br>-32/10  | -19<br>-31/2  | -21<br>-31/2   | -14<br>-32/-3  | -23<br>-36/9  | -16<br>-36/5  | -24<br>-33/7  | -24<br>-37/15  | -14<br>-42/19 | -11<br>-33/10 | -4<br>-26/11  | -3<br>-43/3   |
| LV dp/dtmax (kPa/s)  | -11<br>-32/17 | -20<br>-42/-11 | -29<br>-45/-11 | -34<br>-47/-13 | -37<br>-47/-15 | -37<br>-47/20 | -37<br>-45/-22 | -37<br>-45/-24 | -37<br>-45/25 | -37<br>-43/22 | -37<br>-43/24 | -34<br>-45/-24 | -34<br>-45/25 | -37<br>-45/22 | -34<br>-42/22 | -33<br>-44/22 |
| LV dp/dtmax/pd (1/s) | -9<br>-26/8   | -18<br>-31/-5  | -22<br>-31/8   | -20<br>-27/-9  | -24<br>-33/7   | -25<br>-31/5  | -20<br>-29/5   | -22<br>-29/7   | -24<br>-27/7  | -23<br>-27/2  | -23<br>-27/2  | -25<br>-29/2   | -25<br>-33/0  | -22<br>-33/2  | -22<br>-33/2  | -22<br>-34/0  |
| LV dp/dtmin (kPa/s)  | 0<br>-13/11   | -8<br>-22/2    | -10<br>-23/2   | -13<br>-24/2   | -14<br>-30/4   | -18<br>-29/9  | -16<br>-27/13  | -16<br>-24/12  | -18<br>-24/11 | -18<br>-26/8  | -18<br>-26/8  | -20<br>-28/10  | -15<br>-31/8  | -20<br>-31/5  | -16<br>-28/0  | -18<br>-30/-5 |
| CO (l/min)           | -14<br>-26/15 | -26<br>-46/-9  | -29<br>-38/-12 | -27<br>-36/-20 | -31<br>-42/22  | -32<br>-39/23 | -30<br>-42/21  | -35<br>-41/21  | -35<br>-43/21 | -31<br>-42/24 | -32<br>-44/23 | -37<br>-49/21  | -38<br>-45/19 | -36<br>-44/18 | -34<br>-41/13 | -29<br>-42/11 |
| SV (ml)              | -8<br>-27/-4  | -18<br>-42/-9  | -24<br>-33/-7  | -20<br>-40/-7  | -21<br>-33/-9  | -22<br>-33/-9 | -21<br>-33/13  | -22<br>-33/13  | -19<br>-33/9  | -19<br>-33/9  | -21<br>-33/12 | -19<br>-38/9   | -22<br>-33/15 | -22<br>-38/13 | -22<br>-29/12 | -23<br>-27/-9 |
| SVR (kPa/l/min)      | 20<br>-8/50   | 41<br>15/79    | 46<br>28/67    | 33<br>26/59    | 49<br>22/83    | 43<br>22/68   | 37<br>20/57    | 37<br>22/68    | 35<br>22/61   | 35<br>29/56   | 35<br>24/63   | 41<br>19/93    | 43<br>24/81   | 46<br>24/77   | 46<br>28/68   | 43<br>12/57   |

Values are median (min/max).

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**Table 5b: Effects of (dl)-nebivolol (10 mg/kg orally) in awake, chronically instrumented dogs (data are expressed as median changes versus pre-medication in percentage; n=7).**

