

		0	2.5	10	40
QTc msec	-2	239.5	235.1	210.8	226.2
	4	231.7	229.9	233.3	237.3
	12	227.0	237.4	230.2	228.7
Rate b.p.m.	-2	136.9	134.9	111.3	135.4
	4	144.3	148.4	140.8	144.4
	12	130.4	126.9	99.8	109.0

The only organ weight findings of significance were those of the gonads.

Week	Dosage group mg/kg				
	0	2.5	10	40	
Gonads male	Grams	14.8	16.3	15.5	16.8
	g/10 kg	11.2	14.1	13.3	13.6
Gonads female	Grams	0.631	0.610	0.736	0.765
	g/10kg	0.664	0.616	0.717	0.846

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

The histopathology findings showed an increase in RBC in splenic red pulp in 2/8 control animals and 6/8 HD animals (LD and MD were similar to control). However, there were no corresponding changes in hematology or splenic weight.

**Study title:** *Preliminary Study: Tolerance in white rabbits (oral gavage administration)*

**Key study findings:** The 3 rabbits given 40 mg/kg/day for 5 days lost on average 170 g of body weight compared to the 1 untreated control animal who gained 40 g. No other data was reported although the sponsor notes that no behavioral changes were observed.

**Study no.:** 86-10

**Conducting laboratory and location:** Janssen, Aubervilliers, France

**Date of study initiation:** June 2, 1986

**GLP compliance:** no

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

**Drug, lot #, and % purity:** R67555, batch V8510-201 in — Tween

**Methods** Four female white rabbits were given oral doses of R67555 at doses of 0(n=1) or 40 mg/kg (n=3) for 5 days. The only data provided was weight gain. No behavioral effects were reported.

Summary of weight changes

animal	Body weight gain
Control	40 g
#720 treated	-305 g
#721 treated	-45 g
#723 treated	-160 g

**Study title:** *Oral chronic toxicity study in Wistar rats: repeated dosage for 6 months. Oral administration in the diet.*

**Key study findings:** Three HD f died ahead of schedule. Ptosis was reported for all HD animals. Findings were consistent with other studies in that decreases in HCT, Hb and rbc were seen in the MD and HD of both sexes as well as the usual clinical chemistry findings of increased serum potassium, decreased total protein, albumin, triglycerides, cholesterol and phospholipids. Blood urea nitrogen was increased at MD and HD while creatinine was decreased at the HD. AST and ALT were inconsistently increased. Organ weight and pathology effects were consistent with other studies also. Lung, spleen and heart weights were increased as were pancreas, kidney, brain and adrenal. Absolute gonad weight was decreased at the HD of both groups. Gross observations included swollen adrenals (5/20HD), white foci in the lungs(20/20HD), edematous testes (4/20 HD), soft testes (2/20 HD) swollen testes (1/20HD) and small testes (3/20HD). Control incidence for each of these findings was 0/20. Findings for the females were swollen adrenal glands (10/20 MD, 18/20 HD) white foci on the lungs (10/20 MD, 7/20HD), swollen lungs (15/20HD). The reproductive tract of the females was described as looking "more resting", that is senescent, as characterized by fewer corpora lutea, more atretic follicles, decreased uterine glandular development and thinning of the vaginal epithelium.

**Study no.:** N64890

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

**Date of study initiation:** September 8, 1987

**GLP compliance:** statement included

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

**Drug, lot #, and % purity:** R67555, A0111

**Methods**

R67555 was given to Wistar rats ( 20/sex/group) at doses of 0, 10, 40 and 160 mg/kg for 6 months. Parameters measured included signs, ophthalmoscopy (during and at the end of the study), body weight, food consumption, hematology, clinical chemistry, urinalysis ( prior to euthanasia) from individual samples collected during 16 hours in metabolism cages. At time of euthanasia, organ weights, gross pathology and histopathology were assessed. A standard list of tissues was provided.

## Results

Analysis provided of test article in the diet is summarized below:

Intended dose mg/kg	Actual dose mg/kg
10	8.67(males) 9.5 (females)
40	34.4 (males) 37.7(females)
160	141(males) 154 (females)

There were times when consumption of the test article was estimated to fall outside  $\pm 10\%$  of target levels.

Unscheduled mortality was seen in the female HD group. Three out of 20 females died during the course of the study.

- 1 died after blood sampling week 26. Focal lung lesions, swollen adrenals and hemorrhagic lymph nodes were reported
- 1 died during week 15 with unspecified lung lesions present
- 1 died week 7. Focal lung lesions, swollen adrenals and lymph nodes were reported

Ptosis was reported as occurring in all HD males and females. No other signs were reported. MD and HD males and HD females gained less weight than did the control groups. The difference in weight gain in the HD groups was apparent from the first week. The difference in the male MD group was apparent from week 12. The MD males gained on average 4.6% less than the control while the HDm gained on average 40% ( $p < 0.001$ ) less. HD females gained on average 33% ( $p < 0.001$ ) less weight than the control group. Food consumption was lower in the HD groups from the first week and remained lower until the end of the study.

Decreases in HCT, Hb and rbc were reported for both sexes at the MD and HD.

Parameter		Dosage group ( mg / kg )							
		Control	Males			Females			
			10	40	160	Control	10	40	160
HCT: Haematocrit	%	42.1	42.5	39.5 *	38.1 ***	40.6	41.2	38.6 ***	33.6 ***
HGB: Haemoglobin	g/dl	14.4	14.5	13.6 *	13.7 **	13.9	14.2	13.5 **	12.5 ***
RBC: R.B.C.	10E6/mm3	8.88	8.88	8.20 *	8.12 ***	8.04	8.04	7.64 ***	7.13 ***

Serum potassium levels were increased in both sexes at all doses. Albumin and total protein were decreased, primarily at the MD and HD. Serum triglycerides, phospholipids and cholesterol were decreased at the MD and HD of both sexes. Blood urea nitrogen was increased in all drug-treated groups while creatinine was decreased at the MD and HD. AST and ALT were inconsistently increased.

Parameter	Unit	Dosage group ( mg / kg )							
		Control	Males			Females			
			10	40	160	Control	10	40	160
SOD: Sodium	mEq/l	145	145	144	145	143	144	144	145 *
POT: Potassium	mEq/l	5.0	5.1	5.3 ***	5.8 ***	4.3	4.6	5.0 ***	5.4 ***
CHL: Chloride	mEq/l	103	104	104	106 ***	104	105 *	105	105
CAL: Calcium	mg%	10.6	10.6	10.6	9.7 ***	10.4	10.4	10.0 **	9.2 ***
INP: Inorg. phosphate	mg%	6.3	5.8	6.3	6.2	5.1	5.4	5.1	5.7
TOP: Total protein	g%	6.9	6.8	6.7 **	5.5 ***	6.9	6.7 *	6.6 **	5.5 ***
ALB: Albumin	g%	3.7	3.6	3.6	3.1 ***	4.2	4.0 **	3.9 ***	3.3 ***
HAP: Haptoglobin	mg%	63	68	67	88 *	24	21	38 **	40 *
GLU: Glucose	mg%	151	157	148	131 **	145	152	145	118 ***
CHO: Cholesterol	mg%	104	101	84 **	33 ***	105	101	76 ***	17 ***
TGL: Triglycerides	mg%	135	124	109	42 ***	103	125	140	45 ***
PLP: Phospholipids	mg%	168	165	151 *	86 ***	203	201	181 *	60 ***
BUN: Blood urea nitrog.	mg%	18.0	20.0 **	18.5	23.3 ***	17.0	19.2 *	18.2	23.8 ***
CRS: Creatinine	mg%	0.62	0.63	0.59 *	0.53 ***	0.63	0.63	0.58 ***	0.50 ***
BIL: Total Bilirubin	mg%	0.13	0.12	0.13	0.13	0.13	0.12	0.13	0.13
ALP: Alkal. phosphatase	U/l	140	127	148	173	96	92	99	95
AST: Aspartate aminotr.	U/l	111	93	112	221 ***	127	155	180 *	151 *
ALT: Alanine aminotran.	U/l	78	69	85	201 ***	83	102	130 **	94
CHE: Cholinesterase	ku/l	0.3	0.3	0.3	0.4	2.3	2.2	1.8 **	0.8 ***

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

Urinary creatinine was decreased at the MD and HD for males and the HD for females.

Males	Dosage mg/kg
-------	--------------

	0	10	40	160
Urinary creatinine mg%	169	163	143*	133***
Females				
Urinary creatinine mg%	138	136	148	105**

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

Lung weight in both sexes was increased at the HD. Spleen and heart weights were increased in all drug-treated groups. Normalized pancreas weight was increased in the HD groups as was kidney and brain weight. Absolute and normalized adrenal weight was increased in all drug-treated female groups and MD and HD males. Absolute gonad weight was decreased at the HD in both sexes.

Parameter		Dosage group ( mg / kg )							
		Control	Males			Females			
			10	40	160	Control	10	40	160
WGT: Body weight	g	565	568	545	409 ***	319	310	316	254 ***
LNG: Lungs	mg	2423	2550	2472	2786 *	1747	1677	1830	2894 ***
	mg/kg	4293	4487	4555	6854 ***	5505	5440	5818	11438 ***
SPL: Spleen	mg	1074	1135	1381 ***	1174 *	763	860 *	1014 ***	1145 ***
	mg/kg	1907	2002	2578 ***	2894 ***	2398	2762 **	3216 ***	4539 ***
LIV: Liver	mg	18196	17993	17131	12884 ***	10013	9900	11014 *	9462
	mg/kg	32114	31682	31440	31543	31460	31902	34837 ***	37284 ***
HRT: Heart	mg	1399	1480	1486 *	1219 ***	970	989	1026 *	916 *
	mg/kg	2481	2604 *	2737 ***	2993 ***	3052	3192	3270 *	3615 ***
PNC: Pancreas	mg	1735	1654	1669	1604	1273	1250	1349	1272
	mg/kg	3068	2916	3086	3950 ***	4011	4062	4301	5026 ***
KDN: Kidneys	mg	3671	3535	3463	2949 ***	2164	2069	2315	2115
	mg/kg	6505	6228 *	6354	7230 ***	6803	6671	7349 *	8349 ***
BRN: Brain	mg	2170	2126	2161	2061 ***	1988	1952	1964	1920 **
	mg/kg	3852	3755	3981	5079 ***	6274	6344	6280	7618 ***
THY: Thymus	mg	241	265	249	173 **	244	238	228	183 **
	mg/kg	427	468	457	418	767	763	727	712
ADR: Adrenals	mg	60	62	65	93 ***	81	89 *	109 ***	183 ***
	mg/kg	106	109	119 *	234 ***	253	289 **	348 ***	725 ***
TYR: Thyroids	mg	34	35	34	28 **	25	26	25	22 *
	mg/kg	61	62	62	69	79	84	81	86
GON: Gonads	mg	3654	3691	3782	2978 ***	177	170	175	139 ***
	mg/kg	6477	6507	6965 *	7394 *	562	554	557	543

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

Gross observations were limited to the HD groups. Swollen adrenals (5/20), white foci in the lungs (20/20), edematous testes (4/20), soft testes (2/20), swollen testes (1/20) and small testes (3/20) were reported for the males. Control incidence for each of these was 0/20. Findings for females are summarized below.

Gross observation	Dosage group mg/kg			
	0	10	40	160
Swollen adrenal	0/20	0/20	10/20**	18/20**
Lung: white foci	0/20	0/20	10/20*	7/20*
Lung: swollen	0/20	0/20	0/20	15/20***
Uterus: small	0/20	0/20	0/20	5/20
Uterus: swollen, watery content	8/20	2/20	5/20	1/20*

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

In females at 40 mg/kg and in both males and females at 160 mg/kg adrenal histopathology changes were reported as swollen cortical cells of the zona reticularis and fasciculata. The zona reticularis was also reported to show sinusoidal ectasia with congestion in this area and pigment (unspecified) deposition. The zona glomerulosa was small due to compression.

Lung lesions were described as an increase in foamy cells sometimes with chronic inflammatory reactions present ( MD and HDf and HDm).

