

**Table 2:** Pharmacokinetic parameters of nebivolol in beagle dogs after single (day 1) and after repeated (day 366) oral administration of nebivolol hydrochloride (R067555), provided as a mixture with  $\beta$ -cyclodextrin, at 2.5, 10 or 40 mg(base-equivalents)/kg/day in a twelve-month chronic oral toxicity study. Means  $\pm$  S.D.; n = 4, i.e. 2 male and 2 female dogs, are given for each dose level.

Dose	2.5 mg/kg/day		10 mg/kg/day		40 mg/kg/day	
	single	repeated	single	repeated	single	repeated
$C_{max}$ (ng/ml)	14.5 $\pm$ 4.0	21.1 $\pm$ 6.2	67.6 $\pm$ 18.3	112 $\pm$ 41	186 $\pm$ 62	640 $\pm$ 391
$T_{max}$ (h)	2.5 $\pm$ 1.0	2.5 $\pm$ 1.0	1.5 $\pm$ 0.6	2.5 $\pm$ 1.0	1.8 $\pm$ 0.5	2.0 $\pm$ 0.0
$AUC_{0-\infty}$ (ng.h/ml)	76.1 $\pm$ 7.4	99.0 $\pm$ 14.0 <sup>1)</sup>	285 $\pm$ 32	683 $\pm$ 178 <sup>1)</sup>	1086 $\pm$ 214	4276 $\pm$ 1870 <sup>1)</sup>
$t_{1/2}$ (h)	2.67 $\pm$ 0.72	2.47 $\pm$ 0.38	2.55 $\pm$ 0.15	3.55 $\pm$ 0.31 <sup>2)</sup>	3.85 $\pm$ 0.90	3.81 $\pm$ 0.23

<sup>1)</sup>  $AUC_{0-24 h}$

<sup>2)</sup> n = 3

Again, the striking finding is the level of drug found in the analyzed tissues. The sponsor's summary of tissue to plasma concentrations is shown below. The lung showed the greatest accumulation of material. The sponsor cited the decrease in liver concentration from 10 to 40 mg/kg to indicate no undue accumulation. If one compares the liver findings to the decreases in

**Table 5:** Mean ( $\pm$  S.D.) tissue to plasma concentration ratios of nebivolol at 24 hours after the last dose administration of nebivolol hydrochloride (R067555), provided as a mixture with  $\beta$ -cyclodextrin, at 2.5, 10 or 40 mg(base equivalents)/kg/day in a twelve-month chronic oral toxicity study in beagle dogs (n = 4, i.e. 2 male and 2 female beagle dogs for each dose level).

Dose	Tissue to plasma concentration ratios		
	10 mg/kg/day	40 mg/kg/day	Overall mean (n = 8)
Brain	64.4 $\pm$ 18.4	74.3 $\pm$ 30.1	69.4 $\pm$ 23.7
Heart	21.9 $\pm$ 8.8	15.1 $\pm$ 4.1	18.5 $\pm$ 7.3
Lung	291 $\pm$ 112	205 $\pm$ 46	248 $\pm$ 92
Liver	30.0 $\pm$ 21.3	6.9 $\pm$ 2.4 <sup>1)</sup>	.2) <sup>2)</sup>
Kidney	36.3 $\pm$ 21.3	22.2 $\pm$ 12.4	29.3 $\pm$ 17.8
Spleen	29.5 $\pm$ 15.2	18.2 $\pm$ 7.2	23.9 $\pm$ 12.6
Pancreas	63.1 $\pm$ 31.4	50.3 $\pm$ 20.3	56.7 $\pm$ 25.5
Muscle	10.2 $\pm$ 4.7	5.9 $\pm$ 1.9	8.1 $\pm$ 4.0
Fat	4.4 $\pm$ 1.0	3.0 $\pm$ 1.4	3.7 $\pm$ 1.3

<sup>1)</sup> n = 3.

<sup>2)</sup> overall mean not calculated.

tissue to plasma ratios seen in other tissues going from 10 to 40 mg/kg, it's possible that this reflects variability in both parameters ( tissue and plasma).

***N119664 Toxicokinetics of nebivolol in male and female SPF Albino Swiss mice after single oral administration of aqueous suspensions of nebivolol hydrochloride (R067555) at 10, 40 and 160 mg (base-eq.)/kg in the micronucleus test. 1996***

A satellite group of 44 mice (22/sex) were used as satellite animals to study toxicokinetics in the micronucleus test. The satellite group was divided into 2 groups of 12 (6 of either sex) and 1 group of 20 (10 of either sex). The animals were dosed once to provide doses of 10, 40 or 160 mg (base-eq.)/kg. Blood samples were obtained from the first 3 mice of each sex from each group at 2 hours after dosing and from the remaining 3 per sex per group at 4 hours after dosing. Results: Plasma nebivolol was detectable in all samples at both time points. In both sexes at LD and MD, the 4 hour sample showed lower plasma levels than the 2 hour sample. At the HD. The increase in concentration from LD to MD was proportional. The increase from MD to HD was less than proportional.

Plasma levels of R067555 ng/ml

dose	Time after dosing(h)	Male mice	Female mice
10 mg/kg	2	372±119	258±66
	4	176±51	167±73
40 mg/kg	2	1227±360	814±73
	4	769±304	988±257
160 mg/kg	2	2176±378	2252±434
	4	2699±1100	2705±1040

***N109091 Toxicokinetics of nebivolol in male and female albino Swiss mice at the end of a 3-month subchronic oral toxicity study (Exp No. 1966) with a microcrystalline powder of nebivolol hydrochloride (R067555) admixed in the food at intended nebivolol doses of 10, 40 and 160 mg (base-eq.)/kg/day. 1988-1994***

For three months, the mice of the 3 dosage groups had access to food containing a microcrystalline powder of nebivolol in concentrations to provide daily dosages of 10, 40 or 160 mg/kg. At the end of the 3 months, blood samples were collected from the last 5 mice/sex/group. There is no further information as to the timing of sample collection. The samples were analyzed by HPLC for nebivolol concentration.

Results: Detectable levels were found in all samples. The increase in concentration from LD to MD was greater than proportional in both sexes. The increase from MD to HD was less than proportional in males and proportional in the female mice.

***N108427 Toxicokinetics of nebivolol in albino Swiss mice during an oral 18- to 24-month carcinogenicity study at intended nebivolol doses of 2.5, 10 or 40 mg (base-eg.)/kg/day (Exp.No.1967) administered through the diet as a mixture of nebivolol hydrochloride (R067555) and  $\beta$ -cyclodextrin.1989-1994.***

A satellite group of 3 sets of 30 SPF albino Swiss mice (15/sex) were annexed to the carcinogenicity study. Each set of 30 mice was divided into 3 equal subsets with 5/sex/group. Blood was collected from the satellite mice at 6, 12 and 18 months. Plasma samples were analyzed for nebivolol by HPLC methodology.

Results: Nebivolol was detected at all time points. There was no consistent trend to accumulation or decrease in the single concentration sample per 6 month timepoint. At the LD and MD, the concentration in males was approximately 3x higher than that in females. However, this icepick view of drug exposure does not account for differences in absorptive rates that may possibly exist.

***N108426 Toxicokinetics of nebivolol in SPF Wistar rats in a one-month subchronic oral pilot toxicity study of nebivolol hydrochloride (R067555) provided as a mixture with  $\beta$ -CD admixed in the food at 20, 40 or 80 mg(base-eq.)/kg/day. 1989-1994***

Nebivolol was admixed in the diet to provide dosages of 20, 40 or 80 mg/kg/day. The satellite group consisted of 3 groups of 4 (2/sex) Wistar rats. Blood samples were collected within a 2 hour period on days 2,6 and 21. The animals were euthanized Day 29 and blood collected. Samples were analyzed by HPLC for nebivolol content.

Results: Plasma concentrations were reported to increase to day 21 and sometimes to day 29. Since only 1 sample was taken per sampling day, and the administration was dietary, it can't be said with certainty that there really is an accumulation over time or that the sampling is showing normal variation around absorption.

There were detectable plasma levels at all points of determination.

***N109086Comparative toxicokinetics of rac-, d- and l- nebivolol in SPF Wistar rats in 1-month oral toxicity studies (Exp. Nos. 2335 and 2336) of rac-nebivolol at 20 or 80 mg(base-eq.)/kg/day and of d- and l-nebivolol at 10 or 40 mg (base-eq.)/kg/day with each of the compounds admixed in the food. 1990-1994.***

The satellite group consisted of 4 groups and 2 groups consisting each of 2/sex SPF Wistar rats for each of the toxicology studies (Exps. 2335 and 2336). These groups were dosed with l-nebivolol at 10 or 40 mg/kg/day or with rac-nebivolol at 20 or 80 mg/kg/day. Blood was collected days 2,6,13,21 and 29 of the experiment. Individual and pooled samples were analyzed for rac-,d- or l-nebivolol before and after enzymatic hydrolysis. Levels of unchanged drug were determined by HPLC methods. Concentrations of d- or l-nebivolol plus the hydroxylated metabolites before and after enzymatic hydrolysis were estimated in pooled serum samples by RIA with enantioselective antibodies.

Results There were detectable levels of all drugs at all points of determination.

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**Table 1:** Mean (n=2) 8 a.m. steady-state plasma levels <sup>1)</sup> of *rac*-nebivolol (R067555), *d*- (R067138) and *l*-nebivolol (R067145) during one-month subchronic oral toxicity studies (Exp. Nos. 2335 and 2336) in the SPF Wistar rat (satellite groups) with the drugs admixed in the food (a mixture with  $\beta$ -cyclodextrin) at 20 or 80 mg(base-eq.)/kg/day (*rac*-nebivolol) and at 10 or 40 mg(base-eq.)/kg/day (*d*- and *l*-nebivolol). The figures between brackets represent concentrations recalculated to the intended dose levels.

	Males				Females			
<b>R067555</b>								
Day	20 mg/kg/day		80 mg/kg/day		20 mg/kg/day		80 mg/kg/day	
2	37.2	(31.3)	197	(163)	49.1	(44.8)	240	(242)
6	37.6	(31.6)	279	(231)	72.0	(65.7)	416	(419)
13	24.2	(22.5)	233	(234)	51.4	(55.6)	253	(298)
21	34.1	(38.5)	334	(366)	72.4	(87.8)	355	(442)
29	9.0	(12.1)	102	(135)	25.0	(34.7)	176	(254)
Mean <sup>2)</sup>	33.3 $\pm$ 6.2 (31.0 $\pm$ 6.6)		261 $\pm$ 59 (249 $\pm$ 85)		61.2 $\pm$ 12.7 (63.5 $\pm$ 18.3)		316 $\pm$ 84 (350 $\pm$ 96)	
<b>R067138</b>								
Day	10 mg/kg/day		40 mg/kg/day		10 mg/kg/day		40 mg/kg/day	
2	14.0	(11.0)	52.6	(45.1)	17.4	(16.4)	93.2	(89.6)
6	16.4	(12.9)	71.2	(61.1)	16.3	(15.4)	204	(196)
13	12.1	(12.0)	55.3	(55.7)	15.4	(17.0)	117	(127)
21	14.7	(17.0)	61.5	(72.4)	22.0	(26.6)	148	(185)
29	7.5	(10.3)	34.0	(46.7)	9.5	(13.0)	71.8	(97.0)
Mean <sup>2)</sup>	14.3 $\pm$ 1.8 (13.2 $\pm$ 2.6)		60.2 $\pm$ 8.3 (58.6 $\pm$ 11.4)		17.8 $\pm$ 2.9 (18.9 $\pm$ 5.2)		141 $\pm$ 48 (149 $\pm$ 50)	
<b>R067145</b>								
Day	10 mg/kg/day		40 mg/kg/day		10 mg/kg/day		40 mg/kg/day	
2	16.4	(13.1)	118	(95.8)	13.8	(12.2)	115	(106)
6	13.6	(10.9)	126	(103)	12.6	(11.2)	168	(155)
13	10.2	(9.9)	59.3	(59.0)	13.4	(14.1)	111	(125)
21	11.7	(12.8)	60.7	(66.5)	12.5	(14.4)	72.0	(86.4)
29	6.8	(8.8) <sup>3)</sup>	46.3	(60.8)	8.7	(11.4)	45.3	(59.2)
Mean <sup>2)</sup>	13.0 $\pm$ 2.7 (11.7 $\pm$ 1.5)		91.0 $\pm$ 35.9 (81.1 $\pm$ 21.6)		13.1 $\pm$ 0.6 (13.0 $\pm$ 1.5)		117 $\pm$ 39 (118 $\pm$ 29)	

<sup>1)</sup> All dose levels and plasma concentrations are expressed as base-equivalents.

<sup>2)</sup> The plasma concentrations measured on day 29 (samples collected at 12 h 00 - 12 h 15 p.m. instead of 8 h 00 a.m.) were not taken into account for the calculation of the average steady-state concentrations, as they were 1.5 to 3.7 times lower than their respective average steady-state plasma concentrations calculated between day 2 and day 22 of the study. This was unexpected, since serum concentrations measured at autopsy after withdrawal of the medicated food remained fairly constant for nebivolol as well as for its enantiomers (see 2.5).

<sup>3)</sup> n=1.

***N109031 Toxicokinetics of nebivolol in male and female SPF Wistar rats during a three-month subchronic oral toxicity study of a microcrystalline powder of nebivolol hydrochloride (R067555) admixed in the food at 10, 40 or 160 mg (base-eq.)/100g, intending a dose of 10, 40 or 160 mg (base-eq.)/kg/day. 1986-1994***

A satellite group of 12 SPF Wistar rats (6/sex) were dosed for 16 consecutive days in conjunction with the rats of the 3 month toxicology study, with a microcrystalline powder of nebivolol hydrochloride admixed with food. Blood samples were collected day 2, day 4, day 8 and day 12. Blood was also collected at termination on day 17. The plasma samples were analyzed by HPLC for nebivolol.

### Results

Single samples every few days taken at unspecified times per day after unregulated consumption of a diet/drug admixture are not the most precise way to determine pharmacokinetic parameters. There were detectable levels of drug at all points of determination. Given the variability inherent in the study methodology, the plasma concentrations increased until about day 8.

**Table 2:** Mean (n=2) 8-a.m. plasma levels of nebivolol (base-equivalents) in male and female SPF Wistar rats (added to the rats of the three-month toxicity study Exp. No. 1590) dosed for 16 consecutive days with a microcrystalline powder of nebivolol hydrochloride admixed in the food at a concentration of 10, 40 or 160 mg (base-eq.)/100 g, intending a dose of 10, 40 or 160 mg nebivolol (base-eq.)/kg/day. The plasma concentrations, corrected to the intended intake of nebivolol, are given between brackets.

Time (days)	Plasma concentration (ng/ml)					
	10 mg/kg/day		40 mg/kg/day		160 mg/kg/day	
	Male	Female	Male	Female	Male	Female
2	4.1 (2.9)	6.1 (3.8)	20.6 (14.6)	34.0 (21.6)	187 (133)	167 (109)
4	3.2 (2.3)	5.7 (3.6)	13.0 (9.2)	30.0 (19.1)	259 (184)	271 (177)
8	6.9 (5.7)	7.7 (5.4)	24.8 (20.2)	43.4 (28.5)	358 (277)	287 (165)
12	8.6 (7.1)	9.9 <sup>b)</sup> (6.9) <sup>b)</sup>	18.4 (15.0)	109 (71.6)	163 (126)	235 (135)
17	6.6 (5.8)	20.0 <sup>b)</sup> (15.9) <sup>b)</sup>	19.7 (17.7)	47.2 (32.0)	322 (271)	168 (103)
Average 8-a.m. steady-state plasma concentration	5.9 ± 2.2 (4.8 ± 2.1) (n = 5)	9.9 ± 5.9 (7.1 ± 5.1) (n = 5)	19.3 ± 5.3 (15.3 ± 4.1) (n = 5)	52.7 ± 32.2 (34.6 ± 21.3) (n = 5)	258 ± 84 (198 ± 73) (n = 5)	226 ± 56 (138 ± 33) (n = 5)

<sup>b)</sup> n = 1

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***109345TK of nebivolol in the beagle dog in a 16-day subchronic oral pilot toxicity study EXP No. 2182 with a mixture of nebivolol hydrochloride (R067555) with  $\beta$ -cyclodextrin at 20 and 40 mg nebivolol base- equivalents/kg/day. 1989-1994.***

The sponsor generated the following data from this study:

	20 mg(base-eq)/kg/day		40 mg(base-eq)/kg/day	
	single	repeated	single	Repeated
Cmax (ng/ml)	101±48	124±58	141±77	523±310
Tmax (h)	1.8±0.5	2.5±1.7	2.0±0.0	2.5±1.0
T1/2 (h)	4.1±0.1	5.3±2.7	4.0±0.8	3.8±0.6
AUC* (ng.h/ml)	746±288	859±232	1405±1127	3584±2080

\* AUC<sub>0-∞</sub>(single dose) or AUC<sub>0-24</sub> (repeated administration)

Cmax was increased by 3.7 x at 40 mg/kg with repeated dosing. The AUC values were calculated differently for single and repeat dosing. The sponsor also compares the results of this study with the results of the six-month oral toxicity study of nebivolol given as a microcrystalline powder in gelatin capsules at 20 and 80 mg base-eq/kg/day. The sponsor states that in the present study the Cmax and AUC values were on average 5 times higher than the Cmax and AUC values obtained in the six month study. Since there was no 16-day evaluation in the six-month study, there is a limited value to comparing the results of the studies. The apparent purpose is probably to compare the effects of the different formulations.