| Parameters   | 15 min        | 30 min       | 45 min      | 60 min        | 75 min        | 90 min         | 105 min        | 120 min       | 135 min       | 150 min        | 165 min       | 180 min      | 195 min      | 210 min        | 225 min        | 240 min     |
|--------------|---------------|--------------|-------------|---------------|---------------|----------------|----------------|---------------|---------------|----------------|---------------|--------------|--------------|----------------|----------------|-------------|
| PQ (ms)      | 3<br>-1/22    | 15<br>12/28  | 16<br>5/41  | 15<br>12/42   | 17<br>11/39   | 18<br>12/28    | 16<br>11/27    | 18<br>10/32   | 17<br>8/33    | 13<br>10/29    | 16<br>13/32   | 15<br>8/27   | 16<br>7/42   | 14<br>8/32     | 19<br>8/28     | 13<br>3/29  |
| QRS (ms)     | 0<br>-14/8    | -2<br>-8/6   | -6<br>-10/4 | -2<br>-14/5   | -4<br>-16/0   | -4<br>-16/4    | -7<br>-14/2    | -5<br>-12/4   | -4<br>-14/4   | -4<br>-14/2    | -2<br>-14/6   | -2<br>-12/4  | -6<br>-12/4  | -6<br>-8/8     | -4<br>-10/2    | -6<br>-10/6 |
| QT (ms)      | 1<br>-4/2     | 2<br>-1/4    | 2<br>-3/8   | 1<br>-3/8     | 3<br>-6/8     | 2<br>-2/8      | 2<br>-7/9      | 1<br>-5/9     | 3<br>-1/14    | 2<br>0/13      | 4<br>-1/14    | 4<br>0/16    | 1<br>0/10    | 1<br>-6/9      | 3<br>-9/9      | 5<br>-4/13  |
| QTcB (ms)    | 0<br>-9/4     | -4<br>-8/0   | -4<br>-7/4  | -3<br>-11/1   | -7<br>-10/0   | -5<br>-9/0     | -5<br>-12/2    | -4<br>-11/2   | -5<br>-14/0   | -8<br>-12/1    | -7<br>-12/1   | -9<br>-12/2  | -6<br>-10/1  | -6<br>-10/2    | -6<br>-14/1    | -9<br>-11/3 |
| QTcf (ms)    | -2<br>-5/2    | -3<br>-4/0   | -2<br>-5/6  | -2<br>-6/0    | -5<br>-7/2    | -4<br>-5/3     | -2<br>-10/1    | -2<br>-9/1    | -4<br>-8/4    | -5<br>-6/3     | -4<br>-7/4    | -6<br>-7/4   | -3<br>-7/2   | -4<br>-7/1     | -3<br>-10/0    | -5<br>-8/1  |
| QTcV/DW (ms) | -2<br>-5/1    | -2<br>-2/0   | -1<br>-4/5  | -2<br>-3/2    | -3<br>-6/3    | -2<br>-5/3     | -1<br>-8/2     | -1<br>-7/2    | -4<br>-6/6    | -3<br>-6/4     | -3<br>-5/5    | -3<br>-6/5   | -2<br>-6/1   | -3<br>-4/2     | -2<br>-7/1     | -3<br>-6/3  |
| QTd (ms)     | 27<br>-24/170 | 5<br>-60/170 | 0<br>-73/90 | 20<br>-53/150 | 50<br>-70/150 | -14<br>-68/150 | 20<br>-100/110 | 29<br>-60/144 | 10<br>-80/160 | -27<br>-45/160 | 25<br>-29/140 | 0<br>-73/130 | -8<br>-73/60 | -32<br>-67/150 | -43<br>-67/130 | 0<br>-40/72 |

Values are median (min/max).

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**Table 6a: Effects of (dl)-propranolol (10 mg/kg orally) in awake, chronically instrumented dogs (data are expressed as median changes versus pre-medication in percentage; n=7).**