Genital tract changes for the HD females were reported as a “more resting aspect” characterized by a lower number of corpora lutea, an increase of atretic follicles, reduced development of the uterine glandular system and decreased thickness of the vaginal epithelium. Some of the HD males were described as having testicular degenerative changes resulting in low spermatozoa (visually discerned?) and possibly the presence of cellular debris in the ductules of the epididymus.

The sponsor reports a dose-dependent increase of rbc in the spleen in all treated groups of both sexes compared to the control groups. One MD male was reported as having leukemia of the spleen, lung and lymph nodes. One HD male had a thymoma.

Appears This Way  
On Original

*****				
Males				
Organ or tissue - observation	Dosage group ( mg / kg )			
	Control	10	40	160
Accessory male sex glands				
- focally round cells	0.20	-	-	0.15
- subacute inflammation	0.40	-	-	0.50
Adrenal, cortex				
- ectasia of sinoidal spaces	0.00	0.00	0.00	0.20 *
- extracapsular cortical tissue	0.35	0.10	0.10	0.20
- pigmentation (zona reticularis)	0.00	0.00	0.00	0.15
- swollen cortical cells	0.00	0.00	0.00	0.90 ***
Epididymis				
- cellular debris in ductules	0.00	0.00	0.00	0.30 *
- focally round cells	0.10	0.00	0.05	0.10
- low spermatozoa amount	0.00	0.00	0.00	0.85 *
Lung				
- blood aspiration	0.00	0.15	0.00	0.00
- chronic inflammatory reaction	0.00	0.00	0.00	0.65 ***
- foamy cells	0.05	0.15	0.15	3.05 ***
- focally leucocytes	0.00	0.05	0.00	0.00
Lymph node(s), bronchial				
- erythrophagocytosis	0.00	-	0.06	0.00
- pigmented macrophages	0.00	-	0.00	0.15
Lymph node(s), mesenteric				
- cystic	0.00	0.05	0.00	0.00
- erythrophagocytosis	0.00	0.00	0.05	0.00
- histiocytic reaction	0.25	0.10	0.16	1.35 ***

\*\*\*\*\*

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

Appears This Way  
On Original

Organ or tissue - observation	Males			
	Control	10	40	160
Mammary gland				
- pigmentation	0.00	-	-	0.05
Pancreas, exocrine				
- focally inclusions with halo	0.00	-	-	0.05
- focally round cells	0.05	-	-	0.10
Spleen				
- blood in the red pulp	0.75	1.70 ***	2.30 ***	2.85 ***
- hyperplasia of red pulp	0.10	0.00	0.05	0.05
Testis				
- degenerated tubules	0.05	0.00	0.00	1.10 *
- mineralized tubules	0.00	0.00	0.05	0.00
Thymus				
- involution	1.21	-	-	0.74
Thyroid gland				
- light cell hyperplasia	0.05	-	-	0.00
Trachea				
- focally round cells	0.05	-	-	0.06
Urinary bladder				
- inspissated hyaline material	0.15	0.40	0.10	0.65 *

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

Appears This Way  
On Original

=====  F e m a l e s				
Organ or tissue - observation	Dosage group ( mg / kg )			
	Control	10	40	160
Adrenal, cortex				
- autolysis	0.00	0.00	0.00	0.45
- congested	0.00	0.00	0.00	1.90 ***
- ectasia of sinusoidal spaces	0.00	0.00	0.30 **	1.95 ***
- extracapsular cortical tissue	0.25	0.10	0.10	0.40
- pigmentation (zona reticularis)	0.00	0.00	0.00	1.30 ***
- small zona glomerulosa	0.00	0.00	0.00	0.80 ***
- swollen cortical cells	0.40	0.35	0.75 *	1.15 ***
- vacuolated cells, focally	0.05	0.05	0.00	0.00
Adrenal, medulla				
- autolysis	0.00	-	-	0.47
- medullary hyperplasia	0.00	-	-	0.05
Bone, rib				
- autolysis	0.00	-	-	0.11
Bone, tibia - femoro-tibial joint				
- autolysis	0.00	-	-	0.25
- congested	0.00	-	-	0.10
Diaphragm				
- autolysis	0.00	-	-	0.10
Esophagus				
- autolysis	0.00	-	-	0.15
Eye				
- autolysis	0.00	-	-	0.25
Heart				
- autolysis	0.00	-	-	0.15
- focal endocardial fibrosis	0.00	-	-	0.05
Kidney				
- autolysis	0.00	-	-	0.30
- basophilic tubules	0.05	-	-	0.05
- congested	0.00	-	-	0.05
- cyst	0.00	-	-	0.05
- focally round cells	0.10	-	-	0.00
- hyaline casts	0.15	-	-	0.10
- minerals (pelvis)	0.60	-	-	0.10 *
- parasites (pelvis)	0.00	-	-	0.05
Large intestine, colon				
- autolysis	0.00	-	-	0.45
- parasite in lumen	0.05	-	-	0.10
Liver				
- autolysis	0.00	-	-	0.10
- bile duct proliferation	0.00	-	-	0.15
- centrilobular swelling	0.00	-	-	0.05
- congested	0.00	-	-	0.05
- granulomatous inflammation	0.00	-	-	0.05
Lung				
- autolysis	0.00	0.00	0.00	0.20

=====|

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	10	40	160
Lung				
- chronic inflammatory reaction	0.00	0.00	0.05	0.00
- foamy cells	0.00	0.00	1.05 ***	3.05 ***
- focally leucocytes	0.05	0.00	0.00	0.00
- thick vascular wall	0.00	0.00	0.00	0.05
Lymph node(s), bronchial				
- autolysis	0.00	0.00	0.00	0.16
- chronic inflammation	0.00	0.00	0.05	0.53 ***
- erythrophagocytosis	0.00	0.06	0.00	0.11
- hyperplasia	0.07	0.00	0.00	0.00
- pigmented macrophages	0.00	0.00	0.11	0.11
Lymph node(s), mesenteric				
- autolysis	0.00	0.00	0.00	0.16
- chronic inflammation	0.00	0.00	0.00	0.05
- histiocytic reaction	0.11	0.05	0.20	0.74 **
Mammary gland				
- atrophy	0.00	-	-	0.30
- autolysis	0.00	-	-	0.15
- glandular development	1.05	-	-	1.20
Ovary				
- atretic follicles	0.65	0.80	0.75	0.95 *
- autolysis	0.00	0.00	0.00	0.20
- clear interstitial tissue	0.20	0.10	0.10	0.00
- corpora lutea	2.80	2.65	2.55	1.55 ***
- cystic	0.15	0.05	0.05	0.00
- hemorrhagic follicle	0.00	0.05	0.00	0.00
- tertiary follicles	1.05	1.10	1.00	1.05

===== Females =====				
Organ or tissue - observation	Dosage group ( mg / kg )			
	Control	10	40	160
Spleen				
- autolysis	0.00	0.00	0.00	0.10
- blood in the red pulp	0.25	1.70 ***	2.10 ***	2.90 ***
- prominent pigmentation	0.05	0.00	0.00	0.00

Appears This Way  
On Original

**Study title:** *Twelve-month oral toxicity study in SPF Wistar rats. Administration through the diet.*

**Key study findings:** For the most part the findings in this study were consistent with other rodent studies. The spleen, lungs, liver and reproductive tract were again shown to be affected by drug administration. The hematology findings included increased neutrophils and the decreased lymphocytes in both sexes at all points of determination. This is consistent with adrenal hyperactivity.

**Study no.:** N79297

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

**Date of study initiation:** April 20, 1989

**GLP compliance:** statement included

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

**Drug, lot #, and % purity:** R67555 batch PFA101

Vehicle  $\beta$ CD 880 mg/kg bw/day

LD 5 mg R67555/kg bw and 55 mg  $\beta$ CD/kg bw

MD 20 mg R67555/kgbw and 220 mg  $\beta$ CD/kgbw

HD 80 mg R67555/kgbw and 880 mg  $\beta$ CD/kgbw

#### **Methods**

The drug was administered in the diet as a coprecipitate with  $\beta$ CD continuously for 12 months. Observations were made for signs, mortality, ophthalmoscopy (months 6, 9 and 12), body weight(weekly), food consumption (weekly), hematology, clinical chemistry, urinalysis (month 6: 10/sex/group; month 9: 10/sex/group, terminal: all surviving animals). At time of euthanasia, organ weights were determined and a standard list of tissues was collected for histopath analysis.

#### **Results**

Unscheduled mortality was seen as summarized in the following table.

Dose group mg/kg	males	Females
Control(0)	1	1
Vehicle	0	0
5	0	1
20	0	0
80	5	2

Findings in the HD males who died prematurely included: multiple white foci in the lung, weight loss, tissue mass (psoas muscle), high serum potassium ( 3/5), a left shift in the WBC differential, soft, small, edematous testes(2/5). HD females had findings of pale lungs (2/2), dilated urinary bladder (1/2), grit in the kidney (1/2), urinary calculi (1/2).

The only clinical sign reported was ptosis, identified in the HD groups only (7/20 males, 12/20 females).

Body weight gain was lower for HD animals of both sexes from week 1. This was maintained to the end of the study. The MD females also gained less weight than the controls, apparent from week 22.

#### Summary of differences in body weight gain

	HD males	MD females	HD females
Difference in weight gain vs veh control	157g *** 37%	26g* 12%	93g*** 44%

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

Food consumption was significantly decreased in the HD groups also and to some extent in the MD females as well. There were several occasions when test article intake was outside  $\pm 10\%$  of the target value for each dose level.

It was reported that there were no significant ophthalmoscopic findings although there was a statement that ocular changes were identified in the HD females necessitating the examination of the remainder of the group. An ophthalmologist's report could not be located. There were single animal listings.

The hematology data for week 26 showed some slight decreases in HCT, RBC, and Hb that were statistically significant but of little if any biological significance. An increase in segmented neutrophils and a decrease in lymphocytes were potentially significant as these were maintained to the end of the study. Data for the females showed a more striking, dose-related decrease in HCT, Hb and RBC. As seen in males, the females also showed an increase in segmented neutrophils and a decrease in lymphocytes.

Parameter		Dosage group ( mg / kg )				
		Control	Placebo	5	20	80
HCT: Haematocrit	%	44.4	44.7	42.9	41.6	38.5 ***
HGB: Haemoglobin	g/dl	15.1	15.1	14.4	14.1	12.8 ***
RBC: R.B.C.	10 <sup>6</sup> /mm <sup>3</sup>	8.31	8.27	7.95	7.77 *	7.11 ***

Week 39 data for the males showed some variability and little consistent pattern. The data for the females showed essentially the same pattern as seen at 26 weeks. An increase in segmented neutrophils and a decrease in lymphocytes was beginning to emerge. By week 52, there were few findings of toxicological significance in the data for the males. There was however an increase in segmented neutrophils and a decrease in lymphocytes. Significant decreases in HCT, RBC and Hb were maintained primarily in the HD females. There was also an increase in segmented neutrophils and a decrease in lymphocytes.

The week 26 clinical chemistry data for males showed that the HD group had increases in sodium and potassium. Total protein, albumin, cholesterol, triglycerides, phospholipids and creatinine were decreased. Alkaline phosphatase, AST and ALT were increased. This was maintained at the week 39 and week 52 determinations.

Week 26 data for the females showed an increase in potassium for the HD group, a decrease in calcium for MD and HD, and a dose-related increase in inorganic phosphate.

Total protein, albumin, cholesterol, triglycerides, phospholipids and creatinine were decreased. Alkaline phosphatase and ALT were increased at the HD. This was maintained in the week 39 data. This was maintained in the week 52 determinations. Blood urea nitrogen was also increased. The sponsor's summary of the week 52 data is shown below as an example of the changes.