***N109348 Toxicokinetics of nebivolol in male and female SPF Wistar rats at the end of a 6-month chronic oral toxicity study of nebivolol hydrochloride (R067555) admixed in the food at 10, 40 or 160 mg (base-eq.)/100g, intending a dose of 10, 40 or 160 mg (base-eq.)/kg/day. 1987-1994.***

Three groups of 40 SPF Wistar rats (20/sex) were used. Samples were taken from these main study animals. A microcrystalline powder of nebivolol was used for dosing. Blood samples were collected at time of euthanasia and analyzed for nebivolol.

Results: Nebivolol was detected in the plasma at all points of determination. Increases in the concentration were not proportional, but again, these are single point determinations being compared, not AUC values.

**Table 1:** Mean ( $\pm$  S.D.; n = 9 or 10) actual and normalized (to the intended dose levels) serum concentrations of nebivolol in pooled samples (per 2) of SPF Wistar rats in a six-month chronic oral toxicity study on a microcrystalline powder of nebivolol hydrochloride admixed in the food at a concentration of 10, 40 or 160 mg (base-eq.)/100 g, intending a dose of 10, 40 or 160 mg nebivolol (base-eq.)/kg/day.

	Serum concentrations (ng (base-eq.)/ml)			
	MALES		FEMALES	
	10 mg/kg/day			
Actual	7.70 $\pm$ 3.40	14.0 $\pm$ 5.7		
Normalized	10.2 $\pm$ 4.6	16.2 $\pm$ 6.6		
40 mg/kg/day				
Actual	60.3 $\pm$ 35.5	135 $\pm$ 49		
Normalized	80.6 $\pm$ 46.4	157 $\pm$ 58		
160 mg/kg/day				
Actual	791 $\pm$ 435	972 $\pm$ 351		
Normalized	899 $\pm$ 452	1013 $\pm$ 416		

**109081 TK in a 12-month repeat dose study in Wistar rats.** This was again a single sample study showing that there were detectable plasma levels.

**N109082 Toxicokinetics of nebivolol in SPF Wistar rats in a 24- to 30- month chronic oral carcinogenicity study of nebivolol at intended doses of 2.5, 10 or 40 mg(base.eq)/kg/day given through the diet as a mixture of nebivolol hydrochloride (R067555) and  $\beta$ -cyclodextrin.** Previously reviewed.

**1 a. XBLRPT00960 Method validation of an LC/MS/MS assay for the determination of d-nebivolol and l-nebivolol in mouse plasma**

**1 b. XBL RPT 00961 Method validation of an LC/MS/MS assay for the determination of hydroxyl phenols in mouse plasma**

**1 c. XBL RPT 00971 Method validation of an LC/MS/MS assay for the determination of total nebivolol (conjugated and non-conjugated) in mouse plasma**

The above 3 studies were generated in 2003 by the current sponsor. Each of the 3 studies detailed the same acceptance criteria:

- Calibration curve has a correlation coefficient of  $\geq 0.9900$
- At least 6 back-calculated concentrations for standards are within  $\pm 15\%$  of target values

- Mean values of Intra and Inter batch QC samples at each concentration are within  $\pm 15\%$
- Of target values except for LLOQ —

The 3 studies also detailed the same validation procedures:

- System suitability
- Selectivity/specificity
- Linearity
- Accuracy determined by relative error (RE%) which compares mean values of measured concentrations with nominal concentrations.
  - Intra-day variation
  - Inter-day variation
- Precision determined by coefficient of variance (CV%)
  - Intra-day variation
  - Inter-day variation
- Sensitivity
- Low limit of quantitation (LLOQ)
- Recovery
- Stability
- Multiple freeze-thaw cycles
- Bench top stability
- Long-term
- Extract autosampler
- Dilution (10X, 50 X and 70X. The 50 X and 70X were not used in all studies)

The calibration standards for d- and l-nebivolol were at concentrations from 0.05 – 20 ng/ml in mouse plasma. The standards for the hydroxyl phenols were made at concentrations of 50 to 25000 pg/ml (0.05 – 25 ng/ml). The concentrations for the conjugated species were from 0.05 ng/ml to 20 ng/ml.

The methods for d- and l- nebivolol were reported to be linear within the range 0.05 ng/ml to 20 ng/ml. The methods for the hydroxyl phenols were reported to be linear within the range 0.05 ng/ml to 25 ng/ml. The hydrolysis recovery of nebivolol from nebivolol glucuronides in mouse plasma was calculated at 102% with a reported CV% of 1.64%. The method presented for determination of nebivolol was reported to be linear from 0.05 to 20 ng/ml.

**XBL RPT00962 Method validation of an LC/MS/MS assay for the determination of d-nebivolol and l-nebivolol in rat plasma**

**XBL RPT00959 Method validation of an LC/MS/MS assay for the determination of hydroxyphenols in rat plasma**

**XBL RPT 00964 Method validation of an LC/MS/MS assay for the determination of total nebivolol (conjugated and non-conjugated) in rat plasma**

The above 3 studies were conducted in 2003 by the current sponsor and followed the same acceptance and validation procedure as did the mouse LC/MS/MS studies.

Calibration standards for d- and l- nebivolol in rat plasma were concentrations of 0.05 – 20 ng/ml. The methods were reported to be linear from 0.05 to 20 ng/ml. The methods for the hydroxyl phenols were reported to be linear from 50 to 25000 pg/ml in rat plasma.

**XBL RPT 00963 Method validation of an LC/MS/MS assay for the determination of d-nebivolol and l-nebivolol in dog plasma**

**XBL 00966 Method validation of an LC/MS/MS assay for the determination of hydroxyl phenols in dog plasma**

**XBL RPT 03107 Method validation of an LC/MS/MS assay for the determination of total nebivolol (conjugated and non-conjugated) in dog plasma.**

The same acceptance and validation criteria as were used for the mouse studies (see above) were used here. The methods for d- and l-nebivolol were reported to be linear for the concentration range 0.05 ng/ml to 20 ng/ml. The methods for determination of hydroxyl phenols in dog plasma were reported to be linear from 25 pg/ml to 10000 pg/ml.

The method presented for determination of nebivolol in plasma was reported to be linear in the range of 0.05 ng/ml to 20 ng/ml.

### **3.3.10 Tables and figures to include comparative TK summary**

## **3.4 TOXICOLOGY**

### **3.4.1 Overall toxicology summary**

General toxicology: The spleen and erythron, adrenal glands, lungs and reproductive tract are demonstrated to be target organs in rodents. In dogs, the spleen and adrenals appear to be target organs. There is insufficient information to rule in or rule out the heart (repolarization) and the reproductive tract of dogs as target organs also.

Genetic toxicology: Nebivolol was tested in the Ames assay using strains TA98, TA100, TA1535, TA1537 and E. coli WPuvrA±S9. Under the conditions used there was no increase in revertants. An in vitro mammalian cell gene mutation test (L5178Y/TK<sup>+/+</sup>) also showed no increased frequency of mutations. A chromosome aberration assay using cultured human peripheral lymphocytes had somewhat different results. While no increase in chromosome aberrations was seen under the conditions of the study, increased polyploidy ( $\geq 5\mu\text{g} + \text{S9}$ ) and

endoreduplication ( $\geq 5\mu\text{g/ml}$ , -S9;  $16\mu\text{g/ml}$  +S9) was seen with the test article. The sponsor shows a repeat assay where the polyploidy and endoreduplication repeated in the presence of drug and also in the presence of solvent control. A single oral dose micronucleus test in mice indicated bone marrow toxicity. The sponsor stated that the single oral doses given caused a significant ( $p \leq 0.05-0.001$ ) and dose related decrease in bone marrow proliferation at the 24 hour sampling time. At the 48 hour sampling time a significant ( $p \leq 0.05$ ) decrease in bone marrow proliferation was reported in the 160 mg/kg group. The slight but not statistically significant increase in the micronucleated PCE reported for the 48 hour sampling time with some of the HD mice is described as secondarily related to errors in the process of erythrocyte enucleation or differentiation given the bone marrow toxicity. In female mice, slight increases in micronucleated cells were seen at 24 and 48 hours at the HD. When the results for males and females were combined a slight increase was noted at 24 and 48 hours at the HD.

Carcinogenicity: Leydig cell tumors were present in the male mice : 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. The Executive CAC found the Leydig cell tumors in mice to be drug-related. The CDER statistician did not find evidence of a carcinogenic effect in the rat study. However, there were significant differences in weight gain in the HD groups of both sexes. By the end of the 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 reduction in body weight may also have provided a protective effect for the HD animals

Reproductive toxicology:

As drug was administered later in gestation with each of the reproductive toxicology studies, the dose at which maternal weight gain was affected decreased. That is, the gravid animal seemed more sensitive to the adverse effects of the drug than the non-gravid animal. The sponsor reported cannibalism and dystocia in drug-treated animals but did not give specific details. Two separate SegII studies in rats showed an increase in split thoracic vertebrae in all drug-treated groups, increased rudimentary sternum and an increase in ureteral dilatation. Maternal toxicity was not associated with all the doses at which effects were seen.

The fertility studies were superficial and provided no data about sperm numbers or motility. In light of the scattered reports of testicular pathology and low sperm numbers from the standard toxicology studies, a more detailed examination of fertility would have been advisable.

The developmental landmark data was inconclusive due to the insensitive method used for evaluation. For example, eye opening was not examined until PN day 21 when the process may

happen as early as PN 12. Vaginal opening was not examined until PN42 when it may be observed PN28. Righting on surface was not examined until PN21 but may be seen PN4.

The F1 generation, pups whose dams were dosed with nebivolol, showed decreased birth weight and decreased survival in the first 21 days after birth. This effect repeated in two separate studies at doses of 2.5 mg/kg and 1.25 mg/kg. The exposure relative to the maximum recommended human therapeutic levels on a surface area basis was 2.8X and 1.4X respectively. When the untreated F1 pups became dams, corpora lutea, number of implantations and number of live fetuses was decreased in the offspring of F0 doses of 1.25 and 5 mg/kg.

Special toxicology: The special toxicology studies were half-hearted attempts to address the apparent endocrine findings. One rodent species was used and the examination of circulating hormones was limited. It would have been advisable to include the dog in the special assessment as there are indications that the dog adrenal is also affected. Rats treated with both racemate and enantiomers 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.

### 3.4.2 Single-dose toxicity

**Study title:** *N62289The acute oral toxicity of R67555 in mice (supplement to preclinical Research Report R67555/1)*

**Key study findings:** A dose of 320 mg/kg caused death within 6 hours in females and 3 days in males. LD50 values ( and 95% confidence limits) calculated 14 days after oral administration of the compound were

Males: 243(59.0-999) mg/kg

Females: 178 (136-233) mg/kg

**Study no.:** Serial number R67555/21

**Conducting laboratory and location:** Janssen Research Labs, Beerse, Belgium

**Date of study initiation:** December 6, 1985

**GLP compliance:** no statement found

**QA report:** yes ( ) no ( x )

**Drug, lot #, and % purity:** R67555 batch V8510-171

**Methods :** A single oral dose of R67555 was given by gavage to non-inbred Swiss mice, 10 mice/sex/dose. Doses of 0, 80, 160, 320 and 640 mg/kg were used. Observations included

clinical signs and mortality. Body weights were determined before dosing and days 4, 7, 10 and 14.

**Results:** Lethality is summarized in the reviewer's table.

Summary of mortality at each dose

Dose (mg/kg)	males	Females
0	No mortality	No mortality
80	No mortality	No mortality
160	1 (day 5)	2 (starting day 6)
320	8 (starting day 3)	10 (starting at 6 hours)
640	10(starting at 6 hours)	10 (starting at 6 hours)

A dose related difference in weight was seen from day 4.

Signs reported were death ( $\geq 160$  mg/kg), diarrhea (640 mg/kg), hypothermia ( $\geq 80$  mg/kg), ptosis ( $\geq 80$  mg/kg, 10/10 in each group), prostration ( $\geq 80$  mg/kg), sedation ( $\geq 80$  mg/kg) and tremors ( $\geq 80$  mg/kg). The most commonly reported gross lesions were in the gastrointestinal tract and included irritation and lesions. No organs were collected for histopathological examination.

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On Original

EXPERIMENT: 8539

Acute toxicity study  
R 67555 - OR - MICE

BODY WEIGHT  
Mean values per dosage group in g

Day	Dosage group ( mg / kg )				
	0	80	160	320	640
0	21 N=10	-	21 N=10	22 N=10	21 N=10
4	27 N=10	-	20 N=10	18 N= 7	15 N= 1
7	29 N=10	-	23 N= 9	20 N= 3	-
10	32 N=10	-	25 N= 9	21 N= 3	-
14	33 N=10	-	29 N= 9	27 N= 2	-

  

Day	Dosage group ( mg / kg )				
	0	80	160	320	640
0	23 N=10	21 N=10	22 N=10	23 N=10	22 N=10
4	24 N=10	23 N=10	19 N=10	20 N= 3	17 N= 1
7	26 N=10	25 N=10	20 N= 9	-	-
10	28 N=10	25 N=10	22 N= 8	-	-
14	28 N=10	26 N=10	24 N= 8	-	-

N: Number of animals

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On Original

**Study title:** *The acute intravenous toxicity of R67555 in mice. Supplement to preclinical research report R 67555/1*

**Key study findings:** A dose of 80 mg/kg killed all (10/10m and 10/10f) immediately after injection. A dose of 40 mg/kg killed 5f and 1m immediately after injection. The LD50 values (and 95% confidence limits) calculated 14 days after the intravenous administration of the compound were:

Males: 46.4 (35.5 -60.6) mg/kg  
Females: 40.0 (19.8-80.9) mg/kg

**Study no.:** N62290 Serial # R67555/22

**Conducting laboratory and location:** Janssen Research Laboratories, Beerse, Belgium

**Date of study initiation:** December 5, 1985

**GLP compliance:** no statement found

**QA report:** yes ( ) no ( x )

**Drug, lot #, and % purity:** R67555 batch V8510-171, — H $\beta$ CD in H<sub>2</sub>O was the vehicle.

**Methods:** Swiss mice received single intravenous injections of 0, vehicle, 20, 40 and 80 mg/kg. Mortality and signs were monitored for the next 14 days. Body weights were measured before dosing and at days 4, 7, 10 and 14.

**Results**

Mortality is summarized in the reviewer's table below.

Summary of mortality per dose

Dose mg/kg	males	Females
0	No mortality	No mortality
20	No mortality	No mortality
40	1 immediately after injection	5 immediately after injection
80	10 deaths immediately after injection	10 deaths immediately after injection

Clinical signs reported were: Clonic convulsions ( $\geq 20$  mg/kg), exophthalmos ( $\geq 20$  mg/kg), hypotonia ( $\geq 20$  mg/kg), ptosis ( $\geq 20$  mg/kg), sedation  $\geq 40$  mg/kg, spasms ( $\geq 40$  mg/kg) and tremors ( $\geq 40$  mg/kg).

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Surviving drug-treated females gained less weight than males.

Appears This Way  
On Original

EXPERIMENT: 0537  
 Acute toxicity study  
 R 67555 - IV - NICE

BODY WEIGHT  
 Mean values per dosage group in g

Day	Dosage group ( mg / kg )			
	0	20	40	80
0	23 N=10	24 N=10	22 N=10	21 N=10
4	25 N=10	26 N=10	23 N= 9	-
7	26 N=10	28 N=10	25 N= 9	-
10	28 N=10	31 N=10	26 N= 9	-
14	29 N=10	33 N=10	30 N= 9	-

  

Day	Dosage group ( mg / kg )			
	0	20	40	80
0	23 N=10	22 N=10	22 N=10	21 N=10
4	26 N=10	23 N=10	23 N= 5	-
7	27 N=10	24 N=10	24 N= 5	-
10	28 N=10	25 N=10	24 N= 5	-
14	28 N=10	26 N=10	26 N= 5	-

N: Number of animals

Study title: *The acute oral toxicity of R67555 in rats (supplement to preclinical research report R67555/1)*

**Key study findings:** Doses  $\geq 320$  mg/kg caused death in females starting from Day 1. A dose of 1280 mg/kg killed 10/10 females by day 7. In males 1280 mg/kg and 2560 mg/kg each caused death of 2/10 rats per group. R67555 given orally to adult male Wistar rats at doses of 0, 640, 1280 and 2560 mg/kg produced LD50 values (and 95% confidence limits) calculated 14 days after drug administration of:

Males: >2560 (----) mg/kg  
 Females: 483 (279 -839) mg/kg

**Study no.:** N62179 Serial # R67555/1

**Conducting laboratory and location:** Janssen Research, Beerse, Belgium

**Date of study initiation:** December 6, 1985

**GLP compliance:** statement not found

**QA report:** yes ( ) no ( x )

**Drug, lot #, and % purity:** R67555 batch V8510-171

**Methods** Nebivolol was given in a single oral dose to Wistar rats. Doses used were 0, 160, 320, 640, 1280 and 2560 mg/kg. Mortality and gross clinical signs were monitored for the next 14 days. Body weights were measured before dosing and at days 4,7,10 and 14.

**Results**

Mortality is summarized in the sponsor's table below.