| Parameters            | 15 min       | 30 min        | 45 min        | 60 min        | 75 min        | 90 min        | 105 min       | 120 min       | 135 min       | 150 min       | 165 min       | 180 min       | 195 min       | 210 min       | 225 min       | 240 min       |
|-----------------------|--------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| HR (b/min)            | 1<br>-16/61  | -3<br>-18/45  | -8<br>-29/55  | -6<br>-28/45  | -7<br>-38/23  | -5<br>-41/27  | -11<br>-30/19 | -10<br>-38/34 | -14<br>-34/21 | -15<br>-33/16 | -13<br>-45/23 | -10<br>-32/9  | -6<br>-37/33  | -9<br>-42/20  | -8<br>-41/21  | -13<br>-29/31 |
| AoPs (kPa)            | 5<br>-10/12  | -1<br>-8/14   | -3<br>-15/9   | -5<br>-6/6    | -5<br>-10/3   | -5<br>-14/7   | -6<br>-9/2    | -2<br>-16/7   | 0<br>-7/2     | 0<br>-6/4     | 1<br>-21/9    | -3<br>-18/10  | 3<br>-12/6    | 0<br>-9/11    | -3<br>-10/12  | -4<br>-17/5   |
| AoPd (kPa)            | 3<br>-14/30  | 5<br>-12/11   | -3<br>-29/20  | -6<br>-17/12  | -11<br>-21/5  | -5<br>-29/7   | -11<br>-21/7  | 0<br>-29/9    | -6<br>-13/5   | -9<br>-17/5   | 2<br>-36/14   | -2<br>-31/15  | -2<br>-19/14  | -6<br>-18/20  | -16<br>-20/19 | -9<br>-36/17  |
| PRP (kPa/b/min)       | 11<br>-27/76 | 1<br>-24/41   | -11<br>-31/65 | -11<br>-33/46 | -3<br>-45/14  | 0<br>-51/18   | -17<br>-33/23 | -11<br>-42/26 | -13<br>-38/18 | -14<br>-34/7  | -18<br>-56/22 | -13<br>-38/12 | -8<br>-35/42  | 1<br>-44/15   | -6<br>-42/13  | -10<br>-40/35 |
| L.V dp/dhmax (kPa/s)  | -3<br>-18/6  | -11<br>-22/8  | -16<br>-22/0  | -16<br>-20/0  | -16<br>-23/4  | -18<br>-25/2  | -17<br>-22/6  | -17<br>-26/8  | -18<br>-24/0  | -18<br>-22/4  | -17<br>-32/44 | -18<br>-22/2  | -17<br>-22/6  | -18<br>-22/2  | -18<br>-20/2  | -17<br>-22/0  |
| L.V dp/dtmax/pd (1/s) | -9<br>-19/2  | -12<br>-16/5  | -13<br>-19/2  | -12<br>-18/6  | -9<br>-18/0   | -11<br>-20/0  | -13<br>-18/9  | -12<br>-22/9  | -12<br>-21/2  | -10<br>-19/2  | -12<br>-18/5  | -8<br>-23/13  | -11<br>-20/2  | -9<br>-24/2   | -8<br>-23/2   | -7<br>-25/4   |
| L.V dp/dtmin (kPa/s)  | 0<br>-9/6    | -8<br>-11/0   | -8<br>-21/5   | -8<br>-14/2   | -10<br>-14/0  | -9<br>-17/0   | -8<br>-18/3   | -6<br>-12/2   | -6<br>-11/3   | -6<br>-14/2   | -8<br>-16/4   | -4<br>-20/5   | -4<br>-14/1   | -4<br>-11/2   | -6<br>-11/0   | -8<br>-13/2   |
| CO (l/min)            | -3<br>-24/50 | -11<br>-29/38 | -15<br>-36/44 | -15<br>-41/41 | -17<br>-48/21 | -19<br>-56/25 | -26<br>-44/13 | -20<br>-42/27 | -28<br>-45/21 | -31<br>-43/14 | -31<br>-54/21 | -14<br>-45/2  | -13<br>-48/21 | -16<br>-63/21 | -33<br>-58/20 | -32<br>-52/28 |
| SV (ml)               | -4<br>-9/0   | -7<br>-17/0   | -8<br>-9/0    | -7<br>-14/0   | -8<br>-17/0   | -7<br>-25/0   | -11<br>-17/0  | -7<br>-17/0   | -11<br>-18/0  | -7<br>-22/0   | -8<br>-18/3   | -4<br>-18/0   | -4<br>-23/0   | -9<br>-33/0   | -8<br>-29/0   | -10<br>-40/2  |
| SVR (kPa/l/min)       | 11<br>-23/17 | 15<br>-27/25  | 12<br>-22/57  | 6<br>-27/49   | 7<br>-27/61   | 10<br>-25/73  | 18<br>-22/69  | 38<br>-25/59  | 31<br>-22/71  | 41<br>-20/69  | 50<br>-27/59  | 36<br>-25/61  | 10<br>-31/94  | 15<br>-31/141 | 23<br>-29/114 | 23<br>-30/72  |