Appears This Way  
On Original

Parameter		Dosage group ( mg / kg )				
		Control	Placebo	5	20	80
SOD: Sodium	mEq/l	142	143	143	143	143
POT: Potassium	mEq/l	4.0	3.9	4.1	4.4 **	5.3 ***
CHL: Chloride	mEq/l	101	102	102	102	106 ***
CAL: Calcium	mg%	11.3	11.2	11.3	10.7 **	9.5 ***
INP: Inorg. phosphate	mg%	5.5	5.1	5.7	5.4	6.5 *
TOP: Total protein	g%	7.3	7.2	7.2	6.7 ***	5.9 ***
ALB: Albumin	g%	4.5	4.5	4.4	4.2 *	3.6 ***
HAP: Haptoglobin	mg%	36	22	23	44	49
GLU: Glucose	mg%	138	142	140	133	117 ***
CHO: Cholesterol	mg%	228	157	130	79 ***	25 ***
TGL: Triglycerides	mg%	807	350 *	445	426	108 ***
PLP: Phospholipids	mg%	387	298	283	229 **	86 ***
BUN: Blood urea nitrog.	mg%	17.5	16.4	19.1	19.5	25.7 ***
CRS: Creatinine	mg%	0.59	0.60	0.59	0.56 *	0.53 **
BIL: Total Bilirubin	mg%	0.14	0.12	0.09	0.15	0.15
ALP: Alkal. phosphatase	U/l	85	81	78	77	117 **
AST: Aspartate aminotr.	U/l	149	236	115	213	203 **
ALT: Alanine aminotran.	U/l	83	153 *	69	142 **	119 **
CHE: Cholinesterase	KU/l	2.4	2.4	2.4	2.0 *	1.1 ***

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

There were no findings of toxicological significance in the urinalysis data as presented. The organ weight data was consistent with previous studies in that lung, spleen and heart weight were increased as was pancreas, adrenal and kidney weight. Absolute gonad weight was decreased.

Appears This Way  
On Original

Parameter		Dosage group ( mg / kg )				
		Control	Placebo	Males		
				5	20	80
WGT: Body weight	g	587	599	574	575	433 ***
LNG: Lungs	mg	2510	2482	2351	2343	4625 ***
	mg/kg	4286	4162	4106 *	4085	10786 ***
SPL: Spleen	mg	1073	1163	1235 ***	1447 ***	1763 ***
	mg/kg	1844	1947	2158 ***	2535 ***	4153 ***
LIV: Liver	mg	19586	18791	18848	19364	14141 ***
	mg/kg	33414	31489	32924	33786	32640
HRT: Heart	mg	1555	1542	1549	1581	1379 **
	mg/kg	2650	2579	2710	2758	3201 ***
PNC: Pancreas	mg	1639	1726	1531	1603	1531
	mg/kg	2812	2878	2679	2798	3553 ***
KDN: Kidneys	mg	3544	3579	3554	3700	2991 ***
	mg/kg	6032	6002	6207	6467 *	6931 ***
BRN: Brain	mg	2200	2242	2149	2229	2123 *
	mg/kg	3772	3769	3757	3898	4925 ***
TRY: Thymus	mg	233	234	235	222	173 ***
	mg/kg	399	387	409	388	404
ADR: Adrenals	mg	57	61	61	60	102 ***
	mg/kg	98	101	106	104	236 ***
TYR: Thyroids	mg	32	33	32	32	26 ***
	mg/kg	55	54	56	56	60
GON: Gonads	mg	3676	3804	3615	3742	2857 ***
	mg/kg	6290	6399	6302	6547	6650

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

The same pattern was seen in the females.

Appears This Way  
On Original

Parameter		Dosage group ( mg / kg )				
		Control	Placebo	Females		
				5	20	80
MGT: Body weight	g	352	348	351	321 *	252 ***
LNG: Lungs	mg	1778	1746	1724	1819	4310 ***
	mg/kg	5097	5075	4929	5770	17239 ***
SPL: Spleen	mg	779	774	877	1023 ***	1157 ***
	mg/kg	2203	2233	2495 *	3218 ***	4623 ***
LIV: Liver	mg	12811	11470	11861	11208	9485 ***
	mg/kg	36721	33126	33853	35137	37666
HRT: Heart	mg	1117	1101	1117	1089	1050 **
	mg/kg	3198	3202	3202	3422	3985 ***
PNC: Pancreas	mg	1193	1250	1207	1265	1274
	mg/kg	3417	3632	3456	4007 *	5065 ***
KDN: Kidneys	mg	2463	2350	2382	2233 **	2154 **
	mg/kg	7064	6824	6832	7017	8567 ***
BRN: Brain	mg	2033	2017	2058	2021	1989 *
	mg/kg	5862	5915	5913	6394 *	7933 ***
THY: Thymus	mg	218	208	221	187	121 ***
	mg/kg	629	603	633	584	479 **
ADR: Adrenals	mg	76	79	80	106 ***	369 ***
	mg/kg	217	230	229	332 ***	1500 ***
TYR: Thyroids	mg	25	25	23	23 *	17 ***
	mg/kg	73	73	66	71	69
GON: Gonads	mg	181	171	177	163	147 **
	mg/kg	517	499	506	515	585 *

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

The sponsor again uses a histology reporting method that is difficult to extract incidence information from. However, the summary contained an interesting statement.

Appears This Way  
On Original

Most of the high dosed rats that died or were sacrificed in extremis during the study, showed moderate to severe changes of the lungs, adrenals, urinary tract and testes.

No  $\beta$ -cyclodextrin (880 mg/kg) effects were noted in the vehicle groups.

#### Conclusion

R 67555 did not produce relevant macroscopic changes in male and female rats when dosed at 5 mg/kg body weight/day.

At 20 and 80 mg/kg body weight/day we retain as drug and dose related macroscopic changes:

- swollen adrenals in males at 80 mg/kg and in females at 20 and 80 mg/kg
- lung changes in males at 80 mg/kg and in females at 20 and 80 mg/kg
- testicular changes in males at 80 mg/kg
- spleen changes in females at 80 mg/kg.

No relevant macroscopic changes were noted in the  $\beta$ -cyclodextrin vehicle group (880 mg/kg body weight/day).

The incidence of swollen adrenal glands was listed as 0/20 for all the male groups except the HD (14/20). Pale lungs and swollen lungs each had a reported incidence of 0/20 for all groups except the HD (17/20 for both findings). The summary of testicular findings is shown below.

! Testis : edematous	! 0/20	0/20	0/20	0/20	1/20	!
! Testis : small	! 0/20	0/20	1/20	0/20	7/20*	!
! Testis : soft	! 0/20	0/20	0/20	0/20	8/20**	!
! Thoracic fluid : increase	! 0/20	0/20	0/20	0/20	1/20	!

=====!

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

The incidence of swollen adrenals in the females was 0/20(control), 1/20 (veh), 0/20(LD), 13/20(MD) and 19/20(HD).

Incidence of gross necropsy findings in females

	Dosage group mg/kg				
	0	veh	5	20	80
Lung, pale foci	0/20	0/20	0/20	1/20	0/20
Lung, white foci	0/20	0/20	0/20	6/20	0/20
Lung, pale	0/20	0/20	0/20	2/20	20/20

Lung swollen	0/20	0/20	0/20	1/20	18/20
Uterus small	0/20	0/20	0/20	0/20	3/20

In females, the adrenal cortical histopathology as reported was consistent with previous studies. Findings included swollen cortical cells, ectasia of sinusoidal spaces, vacuolation and nodular hyperplasia.

#### Conclusion

Histological changes due to the administration of R 67555 were:

- an increase of red blood cells in the red pulp of the spleen noted in the 5, 20 and 80 mg/kg dosed males and females
- a decrease of chronic kidney disease in males and females dosed at 80 mg/kg
- the presence of foamy cells in the lungs with sometimes chronic inflammatory reaction was noted in the 20 and 80 mg/kg dosed males and females. No prominent differences were observed between the 5 mg/kg dosed, the vehicle dosed and the control groups
- an increase of erythrophagocytosis with red blood cells in the sinuses and histiocytosis in the mesenteric lymph nodes in the 80 mg/kg dosed females. No prominent differences were observed between the 80 mg/kg dosed males and the 20 and 5 mg/kg dosed, the vehicle dosed and the control males and females
- a disturbance of the adrenal cortex morphology as evidenced by the presence of swollen cortical cells in males dosed at 20 mg/kg, by the presence of swollen cortical cells, ectasia of sinusoidal spaces and congestion in males and females dosed at 80 mg/kg and by the presence of vacuolated zona fasciculata cells, pigmented zona reticularis, small zona glomerulosa and nodular hyperplasia in the females dosed at 80 mg/kg. The 20 mg/kg dosed females and the 5 mg/kg dosed males and females and the vehicle dosed groups were comparable with the control rats
- a decreased activity of the genital tract was noted by changes in the testes (degenerated tubuli and presence of giant cells), in the epididymides (low spermatozoa amount and cellular debris) of 80 mg/kg dosed males and in the ovaries (increase of atretic follicles, decrease of old corpora lutea) and in the uterus (reduced glandular development and granulocyte infiltration) of the 80 mg/kg dosed females. The 5 and 20 mg/kg dosed and the vehicle dosed rats were comparable with the control rats.

**Histological changes related to the administration of  $\beta$ -cyclodextrin were:**

- swollen and vacuolated aspect of the urinary bladder epithelium in the vehicle and 80 mg/kg dosed male and female groups. No prominent differences were observed between the 5 and 20 mg/kg dosed groups and the control groups
- swollen and vacuolated aspect of the renal pelvis epithelium in the vehicle dosed males. No prominent differences were observed between the 5 and 20 mg/kg dosed and the control groups.

The sponsor's link to the ophthalmoscopy results instead linked to clinical observations for the final week of the study. An ophthalmologist's report was not located.

**Study title:** *Chronic toxicity study in Beagle dogs: repeated dosage for 6 months (Administration: orally)*

**Key study findings:** There were few findings of toxicological significance in this study. ECGs, performed at some unspecified time relative to dosing, showed significant increases in PQ interval from week 5 ( first determination after the start of dosing) and increases in QTc from week 11(increases noted in control group as well as dosed). A decrease in heart rate was apparent from week 5. There were no findings of toxicological significance in the hematology, yet the necropsy data showed an increase in splenic weight and an increase in rbc in the splenic red pulp.

**Study no.:** N64891

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

**Date of study initiation:** Sept. 8, 1987

**GLP compliance:** statement included

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

**Drug, lot #, and % purity:** R67555, batch PFA011 in gelatin capsules

#### **Methods**

Beagles, 4/sex/group were given gelatin capsules containing 0, 5, 20 or 80 mg/kg of the test article each day for 6 months. Assessments included mortality, signs, ophthalmoscopy (prior to , during and at the end of the study), heart rate and lead II ECG (prior to the study, periodically during and at the end of the study), body weight, food and water consumption, hematology and clinical chemistry ( prior to, at 2weeks of dosing , monthly thereafter), urinalysis ( collected either by catheterization or use of a metabolism cage), organ weights, gross observations, histopathology for a standard list of tissues.

**Results**

There was no unscheduled mortality. The very few clinical signs reported tended to be isolated in nature and difficult to construe as being drug-related.

We do not know when the ECG data was collected relative to the dosing nor do we know details about the methodology. PQ interval in the dosed animals started at a longer duration than the control animals. The HD group showed a further increase in PQ interval with the onset of dosing. QTc was generated using Bazett's formula. An increase in the QTc was apparent in the dosed animals from week 11. A decrease in heart rate was seen in the dosed groups.

Appears This Way  
On Original

Parameter	Week	Date	Dosage group ( mg / kg )			
			Control	5	20	80
PD m sec	-2	24-08-87	77.3	82.5	85.3 *	82.9
	5	12-10-87	77.4	87.0 *	85.8	91.4 *
	11	30-11-87	72.1	84.5 *	82.3 *	95.0 ***
	26	07-03-88	74.1	81.0	88.8 **	92.1 ***
QRS m sec	-2	24-08-87	28.5	29.9	25.6	27.1
	5	12-10-87	29.9	29.3	30.6	29.5
	11	30-11-87	36.0	40.1	43.4 *	43.5 *
	26	07-03-88	38.9	42.8	42.8	42.6
QT m sec	-2	24-08-87	149.1	151.6	154.0	156.6
	5	12-10-87	156.8	163.0	165.1	170.5
	11	30-11-87	153.6	166.6 *	160.8	178.9 *
	26	07-03-88	155.4	167.5 *	167.9 *	175.1 **
QTc m sec	-2	24-08-87	188.2	202.2	211.0	212.4 *
	5	12-10-87	219.6	202.6	212.2	212.9
	11	30-11-87	198.4	211.1	226.1 **	212.3
	26	07-03-88	205.8	223.2 *	229.6 *	228.2 *
R m volt	-2	24-08-87	1.68	1.92	1.68	2.04
	5	12-10-87	2.02	2.42	2.15	2.54 *
	11	30-11-87	1.45	1.64	1.61	1.78
	26	07-03-88	1.25	1.84 *	1.58	1.54
Rate b.p.m.	-2	24-08-87	97.0	112.4	117.5	116.6
	5	12-10-87	120.8	96.8	100.6	100.9
	11	30-11-87	102.9	100.0	123.3	93.0
	26	07-03-88	105.8	107.9	115.4	106.4

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

Inconsistent with other studies, the HD group showed the greatest weight gain while the MD group lost weight.