Department of Pharmacology

EXPERIMENT: 8538  
 Acute toxicity study  
 R 67 555 - OR - RAT

M O R T A L I T Y
Cumulative incidence per dosage group
LD50

Dosage mg/kg	N	Males			N	Females		
		Day 1	Day 7	Day 14		Day 1	Day 7	Day 14
0	10	0	0	0	10	0	0	0
160	0				10	0	0	0
320	0				10	1	2	2
640	10	0	0	0	10	2	7	7
1280	10	0	2	2	10	7	10	10
2560	10	0	2	2	0			

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 On Original

A dose-related effect on body weight was seen in both sexes.

EXPERIMENT: 0538  
 Acute toxicity study  
 R 67555 - OR - RAT

WEIGHT GAIN  
 Mean values per dosage group in g

Day	Dosage group ( mg / kg )					
	0	160	320	640	1280	2560
Males						
4	42 N=10	-	-	21 ** N=10	-4 *** N= 8	-4 *** N= 8
7	61 N=10	-	-	47 N=10	19 *** N= 8	20 *** N= 8
10	75 N=10	-	-	77 N=10	51 * N= 8	45 ** N= 8
14	97 N=10	-	-	100 N=10	78 N= 8	77 N= 8
Females						
4	31 N=10	22 * N=10	15 ** N= 8	9 ** N= 3	-	-
7	37 N=10	33 N=10	22 ** N= 8	19 * N= 3	-	-
10	46 N=10	41 N=10	32 ** N= 8	33 * N= 3	-	-
14	51 N=10	46 N=10	42 * N= 8	44 N= 3	-	-

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

N: Number of animals

Appears This Way  
 On Original

Clinical signs are summarized in the sponsor's table below.

Observation	Dosage group ( mg / kg )				Females	
	0	160	320	640	1280	2560
AD : Dead	0	0	2	7	10	
CA : Catalepsy	0	2	6	5	10	
CC : Clonic convulsions	0	0	0	3	0	
DIA : Diarrhea	0	6	9	10	10	
EXO : Exophthalmos	0	3	0	0	0	
HT : Hypotonia	0	2	3	7	7	
HTH : Hypothermia	0	0	2	2	10	
PI : Piloerection	0	3	8	3	10	
PP : Palpebral ptosis	0	7	10	9	10	
SE : Sedation	0	2	7	9	10	
TR : Tremors	0	0	4	6	9	
Number of animals	10	10	10	10	10	0

Appears This Way  
On Original

Observation	Dosage group ( mg / kg )				N a l e s	
	0	160	320	640	1280	2560
AD : Dead	0			0	2	2
CA : Catalepsy	0			4	7	10
DIA : Diarrhea	0			9	10	9
HT : Hypotonia	0			7	8	5
HTB : Hypothermia	0			0	7	6
PI : Piloerection	0			1	3	0
PP : Palpebral ptosis	0			10	10	10
SE : Sedation	0			10	10	10
TR : Tremors	0			1	8	8
Number of animals	10	0	0	10	10	10

**Study title:** *The acute oral toxicity of both enantiomers of the  $\beta$ 1 receptor antagonist nebivolol-HCl in female rats in comparison with that of the racemate: A. The toxicity of the racemic mixture*

**Key study findings:** Pharmacological and toxicological effects were studied over a period of 14 days after oral administration of R67555 or its solvent. The doses of 0, 320, 640 and 1280 mg/kg were given to 5 females. The LD50 value (and 95% confidence limits) calculated 14 days after oral administration of the compound was

For racemate: 1194 (881-1618) mg/kg

R85547 >1280 mg/kg

R85548 ~1280 mg/kg

The clinical signs most commonly seen were diarrhea or soft feces, hypotonia and ptosis. Mortality, catalepsy, sedation and tremors were reported for the highest dose tested. Ptosis was seen in all animals at all doses.

**Study no.:** N92876

**Conducting laboratory and location:** Janssen Research, Beerse, Belgium

**Date of study initiation:** February 16, 1993

**GLP compliance:** statement included

**QA report:** yes ( x ) no ( )

**Drug, lot #, and % purity:**

R67555 batch ZR067555PFA141, ref number of preparation 93B10

**Methods** Twenty adult female Wistar rats, 5 per dose level received single oral doses of 0, 320, 640, 1280 mg/kg. The rats were monitored for clinical signs and mortality. Body weights were measured before dosing and at days 4, 7, 10 and 14.

**Results**

Death occurred in 3/5 females at the HD. All deaths were on or before day 6. A profound decrease in body weight was seen in the HD group from day 0-4. By day 7 the difference in weight had been more than compensated.

EXPERIMENT: 2968 / 9302 Acute toxicity study R 67555 - OR - RAT	B O D Y W E I G H T			
	Mean values per dosage group in g			
Day	Dosage group ( mg / kg )			f e m a l e s
	Vehicle	320	640	1280
0	204 N= 5	204 N= 5	203 N= 5	205 N= 5
4	230 N= 5	235 N= 5	239 N= 5	309 N= 3
7	240 N= 5	250 N= 5	240 N= 5	255 N= 2
10	245 N= 5	253 N= 5	250 N= 5	278 N= 2
14	250 N= 5	262 N= 5	258 N= 5	277 N= 2

Clinical signs were reported only for the drug treated groups. These are summarized in the reviewer's table below.

Reviewer's summary of signs

Sign	320 mg/kg	640 mg/kg	1280mg/kg
catalepsy			2/5
diarrhea		2/5	4/5
hypotonia	0	2/5	5/5
Ptosis	5/5	5/5	5/5
Soft feces	0	1/5	2/5

sedation	0	0	4/5
tremors	0	0	4/5

The sponsor distinguished between sedation and ptosis which is shown here.

**Study title:** *The acute oral toxicity of both enantiomers of the  $\beta$ 1-receptor antagonist nebivolol-HCl in female rats in comparison with that of the racemate:B. The toxicity of R85547*

Pharmacological and toxicological effects were studied over a period of 14 days after oral administration of R 85 547 or its solvent. The doses of 0 (control), 320, 640 and 1280 mg/kg were given to 5 females.

The LD<sub>50</sub>-value (and 95 % confidence limits), calculated 14 days after the oral administration of the compound, was:

- for R 85 547 : > 1280 mg/kg.
- in comparison with:
- for the racemate : 1194 (881 - 1618) mg/kg
- for R 85 548 : ~ 1280 (---) mg/kg.

**Key study findings:** The following phenomena occurred most frequently: hypotonia, palpebral ptosis and tremors.

**Study no.:** N92877/serial # R85547

**Conducting laboratory and location:** Janssen Research, Beerse, Belgium

**Date of study initiation:** February 16, 1993

**GLP compliance:** statement included

**QA report:** yes (x) no ( )

**Drug, lot #, and % purity:** R85547 batch ZR085547PFA011 reference number of preparation 93B11

### Methods

Female Wistar rats, 5/dose received single oral doses of R85547 at 0, 320, 640 and 1280 mg/kg. Mortality, clinical signs and weight were monitored as in the study above (Part A.).

### Results

The sponsor's summary of comparative effects between the compounds is shown below.

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On Original

**Table 4: Comparative data for the 3 compounds: Frequency of mortality**

Dose (mg/kg, po)	Frequency of mortality (n/n <sub>tested</sub> )		
	R 67 555	R 85 547	R 85 548
320	0/5	0/5	0/5
640	0/5	0/5	0/5
1280	3/5	0/5	2/5
LD <sub>50</sub> (mg/kg)	1194	> 1280	~ 1280
L.L. (mg/kg)	881	-	-
U.L. (mg/kg)	1618	-	-

The HD group gained weight more slowly than the other groups until day 10.

EXPERIMENT: 2969 / 9303  
 Acute toxicity study  
 R 85547 - OP - RAT

BODY WEIGHT  
 Mean values per dosage group in g

Day	Dosage group (mg / kg)			
	Vehicle	320	640	1280
0	201 N= 5	201 N= 5	201 N= 5	200 N= 5
4	229 N= 5	229 N= 5	231 N= 5	207 N= 5
7	241 N= 5	237 N= 5	246 N= 5	220 N= 5
10	246 N= 5	240 N= 5	253 N= 5	234 N= 5
14	252 N= 5	250 N= 5	259 N= 5	254 N= 5

N: Number of animals

Clinical observations were limited to hypotonia, ptosis and tremors.  
 The sponsor's summary is shown below.

Appears This Way  
 On Original

Observation	Dosage group ( mg / kg )			females
	Vehicle	320	640	1280
BT : Hypotonia	0	0	1	4
PP : Palpebral ptosis	0	5	5	5
TR : Tremors	0	0	1	2
Number of animals	5	5	5	5

**Study title:** *The acute oral toxicity of both enantiomers of the  $\beta$ 1-receptor antagonist nebivolol-HCl in female rats in comparison with that of the racemate: C. The toxicity of R85548.*

**Key study findings:**

The LD<sub>50</sub>-value (and 95 % confidence limits), calculated 14 days after the oral administration of the compound, was:

- for R 85 548 : ~ 1280 mg/kg,
- in comparison with:
- for the racemate : 1194 (881 - 1618) mg/kg
- for R 85 547 : > 1280 (---) mg/kg.

Apart from mortality, the following phenomena occurred most frequently: hypotonia, palpebral ptosis, sedation and tremors.

**Study no.:** N92915

**Conducting laboratory and location:** Janssen Research, Beerse, Belgium

**Date of study initiation:** February 16, 1993

**GLP compliance:** statement included

**QA report:** yes (x) no ( )

**Drug, lot #, and % purity:** R85548, batch ZR085548PFA011

**Methods:** Are the same as for parts A and B of this study ( the 2 studies above).

Results

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On Original

There was a dose-related decrease in weight gain at the day 4 measurement. The difference was no longer apparent at the day 7 measurement.

EXPERIMENT: 2970 / 9304

Acute toxicity study  
R 85548 - CR - RAT

-----  
| B O D Y W E I G H T |  
Mean values per dosage group in g

Day	Dosage group ( mg / kg )			Females
	Vehicle	320	640	1280
0	196 N= 5	197 N= 5	196 N= 5	198 N= 5
4	223 N= 5	221 N= 5	206 N= 5	189 N= 5
7	233 N= 5	230 N= 5	233 N= 5	232 N= 3
10	245 N= 5	248 N= 5	252 N= 5	251 N= 3
14	250 N= 5	252 N= 5	256 N= 5	252 N= 3

A

comparative summary of the clinical signs is shown below.

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On Original

**Table 6: Comparative data for the 3 compounds: Clinical observations (ED<sub>50</sub> values with 95% confidence limits)**

	ED <sub>50</sub> (mg/kg, po) with 95% confidence limits		
	R 67 555	R 85 547	R 85 548
Palpebral ptosis	< 320	< 320	< 320
Hypotonia	686 (506 - 929)	905 (604 - 1357)	520 (347 - 779)
Diarhea / soft defecation	686 (458 - 1029)	> 1280	> 1280
Tremors	1040 (767 - 1409)	~ 1280	520 (347 - 779)
Sedation	1040 (767 - 1409)	> 1280	788 (487 - 1274)
Mortality	1194 (881 - 1618)	> 1280	~ 1280

**Study title:** *The acute intravenous toxicity of R6755 in rats (supplement to preclinical research report R67 555/1)*

**Key study findings:** The LD<sub>50</sub> values (and 95% confidence limits) calculated 14 days after the intravenous administration of the drug were reported as

Males: 56.6 (43.3- 73.9)

Females: 37.4 (28.6-48.8)

Tachypnea, cyanotic tail, tremors and exophthalmos were seen in both sexes. Clonic convulsions were seen in both sexes but with greater incidence in the females.

**Study no.:** N62095

**Conducting laboratory and location:** Janssen Research Labs, Beerse, Belgium

**Date of study initiation:** December 5, 1985

**GLP compliance:**

**QA report:** yes ( ) no ( )

**Drug, lot #, and % purity:** R67555, batch V8510-171. Purity not listed. The solvent used was 15% hydroxypropyl- $\beta$ -cyclodextrin in distilled water.

**Methods**

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R67555 was given intravenously to 30 male and 40 female adult Wistar rats at dose levels of 0 (vehicle control given in the volume used for the highest dose), 40 and 80 mg/kg. Females only also received a dose of 20 mg/kg. Gross behavioral effects and mortality were observed immediately after dosing, periodically during day 1 and once daily until the end of the 14 day observation period. At the conclusion of the study, all surviving animals were euthanized and examined by gross necropsy.

**Results**

All deaths were reported as happening within 1 hour after injection.

Reviewer's summary of mortality

	Dose mg/kg			
	0(vehicle)	20	40	80
males	0		0	10
females	0	0	7	10

Cyanosis and necrosis ( 1 rat) of the tail were reported in survivors.

The males at 40 mg/kg gained approximately 63% of the weight gained by the control group.

EXPERIMENT: 8536  
Acute toxicity study  
R 67555 - IV - RAT

WEIGHT GAIN  
Mean values per dosage group in g

Day	Dosage group ( mg / kg )			
	Placebo	20	40	80
4	21 N=10	-	11 N=10	-
7	39 N=10	-	26 * N=10	-
10	66 N=10	-	43 * N=10	-
14	92 N=10	-	58 ** N=10	-

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On Original

Females in the LD group showed more of an effect than the MD survivors.

Day	Dosage group ( mg / kg )			Females
	Placebo	20	40	80
4	7 N=10	5 N=10	5 N= 3	-
7	15 N=10	7 N=10	15 N= 3	-
10	23 N=10	8 * N=10	17 N= 3	-
14	34 N=10	11 ** N=10	27 N= 3	-

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

N: Number of animals

No clinical signs were reported for the control group of either sex. Signs reported for the drug-treated animals are listed below.

Sign	Males (dose in mg/kg)		Females (dose in mg/kg)		
	40	80	20	40	80
death		10		7	10
Clonic convulsions	1			9	
Cyanosis of the tail	2		1	2	
exophthalmos	10		4	10	
Palpebral ptosis	9				
prostration	10				

tachypnea	10		3	10	
tremors	1	10		5	10
Hypotonia				8	
Loss of righting reflex				2	10

**Study title:** *The acute oral toxicity of R67555 in dogs (supplement to Preclinical Research Report R67555/1)*

**Key study findings:** No unscheduled mortality was seen. The LD50 was thus estimated to be >160 mg/kg. The clinical signs reported for both sexes were diarrhea (4/4 f, 4/4m) and licking (3/4f, 2/4m).

**Study no.:** N62178

**Conducting laboratory and location:** Janssen Research Laboratories, Beerse, Belgium

**Date of study initiation:** December 6, 1985

**GLP compliance:**

**QA report:** yes ( ) no ( )

**Drug, lot #, and % purity:** R67555, batch V8510-171, an aqueous solution with polysorbate 80.

### Methods

Four adult male and 4 adult female mixed-breed dogs were used. R67555 was given by oral gavage at a dose of 160 mg/kg. Observations for gross behavioral effects were made immediately after dosing, periodically during day 1 and at least once daily until the end of the 14 day observation period. Body weights were measured before dosing and at days 4, 7, 10 and 14. After euthanasia, gross necropsy was performed.

### Results

No unscheduled mortality was seen. No vehicle or untreated control group was available for comparison. Therefore, it can't be determined if there were body weight effects from the single administration of drug. Clinical signs are summarized below.

Observation	Dosage group ( mg / kg )	Males
	160	
DE : Defecation	3	
DIA : Diarrhea	4	
LI : Licking	2	
VO : Vomiting	1	
Number of animals	4	

Observation	Dosage group ( mg / kg )	Females
	160	
DE : Defecation	1	
DIA : Diarrhea	4	
LI : Licking	3	

**Study title:** *The acute intravenous toxicity of R67555 in dogs (supplement to preclinical research report R67555/1)*

**Key study findings:** No clinical signs and no unscheduled mortality were reported. The LD50 was estimated to be >10 mg/kg.

**Study no.:** N62267

**Conducting laboratory and location:** Janssen Research, Beerse, Belgium

**Date of study initiation:** December 6, 1985

**GLP compliance:**

**QA report:** yes ( ) no ( )

**Drug, lot #, and % purity:** R67555, batch V5810-171 in a vehicle of — hydroxypropyl- $\beta$ -cyclodextrin.

### Methods

R67555 was given intravenously to 4 male and 4 female adult mixed breed dogs at a dose of 10 mg/kg. The vehicle was given to 1 adult female dog. The dogs were observed for gross behavioral signs immediately after dosing, periodically during the day and at least once a day until the end of the 14 day observation period. Body weights were measured before dosing and at days 4, 7, 10 and 14. At the scheduled euthanasia, gross necropsy observations were made.

### Results

Unscheduled mortality was not seen in the study. There do not appear to be body weight effects, however, the 1 female who received the vehicle started the study at a body weight of 8 kg while the average weight of the females receiving drug was 4.6 kg and the males was 6.0 kg. No behavioral effects or signs were reported.

**Study title:** *The acute intravenous toxicity of R67555 in Beagle dogs*

**Key study findings:** Mortality was seen in  $\frac{1}{4}$  males and  $\frac{1}{4}$  females. Clinical signs included ataxia, defecation, diarrhea, dyspnea, salivation, tremors and vomiting.

**Study no.:** N62268

**Conducting laboratory and location:** Janssen Research, Beerse, Belgium

**Date of study initiation:**

**GLP compliance:** statement included

**QA report:** yes (x) no ( )

**Drug, lot #, and % purity:** R67555, batch PFA-031 in a vehicle of — hydroxypropyl- $\beta$ -cyclodextrin.

### Methods

R67555 was given intravenously to 4 male and 4 female adult Beagles at a dose of 40 mg/kg. The dose was based on the highest soluble concentration of drug (10 mg/ml) and the maximum injection volume for intravenous injection in dogs (4 ml/kg). Observations for lethality and behavioral effects were made immediately after dosing, periodically during day 1 and at least once daily until the end of the 14 day observation period. Body weight was measured before dosing and at days 4, 7, 10 and 14. At scheduled euthanasia, gross necropsy examinations were performed.