Values are median (min/max).

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**Table 6b: Effects of (dl)-propranolol (10 mg/kg orally) in awake, chronically instrumented dogs (data are expressed as median changes versus pre-medication in percentage; n=7).**

| Parameters  | 15 min        | 30 min         | 45 min         | 60 min         | 75 min       | 90 min        | 105 min      | 120 min       | 135 min        | 150 min       | 165 min       | 180 min      | 195 min      | 210 min       | 225 min       | 240 min      |
|-------------|---------------|----------------|----------------|----------------|--------------|---------------|--------------|---------------|----------------|---------------|---------------|--------------|--------------|---------------|---------------|--------------|
| PQ (ms)     | 6<br>-3/20    | 10<br>-2/23    | 7<br>-2/24     | 9<br>0/29      | 8<br>1/24    | 11<br>3/22    | 8<br>4/18    | 9<br>-2/21    | 9<br>-2/21     | 12<br>2/22    | 7<br>4/23     | 9<br>2/21    | 7<br>-2/27   | 7<br>4/20     | 10<br>5/12    | 7<br>-2/17   |
| QRS (ms)    | -2<br>-12/7   | 0<br>-7/9      | 4<br>-7/9      | 4<br>-9/11     | 6<br>-1/09   | -2<br>-7/11   | -2<br>-12/11 | 4<br>-7/11    | 2<br>-3/12     | 2<br>-7/11    | -2<br>-12/18  | -2<br>-9/11  | -6<br>-12/9  | -2<br>-10/14  | 1<br>-8/6     | 2<br>-9/9    |
| QT (ms)     | 0<br>-7/7     | 2<br>-1/9      | 3<br>-3/10     | 4<br>-5/10     | 5<br>0/12    | 4<br>-2/14    | 6<br>1/13    | 7<br>-4/13    | 5<br>0/11      | 5<br>-1/10    | 6<br>-2/12    | 4<br>-5/11   | 1<br>-3/8    | 3<br>2/9      | 4<br>-1/8     | 5<br>1/8     |
| QTcB (ms)   | -2<br>-1/44   | -5<br>-15/7    | -2<br>-16/10   | -1<br>-15/9    | 1<br>-15/6   | -1<br>-10/8   | -4<br>-11/4  | -4<br>-15/8   | -3<br>-15/4    | -5<br>-13/5   | -8<br>-14/5   | -5<br>-11/2  | -6<br>-16/11 | -5<br>-18/4   | -8<br>-15/7   | -5<br>-13/6  |
| QTcF (ms)   | -1<br>-7/3    | -1<br>-10/4    | 0<br>-8/8      | 0<br>-7/7      | 2<br>-7/6    | 2<br>-7/5     | -1<br>-6/3   | 0<br>-6/4     | 0<br>-6/4      | -1<br>-6/4    | -4<br>-6/4    | -4<br>-6/3   | -3<br>-10/6  | -2<br>-9/4    | -4<br>-7/6    | 0<br>-7/4    |
| QTcVDW (ms) | -1<br>-4/3    | 0<br>-9/3      | 0<br>-4/7      | 0<br>-3/7      | 2<br>-7/6    | 3<br>-6/5     | 1<br>-4/3    | 1<br>-4/4     | 0<br>-2/4      | 1<br>-5/4     | -2<br>-4/3    | -2<br>-5/2   | -2<br>-10/4  | -2<br>-4/3    | -2<br>-6/5    | 0<br>-3/3    |
| QTd (ms)    | -8<br>-53/183 | -17<br>-76/133 | -14<br>-47/150 | -17<br>-76/150 | 0<br>-60/133 | -7<br>-36/233 | 7<br>-19/250 | -7<br>-33/106 | -20<br>-44/100 | -8<br>-43/233 | 25<br>-33/217 | 8<br>-33/200 | 8<br>-48/117 | 25<br>-43/117 | -31<br>-75/33 | 5<br>-24/183 |