Summary of body weight changes in kg (percent difference from control)

Week/day	Dosage group mg/kg			
	0	5	20	80
0/-4	11.7	11.9	12.3	11.7
13/87	12.2	12.4	12.6	12.2
27/185	12.9	13.2	13.1	13.5
Δ from baseline	1.2	1.3 (8%)	0.8 (-33%)	1.8 (50%)

Hematology showed a slight decrease in HCT at the beginning of the study that progressed slightly further by week 2. The significance, if any, of these findings is questionable.

Hemoglobin levels did not show any consistent changes. There were no apparent effects upon RBC count. WBC increased in the HD group from weeks 4 through 17: Control group 13.1-14.0 x 1000/mm<sup>3</sup> vs 14.3-18.9 x 1000/mm<sup>3</sup> HD (p<0.05 by the Mann Whitney U test for the highest WBC count).

Triglycerides showed an increase in drug treated animals at one determination point only.

Alkaline phosphatase in the MD group started at a higher level than in other groups and stayed so for the duration of the study. ALT in the HD group showed a transient increase in week 20.

Total protein was decreased in the HD group at the last 3 points of determination.

Reviewer's summary of clinical chemistry changes

parameter	Dosage group mg/kg			
	0	5	20	80
Triglycerides mg/dl week 20	39	71**	58*	57*
ALT U/l week 12	51	50	52	55
Week 17	46	48	47	45
Week 20	41	43	45	100
Week 26	53	53	49	51
Total protein week 17	6.3	6.2	6.2	6.0*
Week 20	6.4	6.1	6.4	6.0*
Week 26	6.4	6.4	6.3	6.1*

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

Occult blood was found in the urine of the MD and HD groups prior to the start of the study.

RBC (frank blood?) was reported for all the groups prior to the start of the study. Occult blood and frank blood were found in each of the treatment groups throughout the study. No explanation was offered for this finding.

No significant ophthalmoscopy findings were reported. Single animal observation sheets were located but no statement from an ophthalmologist. Who conducted the examinations?

Spleen, heart and adrenal weights were primarily affected but not to the extent seen in the rodent studies.

Parameter		Control	Dosage group ( mg / kg )		
			5	20	80
WGT: Body weight	kg	12.93	13.25	13.10	13.53
LNG: Lungs	g	100	103	114	102
	g / 10 kg	78	79	87 *	75
SPL: Spleen	g	36	43	73	67 **
	g / 10 kg	26	32	55 *	50 **
LIV: Liver	g	292	306	318	313
	g / 10 kg	227	231	242	234
HRT: Heart	g	94	107	110	104
	g / 10 kg	73	81	84 *	77
PNC: Pancreas	g	24	24	26	27 *
	g / 10 kg	19	18	20	20
KDN: Kidneys	g	54	55	57	56
	g / 10 kg	41	41	43	41
BRN: Brain	g	75	77	80	80
	g / 10 kg	59	59	62	60
THY: Thymus	g	12	12	13	17
	g / 10 kg	10	9	10	12
ADR: Adrenals	g	1.288	1.338	1.354	1.410
	g / 10 kg	1.019	1.034	1.049	1.069
TYR: Thyroids	g	1.111	1.166	1.135	1.032
	g / 10 kg	0.842	0.885	0.858	0.756
GON: Gonads m	g	17.3	18.0	18.5	17.5
	g / 10 kg	12.6	12.3	13.3	11.7
GOF: Gonads f	g	0.930	1.319	0.722	0.851
	g / 10 kg	0.771	1.093	0.600	0.713
HYP: Hypophysis	g	0.065	0.070	0.066	0.063
	g / 10 kg	0.050	0.053	0.051	0.048
PRS: Prostate	g	8.25	6.50	6.75	7.25
	g / 10 kg	5.92	4.51	4.87	4.83

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

There is not a great deal of information about the histopathology findings. No adrenal changes were reported. An increase in rbc in the splenic red pulp was seen in all drug-treated groups. Other findings were considered referable to the catheterization procedures.

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

**Key study findings:** The ECG findings were reasonably consistent with those of the 6 month dog study. PQ interval increased at week 40. Heart rate decreased in all groups but to a greater

extent in the drug-treated animals. QTc as determined by Bazett's formula did not show a change. Significant increases in QT were seen in all drug-treated groups at week 40. Spleen weight was increased only in the HD group and increases in rbc in the red pulp were reported only for the HD group.

**Study no.:** N79298

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

**Date of study initiation:** April 4, 1989

**GLP compliance:** statement included

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

**Drug, lot #, and % purity:** R67555, batch PFA101/PFA091, as a solid mixture with BCD in a 1/10 ratio

### Methods

Beagles, 4/sex/group, were given gelatin capsules once a day for 12 months. Capsules contained one of the following treatments:

Control ( undosed)

Vehicle: 440 mg  $\beta$ -CD/kg body weight/day

LD: 2.5 mg R67555/kg bw/day

27.5 mg  $\beta$ -CD/kg bw/day

MD: 10 mg R67555/kg bw/day

110 mg  $\beta$ -CD /kg bw/day

HD: 40 mg R67555/kg bw/day

440 mg  $\beta$ -CD/kg bw/day

Parameters studied included mortality, signs, ophthalmoscopy( prior to, ~6 months and at end of study), ECG and heart rate ( lead II, pre-study, 3,6,9 months and end of study), food consumption, body weight, hematology and clinical chemistry (pre-study, 2 weeks and each month thereafter), urinalysis (catheterized, pre-study, 1,3,6, 9months and end of study), organ weights, gross pathology and histopathology. A standard list of tissues was collected for histopathology.

### Results

No unscheduled mortality was reported. Two clinical signs were reported for the HD group. All dogs at 40 mg/kg were reported as showing a moderate increase in salivation during the entire study period. Slightly softened stools were reported for 7/8 HD dogs during the first 3 weeks of the study. No ophthalmoscopic findings were reported.

Bazett's correction factor was used for QTc. The ECG was fairly consistent with that gathered from the 6-month dog study. PQ interval showed an increase in the vehicle and drug-treated

groups. However, QTc showed no consistent changes and gave no particular signal. Uncorrected QT showed a significant increase in the HD group compared to the control before dosing started. Further increases in HD QT were seen from week 12 through the end of the study. Significant increases in QT were seen in all drug-treated groups at week 40. Heart rate did decrease to some extent in all groups, with the effect progressing to week 40. The sponsor's results are shown below.

Appears This Way  
On Original

Parameter	Week	Date	Dosage group ( mg / kg )				
			Control	Placebo	2.5	10	40
PQ m sec	-3	16-03-89	85.0	83.5	75.1	76.6	71.5 *
	12	26-06-89	82.6	86.1	82.4	83.3	88.9
	24	18-09-89	84.0	85.9	84.4	85.0	86.8
	40	08-01-90	84.4	83.6	95.6	95.6	92.9
	52	02-04-90	80.4	90.1 **	93.4	95.4 *	98.0 **
QRS m sec	-3	16-03-89	35.5	35.4	36.5	36.4	36.6
	12	26-06-89	36.6	38.1	37.0	39.4	35.9
	24	18-09-89	38.5	38.5	37.5	39.1	38.4
	40	08-01-90	38.9	40.8	41.6	38.8	42.5
	52	02-04-90	36.9	35.8	35.4	35.8	38.8
QT m sec	-3	16-03-89	152.0	171.0 *	158.0	167.9	173.0 *
	12	26-06-89	157.1	167.9	165.9	165.1	181.3 **
	24	18-09-89	157.6	163.4	158.6	167.5	188.9 *
	40	08-01-90	153.9	165.0	186.1 **	185.0 **	192.6 **
	52	02-04-90	150.6	156.8	159.3	168.1	183.8 **
QTc m sec	-3	16-03-89	230.4	246.5	233.1	238.0	265.2
	12	26-06-89	239.5	239.4	240.5	238.8	251.9
	24	18-09-89	221.9	228.6	230.9	231.8	242.7
	40	08-01-90	224.6	226.9	227.6	226.0	245.8 *
	52	02-04-90	228.3	223.7	223.6	226.6	242.8
R m volt	-3	16-03-89	1.24	1.38	1.46	1.58	1.71 *
	12	26-06-89	1.58	1.65	2.06	1.95	2.11
	24	18-09-89	1.82	1.79	2.10	1.99	1.94
	40	08-01-90	1.78	1.82	2.08	1.99	2.08
	52	02-04-90	1.79	1.93	2.06	2.25	2.19
Rate b.p.m.	-3	16-03-89	138.8	131.8	135.8	123.4	143.3
	12	26-06-89	141.1	124.6	127.9	129.6	116.5 *
	24	18-09-89	123.6	120.1	129.5	117.1	102.5
	40	08-01-90	131.0	114.9	91.6 **	91.4 **	97.8 *
	52	02-04-90	141.1	124.5	119.4	113.5	105.6 **

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

As seen in the 6 month study, the HD group gained the most weight compared to the control group.

Summary of body weight effects (percent difference from control)

Week/day	Dosage group mg/kg				
	0	veh	2.5	10	40
0/4	10.2	10.2	10.0	10.1	10.0
52/360	13.0	12.4	13.1	12.7	13.2
$\Delta$ from baseline	2.8	2.2 (-21%)	3.1(11%)	2.6 (7%)	3.2 (14%)

There were minor changes in the hematology of questionable significance if any. The serum analysis showed that the HD group started with a slightly higher potassium level than the other groups. This was maintained throughout the study. It is difficult to say whether there was any influence of the drug in this. By week 32, there was a decrease in inorganic phosphate in the treated groups. The effect is not large and given the variability and drift in the data, it is difficult to say that there is a real effect. The same could be said about serum cholesterol.

Appears This Way  
On Original

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

PERIOD ANALYSIS  
 IMP: Inorganic phosphate mg%  
 Mean values recorded at stated week and dose

Week	Dosage group ( mg / kg )				
	Control	Placebo	2.5	10	40
-2	6.9	7.2	6.7	7.1	7.1
0	6.8	6.6	6.6	6.6	6.7
2	7.0	6.8	6.8	6.6	7.2
5	6.9	6.4	6.1	5.9 *	6.2
8	6.1	5.8	5.9	5.6	5.9
12	6.0	5.7	4.9 *	4.9 **	5.7
17	5.7	5.0	4.7	5.3	5.1
20	5.3	4.9	4.1 *	4.6	4.9
24	5.0	5.2	5.0	4.7	5.0
28	4.7	4.7	4.4	4.7	4.6
32	5.4	4.6	4.5 *	4.0 *	4.4 *
36	4.6	4.3	3.9	4.4	4.1
40	5.1	5.0	4.2 *	4.6	4.6
44	4.6	4.0	3.9	4.1	4.3
48	4.3	4.5	4.1	4.1	4.4
52	4.4	4.2	4.0	4.0	4.1

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

There were no consistent or distinct findings in the urinalysis data as presented. Spleen weight was increased in the HD group. There was a dose-related increase in absolute and normalized thyroid weight as well.