### Results

One male and 1 female died within an hour of injection. The signs exhibited were ataxia, clonic convulsions, diarrhea and defecation, dyspnea, loss of righting reflex, salivation, tremors and vomiting.

Observation	Dosage group ( mg / kg )	Males
	40	
+D : Dead	1	
AT : Ataxia	4	
CC : Clonic convulsions	1	
DE : Defecation	3	
DIA : Diarrhea	1	
DY : Dyspnea	2	
LR : Loss of righting reflex	2	
SA : Salivation	3	
SD : Soft defecation	2	
TR : Tremors	2	
VD : Vomiting	3	

Observation	Dosage group ( mg / kg )	Females
	40	
+D : Dead	1	
AT : Ataxia	4	
DE : Defecation	2	
DIA : Diarrhea	1	
DY : Dyspnea	2	
LR : Loss of righting reflex	2	
SA : Salivation	3	
TR : Tremors	3	
VD : Vomiting	1	

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On Original

### 3.4.3 Repeat-dose toxicity

**Study title:** *Subchronic oral feeding study in Swiss mice (repeated dosage for 3 months)*

**Key study findings:** A microcytic anemia was seen in the HD of both sexes with insufficient information to discern whether or not it was regenerative. HCT was decreased 6% (MDm), 19%(HDm,  $p<0.001$ ), 2%(MDf) and 21%(HDf,  $p<0.001$ ). Dose-related increases were seen in both sexes in serum  $\text{Na}^+$  (155 mEq/l HDm+f vs 148 and 149 mEq/l for males and female controls respectively) and non-dose-related  $\text{K}^+$  increases. Total protein and albumin showed very slight decreases in the HD of both sexes. Serum glucose, cholesterol, triglycerides and phospholipids were significantly decreased in both sexes. Non-significant increases in AST and ALT were seen at the HD. Lung, spleen, adrenal and liver weight were increased while pancreas, kidney and "gonad" weight were decreased. Leydig cell hyperplasia and "large nucleated tubular cells" in the testes of HD males were reported to have a higher histopathology score than controls. The sponsor also stated that

The presence of large-nucleated tubular cells was more prominent in the 160 mg/kg dosed group than in the control group. These cells are indicative for atrophic changes due to delayed maturation. In one

A decrease in corpora lutea was noted in the HD ovaries. The textual discussion of the histopathology reported adrenal cortical hypertrophic changes in the HD groups of both sexes. Foamy macrophages in the alveolar lumina associated with a reactive inflammatory thickening of the alveolar septae was reported for the male and female HD groups. The sponsor stated that the drug interferes with steroid metabolism causing a hormonal imbalance.

**Study no.:** Exp # 1966, N106653/1

**Conducting laboratory and location:** Janssen Research, Beerse, Belgium

**Date of study initiation:** November 4, 1988

**GLP compliance:**

**QA report:** yes ( ) no ( )

**Drug, lot #, and % purity:** R67555, batch ZR067555PFA081

### Methods

The mice received the drug at dosages of 0, 10, 40 and 160 mg/kg via dietary administration. Food consumption, hematology, clinical chemistry, selected organ weights and some histopathology were included in the analysis of the study. Histopathology was assessed for adrenals, liver and gall bladder, lungs, mesenteric lymph nodes, ovaries, spleen and testes of the HD and control group.

**Results**

Ptoisis was reported for 7/10 HD males and 5/10 HD females.

Food consumption : From 5 weeks onward, food consumption in the MD and HD males and the MD females was influenced by waste of food. Exact consumption was probably lower than that reported due to the wastage.

Test article intake appeared to be within ±10% of target values.

There was no difference in mean weight between the control, LD and MD groups. The mean weight for the HD groups of both sexes was significantly (p<0.001) lower than the control group from week 1 through the end of the study. The difference was 19 % for females at week 1 and 26% at week 14. For HD males, the difference was 27% at week 1 and 24% at week 14.

Hematology showed a dose-related decrease in HCT, Hb and RBC in both sexes. MCV, MCH and MCHC were correspondingly decreased.

Toxicity study  
R 67555 - FOOD - NICE - 3 MONTHS

Terminal, recorded in week 15

Parameter		Dosage group ( mg / kg )							
		Control	Males			Females			
			10	40	160	Control	10	40	160
HCT: Haematocrit	%	37.4	37.9	35.2 *	30.2 ***	40.4	40.6	39.4	32.0 ***
HGB: Haemoglobin	g/dl	13.2	13.7	12.7	11.3 **	14.0	14.1	13.7	11.8 ***
RBC: R.B.C.	10E6/mm <sup>3</sup>	8.34	8.20	7.92 *	7.47 *	8.41	8.49	8.34	7.97
WBC: W.B.C.	1000/mm <sup>3</sup>	7.0	7.9	6.5	5.8	4.2	6.1	6.1	6.7 *
THR: Thrombocytes	1000/mm <sup>3</sup>	843	848	847	962	777	778	844	805
NRC: Normoblasts/100 W.B.C.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MCV: Mean cell volume	fl	45	46	44	41 ***	48	48	47	40 ***
MCH: Mean cell haemogl.	pg	15.8	16.7	16.0	15.2 **	16.6	16.6	16.5	14.8 ***
MHC: Mean cell h. conc.g/dl		35.3	36.1	36.0	37.6 ***	36.6	36.7	34.8	36.7 ***
DIFFERENTIAL COUNT %									
NEB: Band neutrophils		0.3	0.4	0.1	0.3	0.1	0.1	0.0	0.3
NES: Segmented neutrophils		7.5	8.4	5.4	13.1 *	5.6	6.3	8.6	8.6
EOS: Eosinophils		0.3	1.1	0.7	0.7	0.9	1.0	0.3	0.4
LYP: Lymphocytes		91.7	89.6	93.7	85.4 *	92.9	92.3	90.6	90.4
NOC: Monocytes		0.2	0.4	0.1	0.4	0.5	0.2	0.5	0.3

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

Clinical chemistry showed dose-related increases in serum sodium and potassium in both sexes. Total protein and albumin showed very slight decreases while haptoglobin showed an increase. Glucose, cholesterol, triglycerides and phospholipids showed marked decreases while blood urea nitrogen, AST and ALT were increased.

Toxicity study  
R 67555 - FOOD - MICE - 3 MONTHS

Terminal, recorded in week 15

Parameter		Dosage group ( mg / kg )							
		Control	Males				Females		
			10	40	160	Control	10	40	160
SOD: Sodium	mEq/l	149	149	151	155 *	148	149	151	155 *
POT: Potassium	mEq/l	6.2	6.2	6.9	7.1	5.2	6.5 *	6.1	6.7 *
CHL: Chloride	mEq/l	111	111	111	114	113	114	114	113
CAL: Calcium	mg%	9.0	9.0	8.8	8.6	8.9	9.1	9.1	8.6
IMP: Inorg. phosphate	mg%	7.4	6.8	8.0	7.5	6.8	7.6	7.3	8.1
TOP: Total protein	g%	4.9	4.8	4.8	4.4	5.1	4.9	4.8	4.5
ALB: Albumin	g%	3.0	2.9	2.9	2.9	3.3	3.1	3.0	2.9 *
HAP: Haptoglobin	mg%	2	1	1	4	5	4	0	122
GLU: Glucose	mg%	197	188	178	120 **	191	199	168 *	141 **
CHD: Cholesterol	mg%	129	119	109	22 **	90	106	79	16 **
TGL: Triglycerides	mg%	167	149	131	57 **	105	159 *	197 **	66 *
PLP: Phospholipids	mg%	274	256	257	87 **	185	217	191	66 **
BUN: Blood urea nitrog.	mg%	26.7	26.3	28.0	35.7 **	23.1	25.3	28.4	33.1 *
CRS: Creatinine	mg%	0.39	0.38	0.35	0.30 *	0.39	0.38	0.40	0.35 *
BIL: Total Bilirubin	mg%	0.23	0.22	0.18 *	0.25	0.17	0.13	0.10 **	0.15
ALP: Alkal. phosphatase	U/l	56	58	50	107 **	62	67	82	121
AST: Aspartate aminotr.	U/l	112	82	76	174	219	175	108	224
ALT: Alanine aminotran.	U/l	36	42	42	129 **	50	44	42	199
CHE: Cholinesterase	KU/l	6.2	5.6	4.6 *	7.2	8.3	8.3	8.6	7.5

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

Organ weights: Lung weight was increased in both sexes as was spleen weight. Normalized liver weight was increased as was brain weight. Pancreas and kidney weight were both decreased. Adrenal weight was increased. "Gonad" weight was decreased at the HD in both sexes.

Toxicity study  
R 67555 - FOOD - NICE - 3 MONTHS

| Terminal, recorded in week 15 |

Parameter		Dosage group ( mg / kg )							
		Control	Males			Females			
			10	40	160	Control	10	40	160
WGT: Body weight	g	40	41	41	29 ***	35	34	35	27 ***
LNG: Lungs	mg	320	319	315	426 ***	343	319	337	451 ***
	mg / 100 g	797	790	780	1495 ***	993	943	953	1654 ***
SPL: Spleen	mg	98	103	114 **	137 *	110	112	151 ***	184 **
	mg / 100 g	244	254	283 *	468 ***	318	333	426 ***	665 ***
LIV: Liver	mg	2049	2047	2019	1680 ***	1733	1672	1953 **	1791
	mg / 100 g	5088	5031	4981	5800 **	4995	4923	5519 **	6536 ***
HRT: Heart	mg	192	208	213 *	158 **	163	159	164	136 ***
	mg / 100 g	477	513	528	548 *	471	467	465	495
PNC: Pancreas	mg	400	399	408	307 **	387	347	377	304 ***
	mg / 100 g	989	984	1005	1054	1116	1021	1067	1113
KDN: Kidneys	mg	664	702	698	505 ***	438	422	476 *	383 *
	mg / 100 g	1653	1734	1720	1752	1264	1245	1346	1396 *
BRN: Brain	mg	500	490	496	442 ***	505	505	511	454 ***
	mg / 100 g	1245	1212	1225	1551 ***	1460	1495	1447	1662 **
THY: Thymus	mg	30	32	32	22 **	39	41	40	29 **
	mg / 100 g	73	79	79	77	114	121	111	106
ADR: Adrenals	mg	5	5	5	10 ***	11	11	10	14 **
	mg / 100 g	13	12	12	36 ***	32	31	28	52 ***
GON: Gonads	mg	278	274	285	261	47	50	49	32 **
	mg / 100 g	693	682	704	905 ***	135	148	140	115

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

Histopathology

The sponsor uses a scale rather than incidence and severity to report histopathological findings. This potentially has a smoothing effect on histological findings. However, in both sexes there were findings of significance in the adrenals, liver, lung, lymph nodes and gonads.

Appears This Way  
On Original

Males				
Organ or tissue - observation	Dosage group ( mg / kg )			
	Control	10	40	160
Adrenals				
- cortical hypertrophy	0.00	-	-	1.10 ***
- prominent red blood cells	0.00	-	-	0.10
- spindle-cell hyperplasia	0.20	-	-	0.00
Liver				
- RES-aggregates	0.30	-	-	0.10
- centrilobular swelling	0.00	-	-	0.10
Lung				
- RES-aggregates	0.10	-	-	0.00
- focally alveolar macrophages	0.00	-	-	2.40 ***
- thickened septae (reactive)	0.00	-	-	3.40 ***
Spleen				
- atrophy	0.00	-	-	1.00 ***
- prominent red blood cells	0.00	-	-	0.70 **
Testes				
- focal Leydig-cell hyperplasia	0.00	-	-	0.10
- large-nucleated tubular cells	0.40	-	-	1.50 **

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

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On Original

*****				
===== Females =====				
*****				
Organ or tissue - observation	Dosage group ( mg / kg )			
	Control	10	40	160
-----				
Adrenals				
- X-zone	0.80	-	-	0.00 *
- cortical hypertrophy	0.00	-	-	1.60 ***
- spindle-cell hyperplasia	0.30	-	-	0.00
Liver				
- RES-aggregates	0.10	-	-	0.10
- centrilobular swelling	0.00	-	-	0.10
- focal necrosis	0.10	-	-	0.40
- inflammation	0.00	-	-	0.30
- prominent myelopoiesis	0.00	-	-	0.20
Lung				
- blood in alveoles	0.20	-	-	0.00
- focal hyperplasia	0.10	-	-	0.00
- focally alveolar macrophages	0.00	-	-	2.10 ***
- thickened septae (reactive)	0.00	-	-	3.00 ***
Lymph node(s), mesenteric				
- atrophy	0.00	-	-	0.10
- histiocytosis	0.00	-	-	0.40
Ovaries				
- corpora lutea	2.20	-	-	0.70 **
- cystic	0.40	-	-	0.00 *
- pigmentation (interstitium)	0.00	-	-	0.10
- tertiary follicles	1.80	-	-	2.10
Spleen				
- atrophy	0.00	-	-	1.00 ***
- atrophy (white pulp)	0.00	-	-	0.10
- prominent hematopoiesis	0.00	-	-	0.10
- prominent myelopoiesis	0.00	-	-	0.10
- prominent red blood cells	0.00	-	-	0.40 *

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

Summary

Changes found were indicative of loss of liver function and also endocrine disruption. The foamy macrophages in the pulmonary alveoli were associated with reactive inflammatory thickening. The sponsor gives little detail about the gonadal histopathology but notes that there was a decrease of corpora lutea. A dose-response can't be assessed given the method of reporting (a histo score rather than an incidence table) and lack of assessment of the LD and MD groups. The testicular changes were reported as atrophy due to delayed maturation. In one animal, Leydig cell hyperplasia was noted. The sponsor's summary is interesting and is shown below.

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On Original

**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 alfa-adrenolytic activity of the test article.

**Study title: *Pilot subchronic toxicity study in Wistar rats***

**Key study findings:** One control rat died in this pilot study. Sedation manifested as ptosis was seen at the HD of 80 mg/kg/day in both males (2/5) and females (3/5). High dose males gained on average 25% less than the vehicle control and HDf gained on average 8% less than vehicle. Significant dose related decreases in HCT, Hb and RBC count were seen in both sexes, consistent with other studies. Significant increases in serum potassium were seen in drug-treated animals of both sexes. Other clinical chemistry findings seen in both sexes were decreased cholesterol, triglycerides, phospholipids, total protein and albumin. Lung and pancreatic weight were increased in both males and females. Absolute and normalized adrenal weight was increased in both sexes. Normalized kidney weight was increased in males while absolute and normalized kidney weight was decreased in females. No histopathology was provided.

**Study no.:** N76736

**Conducting laboratory and location:** Janssen, Beerse, Belgium

**Date of study initiation:** March 8, 1989

**GLP compliance:** no

**QA report:** yes ( ) no ( )

**Drug, lot #, and % purity:** R67555 combined with  $\beta$ -cyclodextrin powder  
Drug was administered orally, mixed with the food.

**Methods**

Five Wistar rats per sex per group were assigned to untreated control, vehicle or 20, 40 and 80 mg/kg/day dosage groups. Animals were observed daily for mortality. Weekly observations were made for signs, food consumption and body weight. At time of euthanasia, blood was collected for hematology and clinical chemistry. Urinalysis was also performed. The methodology for the urinalysis was not described. Necropsy was conducted with observations of gross lesions and organ weights for selected organs. Histopathology was not conducted.

**Results**

One control male died prior to scheduled euthanasia. No clinical signs were reported for the control, vehicle, LD and MD groups. Ptosis was reported for 2/5 HDm and 3/5 HDF.

Average weight gain per group was decreased with drug administration.

EXPERIMENT: 2183  
 Toxicity study  
 R 67555 - FOOD - RAT - 1 MONTH

BODY WEIGHT  
 Mean values per dosage group in g

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

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

The food consumption pre-test was not shown. From the first reported time point of 1 week, the HD group had lower food consumption than the other groups. Test article intake was reported to be within ±10% of the target levels.

The MD and HD groups of both sexes showed decreases in the HCT, Hb and RBC count.

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 On Original

Parameter		Dosage group ( mg / kg )				
		Control	Placebo	Males		
				20	40	80
HCT: Haematocrit	%	46.1	46.7	46.5	44.0	42.0 * <sup>aa</sup>
HGB: Haemoglobin	g/dl	15.3	15.6	15.6	14.7	14.2 * <sup>aa</sup>
RBC: R.B.C.	10E6/mm <sup>3</sup>	8.24	8.15	8.27	7.60	7.63 * <sup>a</sup>

Parameter		Dosage group ( mg / kg )				
		Control	Placebo	Females		
				20	40	80
HCT: Haematocrit	%	44.6	45.8	44.4	41.3	37.8 ** <sup>aa</sup>
HGB: Haemoglobin	g/dl	14.8	15.4	15.0	14.2	13.1 ** <sup>aa</sup>
RBC: R.B.C.	10E6/mm <sup>3</sup>	7.97	8.16	8.02	7.51	7.09 * <sup>aa</sup>

Serum potassium levels were increased in drug treated animals of both sexes. Total protein, albumin, cholesterol, triglycerides and phospholipids were decreased in both sexes also.