Values are median (min/max).

The data shows changes expected from the pharmacology. Heart rate is decreased in both nebivolol and propranolol compared to the control. LV dp/dtmax and LV dp/dtmax/pd both decreased compared to the control with both drugs. The magnitude of change, indicating decreased contractility, was greater with nebivolol. Cardiac output was also decreased with both drugs. All of these changes persisted for the 6 hour period of sampling and might possibly have persisted longer. The data provided do not indicate prolongation of QTc. This is consistent with other acute dosing studies that also show no effects on QTc.

Effects, if any, on ST segment magnitude, as an index of perfusion adequacy were not disclosed.

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***N59900 The comparison of the cardiac and hemodynamic effects of cumulative intravenous injections of R67138(d) with those of R67145(l), the two enantiomers of nebivolol in closed chest anesthetized mongrel dogs. (1987)***

Fourteen mixed-breed dogs of both sexes and varying ages were divided into 2 groups and randomly treated with either d-(n=7) or l-(n=7) nebivolol. The animals were anesthetized, intubated and intermittent positive pressure ventilation was performed with a volume controlled ventilator. Dogs were instrumented for monitoring. After a recorded control period of 20 minutes, the drugs studied were injected intravenously in each 7 dogs in cumulative doses of 0.0025, 0.01, 0.04, 0.16 and 0.63 mg/kg at 30 minute intervals. The methods are not clear as to time points of data determination but the statistical analysis section notes that

The changes in the values of the various variables 1, 2, 5, 10, 20, and 30 min after administration of the various doses of the drugs under investigation, as compared with the control values (i.e. the median value of the values obtained just prior to, and 5 and 10 min before the first injection of the compound ), were evaluated for statistical

Results: The presentation of the results is suboptimal in that it is entirely graphical.

d-enantiomer: The sponsor notes that the pressure needed to ventilate the lungs at a constant minute volume did not change after the administrations of the d-enantiomer.

After 0.01 mg/kg ↓LV dp/dt max, LV dp/dt max/Pd and Ao dv/dt max

After 0.16 mg/kg ↓ AoPs, Aov max, SV, CO, LVSW and external mechanical efficiency

Increases were reported for pulmonary arterial pressure, total pulmonary resistance, total systemic resistance and time of relaxation after 0.01 and 0.04 mg/kg. All changes were reported as lasting up to and through the highest dose administered.

The decrease in contractility (LV dp/dt max/p) was on average ~10% after the lowest dose and ~40% after the highest. The decrease in stroke volume was ~10%(0.0025 mg/kg) to 50%(0.63 mg/kg) and showed a dose response.

l-enantiomer: The pressure needed to ventilate the lungs at a constant minute volume did not change after the administrations of the d-enantiomer. Decreases were observed in TSR after injection of 0.0025 and 0.01 mg/kg, in AoPd, PRP and VO2 following 0.01, 0.04 and 0.16 mg/kg and in AoPs and LV dp/dt max after administration of 0.63 mg/kg.

0.63 mg.kg<sup>-1</sup> of the compound. A significant increase was noted in LVEDP, LV dp/dt

max/Pd, Ao dv/dt max, SV, CO, LVSW and EME starting at the lowest dose injected up to and including 0.01 or 0.04 mg.kg<sup>-1</sup>.