Appears This Way  
 On Original

Parameter		Control	Placebo	Dosage group ( mg / kg )		
				2.5	10	40
WGT: Body weight	kg	13.04	12.44	13.11	12.73	13.21
LNG: Lungs	g	101	100	97	97	97
	g / 10 kg	80	80	77	76	75
SPL: Spleen	g	43	26 **	40	34 a	65 aa
	g / 10 kg	34	21 **	32 a	27 aa	48 aaa
LIV: Liver	g	314	279	283	305	315
	g / 10 kg	246	226	219	240	242
HRT: Heart	g	101	98	107	105	111
	g / 10 kg	79	79	84	84	85
PNC: Pancreas	g	24	26	24	23	24
	g / 10 kg	19	21	18	18 a	19
KDN: Kidneys	g	58	54	52	53	64
	g / 10 kg	45	44	41	42	48
BRN: Brain	g	74	72	74	74	77
	g / 10 kg	58	59	59	60	61
THY: Thymus	g	15	12	17	14	18
	g / 10 kg	11	10	13	11	13
ADR: Adrenals	g	1.149	1.163	1.141	1.161	1.142
	g / 10 kg	0.893	0.938	0.917	0.925	0.898
TYR: Thyroids	g	0.810	0.737	0.880	0.864	0.925
	g / 10 kg	0.618	0.592	0.658	0.677	0.692 a
GOM: Gonads m	g	19.0	17.5	17.8	17.5	16.0
	g / 10 kg	13.6	13.1	12.3	13.3	11.3
GOF: Gonads f	g	0.901	1.124	0.735	0.614 *	1.313
	g / 10 kg	0.805	1.032	0.677	0.531	1.057
HYP: Hypophysis	g	0.059	0.060	0.058	0.062	0.072
	g / 10 kg	0.046	0.047	0.047	0.048	0.057
PRS: Prostate	g	9.75	10.25	10.25	8.50	10.60
	g / 10 kg	6.71	8.08	7.39	6.57	7.59

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

The only pertinent gross observation was that of swollen spleen. The incidence was reported as 0/8 for control, vehicle and MD groups, 1/8 (LD) and 4/8 (HD). The only drug-associated histological sign reported was an increase in rbc in the splenic red pulp of the HD group animals.



**Conducting laboratory and location:** Dept of Toxicology, Janssen, Beerse, Belgium

**Date of study initiation:** unknown. Signed June 1988

**GLP compliance:** statement included

**QA reports:** yes ( ) no ( )

**Drug, lot #, and % purity:** R67555 batch pfa011, purity —

Positive controls were sodium azide, 2-nitrofluorene and 2-aminoanthracene

**Methods** The Ames reverse mutation assay was conducted in triplicate using tester strains TA1535, TA1538, TA97, TA98 and TA100  $\pm$ S9 activation. A preliminary study used concentrations of 5, 10, 25, 75, 100, 200, 300, 400 and 500  $\mu$ g per plate  $\pm$ S9. Concentrations of 5, 10, 25, 50, 100, 250 and 300  $\mu$ g per plate  $\pm$  S9 were used in the more extensive assay.

**Results** Thinning of the bacterial lawn was seen at concentrations  $\geq$ 75  $\mu$ g  $\pm$ S9. At concentrations  $\geq$ 100 mg per plate, toxicity was manifested as pinpoint colonies or absence of bacterial background both  $\pm$ S9. It was thus decided to limit the concentrations tested in the main assay to  $\leq$ 300  $\mu$ g per plate. The preliminary assay was conducted only in TA100.

A mild increase in revertants was seen in TA97 without S9. This slight effect was repeated.

Appears This Way  
On Original

First study : in the absence of a metabolic activation system

			Number of His <sup>+</sup> colonies / plate														
Dosage group	ug/plate	Solvent	TA 97			TA 98			TA 1538			TA 100			TA 1535		
			Indiv. count	Mean	S.D.	Indiv. count	Mean	S.D.	Indiv. count	Mean	S.D.	Indiv. count	Mean	S.D.	Indiv. count	Mean	S.D.
Control	0.00	DMSO	87			12			8			155			10		
			70	76	9.5	10	11	1.2	6	6	1.5	136	139	15.2	16	12	3.2
			71			10			5			125			11		
R 67555	5.00	DMSO	84			13			9			144			11		
			77	77	7.5	12	13	0.6	7	7	2.0	137	129	20.2	11	12	1.7
			69			13			5			106			14		
R 67555	10.00	DMSO	74			13			3			119			10		
			74	79	9.2	11	11	1.5	5	5	1.5	182	137	39.6	2	6	4.0
			90			10			6			109			7		
R 67555	25.00	DMSO	79			7			4			128			14		
			84	95	22.9	10	12	5.7	4	5	1.2	153	135	15.7	14	12	4.0
			121			18			6			124			7		
R 67555	50.00	DMSO	65 *			17 *			6 *			104 *			9 *		
			71 *	88	34.8	9 *	12	4.6	7 *	8	3.2	131 *	130	25.0	9 *	10	1.2
			128 *			9 *			12 *			154 *			11 *		
R 67555	100.00	DMSO	43 @			@			1 @			47 @			1 @		
			71 @	59	14.3	@	-	-	4 @	5	4.0	56 @	47	9.5	4 @	6	6.2
			62 @			@			9 @			37 @			13 @		
R 67555	250.00	DMSO	T			@T			T			T			T		
			T	-	-	@T	-	-	T	-	-	T	-	-	T	-	-
			T			@T			T			T			T		
R 67555	300.00	DMSO	T			T			T			T			T		
			T	-	-	T	-	-	T	-	-	T	-	-	T	-	-
			T			T			T			T			T		
2-nitrofluor ene	5.00	DMSO	592			1426			1702								
			480	493	92.7	1360	1398	34.1	2384	1794	549.8						
			408			1408			1296								
sodiumazide	1.00	Water										688			1056		
												608	629	51.4	656	757	263.1
												592			560		

C: Contamination, P: Precipitation, \*: Thinning of background lawn, T: Toxic, @: Pinpoints

Repeated study : in the absence of a metabolic activation system

			Number of His+ colonies / plate														
Dosage group	ug/plate	Solvent	TA 97			TA 98			TA 1538			TA 100			TA 1535		
			Indiv. count	Mean	S.D.	Indiv. count	Mean	S.D.	Indiv. count	Mean	S.D.	Indiv. count	Mean	S.D.	Indiv. count	Mean	S.D.
			Control	0.00	DMSO	115 145 123	128	15.5	13 12 20	15	4.4	11 15 6	11	4.5	129 145 141	138	8.3
R 67555	5.00	DMSO	126 176 174	159	28.3	19 16 10	15	4.6	9 6 6	7	1.7	176 167 151	165	12.7	21 29 32	27	5.7
R 67555	10.00	DMSO	156 135 170	154	17.6	10 10 12	11	1.2	6 10 6	7	2.3	175 183 137	165	24.6	29 20 30	26	5.5
R 67555	25.00	DMSO	172 117 170	153	31.2	10 16 11	12	3.2	2 10 4	5	4.2	153 121 171	148	25.3	32 21 23	25	5.9
R 67555	50.00	DMSO	137 * 181 * 168 *	169	27.6	12 * 10 * 18 *	13	4.2	1 * 6 * 7 *	5	3.2	146 * 137 * 136 *	140	5.5	17 * 24 * 27 *	23	5.1
R 67555	100.00	DMSO	64 @ 40 @ 37 @	47	14.8	1 @ 3 @ 1 @	2	1.2	@ @ @	-	-	52 @ 72 @ 63 @	62	10.0	3 * 4 * 5 *	4	1.0
R 67555	250.00	DMSO	T T T	-	-	T T T	-	-	T T T	-	-	T T T	-	-	T T T	-	-
R 67555	300.00	DMSO	T T T	-	-	T T T	-	-	T T T	-	-	T T T	-	-	T T T	-	-
2-nitrofluor ene	5.00	DMSO	1040 944 752	912	146.6	600 680 600	627	46.2	1968 1572 1536	1692	239.7						
sodiumazide	1.00	Water										464 672 720	619	136.1	768 936 808	837	87.8

C: Contamination, P: Precipitation, \*: thinning of background lawn, T: Toxic, @: Pinpoints

The values were outside the historical mean for the strain but within the historical range.

Appears This Way  
On Original

Cumulative historical control data on spontaneous and solvent control revertants in the Ames reverse mutation test with *Salmonella typhimurium*

	Spontaneous revertants	Solvent control revertants				
		DMSO		Water		
		-S9	+S9	-S9	+S9	
TA97	Number of plates	87	77	66	6	6
	Total revertants	10774	10163	9096	945	865
	Mean rev./plate	123.8	134.0	137.4	157.5	134.2
	Range of revertants	70-180	70-195	73-201	133-173	107-162

The study is adequate.

**Study title:** *Bacterial reverse mutation assay*

**Key findings:** Under the conditions of the assay, the test article did not produce an increase in revertants either with or without metabolic activation.

**Study no.:** R067555-5256

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** September 11, 2001

**GLP compliance:** statement included

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

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

**Methods** The test article was tested in *Salmonella* strains TA98, TA100, TA1535, TA1537 and *E.coli* WP2uvrA ±S9. In the initial assay, concentration levels were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate. Precipitate was observed at concentrations ≥500 µg per plate. Toxicity was reported as starting at 500 or 1500 µg per plate. Based on this initial assay, the highest concentration used was 1800 µg per plate. Concentrations used in the second assay were 7.5, 25, 75, 200, 600 and 1800 µg per plate. Precipitate was observed beginning at 600 µg per plate. Toxicity was observed beginning at 200, 600 or 1800 µg per plate. Positive controls included sodium azide and 2-aminoanthracene, 2-nitrofluorene, 9-aminoacridine and methyl methanesulfonate.

The difference in concentration at which toxicity was observed varied with the bacterial strain.

**Results** The positive controls produced appropriate responses. The test article did not produce an increase in revertants either with or without metabolic activation.

The study was adequate.

**Study title:** *Evaluation of the mutagenic activity of nebivolol hydrochloride in an in vitro mammalian cell gene mutation test with L5178Y mouse lymphoma cells (with independent repeat)*

**Key findings:** The study did not use sufficiently high concentrations to achieve adequate cellular toxicity. The study was inadequate.

**Study no.:** N99905

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** February 23, 1993

**GLP compliance:** statement included

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

**Drug, lot #, and % purity:** nebivolol, ZR067555PFA141, DMSO used as the vehicle

Positive controls were ethylmethanesulphonate and dimethylnitrosamine

**Methods** The preliminary test used concentrations of 1.1, 3.6, 10.9, 36.4, 109.3 and 364.0 compared to the solvent control. At concentrations  $\geq 36.4$   $\mu\text{g/ml}$  there was total cell death -S9. Total cell death

occurred at  $\geq 109.3$   $\mu\text{g/ml}$  +S9. For the main studies, concentrations of 1.1, 6.1 and 10.9  $\mu\text{g/ml}$  were used -S9. The Concentrations -S9 were 19.7, 36.4, 45.9 61.2  $\mu\text{g/ml}$ .

**Results**

The concentrations used did not go far enough into the range of cytotoxicity based upon the data from the preliminary assay.