Appears This Way  
On Original

Parameter		Dosage group ( mg / kg )				
		Control	Placebo	Males		
				20	40	80
SOD: Sodium	mEq/l	142	142	141	141	143
POT: Potassium	mEq/l	5.1	4.9	5.5	5.5	5.7
CHL: Chloride	mEq/l	103	103	104	104	105
CAL: Calcium	mg%	10.5	10.4	10.1	10.0	9.7
INP: Inorg. phosphate	mg%	8.6	8.3	8.2	8.6	8.3
TOP: Total protein	g%	5.5	5.6	5.5	5.2	5.0
ALB: Albumin	g%	3.4	3.5	3.5	3.3	3.1
HAP: Haptoglobin	mg%	28	21	29	30	42
GLU: Glucose	mg%	169	186 *	176	171	170
CHO: Cholesterol	mg%	68	62	57	56 *	37 *
TGL: Triglycerides	mg%	132	126	102	121	96
PLP: Phospholipids	mg%	139	133	121	130	99 *
BUN: Blood urea nitrog.	mg%	15.9	15.6	18.1	16.8	21.7 *
CRS: Creatinine	mg%	0.50	0.49	0.48	0.46	0.42 *
BIL: Total Bilirubin	mg%	0.11	0.06	0.10	0.06	0.08
ALP: Alkal. phosphatase	U/l	244	312	270	240	248
AST: Aspartate aminotr.	U/l	139	131	394	114	118
ALT: Alanine aminotran.	U/l	60	65	337	59	73
CHE: Cholinesterase	ku/l	0.3	0.2	0.2	0.3	0.3

Significance computed by Mann-Whitney U test (two tailed)

Control versus all groups : \* P < .05 \*\* P < .01 \*\*\* P < .001  
 Placebo versus other groups : @ P < .05 @@ P < .01 @@@ P < .001

Urinalysis for the males showed occult blood in the MD and HD groups. Frank RBCs were reported for the vehicle and the HD groups.

Appears This Way  
On Original

Parameter		Dosage group ( mg / kg )				
		Control	Placebo	Females		
				20	40	80
SOD: Sodium	mEq/l	144	143	145	145	144
POT: Potassium	mEq/l	4.3	4.3	4.4	4.9	5.4 ** @
CHL: Chloride	mEq/l	105	104	105	104	105
CAL: Calcium	mg%	10.8	10.9	10.8	10.8	10.3
INP: Inorg. phosphate	mg%	7.6	8.0	8.5	9.0	9.1
TOP: Total protein	g%	5.9	6.0	5.7	5.5 @	5.5 @
ALB: Albumin	g%	3.8	3.9	3.6	3.5 @	3.6 @
HAP: Haptoglobin	mg%	55	45	31	24	45
GLU: Glucose	mg%	159	163	180 *	155	153
CHO: Cholesterol	mg%	79	76	68	45 * @	24 ** @
TGL: Triglycerides	mg%	59	60	82	66	76
PLP: Phospholipids	mg%	146	146	142	112	77 ** @
BUN: Blood urea nitrog.	mg%	16.1	16.6	19.5	19.7	20.7
CRS: Creatinine	mg%	0.50	0.52	0.47	0.44 @	0.42 * @
BIL: Total Bilirubin	mg%	0.09	0.08	0.09	0.07	0.07
ALP: Alkal. phosphatase	U/l	243	235	214	172	213
AST: Aspartate aminotr.	U/l	126	149	96 * @	105	93 @
ALT: Alanine aminotran.	U/l	45	55	46	55 *	52
CHE: Cholinesterase	ku/l	1.1	1.6	1.2	1.1	1.0 @

Significance computed by Mann-Whitney U test (two tailed)

Control versus all groups : \* P < .05 \*\* P < .01 \*\*\* P < .001  
 Placebo versus other groups : @ P < .05 @ @ P < .01 @ @ @ P < .001

Urinary creatinine and urinary specific gravity were decreased in the drug-treated females. Urinary volume was increased in the vehicle and drug-treated females.

Appears This Way  
 On Original

Parameter		Dosage group ( mg / kg )				
		Control	Placebo	Females		
				20	40	80
CRU: Creatinine	mg%	98	95	86	56 *	56 **
SGR: Specific gravity		1.056	1.056	1.053	1.040	1.035 **
UBG: Urobilinogen Ehrlich U		0.10	0.18	0.10	0.18	0.18
ACI: pH		6.10	5.90	6.10	6.10	6.40 a
VOL: Volume	ml / 16 h	1.8	2.4	2.6	5.6 *	3.9 *

Organ weights

Males: Normalized lung weight was increased in HD males. Normalized pancreatic, brain, adrenal, gonadal and kidney weight were increased in vehicle and drug-treated animals.

Females: Lung weight showed a non-dose-related increase. Absolute and normalized pancreas weight were increased. Absolute and normalized kidney weight were decreased. Absolute and normalized adrenal weights showed significant increases.

Parameter		Dosage group ( mg / kg )				
		Control	Placebo	Females		
				20	40	80
WGT: Body weight	g	191	196	205	194	190
LNG: Lungs	mg	1351	1434	1453	1473	1432
	mg/kg	7087	7307	7122	7620	7530
SPL: Spleen	mg	557	503	651 a	671 a	650
	mg/kg	2917	2554	3185 aa	3463 aa	3426 a
LIV: Liver	mg	7508	7855	8773	8384	8229
	mg/kg	39315	39898	42891	43256	43421
HRT: Heart	mg	737	717	742	697	721
	mg/kg	3876	3665	3643	3596	3790
PNC: Pancreas	mg	927	957	1080 *	1203 *	1171 *
	mg/kg	4907	4818	5292	6198 *	6178 a
KDN: Kidneys	mg	1851	1647	1719	1761	1740
	mg/kg	9738	8364	8393	9078	9164 a
BRN: Brain	mg	1750	1807	1705	1786	1781
	mg/kg	9230	9235	8369	9221	9402
THY: Thymus	mg	354	312	353	314	326
	mg/kg	1853	1594	1723	1625	1716
ADR: Adrenals	mg	86	89	90	119 **	168 ** aa
	mg/kg	453	451	444	614 * a	886 ** aa
GON: Gonads	mg/kg	76	84	91	73	88
	mg	2575	2968	3097	3065	2993
	mg/kg	8049	9331	10212	9873	10814

Significance computed by Mann-Whitney U test (two tailed)

Control versus all groups : \* P < .05 \*\* P < .01 \*\*\* P < .001  
 Placebo versus other groups : a P < .05 aa P < .01 aaa P < .001

**Study title:** *d-nebivolol (R85547) in comparison with dl-nebivolol (R67555): Experiment No. 2336 One-month toxicity study in SPF Wistar rats*

**Key study findings:** Both the d-isomer and the racemic mixture produced similar effects. Body weight gain was decreased only in the animals receiving R67555, the racemic mixture. HDm and HDf gained on average 18% and 26% less weight than the vehicle groups. HCT, Hb, RBC were decreased in males treated with R85547 and HDR67555. Decreased HCT was seen in females at both doses of R67555. Hb and RBC were decreased in HDf treated with R85547. All drug treated males showed increases in serum sodium and inorganic phosphate and decreases in total protein and albumin. The R67555 HDm also showed decreased cholesterol and phospholipids. Serum potassium and inorganic phosphorous were increased in drug-treated females. Total protein and albumin were decreased in the R67555 HD group. Cholesterol and phospholipids were decreased in all drug-treated groups. In both sexes, spleen, liver, lungs, pancreas and adrenal weights were increased. Kidney weight in females was decreased. "Gonad" weight in males was increased. Histologic changes were reported as foam cells in the lungs (80 mg/kg R67555) of both sexes and focal septal thickening in females. Increased rbc were seen in the mesenteric lymph nodes(80 mg/kg R67555) of both sexes. Increased rbc in the spleen was also reported for both drugs, all doses, both sexes except for the males, 10 mg/kg R85547. Adrenal changes included swollen cortical cells in both sexes and ectasia of sinusoidal spaces and small zona glomerulosa in females.

Overall, the effects noted in this study were somewhat more pronounced with the racemic mixture than the d-enantiomer.

**Study no.:** N92595

**Conducting laboratory and location:** Janssen Research, Beerse, Belgium

**Date of study initiation:** May 16, 1990

**GLP compliance:** statement included

**QA report:** yes (x) no ( )

**Drug, lot #, and % purity:** d-nebivolol (R85547) batch ZR085547PFA011  
dl-nebivolol (R67555) batch ZR067555PFA091

**Methods**

Five groups of 10 males and 10 females were assigned to each of the following groups:

Control :  $\beta$ -cyclodextrin (880 mg/kg/body weight)

R88547 10 mg/kg/day

R85547 40 mg/kg/day

R67555 20 mg/kg/day

R67555 80 mg/kg/day

Drug was given orally via the diet for 1 month. The drug was given as a co-precipitate with  $\beta$ -cyclodextrin in a 1/10 ratio (11 gram coprecipitate = 1 gram nebivolol). The sponsor's explanation for the dose selection is shown below.

#### JUSTIFICATION FOR SELECTION OF DOSES

The doses used in this study were chosen based upon the available information on the toxicity of nebivolol (R 67555) in rats. In two studies in which a similar  $\beta$ -cyclodextrin formulation has been mixed with the food, doses were 20, 40 and 80 mg/kg body weight/day in a one-month (pilot) oral toxicity study (Exp.No.2183) and 5, 20 and 80 mg/kg body weight/day in a twelve-month oral toxicity study (Exp.No.1964). The dose of 5 mg/kg body weight/day was considered as non-toxic. In both studies, 20 mg/kg body weight/day was found to be slightly toxic. Eighty mg/kg body weight/day was found to be moderately toxic after one month of dosing with the adrenals and spleen (in females) as target organs. Pronounced toxicity was seen at this dose after 12 months of dosing with the spleen, adrenals, lungs, lymph nodes and male and female genital tract as target organs. It was therefore decided to select these doses (20 and 80 mg/kg body weight/day) in the present 1 month comparative study. Since R 67555 is the racemic (50% - 50%) mixture of d-nebivolol (R 85547) and l-nebivolol (R 85548), the doses chosen for d-nebivolol in this study were also 50% of the doses chosen for dl-nebivolol.

Stability data for the test articles in the diet was provided for 6 and 11 weeks. Concentrations of 79% or greater of the nominal concentration were reported for R85547. Concentrations of  $\geq 86\%$  of the nominal concentration were found for R67555.

Animals were observed once a day for signs and mortality. Body weight was determined weekly. Ophthalmic exams were performed at the end of the study for all control animals, HD animals and the R67555 LD females. Hematology was conducted several days before the end of the study. Clinical chemistry samples were collected at euthanasia. Terminal urinalysis determinations were made on freshly collected individual samples of all animals a few days before euthanasia. Sixteen hour collections were made using metabolism cages. Weights were recorded for a limited number of organs. A standard list of tissues was collected for histopathology. Only control and HD samples were examined.

#### Results

No unscheduled mortality was reported. The only clinical sign reported was ptosis in the HD females (10/10). Food consumption was significantly decreased in the HD groups of both sexes. Differences in weight gain between controls and the HD m and the MD f and HDf were apparent from the first week.

Mean weight gain (g)

Week	Males mg/kg		Females mg/kg		
	vehicle	R67555: 80	vehicles	R67555: 20	R67555:80
1	60	44***	35	30	23***
2	122	100***	60	56	48**
3	178	147***	89	77*	60***
4	205	167***	101	89	75***

Significance by Mann-Whitney U test \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

Hematology: HCT, Hb and RBC count were decreased in males treated with R85547 and the 80 mg/kg group of R67555. In females, a decrease in HCT was seen in both doses of R67555. Hb and RBC were decreased in females receiving 80 mg/kg R67555.

Clinical Chemistry: Serum sodium and inorganic phosphate were increased in all drug treated groups of males. Total protein and albumin were decreased in all treated groups of males. Serum cholesterol and phospholipids were significantly decreased in the R67555 HD group.

In the females, serum potassium was increased in all drug-treated groups, significantly so in the HD R67555. Inorganic phosphorous was non-significantly increased. Total protein and albumin were significantly decreased in the R67555 HD group. Cholesterol and phospholipids were decreased in all drug-treated groups, significantly so in the R67555 groups.

There were no significant effects in the urinalysis.

In the male organ weight data, lungs, spleen, heart, pancreas, adrenals and gonads were affected as shown in the sponsor's summary below.

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On Original

Parameter		Dosage group ( mg / kg )				
		Vehicle	Males			
			R 85547 10	R 85547 40	R 67555 20	R 67555 80
WGT: Body weight	g	365	362	359	363	327 *
LNG: Lungs	mg	1914	1888	1870	1954	1963
	mg/kg	5257	5229	5219	5397	5996 **
SPL: Spleen	mg	800	818	882	888	894
	mg/kg	2196	2269	2461	2451	2727 **
LIV: Liver	mg	16184	16034	15573	15892	15600
	mg/kg	44406	44155	43473	43868	47584 *
HRT: Heart	mg	1171	1212	1208	1233	1169
	mg/kg	3208	3351 *	3369 **	3400 *	3513 **
PNC: Pancreas	mg	1308	1255	1176	1216	1347
	mg/kg	3598	3468	3273	3360	4099 *
KDN: Kidneys	mg	2894	2973	2808	2863	2717
	mg/kg	7943	8228	7833	7949	8310
BRN: Brain	mg	2078	2031	2045	2042	1999 *
	mg/kg	5728	5633	5724	5641	6124
THY: Thymus	mg	600	686	640	728 *	618
	mg/kg	1639	1902	1786	2002 **	1885
ADR: Adrenals	mg	56	58	58	60	98 ***
	mg/kg	154	160	162	166	300 ***
TYR: Thyroids	mg	25	22	25	24	22
	mg/kg	68	59	69	66	66
GON: Gonads	mg	3089	3005	3191	3175	3040
	mg/kg	8496	8332	8925	8749	9304 **

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

Female organ weight data showed effects in the lungs, spleen, liver, heart, pancreas, kidneys, brain and adrenals. Gonad weight was increased non-significantly.

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On Original

Parameter		Dosage group ( mg / kg )				
		Vehicle	Females			
			R 85547 10	R 85547 40	R 67555 20	R 67555 80
WGT: Body weight	g	249	233	241	233	222 **
LNG: Lungs	mg	1528	1497	1509	1518	1583
	mg/kg	6166	6428	6263	6504	7138 **
SPL: Spleen	mg	622	628	647	592	644
	mg/kg	2507	2693	2687	2546	2909 *
LIV: Liver	mg	10028	9496	9888	9685	10328
	mg/kg	40261	40709	40966	41526	46586 ***
HRT: Heart	mg	855	869	906	893	849
	mg/kg	3449	3730 *	3780	3839 **	3828 **
PNC: Pancreas	mg	1075	970	1002	974	1252 *
	mg/kg	4309	4166	4159	4200	5657 ***
KDN: Kidneys	mg	1908	1824	1897	1929	1955
	mg/kg	7710	7828	7916	8285 *	8819 **
BRN: Brain	mg	1949	1880 *	1867	1893	1918
	mg/kg	7898	8091	7904	8148	8669 *
THY: Thymus	mg	614	646	682	595	597
	mg/kg	2481	2762	2485	2540	2678
ADR: Adrenals	mg	75	71	77	77	164 ***
	mg/kg	304	305	323	329	737 ***

Histopathologic changes reported were foam cells in the lungs and increased rbc in the lymph nodes. Increased rbc in the spleen was also reported for both drugs, all doses, males and females. Adrenal changes included swollen cortical cells and ectasia of the sinusoidal spaces.

The overall level of reporting is suboptimal in that there is only a superficial discussion of the histopathology.

The ophthalmologist's report could not be located.

**Study title: *l*-nebivolol (R85548) in comparison with *dl*-nebivolol (R67555)**

**Key study findings:** Ptosis was reported for 6/10 f receiving R85548 40 mg/kg and 10/10f receiving R67555 80 mg/kg. Food consumption was decreased in the HD of R67555 for both sexes. Drug-treated animals gained less weight than the control groups: 7%(R85548 HD m), 15%(R67555 HD m), 10% (R85548 HDf) and 22% (R67555 HDf).

In both males and females treatment with R88548 at 40 mg/kg caused a decreased HCT ( $p < 0.001$ ), Hb ( $p < 0.001$ ) and RBC ( $p < 0.01-0.001$ ). Changes in renal, adrenal and liver function were suggested by the increase in serum potassium in both sexes with both drugs, the decreases in calcium, total protein, albumin, cholesterol and triglycerides, increased BUN, alk phos and

ALT. Urinalysis showed decreased creatinine excretion for both drugs and increases in urinary volume in males. The organs that were reported to have altered weight were consistent with the findings in other studies. That is, in both sexes, with both drugs, there was an increase in spleen, liver, kidney, pancreas and adrenal weights. Lung weights were increased in both sexes with the racemic mix and in females only with R85548. The histologic scoring system makes interpretation of the findings difficult. Both males and females of the HD of both drugs were indicated to have changes in the adrenals, liver and lungs. Findings were also suggested for males in the epididymides, kidneys and urinary bladder. Other pathology was indicated for the ovaries of HDf for both drugs. Histopathology was described as similar in both sexes for the adrenal gland. Changes were present following treatment with both drugs and included swollen and/or vacuolated cortical cells and small zona glomerulosa. The hepatic findings were described as centrilobular swelling. Lungs contained foamy cells, described as "females more than males" associated with septal thickening in females. The sponsor concluded that the toxic effects of nebivolol were primarily due to the l-enantiomer.