Aov max, RVSW, and PAPs showed a significant increase starting at 0.01 mg.kg<sup>-1</sup> up to and including 0.16, 0.04, and 0.63 mg.kg<sup>-1</sup>

i.v., respectively. PAPd and TPR increased significantly after the injection of the higher doses of the compound. HR and T of relaxation did not show consistent significant changes in the dose range tested (figures 6 through 10).

At the highest dose, there was on average a 10% loss of contractility. A dose response effect was not seen for the doses that did not decrease contractility.

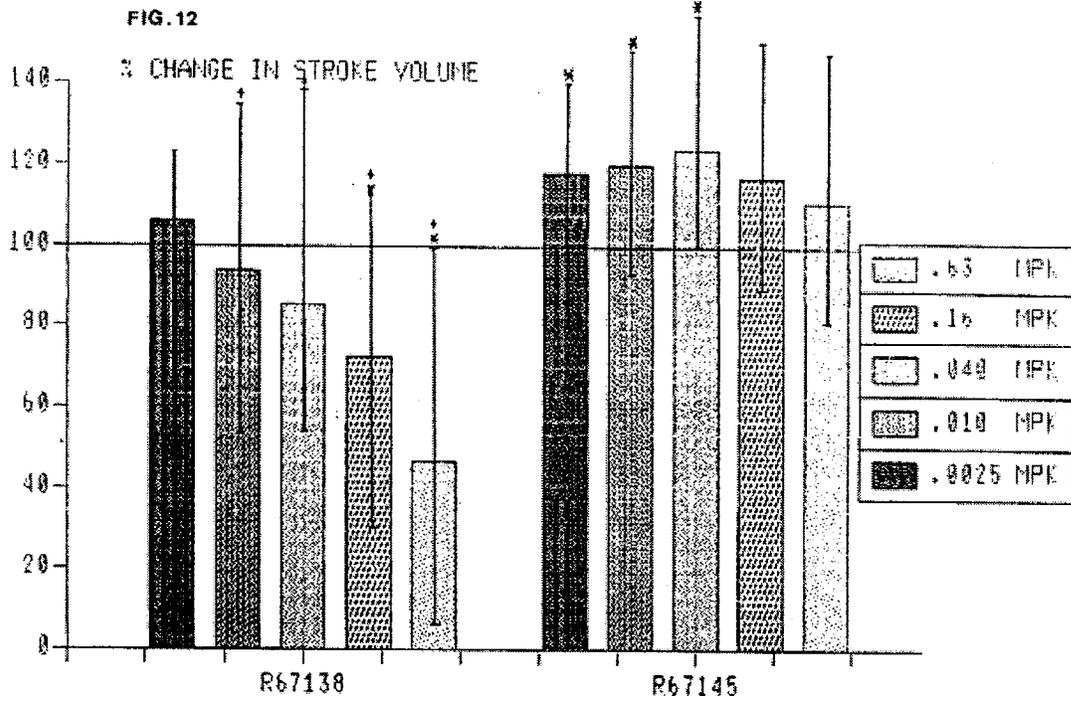
Under the conditions of this study the d-enantiomer caused a decrease in cardiac function starting at 0.01 mg/kg given intravenously as shown by LV dp/dt max, LV dp/dt max/Pd, Aov max, Ao dv/dt max and SV in the absence of heart rate changes and pre-load. The sponsor also notes peripheral and pulmonary vasoconstriction:

diastolic pressure (preload). Starting at 0.01 and 0.04 mg.kg<sup>-1</sup> i.v., respectively, the compound acutely induces peripheral systemic and pulmonary vasoconstriction as indicated by the significant increase in total systemic and total pulmonary vascular resistance. Since the decrease in systolic aortic blood pressure after the highest dose injected (0.63 mg.kg<sup>-1</sup>) is associated with an increase in systemic vascular resistance,