TABLE 5 L5178Y MOUSE LYMPHOMA TEST SYSTEM - CLONING EFFICIENCY DAY 0

EXPERIMENT 1

Dose ( $\mu\text{g/ml}$ )	No. of colonies/ cloning plate			Mean No. of colonies/plate	Cloning efficiency	
	1	2	3		absolute	relative (% of control)
Without S9-mix						
Solvent control	120	120	107	116	58	100
1.1	130	149	111	130	65	112
3.6	130	141	139	137	69	118
6.1	103	113	117	111	56	96
10.9	51	67	50	56	28	48
14.5	a)					
19.7	a)					
EMS	117	128	129	125	63	108
-----						
With S9-mix						
Solvent control	132	139	141	137	69	100
10.9	117	123	120	120	60	88
19.7	115	114	124	118	59	86
36.4	81	78	FI	80	40	58
45.9	84	92	78	85	43	62
61.2	56	60	74	63	32	46
82.0	3	5	2	3	2	2
109.3	a)					
DMN	55	65	71	64	32	47

a) Cell killing directly after treatment  
FI = Plate infected with fungi

3.6,

and

TABLE B L5178Y MOUSE LYMPHOMA TEST SYSTEM - CLONING EFFICIENCY DAY 0

## EXPERIMENT 2

Dose (ug/ml)	No. of colonies/ cloning plate			Mean No. of colonies/plate	Cloning efficiency	
	1	2	3		absolute	relative (% of control)
Without S9-mix						
Solvent control	125	136	131	131	66	100
1.1	137	128	121	129	65	98
3.6	148	128	122	133	67	102
6.1	136	137	133	135	68	103
10.9	120	118	112	117	59	89
14.5	a)					
19.7	a)					
EMS	112	128	129	123	62	94
-----						
With S9-mix						
Solvent control	152	168	143	154	77	100
8.2	144	171	165	160	80	104
10.9	139	150	151	147	74	95
14.5	141	165	158	155	78	101
19.7	143	137	152	144	72	94
26.2	139	141	151	144	72	94
36.4	161	138	139	146	73	95
45.9	146	160	155	154	77	100
61.2	89	112	81	94	47	61
82.0	a)					
DMN	61	57	51	56	28	36

a) Cell killing directly after treatment

Appears This Way  
On Original

## EXPERIMENT 1

Dose µg/ml	Number of colonies/Selection plate										Total No.	No. of colonies /cloning plate			Mean	MF
	1	2	3	4	5	6	7	8	9	10		1	2	3		
Without Metabolic Activation (-S9-mix)																
Solvent control	2	0	2	0	0	1	3	2	1	FI	11	180	179	165	175	0.9
1.1	2	3	3	1	0	5	1	FI	FI	FI	15	166	188	189	181	1.6
3.6	1	2	1	0	6	1	0	1	0	FI	12	145	155	168	156	1.1
6.1	6	1	2	0	3	3	1	2	0	FI	18	167	180	171	173	1.5
10.9	6	4	2	3	1	3	5	4	2	1	31	167	179	180	175	2.4
EMS	20	21	21	20	18	18	20	24	12	FI	174	180	155	161	165	15.6
With Metabolic Activation (+S9-mix)																
Solvent control	0	4	1	1	2	2	1	1	FI	FI	12	155	157	190	167	1.2
19.7	1	1	2	3	2	2	0	0	1	FI	12	180	179	165	175	1.0
36.4	0	1	0	1	8	4	3	2	FI	FI	19	166	173	175	171	1.9
45.9	0	1	0	2	2	0	0	2	3	0	10	180	179	177	179	0.7
61.2	0	3	2	2	1	0	1	2	1	2	14	167	168	175	170	1.1
DMN	13	11	12	10	18	9	9	12	25	12	131	125	141	135	134	13.0

MF = Mutant frequency per 10<sup>5</sup> survivors  
 FI = Plate infected with fungi

## EXPERIMENT 2

Dose µg/ml	Number of colonies/Selection plate										Total No.	No. of colonies /cloning plate			Mean	MF
	1	2	3	4	5	6	7	8	9	10		1	2	3		
Without Metabolic Activation (-S9-mix)																
Solvent control	1	0	0	0	1	0	1	2	2	0	7	121	122	132	125	0.7
1.1	1	0	3	0	1	0	0	1	0	2	8	113	115	126	118	0.9
3.6	1	2	2	1	1	0	1	2	4	2	16	110	112	118	113	1.9
6.1	0	2	2	1	0	0	1	0	0	2	8	123	120	112	118	0.9
10.9	2	2	2	4	5	4	4	3	2	1	29	126	130	125	127	3.0
EMS	19	40	33	33	40	19	26	34	28	29	301	106	116	118	113	35.5
With Metabolic Activation (+S9-mix)																
Solvent control	0	0	1	2	0	1	1	2	1	2	10	137	138	141	139	1.0
26.2	1	0	2	0	1	1	0	2	1	0	8	151	137	143	144	0.7
36.4	1	1	1	3	1	1	2	2	1	2	15	118	129	118	122	1.6
45.9	0	1	1	0	1	2	0	0	1	FI	6	145	137	131	138	0.6
61.2	2	0	0	0	1	1	0	0	2	2	8	151	137	136	141	0.8
DMN	5	6	12	6	7	5	6	5	9	9	70	101	103	88	97	9.6

MF = Mutant frequency per 10<sup>5</sup> survivors  
 FI = Plate infected with fungi

The study is inadequate and uninformative.

**Study title:** *In vitro* mammalian cell gene mutation test (L5178Y/TK<sup>±</sup> mouse lymphoma assay)

**Key findings:** Under the conditions of the assay, the test article did not produce an increase in mutations.

**Study no.:** R067555-5258

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** September 12, 2001

**GLP compliance:** statement included

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

**Drug, lot #, and % purity:** nebivolol, ZR067555PUA711, DMSO was used as the vehicle. Positive controls were methyl methanesulfonate and 7,12-dimethyl-benz(a)anthracene (7,12-DMBA)

**Methods** There were 4 hour and 24 hour exposure periods. Concentrations used are summarized in the reviewer's table below.

assay	Concentrations used (µg/ml)	
	-S9	+S9
Preliminary: 4 hour	0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1400 ≥15 µg/ml: 100% cell death	0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1400 ≥50 µg/ml: 100% cell death
Preliminary: 24 hour	0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1400 ≥15µg/ml: 100% cell death ≥50 µg/ml caused precipitation under all of the above conditions	
Initial assay: 4 hours	2.5, 5,6,7,8,9	30, 32.5, 35, 37.5, 40, 50
Initial assay: 24 hours	0.5, 1,2,3,4,5,6	

**Results**

Appears This Way  
On Original

TABLE 1

## PRELIMINARY TOXICITY ASSAY USING Nebivolol, lot ZR087555PUA711

Test Article Concentration (µg/mL)	Cell Concentration (X10 <sup>6</sup> ) <sup>a</sup>		Suspension Growth % of	
	Day 1	Day 2	Total <sup>b</sup>	Control <sup>c</sup>
WITHOUT ACTIVATION (4-Hour)				
Solvent 1	1.735	1.440	27.8	
Solvent 2	1.718	1.356	25.9	
.15	1.737	1.215	23.5	87
.5	1.649	1.431	26.2	98
1.5	1.557	1.273	22.0	82
5	1.370	1.431	21.8	81
15	0.005	0.027	0.0	0
50*	0.014	0.019	0.0	0
150*	0.001	0.018	0.0	0
500*	0.000	0.025	0.0	0
1400*	0.002	0.021	0.0	0
WITH S9 ACTIVATION (4-Hour)				
Solvent 1	1.239	1.317	18.1	
Solvent 2	1.227	1.363	18.6	
.15	1.243	1.335	18.4	100
.5	1.114	1.452	18.0	98
1.5	1.225	1.334	18.2	99
5	1.179	1.324	17.4	95
15	1.200	1.225	16.3	89
50*	0.025	0.098	0.0	0
150*	0.021	0.092	0.0	0
500*	0.003	0.026	0.0	0
1400*	0.017	0.024	0.0	0
WITHOUT ACTIVATION (24-Hour)				
Solvent 1	1.108	1.201	14.8	
Solvent 2	1.113	1.220	15.1	
.15	1.133	1.357	17.1	114
.5	0.906	1.371	13.8	92
1.5	1.304	1.159	16.8	112
5	0.880	0.934	9.1	61
15	0.132	0.056	0.0	0
50*	0.171	0.120	0.0	0
150*	0.052	0.043	0.0	0
500*	0.010	0.001	0.0	0
1400*	0.023	0.030	0.0	0

Solvent = DMSO

1 and 2 are duplicate cultures

\* - Precipitating dose

<sup>a</sup> - Cultures containing <0.3x10<sup>6</sup> cells/mL on day 1 and 2 are considered as having 0% total suspension growth.

<sup>b</sup> - Total suspension growth = (Day 1 cell conc. / 0.3x10<sup>6</sup> cells/mL) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

<sup>c</sup> - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

Appears This Way  
On Original

**CLONING DATA FOR LS178Y/TK<sup>+</sup> MOUSE LYMPHOMA CELLS  
TREATED WITH Nebivolol, lot ZR067565PUA711  
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION  
Initial Assay (4-hour exposure)**

Test Article Concentration (µg/mL)	TFT Colonies				VC Colonies				Mutant Freq. <sup>a</sup>	Induced Mutant Freq. <sup>b</sup>	% Total Growth <sup>c</sup>
	Counts	Mean	Counts	Mean	Counts	Mean					
Solvent 1	50 35 30	38 ±8	160 176 159	165 ±8	46						
Solvent 2	42 41 40	41 ±1	175 183 170	176 ±5	47						
Mean Solvent Mutant Frequency= 47											
2.5 A	55 58 30	48 ±13	139 155 122	139 ±13	69	22	69				
2.5 B	30 27 +	29 ±1	122 135 138	132 ±7	43	-3	69				
5 A	41 28 46	38 ±8	140 116 151	136 ±15	57	10	61				
5 B	44 42 58	48 ±7	110 153 166	143 ±24	67	21	66				
6 A	40 30 35	35 ±4									
6 B	28 27 32	29 ±2	219 243 283	248 ±26	23	-23	92				
7 A	33 42 30	35 ±5	172 150 153	158 ±10	44	-2	36				
7 B	39 26 +	33 ±5	179 142 173	165 ±16	39	-7	34				
8 A	38 51 31	40 ±8	156 136 144	145 ±8	55	9	25				
8 B	37 37 41	38 ±2	119 130 153	134 ±14	57	11	16				
Positive Control - Methyl Methanesulfonate (µg/mL)											
10	223 209 194	209 ±12	104 122 107	111 ±8	376	329	37				
20	160 149 168	159 ±8	42 45 52	46 ±4	686	640	12				

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

+ - Culture lost

<sup>a</sup> - Mutant frequency (per 10<sup>5</sup> surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

<sup>b</sup> - Induced mutant frequency (per 10<sup>5</sup> surviving cells) = mutant frequency - average mutant frequency of solvent controls

<sup>c</sup> - % total growth = (% suspension growth x % cloning growth) / 100

Appears This Way  
On Original

**TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK\* MOUSE LYMPHOMA CELLS  
TREATED WITH Nebivolol, lot ZR067666PUA711  
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION  
Initial Assay (4-hour exposure)**

Test Article Concentration (µg/mL)		Cell Concentration (X 10 <sup>6</sup> ) <sup>a</sup>		Susp Growth		Cloning Growth		% Total Growth <sup>e</sup>
		Day 1	Day 2	Total <sup>b</sup>	%Cntl <sup>c</sup>	Avg VC	%Cntl <sup>d</sup>	
Solvent	1	1.228	1.643	22.4		165		
Solvent	2	1.224	1.539	20.9		176		
2.5	A	1.041	1.594	18.4	85	139	81	69
2.5	B	1.154	1.510	19.4	89	132	77	69
5	A	0.967	1.558	16.7	77	136	80	61
5	B	1.083	1.420	17.1	79	143	84	66
6	A	0.805	1.336	11.9	55	+		
6	B	0.794	1.558	13.8	63	248	146	92
7	A	0.558	1.372	8.5	39	158	93	36
7	B	0.505	1.373	7.7	36	165	97	34
8	A	0.417	1.350	6.3	29	145	85	25
8	B	0.315	1.249	4.4	20	134	79	16
9	A	0.087	0.414	1.4	6	++		
9	B	0.093	0.446	1.5	7	++		
-----								
Positive Control - Methyl Methanesulfonate (µg/mL)								
	10	0.857	1.287	12.3	57	111	65	37
	20	0.822	1.035	9.5	44	46	27	12
-----								

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

+ - Culture lost

++ - Too toxic to clone

<sup>a</sup> - Cultures containing <0.3x10<sup>6</sup> cells/mL on day 1 and 2 are considered as having 0% total suspension growth.