**Study no.:** N92594/1

**Conducting laboratory and location:** Janssen, Beerse, Belgium

**Date of study initiation:** May 14, 1990

**GLP compliance:** statement included

**QA report:** yes (x) no ( )

**Drug, lot #, and % purity:** l-nebivolol (R85548), batch  
dl-nebivolol (R67555)

### Methods

Five groups of 10 males and 10 female SPF Wistar rats were dosed with

Control ;  $\beta$ -cyclodextrin 880 mg/kg/day

R85548: 10 mg/kg/day

R85548: 40 mg/kg/day

R67555: 20 mg/kg/day

R67555: 80 mg/kg/day

The drug was given via the diet for 1 month. The test article was given as a co-precipitate in a 1/10 ratio.

Clinical observations were made daily. Body weight and food consumption were determined weekly. Ophthalmoscopy was performed at the end of the study for HD and control animals. Blood for hematology was collected a few days before termination. Blood for clinical chemistry was obtained at euthanasia.

Urinalysis was determined on individual samples obtained from 16 hour collections using metabolism cages. Organ weights were determined for a limited number of organs. A standard list of tissues was collected for histopathology. Only control and HD animals were examined. Only target organs in the LD animals were examined.

### Results

No unscheduled mortality was reported. No clinical signs were reported for males. Ptosis was reported for females getting R85548, 40 mg/kg (6/10) and R67555 80 mg/kg (10/10).

Body weight was affected in both sexes receiving R85548 40 mg/kg and R67555 80 mg/kg.  
Summary of weight gain (changes)

Week	Dosage group ( mg/kg)					
	males			females		
	vehicles	R85548 40	R67555 80	vehicle	R85548 40	R67555 80
1	57	48**	38***	28	26	16**
2	123	113*	96***	55	48	34*
3	169	157*	144***	78	64	59*
4	199	185**	170***	91	82	71*

Mann-Whitney U test: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

Food consumption was decreased in the R67555 HD of both sexes. The consumption of drug decreased markedly away from the target levels as the study progressed.

Department of toxicology

EXPERIMENT: 2335  
Toxicity study  
R 85548 - R 67555 - FOOD - RAT - 1 MONTH

-----  
| TEST ARTICLE INTAKE |  
Mean values per dosage group in mg/kg /day

Week	Dosage group ( mg / kg )							
	Males				Females			
	R 85548 10	R 85548 40	R 67555 20	R 67555 80	R 85548 10	R 85548 40	R 67555 20	R 67555 80
1	12.5	49.0	23.8	96.9	11.3	43.2	21.9	79.5
2	10.3	40.2	21.5	79.7	9.48	35.5	18.5	67.8
3	9.16	36.5	17.7	72.9	8.67	33.4	16.5	64.3
4	7.70	30.5	14.8	60.9	7.67	30.6	14.4	55.6
Mean	9.93	39.0	19.5	77.6	9.27	35.7	17.8	66.8

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Consistent with other studies, HCT, Hb and PCV were decreased with drug treatment. In both males and females treatment with R88548 at 40 mg/kg caused a decrease in HCT ( $p < 0.001$ ), Hb ( $p < 0.001$ ) and RBC ( $p < 0.01 - 0.001$ ).

Treatment with R67555 decreased HCT in males ( $p < 0.01$ ) at 80 mg/kg and in females ( $p < 0.01 - 0.001$ ) at 20 and 80 mg/kg. Hb was decreased in males ( $p < 0.001$ ) at 80 mg/kg and females ( $p < 0.01 - 0.001$ ) at 20 and 80 mg/kg. RBC were decreased in males ( $p < 0.05$ ) at 80 mg/kg and females ( $p < 0.05$ ) at 20 mg/kg but not at 80 mg/kg.

In both males and females serum potassium levels were increased with both drugs while calcium, total protein, albumin, cholesterol and triglycerides were decreased. Blood urea nitrogen increased in both sexes while creatinine was decreased. Alkaline phosphatase and ALT were both increased. Changes were seen with both drugs and showed an apparent dose-relationship.

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Parameter	Dosage group ( mg / kg )	Males				
		Vehicle	R 85548 10	R 85548 40	R 67555 20	R 67555 80
SOD: Sodium	mEq/l	144	144	143 *	143	143
POT: Potassium	mEq/l	4.8	4.8	5.7 ***	5.0	5.6 ***
CHL: Chloride	mEq/l	100	100	102 **	101	102 **
CAL: Calcium	mg%	11.2	10.7 **	10.3 ***	11.0	10.6 **
INP: Inorg. phosphate	mg%	9.1	9.8	9.5	9.3	9.2
TOP: Total protein	g%	6.3	6.0	5.5 ***	6.0	5.5 ***
ALB: Albumin	g%	3.7	3.6	3.3 ***	3.6	3.4 ***
HAP: Haptoglobin	mg%	27	17	25	15	25
GLU: Glucose	mg%	168	165	162	164	156 *
CHO: Cholesterol	mg%	81	72	34 ***	72	32 ***
TGL: Triglycerides	mg%	146	138	131	179	120
PLP: Phospholipids	mg%	166	157	109 ***	164	110 ***
BUN: Blood urea nitrog.	mg%	12.4	13.9	14.7 **	13.6	14.2 *
CRS: Creatinine	mg%	0.49	0.48	0.45 ***	0.48	0.44 ***
BIL: Total Bilirubin	mg%	0.08	0.06	0.07	0.07	0.07
ALP: Alkal. phosphatase	U/l	457	488	554	414	585
AST: Aspartate aminotr.	U/l	111	111	129	106	122
ALT: Alanine aminotran.	U/l	44	62 **	63 *	47	78 ***
ChE: Cholinesterase	KU/l	0.2	0.2	0.2	0.2	0.2

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

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Parameter	Dosage group ( mg / kg ) Females				
	Vehicle	R 85548 10	R 85548 40	R 67555 20	R 67555 80
SOD: Sodium mEq/l	143	144	143	143	143
POT: Potassium mEq/l	4.4	4.6	5.2 ***	4.8 **	5.6 ***
CHL: Chloride mEq/l	100	101	102 **	101	102 *
CAL: Calcium mg%	10.8	10.6	9.9 ***	10.8	10.1 **
INP: Inorg. phosphate mg%	8.4	8.8	8.4	9.1	8.5
TCP: Total protein g%	6.5	6.2 *	5.6 ***	6.0 **	5.4 ***
ALB: Albumin g%	4.0	3.8 *	3.3 ***	3.6 ***	3.3 ***
HAP: Haptoglobin mg%	10	3	8	15	18
GLU: Glucose mg%	164	160	148	162	149
CHD: Cholesterol mg%	80	71	23 ***	71 *	22 ***
TGL: Triglycerides mg%	87	76	83	103	91
PLP: Phospholipids mg%	150	138	73 ***	145	73 ***
BUN: Blood urea nitrog. mg%	12.6	12.3	15.8 **	14.2	16.2 **
CRS: Creatinine mg%	0.53	0.51	0.43 ***	0.49 *	0.43 ***
BIL: Total Bilirubin mg%	0.09	0.10	0.08	0.09	0.06
ALP: Alkal. phosphatase U/l	304	286	272	335	276
AST: Aspartate aminotr. U/l	110	112	108	112	122
ALT: Alanine aminotran. U/l	44	47	51	49	70 **
CHE: Cholinesterase KU/l	0.9	0.9	0.6 ***	0.7 *	0.6 **

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

Urinalysis showed significant decreases in creatinine excretion for both drugs and increases in urinary volume for the males. This was not seen in the females.

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## Organ weight changes in males

	Dosage group ( mg/kg)				
	vehicle	R85548 10	R85548 40	R67555 20	R67555 80
Lungs: abs Norm.	1910 5613				1883 6071*
Spleen: abs norm	764 2249	794 2285	773 2370	864 2529	808 2597*
Liver: abs norm	14717 43252		14853 45620		14424 46377*
Heart:abs norm	1119 3290		1127 3466		1114 3579**
Pancreas: ab norm	1238 3646	1307 3778	1356 4162*	1226 3590	1375 4450*
Kidneys:ab norm	2638 7743	2699 7769	2731 8388*	2773 8123	2591 8340
Brain:abs norm	1970 5799	2006 5784	2027 6229**	2036 5966	1966 6334**
Adrenals:ab norm	54 158	54 157	77*** 236***	56 163	78*** 251***

## Organ weight changes in females

	Dosage group ( mg/kg)				
	vehicle	R85548 10	R85548 40	R67555 20	R67555 80
Lungs: abs Norm.	1452 6493	1541 6644	1597 7363**	1498 6490	1539 7526***
Spleen: abs norm	568 2566	583 2505	593 2738	613 2656	625 3057**
Liver: abs norm	8921 40112	9604 41286	10020* 46217***	9886 42813	9574 46882***
Pancreas: ab norm	1091 4929	1062 4595	1119 5147	1046 4529	1233 6059**
Kidneys:ab norm	1760 7911	1901 8182	1833 8458*	1951* 8447*	1788 8786**
Brain:abs norm	1893 8549		1825* 8431		1849 9093
Adrenals:ab norm	69 310	74 322	129*** 597***	80* 346	141*** 695***

NDA21742

Reviewer: E.A. Hausner, D.V.M.

Mann-Whitney U test \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

The histologic scoring system again obscures the incidence and severity of findings. The summary table for the males indicates histologic findings in the adrenals, epididymides, kidneys, lungs, spleen, urinary bladder and liver.

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Organ or tissue - observation	Males				
	Dosage group ( mg / kg )				
	Vehicle	10	40	20	80
Adrenals, cortex					
- ectasia of sinusoidal spaces	0.10	0.00	0.30	0.10	0.00
- large vacuoles	0.20	0.00	0.00	0.00	0.00
- mineral deposits	0.00	0.20	0.00	0.00	0.00
- prominent sinusoidal lining cells	0.10	0.00	0.20	0.00	0.10
- swollen/vacuolated cort. cells	0.00	0.00	0.10	0.00	0.10
Brain					
- vacuolization, large	0.90	-	0.90	-	1.00
Epididymides					
- cellular debris in lumen	0.30	-	0.00	-	0.00
- focal chronic inflammatory cells	0.50	-	0.80	-	0.30
- perivascular inflammatory cells	0.30	-	0.50	-	0.40
- reduced spermatozoa amount	0.40	-	0.00	-	0.00
- vacuolated epithelium	0.50	-	0.40	-	0.70
Exorbital lacrimal gland					
- focal atrophy	0.10	-	0.00	-	0.00
Kidneys					
- atrophic glomerulus	0.10	-	0.20	-	0.30
- basophilic tubuli	0.40	-	0.30	-	0.40
- blood in pelvis	0.30	-	0.20	-	0.50
- cyst(s)	0.10	-	0.10	-	0.00
- dilated pelvis	0.00	-	0.20	-	0.00
- focal chronic inflammatory cells	0.60	-	0.30	-	0.60
- inflamed pelvis	0.00	-	0.00	-	0.10
- swollen/vacuolated pelvis epith.	0.10	-	0.00	-	0.20
Liver					
- RES-aggregates	0.60	-	0.60	1.20	0.30
- centrilobular swelling	0.10	-	0.10	0.00	0.60 *
- focal necrosis	0.00	-	0.10	0.00	0.10
- focal postnecrotic regeneration	0.00	-	0.00	0.00	0.20
- focal swollen hepatocytes	0.00	-	0.10	0.00	0.00
- granulocyte infiltration	0.00	-	0.20	0.00	0.00
- hydropic aspect	0.00	-	0.20	0.00	0.00
- large vacuoles	0.10	-	0.00	0.00	0.00
- mineral deposits	0.00	-	0.00	0.00	0.10
- perivascular inflammatory cells	0.00	-	0.00	0.20	0.00
- prominent Kupffer's cells	0.00	-	0.10	0.20	0.30
Lungs					
- blood in lumen	0.60	0.10	0.10	0.50	0.00
- emphysematous	0.00	0.10	0.00	0.00	0.00
- foamy cells	0.20	0.00	1.40 **	0.00	1.40 ***
- focal alveolar macrophages	0.40	0.20	0.20	0.20	0.00 *
- focal septal thickening	0.50	0.20	0.50	0.20	0.30
Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** p < .01 *** p < .001					
Spleen					
- red blood cells in red pulp	0.20	0.80 **	0.90 **	1.30 ***	1.50 ***

Organ or tissue - observation	Males			
	Dosage group ( mg / kg )			
	R 85548 Vehicle	R 85548 10	R 67555 40	R 67555 20 80
Thyroid glands				
- desquamated epithelium	0.10	-	0.10	- 0.00
- small follicles	0.10	-	0.20	- 0.11
- squamous follicle	0.50	-	0.20	- 0.11
- thymic remnants	0.00	-	0.30	- 0.11
Urinary bladder				
- desquamated epithelium	0.11	-	0.00	- 0.00
- inspissated material	0.69	-	0.70	- 1.10
- swollen epithelium	0.00	-	0.00	- 0.10

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

The summary table for the females indicates findings in the adrenals, liver, lungs, ovaries and spleen

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Females					
Organ or tissue - observation	Dosage group ( mg / kg )				
	Vehicle	10	40	20	80
Adrenals, cortex					
- clear cell plaque	0.00	0.10	0.00	0.00	0.00
- congestion	0.00	0.00	0.20	0.00	0.10
- ectasia of sinusoidal spaces	0.20	0.00	0.60	0.00	0.20
- extracapsular tissue	0.00	0.00	0.00	0.00	0.10
- large vacuoles	0.10	0.00	0.00	0.00	0.00
- prominent sinusoidal lining cells	0.00	0.00	0.30	0.00	0.60 **
- small zona glomerulosa	0.00	0.00	0.80 **	0.00	0.60 **
- swollen/vacuolated cort. cells	0.00	0.10	1.30 ***	0.40 *	1.20 ***
Brain					
- vacuolization, large	0.70	-	0.70	-	0.80
Eye					
- conjunctivitis	0.00	-	0.00	-	0.10
Kidneys					
- atrophic glomerulus	0.00	-	0.10	-	0.00
- basophilic tubuli	0.30	-	0.10	-	0.30
- blood in pelvis	0.30	-	0.30	-	0.30
- focal chronic inflammatory cells	0.60	-	0.60	-	0.30
- inflamed pelvis	0.10	-	0.00	-	0.00
- swollen/vacuolated pelvis epith.	0.00	-	0.10	-	0.00
Liver					
- RES-aggregates	0.70	-	0.60	-	0.50
- centrilobular swelling	0.20	-	0.00	-	0.30
- focal chronic inflammatory cells	0.00	-	0.00	-	0.10
- focal necrosis	0.00	-	0.20	-	0.00
- large vacuoles	0.30	-	0.00	-	0.00
- prominent Kupffer's cells	0.70	-	0.60	-	0.60
Lungs					
- blood in lumen	0.00	0.10	0.00	0.00	0.30
- emphysematous	0.10	0.00	0.00	0.00	0.10
- foamy cells	0.10	0.00	2.10 ***	0.00	1.90 ***
- focal alveolar macrophages	0.00	0.00	0.00	0.10	0.00
- focal septal thickening	0.10	0.00	0.70 **	0.30	0.60 *
- granulocyte infiltration	0.50	0.50	0.40	0.70	0.40
- prominent lymphoid tissue	0.10	0.00	0.00	0.00	0.00
- thick vessel wall	0.10	0.20	0.30	0.20	0.10
Lymph node(s), mesenteric					
- atrophic aspect	0.00	-	0.00	-	0.10
- red blood cells in sinuses	0.00	-	0.10	-	0.10
- starry sky appearance	0.00	-	0.10	-	0.00
Mammary gland					
- fibrosis	0.00	-	0.00	-	0.10
- focal chronic inflammatory cells	0.10	-	0.00	-	0.00
Nose (nasal turbinates)					
- blood in lumen	0.10	-	0.00	-	0.00

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

month toxicity study in SPF Wistar rats: intravenous administration

Study  
title:  
One

**Key study findings:** The body weight effects and some of the hematology and clinical chemistry findings typical of oral administration studies were not present in this report. The absence of the usual decrease in HCT and RBC is somewhat unusual in light of the increased RBC in the spleen. Clinical chemistry effects that were present included a decrease in cholesterol and phospholipids in the MD and HD males. Serum potassium was increased in HDf while serum albumin was decreased in HDf. It is not clear if there are significant metabolic differences between oral and IV administration. A PK study (N92692) comparing IV and oral parameters examined only parent drug, not metabolites. There is no characterization of metabolism in rats after IV administration. Histologic changes were reported for several organs. An increase in rbc in the spleen was reported for HDm, MDf and HDf. Vehicle related changes were reported for the kidneys and urinary bladder of the veh and HD rats characterized by swollen and granular cortical tubuli and swollen, vacuolated epithelium in the renal pelvis and urinary bladder. This was regarded as adaptive to the osmotic effects associated with  $\beta$ -CD. Changes seen in the lungs of HDm, MDf and HDf were described as an increase in focal alveolar macrophages. There were no overt signs of toxicity in this study, excluding the irritant effects at the injection site. However, there were clinical chemistry changes in both sexes as well as organ weight and histologic effects. Sex-related differences in metabolism are suggested in that neither adrenal effects nor serum cholesterol, phospholipids, albumin etc. effects seen in other rat studies were reported for the females

**Study no.:** N88268

**Conducting laboratory and location:** Janssen, Beerse, Belgium

**Date of study initiation:**

**GLP compliance:**

**QA report:** yes ( ) no ( )

**Drug, lot #, and % purity:** R67555 batch PFA141 in a vehicle of hydroxypropyl- $\beta$ -CD

**Methods** Nebivolol, R67555, was given intravenously to SPF Wistar rats (5/sex/group) at a daily dosage of 0 (saline control), 00 (vehicle control) 0.31, 1.25 and 5 mg/kg for 1 month. Parameters studied included mortality, signs, body weight, food and water consumption, hematology, clinical chemistry, urinalysis, organ weights, gross pathology and histopathology.