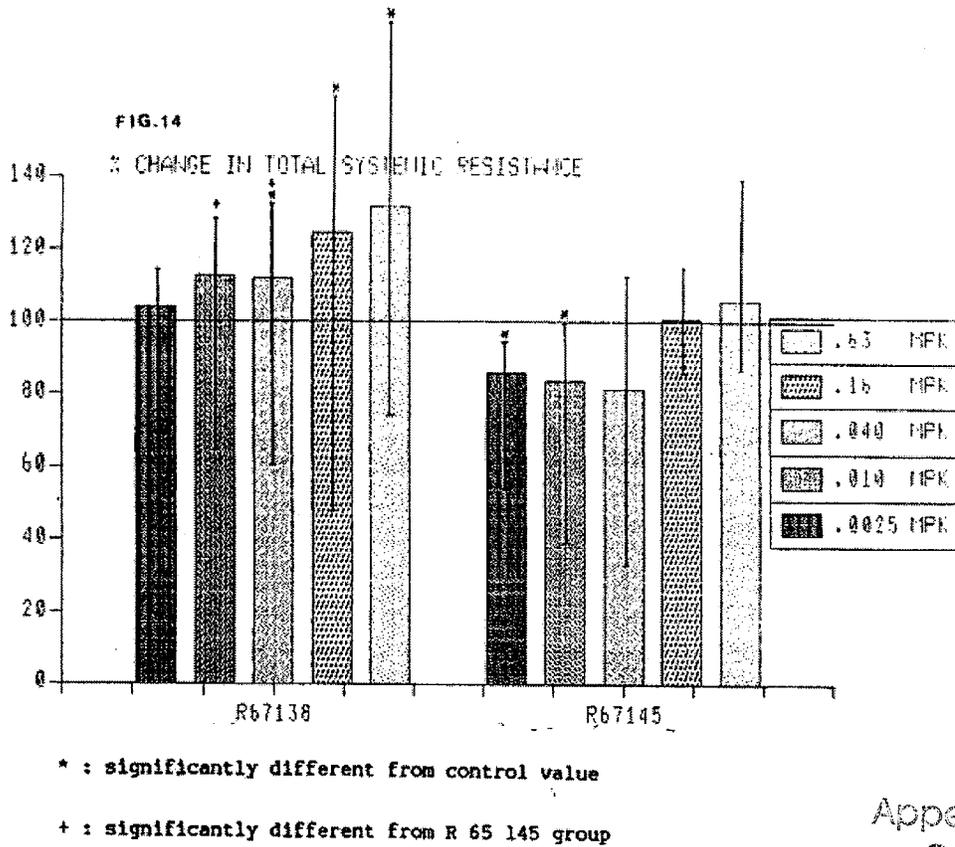
How do we eliminate any kind of coronary vasoconstriction from consideration?

The l-enantiomer was reported to cause an acute decrease in total systemic resistance after 0.0025 and 0.01 mg/kg iv. However, after the highest dose, 0.63 mg/kg, the l-enantiomer caused peripheral vasoconstriction with a decrease in cardiac performance. Therefore, both enantiomers cause vasoconstriction and decreased cardiac performance. There is a window of doses in which one of the enantiomers will cause vasodilation and some increase in cardiac performance. Information was not provided for the system over time or the vehicle. A very interesting point is that dose response effects were seen for the d-enantiomer but were not present for any of the parameters affected by the l-enantiomer.