<sup>b</sup> - Total suspension growth = (Day 1 cell conc. / 0.3x10<sup>6</sup> cells/mL) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

<sup>c</sup> - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

<sup>d</sup> - % control cloning growth = (average VC of treated culture / average VC of solvent control) x 100

<sup>e</sup> - % total growth = (% suspension growth x % cloning growth) / 100

Appears This Way  
On Original

**TREATED WITH Nebivolol, lot ZR067565PUA711  
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION  
Initial Assay (4-hour exposure)**

Test Article Concentration (µg/mL)	Cell Concentration (X 10 <sup>6</sup> ) <sup>a</sup>	Cell Concentration		Susp Growth Total <sup>b</sup>	%Cntl <sup>c</sup>	Cloning Growth		% Total Growth <sup>e</sup>
		Day 1	Day 2			Avg VC	%Cntl <sup>d</sup>	
Solvent 1	1.228	1.643	22.4		165			
Solvent 2	1.224	1.539	20.9		176			
2.5 A	1.041	1.594	18.4	85	139	81	69	
2.5 B	1.154	1.510	19.4	89	132	77	69	
5 A	0.967	1.558	16.7	77	136	80	61	
5 B	1.083	1.420	17.1	79	143	84	66	
6 A	0.805	1.336	11.9	55	+			
6 B	0.794	1.558	13.8	63	248	146	92	
7 A	0.558	1.372	8.5	39	158	93	36	
7 B	0.505	1.373	7.7	36	165	97	34	
8 A	0.417	1.350	6.3	29	145	85	25	
8 B	0.315	1.249	4.4	20	134	79	16	
9 A	0.087	0.414	1.4	6	++			
9 B	0.093	0.446	1.5	7	++			
-----								
Positive Control - Methyl Methanesulfonate (µg/mL)								
10	0.857	1.287	12.3	57	111	65	37	
20	0.822	1.035	9.5	44	46	27	12	
-----								

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

+ - Culture lost

++ - Too toxic to clone

<sup>a</sup> - Cultures containing <0.3x10<sup>6</sup> cells/mL on day 1 and 2 are considered as having 0% total suspension growth.

<sup>b</sup> - Total suspension growth = (Day 1 cell conc. / 0.3x10<sup>6</sup> cells/mL) x (Day 2 cell

Appears This Way  
On Original

-----  
 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION  
 Independent Repeat Assay (24-hour exposure)  
 -----

Test Article Concentration (µg/mL)	FFT Colonies				VC Colonies				Mutant Freq. <sup>a</sup>	Induced Mutant Freq. <sup>b</sup>	Total Growth <sup>c</sup>
	Counts	Mean	Counts	Mean	Counts	Mean	Counts	Mean			
Solvent 1	55	39	55	50 ±8	143	139	158	147 ±8	68		
Solvent 2	34	39	20	31 ±8	138	122	118	126 ±9	49		
Mean Solvent Mutant Frequency= 58											
.5 A	39	57	34	43 ±10	140	136	129	135 ±5	64	6	102
.5 B	33	35	25	31 ±4	129	143	144	139 ±7	45	-14	107
1 A	41	30	34	35 ±5	150	143	127	140 ±10	50	-8	116
1 B	35	32	41	36 ±4	162	181	148	164 ±14	44	-14	122
2 A	31	42	27	33 ±6	111	126	145	127 ±14	52	-6	87
2 B	31	35	45	37 ±6	163	124	140	142 ±16	52	-6	87
3 A	24	30	20	25 ±4	151	126	122	133 ±13	37	-21	61
3 B	35	22	20	26 ±7	127	169	140	145 ±18	35	-23	66
4 A	21	30	27	26 ±4	98	87	104	96 ±7	54	-4	23
4 B	39	38	28	35 ±5	119	139	127	128 ±8	55	-4	32
5 A	31	32	38	34 ±3	111	114	123	116 ±5	58	0	14
5 B				++				++			
----- Positive Control - Methyl Methanesulfonate (µg/mL) -----											
2.5	140	133	130	134 ±4	110	104	112	109 ±3	247	189	85
5	178	196	186	187 ±7	109	132	124	122 ±10	307	248	84
----- Solvent = DMSO -----											

**Study title:** *Chromosome aberrations in cultured human peripheral lymphocytes*

**Key findings:** 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 ( $\geq 5\mu\text{g/ml}$ ,  $-\text{S9}$ ;  $16\mu\text{g/ml} +\text{S9}$ ) was seen with the test article. The historical control data provided did not include values for polyploidy and endoreduplication.

**Study no.:** N92628

**Conducting laboratory and location:** Dept. Toxicology, Janssen, Beerse, Belgium

**Date of study initiation:** June 24, 1991

**GLP compliance:** statement included

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

**Drug, lot #, and % purity:** R67555, ZR067555PFA111

Positive controls were mitomycin C ( $-\text{S9}$ ) and cyclophosphamide ( $+\text{S9}$ )

### Methods

In the dose selection/first test, human peripheral lymphocytes were incubated  $\pm$  S9 with R67555 with approximate concentrations of 5,7,10, 16,23,35,53,79,119,178,267 and 400 µg/ml culture. 50 metaphase spreads per slide per observer (200 per dosage group) of human peripheral lymphocytes of 5, 10 and 16µg R67555/ml culture ( $-\text{S9}$ ) and 5,16 and 35 µg R67555/ml culture

(+S9) were analyzed for the occurrence of chromosome aberrations. A repeat assay was conducted using the same concentrations and higher concentrations of S9.

The incubation time in cultures was:

- I. Without activation 21 hour (first test)  
21 hour and 45 hours (repeat test)
- With activation 2 hours (first and repeat test)

Harvest times after initiation of cultures:

- 69hours(first test)
- 69hours and 93 hours (repeat test)

**Results**

In the first chromosome aberration test (69 hour harvest time) a decrease of 41% and 46% in the mitotic index was found at 16 and 35 µgR67555/ml culture both ±S9 respectively.

Department of Toxicology

EXPERIMENT: 2603  
Mutagenicity study  
CHROMOSOME ABERRATION TEST  
R 67555 - HUMAN LYMPHOCYTES

FIRST AND REPEAT CHROMOSOME ABERRATION TEST
MITOTIC INDEX

DOSAGE GROUP	CONC. (µg/ml)	MITOTIC INDEX per 1000 cells								
		69 h. CULTURE TIME						93 h. CULTURE TIME		
		WITHOUT S9-MIX			WITH S9-MIX			WITHOUT S9-MIX		WITH S9-MIX
		Culture A	B	% of solv. contr.	Culture A	B	% of solv. contr.	Culture A	B	% of solv. contr.

At concentrations of 5, 10 and 16 µg/ml -S9 and at concentrations of 5, 16 and 35 µg/ml +S9, R67555 didn't cause a perceptible increase in the number of cells with chromosome aberrations.

FIRST TEST	Solvent control (DMSO : 20 µl/ml)	-	34	37	100	93	75	100				
	Positive control: - Mitomycin C	0.2	17	25	59	-	-	-				
	- Cyclophosphamide	20	-	-	-	39	43	49				
	Test article R 67555	5	39	34	103	87	64	90				
		10	21	38	83							
		16	23	19	59	72	65	82				
		35				48	43	54				

In the preliminary study, total number of metaphases were decreased by ~85% and 46% by concentrations of 23 (-S9) and ~48% (+S9). Total inhibition of mitosis was seen at 35µg (-S9) and 53µg(+S9).

REPEAT TEST	Solvent control (DMSO : 20 µl/ml)	-	46	35	100	74	83	100	31	18	100	74	58	100
	Positive control: - Mitomycin C	0.2	32	39	88	-	-	-	-	-	-	-	-	-
	- Cyclophosphamide	20	-	-	-	24	20	28	-	-	-	-	-	-
	Test article R 67555	5	28	29	70	84	80	104	19	12	63	91	88	136
		10	27	29	69				18	27	92			
		16	18	23	51	71	85	99	9	13	45	72	52	94
		35				73	68	90				77	75	115

Department of Toxicology

EXPERIMENT: 2603  
 Mutagenicity study  
 CHROMOSOME ABERRATION TEST  
 R 67555

<b>FIRST AND REPEAT CHROMOSOME ABERRATION TEST</b>
<b>SUMMARIZED RESULTS</b>

DOSAGE GROUP	CONC. (µg/ml)	NUMBER OF CELLS WITH CHROMOSOME ABERRATIONS SCORED ON A TOTAL OF 200 CELLS							
		69 h. CULTURE TIME				93 h. CULTURE TIME			
		WITHOUT S9-MIX		WITH S9-MIX		WITHOUT S9-MIX		WITH S9-MIX	
		+ gaps	- gaps	+ gaps	- gaps	+ gaps	- gaps	+ gaps	- gaps

<b>F I R S T  T E S T</b>	Solvent control (DMSO : 20 µl/ml)	-	7	4	11	0				
	Positive control: - Mitomycin C	0.2	p=0.000 22***	p=0.000 13***						
	- Cyclophosphamide	20			p=0.000 23***	p=0.000 17***				
	Test article R 67555	5	12	4	16	2				
		10	10	3						
		16	8	2	15	3				
35				12	3					

<b>R E P E A T  T E S T</b>	Solvent control (DMSO : 20 µl/ml)	-	8	1	16	4	8	3	5	3
	Positive control: - Mitomycin C	0.2	p=0.000 33***	p=0.000 25***						
	- Cyclophosphamide	20			p=0.000 36***	p=0.000 33***				
	Test article R 67555	5	8	1	12	6				
		10	12	4						
		16	11	5	19	7	12	2		
35				9	3			9	1	

Significantly increased when compared to the solvent control group (Fisher Exact Probability Test; one tailed): \* p≤0.05, \*\* p≤0.01, \*\*\* p≤0.001.

NDA21742

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

Appears This Way  
On Original

Appears This Way  
On Original

Department of Toxicology

EXPERIMENT : 2603  
 Mutagenicity study  
 CHROMOSOME ABERRATION TEST  
 R 67555 - HUMAN LYMPHOCYTES

FIRST CHROMOSOME ABERRATION TEST	
69 h. culture time	without S9-mix
Detailed results on chromosome aberrations	

Concentration µg/ml	Solvent control			Positive control			Test article R 67555									
	DMSO 20 µl/ml			Mitomycin C 0.2 µg/ml			5 µg/ml			10 µg/ml			16 µg/ml			
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	
No. of cells scored	100	100	200	25	25	50	100	100	200	100	100	200	100	100	200	
No. of cells with aberr. (+gaps)	5	2	7	12	10	22 *** p=0.000	8	4	12 p=0.174	5	5	10 p=0.311	6	2	8 p=0.500	
No. of cells with aberr. (-gaps)	4	0	4	7	6	13 *** p=0.000	3	1	4 p=0.638	1	2	3 p=0.776	2	0	2 p=0.892	
Gap'	1	2	3	9	8	17	4	2	6	3	4	7	5	2	7	
Break	4		4	2	2	4	2	1	3	1	1	2	2		2	
Exch.				5	3	8										
Gap*				1	1		1	1	2	1		1				
Delet.	1		1	1	1	2	1		1	1		1				
Trans.																
C.R.																
Di:																
M	Mult.					1	1									
i	Puv.															
s	Poly.									1	1		4	4		
c.	Endo.						1	1								
Total aberr. (+gaps)	6	2	8	17	25	42	8	4	12	5	6	11	7	2	9	
Total aberr. (-gaps)	5	0	5	8	16	24	3	1	4	1	2	3	2	0	2	

The various types of aberrations are described in appendix A 6.1 - A 6.2  
 Arbitrary, each cell with multiple aberrations was accounted for ten aberrations (gaps excluded)  
 and a cell with one or more pulverized chromosomes as one aberration.  
 Numerical variations as polyploidy (Poly.) and endoreduplication (Endo.) were not counted as aberrations.

† : One-tailed Fisher Exact Probability Test of A + B, treatment versus solvent control.  
 \* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001.

NDA21742

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

Appears This Way  
On Original

Appears This Way  
On Original

Department of Toxicology

EXPERIMENT : 2803  
 Mutagenicity study  
 CHROMOSOME ABERRATION TEST  
 R 67555 - HUMAN LYMPHOCYTES

FIRST CHROMOSOME ABERRATION TEST	
69 h. culture time	with S9-mix
Detailed results on chromosome aberrations	

Concentration µg/ml	Solvent control			Positive control			Test article R 67555								
	DMSO 20 µl/ml			Cyclophosphamide 20 µg/ml			5 µg/ml			16 µg/ml			35 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	25	25	50	100	100	200	100	100	200	100	100	200
No. of cells with aberr. (+gaps)	3	8	11	9	14	23 † *** p=0.000	7	9	16 † p=0.213	10	5	15 † p=0.272	4	8	12 † p=0.500
No. of cells with aberr. (-gaps)	0	0	0	8	9	17 † *** p=0.000	1	1	2 † p=0.249	2	1	3 † p=0.124	0	3	3 † p=0.124
Gap*	3	9	12	2	10	12	6	10	16	8	4	12	4	9	13
Break				8	4	12	1	1	2	2		2			3
Exch.				6	6	12									
Gap*		1	1	1		1	1	1							
Delet.					4	4									
Trans.															
C.R.															
Dic.															
M i s c.	Mult.			1		1									
	Pulv.									1	1				
	Poly.												3	1	4
	Endo.				1		1			1	1				
Total aberr. (+gaps)	3	10	13	27	24	51	7	12	19	10	5	15	4	12	16
Total aberr. (-gaps)	0	0	0	24	14	38	1	1	2	2	1	3	0	3	3

The various types of aberrations are described in appendix A 6.1 - A 6.2  
 Arbitrary, each cell with multiple aberrations was accounted for ten aberrations (gaps excluded) and a cell with one or more pulverized chromosomes as one aberration.  
 Numerical variations as polyploidy (Poly.) and endoreduplication (Endo.) were not counted as aberrations.