## Results

Irritation and swelling of the tail at the site of injection were reported for both sexes of HD animals. There were no other signs reported. There were no significant differences in weight gain between the drug-treated groups and control groups of either sex. The vehicle and drug-treated females ate somewhat less than did the control groups but not in a dose-related manner.

The decreases in HCT, Hb and RBC typically seen in the oral administration studies were not seen in this study.

The clinical chemistry changes in males included a decrease in serum cholesterol, phospholipids. This is summarized in the reviewer's table below.

Summary of clinical chemistry changes in males

	Dosage ( mg/kg)				
	0	00 (vehicle)	0.31	1.25	5
Cholesterol mg/dl	78	85	82	66**	64*
Phospholipids Mg/dl	158	165	182	150	140*

Vehicle vs other groups: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

Clinical chemistry changes in females included increases in serum potassium and a decrease in albumin. Cholesterol and phospholipids showed no substantive changes compared to the controls.

Summary of clinical chemistry changes in females

	Dosage (mg/kg)				
	0	00 (vehicle)	0.31	1.25	5
Potassium mEq/l	4.4	4.6	4.6	4.6	5.0*
Albumin g/dl	4.3	4.0*	4.2@	4.1	3.7**@

Control (0) vs other groups: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

Vehicle vs other groups: @p<0.05

There were no findings of toxicologic significance apparent in the urinalysis data as presented. Organ weight data for the males showed an increase in spleen weight for the MD and HD groups. There was a non-dose-related increase in liver weight in the vehicle and drug-treated groups. A slight (4%) increase in normalized kidney weight was seen at the HD. An increase in absolute and normalized adrenal weight was seen at the HD.

Adrenal weight changes in males

	control	vehicle	5 mg/kg
Absolute adrenal weight mg	61	58	68
Normalized adrenal weight mg/kg	153	145	172

Organ weight data for females showed no significant effects except for the gonads. A decrease in weight was seen at the HD. Adrenal weight changes were not reported for females.

Gonad weight changes in females

	control	vehicle	5 mg/kg
Absolute gonad weight mg	157	168	141**
Normalized gonad weight mg/kg	629	682	578**

\*\*p<0.01 versus vehicle by the two-tailed Mann-Whitney U test

Gross observations in the males included dilated kidneys, swollen lymph nodes and hemorrhagic lymph nodes. The gross observations for the females were more pronounced liver lobulation (1 HDf) and watery uterine content (1 control f, 1 LD f).

Summary of gross necropsy findings in males

	Dosage ( mg/kg)				
	0	00 (vehicle)	0.31	1.25	5
Dilated kidneys	0/5	0/5	1/5	1/5	1/5
Swollen lymph nodes	0/5	0/5	0/5	1/5	2/5
Swollen, hemorrhagic lymph nodes	0/5	0/5	0/5	1/5	1/5

Vehicle vs other groups: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

Histologic changes were reported for the kidneys, lungs, urinary bladder and spleen.

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-----+-----					
Males					
Organ or tissue - observation	Dosage group ( mg / kg )				
	Control	Placebo	0.31	1.25	5
-----+-----					
Administration site					
- edematous	0.00	0.00	0.00	0.20	1.00
- exudative inflammation (skin)	0.00	0.00	0.00	0.00	0.60
- hair in vessel	0.00	0.20	0.00	0.00	0.20
- inflammatory cell infiltration	0.20	0.80	0.20	0.60	0.80
- perivascular bleeding(s)	0.20	0.00	0.00	0.20	0.40
- perivascular fibrosis	0.20	0.80	0.40	0.80	0.80
- perivascular inflammation	0.00	0.40	0.00	0.20	0.60
- thrombosis	0.00	0.00	0.00	0.00	0.20
- vasculopathy	0.00	0.00	0.00	0.00	0.20
Adrenals, cortex					
- ectasia sinusoidal spaces	0.20	-	-	-	0.20
- extracapsular tissue	0.00	-	-	-	0.20
- swollen cortical cells	0.20	-	-	-	0.20
Kidneys					
- diffuse swollen/granular tubuli	0.00	0.20	0.00	0.00	0.20
- dilated pelvis	0.00	0.00	0.00	0.60	0.20
- focal basophilic tubuli	0.60	0.20	0.40	0.20	0.60
- focal chronic infl. cells	0.40	0.40	0.60	0.40	0.40
- focal dilated cortical tubuli	0.20	0.00	0.00	0.20	0.00
- focal swollen/granular tubuli	0.20	0.60	0.20	0.20	1.00 *
- inflammation, pelvis	0.40	0.00	0.40	0.20	0.00
- swollen/vacuol. pelvis epithelium	0.00	0.40	0.00	0.00	0.60 *
Liver					
- RES-aggregates	1.00	-	-	-	1.20
- granulocyte infiltration	0.40	-	-	-	0.00
- hydropic aspect	0.00	-	-	-	0.20
- large vacuoles	0.20	-	-	-	0.20
- prominent Kupffercells	0.20	-	-	-	0.20
Lungs					
- blood in lumen	0.20	0.20	0.20	0.40	0.20
- focal alveolar macrophages	0.20	0.60	0.40	0.20	0.20
- focal chronic infl. reaction	0.00	0.00	0.00	0.40	0.60
- focal septal thickening	0.00	0.20	0.40	0.00	0.00
- focal swollen alveolar macrophages	0.00	0.20	0.20	0.40	0.60 *
- granulocyte infiltration	0.00	0.80 *	1.00 **	0.20	0.60 *
- hair in vessel	0.00	0.00	0.20	0.20	0.20
- thick vessel wall	0.00	0.20	0.40	0.00	0.00
Spleen					
- red blood cells in red pulp	1.20	1.80	1.80	1.80	2.60 *
Urinary bladder					
- inspissated hyalin material	0.40	0.40	0.00	0.80	0.80
- prominent subepith. infl. cells	0.20	0.00	0.20	0.00	0.00
- swollen/vacuolated epithelium	0.00	0.80 *	0.00	0.00	1.00 **

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

----->>>>					
! Females !					
! Organ or tissue - observation	! Dosage group ( mg / kg )				
	! Control	! Placebo	! 0.31	! 1.25	! 5
!----->>>>					
! Administration site					
! - edematous	! 0.00	! 0.20	! 0.20	! 0.20	! 1.40 *
! - hair in vessel	! 0.00	! 0.00	! 0.00	! 0.00	! 0.20
! - inflammatory cell infiltration	! 0.00	! 0.20	! 0.20	! 0.20	! 1.60 **
! - perivascular bleeding(s)	! 0.20	! 0.40	! 0.40	! 0.40	! 0.80
! - perivascular fibrosis	! 0.00	! 0.40	! 0.00	! 0.00	! 1.00 *
! - perivascular inflammation	! 0.00	! 0.60	! 0.00	! 0.00	! 0.60
! - vasculopathy	! 0.00	! 0.00	! 0.00	! 0.00	! 0.20
! Adrenals, cortex					
! - ectasia sinusoidal spaces	! 0.20	-	-	-	! 0.20
! - swollen cortical cells	! 0.20	-	-	-	! 0.20
! Kidneys					
! - diffuse swollen/granular tubuli	! 0.00	! 0.60 *	! 0.00	! 0.00	! 0.60 *
! - dilated pelvis	! 0.00	! 0.00	! 0.20	! 0.00	! 0.00
! - focal basophilic tubuli	! 0.00	! 0.00	! 0.20	! 0.40	! 0.40
! - focal chronic infl. cells	! 0.20	! 0.20	! 0.20	! 0.40	! 0.20
! - focal swollen/granular tubuli	! 0.00	! 0.20	! 0.20	! 0.00	! 0.60 *
! - swollen/vascul. pelvis epithelium	! 0.00	! 0.60 *	! 0.00	! 0.00	! 0.40
! Liver					
! - RES-aggregates	! 0.60	-	-	-	! 0.80
! - focal necrosis	! 0.20	-	-	-	! 0.00
! - hydropic aspect	! 0.40	-	-	-	! 0.00
! - large vacuoles	! 0.40	-	-	-	! 0.40
! - prominent Kupffercells	! 0.60	-	-	-	! 0.40
! Lungs					
! - focal alveolar macrophages	! 0.20	! 0.80	! 0.60	! 0.40	! 0.20
! - focal chronic infl. reaction	! 0.20	! 0.20	! 0.00	! 0.00	! 0.20
! - focal septal thickening	! 0.00	! 0.20	! 0.00	! 0.00	! 0.00
! - focal swollen alveolar macrophages	! 0.20	! 0.20	! 0.00	! 0.20	! 0.60
! - granulocyte infiltration	! 0.60	! 0.60	! 0.20	! 0.20	! 0.40
! - hair in vessel	! 0.00	! 0.00	! 0.00	! 0.00	! 0.40
! - thick vessel wall	! 0.00	! 0.00	! 0.20	! 0.00	! 0.00
! Spleen					
! - red blood cells in red pulp	! 1.00	! 1.40	! 1.80	! 2.00 *	! 2.20 *
! Urinary bladder					
! - inspissated hyalin material	! 0.00	! 0.00	! 0.20	! 0.00	! 0.00
! - prominent subepith. infl. cells	! 0.00	! 0.00	! 0.20	! 0.40	! 0.00
! - swollen/vacuolated epithelium	! 0.00	! 1.00 **	! 0.00	! 0.00	! 1.00 **

Significance computed by Mann-Whitney U test (two tailed) ; \* p < .05 \*\* p < .01 \*\*\* p < .001

**Study title: Subchronic toxicity study in Wistar Rats: Repeated dosage for 3 months administered orally through the diet**

**Key study findings:** No unscheduled mortality or behavioral changes were reported. HD males gained on average 9% (34 g) less than the other groups while HD f gained on average 3% (5g) less than the control group. The usual clinical chemistry changes indicating serum electrolyte

(K+) changes and decreased liver and kidney function were present but without the hematology changes (HCT, Hb, and RBC) usually seen.

Reported histologic changes were centered on the lungs and spleen. An increase of foci with foam cells was observed in the HD f group. The reproductive organs were reported were described as similar to the control group. A dose dependent increase in rbc in the splenic red pulp was reported for all treated groups compared to the control group.

**Study no.:** N54353

**Conducting laboratory and location:** Janssen, Beerse, Belgium

**Date of study initiation:** March 17, 1986

**GLP compliance:** statement included

**QA report:** yes ( ) no ( )

**Drug, lot #, and % purity:** R67555, batch A0301

### **Methods**

Four groups of SPF Wistar rats, 20/sex/group, were given nebivolol in their diets at levels calculated to give 0, 10mg/100 g food, 40 mg/100 g food and 160 mg/100 g food. Parameters studied were mortality, signs, body weight (weekly) and food (weekly) consumption, test article intake, water consumption, hematology, clinical chemistry, urinalysis, organ weights, gross pathology and a basic list of tissues for histopathology analysis. Ophthalmic exams were performed prior to, once during and at the end of the study on at least 10 animals of each sex in the HD and control groups.

### **Results**

There was no unscheduled mortality. No signs were reported. Food wastage was increased in the HD group (14/20 m vs 1/20 for the male control group and 17/20 HDf vs 0/20 for the female controls). Drug-treated males showed lower average weight gain compared to the other groups from the first week of the study. Only minor differences were seen in the females.

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EXPERIMENT: 1590  
 Toxicity study  
 R 67555 - FOOD - RAT - 3 MONTH

! BODY WEIGHT  
 ! Mean values per dosage group in g

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

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

What was perplexing was the analysis of drug in food. The nominal concentration for the LD appeared to be greater than the nominal concentration for the HD. The sponsor did not seem to find this a problem.

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<u>Date of</u>		<u>Nominal concentration in mg/100 g food</u>		
		10	40	160
<u>mixing</u>	<u>analysis</u>	<u>concentration found in %</u>		
86.03.13	86.05.13	144	126	126
86.05.16	86.08.04	126	131	98
	repetition	126	129	94

ref.chem.act.Nos.6682, 6752

As appears from these data, the concentration of the test article found in the various dose levels was somewhat different from the nominal concentration. However, the rats of the various dosage groups were certainly not under-dosed.

Because of the food wastage, it is difficult to assess the consumption data. However, since there were no marked effects on body weight, it's probably safe to assume that there were no major perturbations in consumption.

There was a slight decrease in HCT(39.1% vs 41.9% for control,  $p < 0.001$ ), Hb(13.5 vs 14.3g/dl for control,  $p < 0.01$ ) and RBC ( $7.81$  vs  $8.33 \times 10^6$  for control,  $p < 0.001$ ) in the HD females but not in the males and not the dose-related pattern that is usually seen in oral administration studies. While the changes are listed as statistically significant, the biological significance is questionable.

Increases in serum potassium were seen in the HD males and all groups of drug-treated females. Inorganic phosphate was increased in both sexes also. Total protein and albumin were decreased in the HD groups of both sexes. Cholesterol showed dose-related decreases while phospholipids were decreased only at the HD.

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Parameter		Dosage group ( mg / 100 g food )							
		Control	Males			Females			
			10	40	160	Control	10	40	160
SOD: Sodium	mEq/l	150	149	150	150	148	147	148	147
POT: Potassium	mEq/l	4.9	4.7	4.9 M	5.1 MM	4.5	4.6	4.8 M	4.8 MM
CHL: Chloride	mEq/l	104	104	103	104	109	108	109	109
CAL: Calcium	mgZ	11.1	11.3	11.3	11.1	10.7	10.7	10.9	10.4 M
INP: Inorg. phosphate	mgZ	6.8	6.9	7.2	7.4	5.7	5.8	6.4	6.9 MM
TOP: Total protein	gZ	6.8	6.8	6.7	6.5	6.7	6.6	6.6	6.4 MM
ALB: Albumin	gZ	4.1	4.1	4.1	3.9 M	4.3	4.3	4.3	4.0 MM
HAP: Haptoglobin	mgZ	70	77	75	85	32	42 M	36	50 MM
GLU: Glucose	mgZ	173	180	177	164	163	169	169	153
CHD: Cholesterol	mgZ	90	89	86	64 MM	95	86	84 M	63 MM
TGL: Triglycerides	mgZ	145	145	153	117	86	95	107	113 M
PLP: Phospholipids	mgZ	164	165	167	140 MM	179	174	177	155 M

The urinalysis data showed granular casts in the treated males and to some extent in the females.

Parameter		Dosage group ( mg / 100 g food )							
		Control	Males			Females			
			10	40	160	Control	10	40	160
EPITHELIAL CELLS:	score								
SQN: Squamous	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CYL: Cylindrical	0-3	0.15	0.15	0.10	0.05	0.05	0.25	0.10	0.10
REN: Renal	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CASTS:	score								
HYL: Hyaline	0-3	0.20	0.05	0.10	0.15	0.10	0.05	0.10	0.10
GRN: Granular	0-3	0.05	0.15	0.25	0.35 MM	0.00	0.15	0.05	0.10

The organ weight data showed an increase in average spleen weight in both sexes despite the lack of change in the hematology data. Normalized pancreas weight was increased in both sexes

also. Adrenal weight was increased in both sexes at the HD only. Gonadal weight was increased in the males and decreased in the females.

Parameter	Unit	Dose group ( mg / 100 g food )							
		Control	Males			Females			
			10	40	160	Control	10	40	160
NWT: Body weight	g	498	505	501	463 <sup>NS</sup>	268	282 <sup>N</sup>	275	262
LNG: Lungs	mg	2265	2282	2323	2178	1548	1610	1629	1680
	mg / kg	4557	4526	4632	4703	5786	5710	5923	6419 <sup>NS</sup>
SPL: Spleen	mg	998	1087 <sup>NS</sup>	1092 <sup>NS</sup>	1221 <sup>NS</sup>	656	720 <sup>N</sup>	765 <sup>NS</sup>	868 <sup>NS</sup>
	mg / kg	2008	2158 <sup>N</sup>	2184 <sup>NS</sup>	2639 <sup>NS</sup>	2451	2558	2783 <sup>NS</sup>	3323 <sup>NS</sup>
LIV: Liver	mg	17333	17381	17141	16266	8815	9169	9221	9320
	mg / kg	35185	34448	34111	33127	32889	32466	33418	35592 <sup>NS</sup>
HRT: Heart	mg	1339	1385	1397	1314	902	946	926	892
	mg / kg	2688	2745	2790 <sup>N</sup>	2836 <sup>N</sup>	3371	3351	3367	3415
PNC: Pancreas	mg	1649	1648	1615	1598	1109	1230 <sup>NS</sup>	1169	1185
	mg / kg	3317	3264	3230	3449	4144	4361	4249	4539 <sup>NS</sup>
KID: Kidneys	mg	3507	3378	3305	3259 <sup>N</sup>	1875	1895	1817	1838
	mg / kg	7041	6690	6606 <sup>N</sup>	7033	7012	6720	6603 <sup>N</sup>	7038
BRN: Brain	mg	2122	2076	2126	2092	1907	1887	1873	1880
	mg / kg	4273	4129	4264	4524 <sup>N</sup>	7148	6703 <sup>N</sup>	6833	7212
THY: Thymus	mg	426	427	450	411	324	366	338	323
	mg / kg	859	853	896	886	1204	1299	1229	1236
ADR: Adrenals	mg	58	59	60	66 <sup>N</sup>	80	83	82	108 <sup>NS</sup>
	mg / kg	117	118	121	143 <sup>NS</sup>	301	296	299	418 <sup>NS</sup>
TYR: Thyroids	mg	27	28	28	27	21	21	21	19
	mg / kg	55	55	55	58	77	76	77	72
GON: Gonads	mg	3574	3629	3636	3541	173	166	165	162
	mg / kg	7188	7216	7283	7633 <sup>NS</sup>	648	591	603	623

Significance computed by Mann-Whitney U test (two tailed) : <sup>N</sup> P < .05 <sup>NS</sup> P < .01 <sup>NSNS</sup> P < .001

There were few significant gross necropsy findings in the males. The HD females had changes in the adrenals and lungs as summarized in the sponsor's table below.