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*N184228: Electrophysiological evaluation of d,l-nebivolol in isolated rabbit Purkinje fibers in a condition of a normal rhythm and of a bradycardia: a comparison with solvent, d,l-propranolol and d,l-sotalol (March 2003).*

In the present study the sponsor investigated the potential electrophysiological effects of solvent, d,l-nebivolol (a selective  $\beta_1$ -adrenoceptor blocker), d,l-propranolol and d,l sotalol (two reference  $\beta$ -adrenoceptor blockers) on isolated rabbit Purkinje fibers, in a condition of a normal rhythm (stimulation rate of 1 Hz) and of bradycardia (0.2 Hz). d,l-Nebivolol and d,l-propranolol were tested at concentrations of  $1 \times 10^{-8}$  M to  $1 \times 10^{-5}$  M, and d,l-sotalol was tested at concentrations of  $1 \times 10^{-7}$  M to  $1 \times 10^{-4}$  M. The concentrations of the compounds were selected according to their  $pIC_{50}$  for binding to  $\beta_1$ -adrenoceptors in vitro ( $pIC_{50}$ : 8.5, 8.3 and 5.6 for d,l-nebivolol, d,l-propranolol and d,l-sotalol, respectively), and according to the

effects of two reference compounds (d,l-propranolol and d,l-sotalol) on the duration of the action potential (APD) in vitro.

Electrophysiological experiments were performed on isolated rabbit Purkinje fibers using conventional microelectrode techniques to record transmembrane action potentials. Cardiac tissue was isolated shortly after euthanasia of the animal and stored in a tissue bath. The Purkinje fibers were perfused with gassed (95% O<sub>2</sub> and 5% CO<sub>2</sub>) Tyrode solution (KCl 4mM). The preparations were fixed to the bottom of a perfusion organ bath and continuously superfused with oxygenated Tyrode solution in a 34-37 °C bath. Purkinje fibers were stimulated at a basal rate of 1 Hz. Stimuli were regular pulses of 0.5 to 1.0 ms duration delivered at an intensity of two times the diastolic threshold. The amplitude of action potential (AAP in mV), action potential duration at 50% or at 90% (APD<sub>50</sub> or APD<sub>90</sub> in ms) were determined from the recordings. Control values (baseline values before solvent or compound) were determined after a stabilization period ( $\pm 90$  min) when the measured variables had reached a steady-state. Abnormal action potentials in terms of triggered activities (TAs) and spontaneous activity were also recorded during the experiments. TAs are caused by early afterdepolarizations (EADs) or delayed afterdepolarizations (DADs). An EAD was identified as a small afterpotential that interrupts or delays the normal repolarization of the action potential; a DAD was identified as a small afterpotential that follows its complete repolarization. Preparations in which electrical stimulation was not consistently followed by an action potential, or preparations with APD<sub>90</sub> being  $\leq 360$  ms or  $\geq 160$  ms; V<sub>max</sub>  $\leq 100$  V/s or  $\geq 1000$  V/s, or preparations that had abnormal action potentials (such EADs or DADs during the control period), were excluded from the study. All the electrophysiological parameters were recorded during the entire experimental period.

The 3 drugs were dissolved in DMSO to make stock solutions that were further diluted with perfusion solution to make the final concentrations.

After recording baseline values, one of following solutions was continuously infused into the superfusion medium for 60 min:

- 1) solvent (n = 7);
- 2) d,l-nebivolol at  $1 \times 10^{-8}$  M,  $1 \times 10^{-7}$  M,  $1 \times 10^{-6}$  M and  $1 \times 10^{-5}$  M (15 min for one concentration; n = 8);
- 3) d,l-propranolol at  $1 \times 10^{-8}$  M,  $1 \times 10^{-7}$  M,  $1 \times 10^{-6}$  M and  $1 \times 10^{-5}$  M (15 min for one concentration; n = 8);
- 4) d,l-sotalol at  $1 \times 10^{-7}$  M,  $1 \times 10^{-6}$  M,  $1 \times 10^{-5}$  M and  $1 \times 10^{-4}$  M (15 min for one concentration; n = 7);

The Purkinje fibers were stimulated at a normal rhythm (1 Hz: 60 pulses/min) during the experimental period. Thereafter, the electrical stimulation rate was reduced to 0.2 Hz (12 pulses/min) for another 5 min (total time 65 min).

## Results

The solvent itself produced increases in APD<sub>50</sub> and APD<sub>90</sub> and decreases in V<sub>max</sub>. The changes are summarized below.