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Organ or tissue : observation	Females			
	0	10	40	160
	X/ N	X/ N	X/ N	X/ N
Adrenal gland : swollen	0/19	0/19	1/19	9/19M
Lung : aspiration bleedings	0/19	0/19	2/19	1/19
Lung : focus, white	0/19	0/19	0/19	8/19M
Thymus : petechia	0/19	0/19	1/19	0/19
Uterus : swollen	2/19	3/19	2/19	2/19

Significance computed by Chi square test (two tailed) : \* P < .05 \*\* P < .01 \*\*\* P < .001

Reported histologic changes were centered on the lungs and spleen. An increase of foci with foam cells was observed in the HD f group. The reproductive organs were reported were described as similar to the control group. A dose dependent increase in rbc in the splenic red pulp was reported for all treated groups compared to the control group.

**Study title:** *Subchronic toxicity study in Beagle dogs (repeated dosage for 2 weeks)*

**Key study findings:** Within the parameters reported, the only effect was vomiting in the 20 and 40 mg/kg animals in the first week of the study. Heart rate decreased in both drug-treated groups as might be expected from the pharmacology of the drug.

**Study no.:** N76735

**Conducting laboratory and location:**

**Date of study initiation:** March 7, 1989

**GLP compliance:** statement not found

**QA report:** yes ( ) no (x)

**Drug, lot #, and % purity:** nebivolol, batch PFA091

### Methods

Beagles, 2/sex/group were given either control, 20 or 40 mg/kg nebivolol in  $\beta$ -cyclodextrin, orally administered in gelatin capsules for 2 weeks. Parameters studied were listed as mortality, signs, heart rate and ECG (week 0, 2), body weight, hematology, clinical chemistry, urinalysis, organ weights, gross pathology and histopathology (control and HD only) for heart, lung, liver, kidney, spleen and adrenal.

### Results

Vomiting during the first week was reported for the 20 and 40 mg/kg groups. There were no apparent effects upon body weight. No effects were apparent in the hematology data.

No effects were apparent in the clinical chemistry data.

No effects were apparent in the urinalysis data.

There were no apparent effects upon ECG. However, it is not specified when relative to dosing (Cmax) the ECG was determined. Bazett's correction formula was used.

Heart rate showed a decrease in the LD group.

Heart rate in bpm

week	Dose mg/kg		
	vehicle	20	40
-2	126	130	109
2	134.5	91.8	98.5*

\*p<0.05 by Mann Whitney U test

No gross necropsy data, organ weight or histopathology information was located.

**Study title:** *One month comparative toxicity study in Beagle dogs (Administration: orally(capsules)).*

**Key study findings:** Dogs given the racemic drug did not gain weight while those receiving the isomers gained as much or more than the control animals. Serum potassium was increased compared to control in dogs given the racemate and the d-isomer. A decrease in heart rate and increase in PQ interval was also seen with the racemate and d-isomer. QTc was increased in all groups including the control. We do not have details about when the ECG was collected relative to dosing and we were not told the correction used to calculate QTc. Splenic weights were increased even though the hematology changes seen in other studies were not apparent. Reported histopathology results were limited to the increased rbc in the splenic red pulp.

**Study no.:** N92989

**Conducting laboratory and location:** Janssen, Beerse, Belgium

**Date of study initiation:** Feb 25, 1992

**GLP compliance:** statement included

**QA report:** yes ( x ) no ( )

**Drug, lot #, and % purity:** R67555(91J28/F21: ZRO67555PFA111), R85548(GSV-244; ZRO85548PFA011), R85547(GSV-244; ZRO85547PFA011)

### Methods

Beagles, 2/sex/group were given gelatin capsules containing test article.

Summary of dosage groups

Dosage groups	
Vehicle control	$\beta$ -cyclodextrin (880 mg/kg)
R67555 80 mg/kg*	960 mg/kg $\beta$ -CD coprecipitate
R88548 40 mg/kg*	480mg/kg $\beta$ -CD coprecipitate
R85547 40 mg/kg	480mg/kg $\beta$ -CD coprecipitate

\*doses expressed as base equivalents

Parameters that were studied were listed as : mortality, signs, ECG and heart rate, body weight, hematology, clinical chemistry, urinalysis, organ weights, gross pathology and histopathology of the spleen only.

### Results

Soft feces were reported as having occurred during the entire study in all dogs including vehicle control dogs.

The R67555 group on average did not gain weight during the study while the other groups gained from 0.2(control) to 0.4 (R85548 and R85547) kg.

PQ interval was increased in the R67555/ 80 mg/kg and R85547/40 mg/kg groups. QTc was increased in all groups, including the control. Heart rate was significantly decreased in the R67555/80mg/kg and R85547/40 mg/kg.

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Parameter	Week	Date	Control	Dosage group ( mg / kg )		
				R 67555 80	R 85548 40	R 85547 40
PQ m sec	-2	17-02-92	76.8	76.3	77.8	77.3
	4	23-03-92	75.5	87.0	77.3	87.8
QRS m sec	-2	17-02-92	35.5	30.0	26.0	36.3
	4	23-03-92	36.0	34.5	34.5	33.0
QT m sec	-2	17-02-92	150.8	137.0	140.5	142.5
	4	23-03-92	162.0	185.3	159.0	179.8 *
QTc m sec	-2	17-02-92	243.9	230.3	228.7	226.4
	4	23-03-92	254.9	242.7	224.9	238.0
R m volt	-2	17-02-92	1.87	1.69	1.95	2.24
	4	23-03-92	2.01	1.89	1.99	2.42
Rate b.p.m.	-2	17-02-92	157.0	172.8	160.8	151.3
	4	23-03-92	149.0	103.3 *	120.3	105.3 *

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

There were no toxicologically significant findings in the hematology data as presented.

Serum potassium was slightly elevated in all drug-treated groups before dosing started. With drug-treatment serum potassium increased relative to the control group in week 2 and remained elevated week 4 but not to the same extent.

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EXPERIMENT: 2671  
 Toxicity study  
 R 67555/R 85548/R 85547 - OR - DOG - 1 MONTH

Mean values per dosage group  
 Recorded in week -1

Parameter		Control	Dosage group ( mg / kg )		
			R 67555 80	R 85548 40	R 85547 40
SOD: Sodium	mEq/l	150	150	148	151
POT: Potassium	mEq/l	4.8	5.1	5.0	5.4

EXPERIMENT: 2671  
 Toxicity study  
 R 67555/R 85548/R 85547 - OR - DOG - 1 MONTH

Mean values per dosage group  
 Recorded in week 2

Parameter		Control	dosage group ( mg / kg )		
			R 67555 80	R 85548 40	R 85547 40
SOD: Sodium	mEq/l	149	150	148	147
POT: Potassium	mEq/l	4.8	5.6 *	5.4	5.6 *

EXPERIMENT: 2671  
 Toxicity study  
 R 67555/R 85548/R 85547 - OR - DOG - 1 MONTH

Mean values per dosage group  
 Recorded in week 4

Parameter		Control	Dosage group ( mg / kg )		
			R 67555 80	R 85548 40	R 85547 40
SOD: Sodium	mEq/l	149	150	149	149
POT: Potassium	mEq/l	4.7	5.4 *	5.0	5.5 *

Spleen weight was increased in R67555 and R85547. Liver and pancreas weight were increased in all drug-treated groups. Adrenal weight was slightly increased in the R85547 group. Gonad weight in the females in this group was also increased. Prostate weight was increased in the R85548 group. In the sponsor's table below, organ weights were combined for the sexes with the exception of the gonad weights.

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Parameter		Control	Dosage group ( mg / kg )		
			R 67555 80	R 85548 40	R 85547 40
WGT: Body weight	kg	9.78	9.30	10.20	9.98
LNG: Lungs	g	85	81	89	82
	g / 10 kg	87	86	87	84
SPL: Spleen	g	20	29	23	36
	g / 10 kg	21	29	22	36 *
LIV: Liver	g	223	257	269	272
	g / 10 kg	229	278 *	263 *	269
HRT: Heart	g	75	72	77	74
	g / 10 kg	77	77	76	75
PNC: Pancreas	g	21	23	26	27
	g / 10 kg	21	25	25 *	27 *
KDN: Kidneys	g	41	46	47	45
	g / 10 kg	42	49	45	45
BRN: Brain	g	74	69	72	76
	g / 10 kg	75	77	73	77
THY: Thymus	g	10	9	10	10
	g / 10 kg	10	9	9	10
ADR: Adrenals	g	0.944	0.881	0.991	1.045
	g / 10 kg	0.957	0.962	0.997	1.043
TYR: Thyroids	g	0.774	0.906	0.817	0.924
	g / 10 kg	0.794	0.991	0.809	0.945
GOM: Gonads m	g	13.0	13.0	13.5	13.0
	g / 10 kg	13.3	13.1	12.3	11.6
GOF: Gonads f	g	0.641	0.408	0.621	0.703
	g / 10 kg	0.670	0.505	0.696	0.803
HYP: Hypophysis	g	0.044	0.052	0.063	0.045
	g / 10 kg	0.045	0.058	0.062	0.045
PRS: Prostate	g	3.15	3.55	4.65	3.25
	g / 10 kg	3.19	3.61	4.08	2.89

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

The only histopathology results provided were for the spleen. Splenic findings were assessed on a scale of 0-5 (absent – severe). The presence of RBC in the spleen (a normal situation) was listed as a mean score of 1 in the control group, 3.3 (R67555), 1.5(R85548) and 3.3 (R85547).

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**Study title:** *One month toxicity study in Beagle dogs (intravenous administration)*

**Key study findings:** This preliminary subchronic intravenous dosing study was somewhat short on methodological details especially pertaining to the ECG data. No clinical signs were reported and there were no findings of toxicological significance.

**Study no.:** N88172

**Conducting laboratory and location:** Janssen, Beerse, Belgium

**Date of study initiation:** not stated

**GLP compliance:** no

**QA report:** yes ( ) no ( x )

**Drug, lot #, and % purity:** R67555 batch PFA141 in a vehicle of hydroxypropyl  $\beta$ -CD

**Methods** R67555 was given intravenously to Beagles (2/sex/group) at daily dosages of 0, 0.31, 1.25 and 5 mg/kg body weight for 1 month. HP- $\beta$ -CD was present at dosages of 80, 5, 20 and 80 mg/kg bw/day. Parameters studied were mortality, signs, body weight, ophthalmoscopy, heart rate and ECG, hematology, serum analysis, urinalysis, organ weights, gross pathology and histopathology.

## Results

There were no significant differences in body weight. However, the control group lost weight and the treated groups gained weight.

Summary of mean body weight changes (kg)

week	Dosage group mg/kg			
	0	0.31	1.25	5
1	0.1	0.2	0.4	0.3
2	0	0.3	0.4	0.3
3	-0.1	0.1	0.2	0.2
4	-0.1	0	0.4	0.4

There were no toxicologically significant findings in the hematology data as presented.

There were no toxicologically significant findings in the clinical chemistry data as presented.

There were no toxicologically significant findings in the urinalysis data as presented.

There were no significant organ weight findings, gross observations or histopathology.

PQ interval was increased in all drug-treated groups. Heart rate, QT and QTc were reported as decreased. We do not know when relative to dosing the ECGs were obtained and we do not know the methodology used. The correction factor used was  $QTc=QT(HR/60)$ . The data is summarized in the reviewer's table below.

parameter	week	Dosage group mg/kg			
		0	0.31	1.25	5
PQ m sec	-2	76.3	83.0	77.5	81.3
	4	76.3	88.3	81.8	92.3*
QTc msec	-2	223.4	233.2	238.7	239.2
	4	225.2	219.6	229.2	226.0
Rate b.p.m.	-2	138.3	132.5	126.3	138.0
	4	142.0	118.8	122.5	117.3

Mann-Whitney U test ( two tailed) \*p<0.05

**Study title:** *Subchronic toxicity study in Beagle dogs: repeated dosage for 3 months with oral administration*

**Key study findings:** The HD group gained on average 0.7(30%)Kg less than the control group. There were trends to decreased total protein, increased serum potassium and increased urea nitrogen. Absolute and normalized male gonad weight was increased in all drug-treated groups. Increased absolute and normalized female gonad weight was seen only in the MD and HD groups. While there were no corresponding hematology findings or changes in organ weight, the histopathology findings showed an increase in RBC in splenic red pulp in 2/8 control animals and 6/8 HD. LD and MD were reported as similar to control.  $QT_C$  was increased inconsistently throughout the groups.

**Study no.:** N54352

**Conducting laboratory and location:** Janssen, Beerse, Belgium

**Date of study initiation:** March 18, 1986

**GLP compliance:** statement included

**QA report:** yes ( x ) no ( )

**Drug, lot #, and % purity:** R67555 batch A0301 in gelatin capsules ( HP- $\beta$ -CD not listed as vehicle)

**Methods**

R67555 in gelatin capsules was given to Beagles (4 /sex/group) at doses of 0, 2.5, 10 and 40 mg/kg for 90 consecutive days. Parameters studied included signs, mortality, ophthalmoscopy (prior to, during and at the end of the study), food consumption (estimated), body

weight(weekly), hematology and clinical chemistry (prior to study, week 2, then monthly), urinalysis(prior, to , once during and at the end of the study). At euthanasia, organ weight, gross observations and histopathology from a standard list of tissues was assessed. ECGs were determined week -2, week 4 and week 12.

### Observation times and results

There was no unscheduled mortality. The following clinical findings were reported:

1m LD – urolithiasis from 3 month to end of study

1m MD – prostatitis from month 3 to end of study

1m MD – sporadic emesis in week 2

The HD group gained slightly less weight than did the control group. A difference in weight gain was apparent from week 2 of the study. This is summarized in the reviewer's table.

Summary of mean body weight gain (kg)

Week	Dosage mg/kg			
	0	2.5	10	40
1	0.3	0.2	0.2	0.2
2	0.6	0.5	0.5	0.3
3	0.9	0.8	0.8	0.6
4	1.3	0.9	1.2	0.7
5	1.7	1.3	1.4	1.1*
6	1.4	1.3	1.4	1.0
7	2.0	1.6	1.8	1.4
8	2.0	1.6	1.7	1.4
9	2.0	1.7	2.0	1.4
10	2.3	1.9	2.1	1.6
11	2.4	2.1	2.1	1.6
12	2.5	2.1	2.2	1.8
13	2.4	2.1	2.3	1.7

Mann-Whitney U test \*p<0.05

There were no findings of toxicological significance in the hematology as reported.

The clinical chemistry findings showed few changes of significance. Serum potassium showed a significant increase in week 3 and 8.

Summary of serum potassium (mEq/l)

Week	Dosage group mg/kg			
	0	2.5	10	40
-2	5.3	5.7*	5.6	5.6
0	5.3	5.3	5.1	5.4

3	5.2	5.3	5.7*	5.7**
4	5.1	5.2	5.3	5.4
8	5.1	5.2	5.3	5.4**
12	5.4	5.1	5.3	5.5

Mann-Whitney U test \*p<0.05, \*\*p<0.01

Total protein in the drug-treated animals showed a tendency to lower values than the control group. Albumin values showed only minor differences between the groups.

#### Summary of serum total protein (g/dl)

Week	Dosage group mg/kg			
	0	2.5	10	40
-2	5.8	5.7	5.9	5.9
0	6.0	6.0	5.8*	5.8
3	6.1	6.0	5.9	6.0
4	6.2	6.1	6.1	6.0*
8	6.1	6.0	6.0	5.8
12	6.4	6.0	6.3	6.0*

Mann-Whitney U test \*p<0.05, \*\*p<0.01

Blood urea nitrogen was increased in the MD and HD groups from week 3. There were no differences between groups in the creatinine values.

#### Summary of blood urea nitrogen

Week	Dosage group mg/kg			
	0	2.5	10	40
-2	12.9	13.6	12.4	12.9
0	12.7	12.0	11.1	13.3
3	15.8	15.6	17.1	19.3
4	13.6	15.4	15.5	16.6
8	15.7	15.8	16.7	18.9*
12	17.9	16.5	19.5	18.9

Mann-Whitney U test \*p<0.05, \*\*p<0.01

There were no findings of significance in the urinalysis data as reported.

ECG: There was a lengthening of PQ interval in both control and drug-treated animals. QTc was inconsistently increased, from week 4 of the study. Heart rate was increased in week 4 and decreased in week 12. The data is summarized in the reviewer's table below.

parameter	week	Dosage mg/kg
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