† : One-tailed Fisher Exact Probability Test of A + B, treatment versus solvent control.  
 \* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001.

Appears This Way  
On Original

Appears This Way  
On Original

Department of Toxicology

EXPERIMENT : 2603  
 Mutagenicity study  
 CHROMOSOME ABERRATION TEST  
 R 67555 - HUMAN LYMPHOCYTES

REPEAT CHROMOSOME ABERRATION TEST	
69 h. culture time	without S9-mix
Detailed results on chromosome aberrations	

Concentration µg	Solvent control			Positive control			Test article R 67555								
	DMSO 20 µl/ml			Mitomycin C 0.2 µg			5 µg			10 µg			16 µg		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	25	25	50	100	100	200	100	100	200	100	100	200
No. of cells with aberr. (+gaps)	4	4	8	17	16	33 † *** p=0.000	4	4	8 † p=0.600	5	7	12 † p=0.246	5	6	11 † p=0.320
No. of cells with aberr. (-gaps)	0	1	1	13	12	25 † *** p=0.000	1	0	1 † p=0.751	3	1	4 † p=0.186	1	4	5 † p=0.108
Gap'	4	3	7	10	13	23	4	4	8	5	6	11	4	4	8
Break				10	6	16	1		1	2	1	3	1	4	5
Exch.				5	6	11									
Gap*				1		1									
Delet.		2	2	4	4	8									
Trans.															
C.R.															
Dic.										1		1			
M i s c.	Mult.			1	1	2									
	Pulv.														
	Poly.						1	1		2	1	3	1	1	2
	Endo.														
Total aberr. (+gaps)	4	5	9	40	39	79	5	4	9	8	7	15	5	8	13
Total aberr. (-gaps)	0	2	2	29	26	55	1	0	1	3	1	4	1	4	5

The various types of aberrations are described in appendix A 6.1 - A 6.2  
 Arbitrary, each cell with multiple aberrations was accounted for ten aberrations (gaps excluded)  
 and a cell with one or more pulverized chromosomes as one aberration.  
 Numerical variations as polyploidy (Poly.) and endoreduplication (Endo.) were not counted as aberrations.

† : One-tailed Fisher Exact Probability Test of A + B, treatment versus solvent control.  
 \* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001.

NDA21742

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

Appears This Way  
On Original

Appears This Way  
On Original

Department of Toxicology

EXPERIMENT : 2603  
 Mutagenicity study  
 CHROMOSOME ABERRATION TEST  
 R 67555 - HUMAN LYMPHOCYTES

REPEAT CHROMOSOME ABERRATION TEST	
69 h. culture time	with S9-mix
Detailed results on chromosome aberrations	

Concentration µg	Solvent control			Positive control			Test article R 67555								
	DMSO 20 µl/ml			Cyclophosphamide 20 µg			5 µg			16 µg			35 µg		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	25	25	50	100	100	200	100	100	200	100	100	200
No. of cells with aberr. (+gaps)	6	10	16	18	18	36 † *** p=0.000	6	6	12 † p=0.836	10	9	19 † p=0.362	5	4	9 † p=0.952
No. of cells with aberr. (-gaps)	1	3	4	17	16	33 † *** p=0.000	4	2	6 † p=0.375	5	2	7 † p=0.272	2	1	3 † p=0.776
Gap'	6	7	13	12	6	18	5	6	11	5	7	12	3	3	6
Break	1	3	4	23	13	36	3	2	5	4	2	6	2	1	3
Exch.				13	13	26									
Gap*				1	2	3							1		1
Delet.				7	2	9	1		1	1		1			
Trans.															
C.R.															
Dc.															
M i s c.	Mult.			3	5	8									
	Pulv.														
	Poly.						1		1	1		1			
	Endo.														
Total aberr. (+gaps)	7	10	17	86	86	172	9	8	17	10	9	19	6	4	10
Total aberr. (-gaps)	1	3	4	73	78	151	4	2	6	5	2	7	2	1	3

The various types of aberrations are described in appendix A 6.1 - A 6.2  
 Arbitrary, each cell with multiple aberrations was accounted for ten aberrations (gaps excluded) and a cell with one or more pulverized chromosomes as one aberration.  
 Numerical variations as polyploidy (Poly.) and endoreduplication (Endo.) were not counted as aberrations.

† : One-tailed Fisher Exact Probability Test of A + B, treatment versus solvent control.  
 \* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001.

Department of Toxicology

EXPERIMENT : 2603  
 Mutagenicity study  
 CHROMOSOME ABERRATION TEST  
 R 67555 - HUMAN LYMPHOCYTES

REPEAT CHROMOSOME ABERRATION TEST	
93 h. Culture time	Without and with S9-mix
Detailed results on chromosome aberrations	

Conc. µg/ml	Without S9-mix						With S9-mix					
	Solvent control (DMSO : 20 µl/ml)			Test article R 67555 16 µg/ml			Solvent control (DMSO : 20 µl/ml)			Test article R 67555 35 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	100	100	200	100	100	200	100	100	200
No. of cells with aberr. (+gaps)	4	4	8	6	6	12	2	3	5	3	6	9
	p=0.248						p=0.208					
No. of cells with aberr. (-gaps)	1	2	3	1	1	2	2	1	3	0	1	1
	p=0.814						p=0.938					
Gap <sup>+</sup>	3	2	5	5	5	10		2	2	3	5	8
Break		1	1	1		1	2	1	3		1	1
Exch.												
Gap <sup>-</sup>												
Delet.	1	1	2		1	1						
Trans.												
C. R.												
Dic.												
M i s c.	Mult.											
	Pulv.											
	Poly.			1	1	2	2	2	4		3	3
Endo.												
Total aberr. (+gaps)	4	4	8	6	6	12	2	3	5	3	6	9
Total aberr. (-gaps)	1	2	3	1	1	2	2	1	3	0	1	1

The various types of aberrations are described in appendix A6.1-A6.2.  
 Arbitrary, each cell with multiple aberrations was accounted for ten aberrations (gaps excluded) and a cell with one or more pulverized chromosomes as one aberration.  
 Numerical variations as polyploidy (Poly.) and endoreduplication (Endo.) were not counted as aberrations.

One-tailed Fisher Exact Probability Test of A + B, treatment versus solvent control  
 \* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001

**Study title:** *Micronucleus test in mice: Single oral dose administration.*

**Key findings:** Under the conditions of the assay, when R67555 was given at a dose that caused an unspecified amount of weight loss ( $p < 0.001$ ), there was no reported increase in micronuclei.

**Study no.:** N56071

**Conducting laboratory and location:** Dept. Tox., Janssen, Beerse, Belgium

**Date of study initiation:** February 4, 1987

**GLP compliance:** Statement included

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

**Drug, lot #, and % purity:** R67555, PFA011

Cyclophosphamide was used as the positive control

**Methods**

A preliminary study used doses of 20, 80 and 320 mg/kg given to Swiss albino mice. The animals were euthanized at 18, 24, 30, 48 and 72 hours after dosing. In the main study, male and female Swiss albino mice, 5/sex/group were given single oral doses of 10, 40 and 160 mg R 67555/kg body weight, 30 hours prior to preparation of the bone marrow. A total of 1000 polychromatic erythrocytes per animal were evaluated.

**Results:** The sponsor reported no decrease in bone marrow proliferation and no increase in the number of micronucleated PCE in the preliminary study.

In the main study, the sponsor reported a significant weight loss in the HD animals. Data was not provided to support this. The positive control produced an appropriate response. No increase in micronucleated cells was seen with the test article when results were combined for the sexes. There was no significant difference in the results for the males and females.

**Study title:** *Micronucleus test in mice: Single oral dose*

**Key findings:** The sponsor states 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 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 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.

**Study no.:** N122168

**Conducting laboratory and location:**

**Date of study initiation:** May 8, 1996

**GLP compliance:** statement included

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

**Drug, lot #, and % purity:** R067555, ZR067555PUA061

cyclophosphamide was used as a positive control

**Methods** Male and female mice were dosed with 10, 40 and 160 mg R067555/kg body weight at 24 and 48 hours prior to preparation of bone marrow. A total of 2000 polychromatic erythrocytes per mouse and per time point were screened for the presence of micronuclei. Plasma levels of test article were analyzed at 2 and 4 hours after dosing using satellite animals treated with R067555. This was described in an appendix.

### **Results**

Detectable plasma concentrations were found for both sexes, each dose group at both time points.

The HD groups gain less weight on average than did the control groups.

Appears This Way  
On Original

Sex : M = male (no. 1 - 5)  
 : F = female (no. 6 - 10)

Weight of animals (in g) at (T=) treatment and (S=) sacrifice + 24 h  
 (D=) Weight change

Animal	Negative control			Positive control			R067555 10 mg / kg			R067555 40 mg / kg			R067555 160 mg / kg		
	T	S	D	T	S	D	T	S	D	T	S	D	T	S	D
M 1	34	34	0				37	34	-3	39	38	-1	36	32	-4
M 2	35	33	-2				37	36	-1	37	34	-3	36	34	-2
M 3	34	33	-1				37	35	-2	32	31	-1	34	30	-4
M 4	33	32	-1				36	36	0	35	33	-2	35	34	-1
M 5	39	36	-3				40	39	-1	37	35	-2	34	32	-2
MEAN	35.0	33.6	-1.4				37.4	36.0	-1.4	36.0	34.2	-1.8	35.0	32.4	-2.6
Sign.															
F 6	30	28	-2				29	29	0	29	26	-3	29	28	-1
F 7	29	27	-2				30	26	-4	27	24	-3	29	26	-3
F 8	29	28	-1				30	29	-1	26	24	-2	27	27	0
F 9	29	28	-1				30	30	0	30	28	-2	27	27	0
F 10	28	26	-2				30	27	-3	28	27	-1	28	27	-1
MEAN	29.0	27.4	-1.6				29.8	28.2	-1.6	28.0	25.8	-2.2	28.0	27.0	-1.0
Sign.															
TOTAL															
MEAN	32.0	30.5	-1.5				33.6	32.1	-1.5	32.0	30.0	-2.0	31.5	29.7	-1.8
Sign.															

Statistics on weight change computed by Mann-Whitney U test (two tailed probability) \* p < .05 \*\* p < .01 \*\*\* p < .001

Appears This Way  
 On Original

Sex : M = male (no. 1 - 5)  
 : F = female (no. 6 - 10)

Weight of animals (in g) at (T=) treatment and (S=) sacrifice + 48 h  
 (D=) Weight change

Animal	Negative control			Positive control			R067555 10 mg / kg			R067555 40 mg / kg			R067555 160 mg / kg		
	T	S	D	T	S	D	T	S	D	T	S	D	T	S	D
M 1	35	35	0	32	32	0	34	32	-2	36	34	-2	33	30	-3
M 2	40	37	-3	34	34	0	34	34	0	36	32	-4	35	31	-4
M 3	36	32	-4	36	35	-1	39	38	-1	37	34	-3	33	31	-2
M 4	33	33	0	33	34	1	37	36	-1	34	32	-2	37	33	-4
M 5	35	33	-2	34	34	0	34	32	-2	31	29	-2	35	30	-5
MEAN	35.8	34.0	-1.8	33.8	33.8	0.0	35.6	34.4	-1.2	34.8	32.2	-2.6	34.6	31.0	-3.6
Sign.															
F 6	28	27	-1	26	25	-1	28	27	-1	30	28	-2	29	25	-4
F 7	25	25	0	29	26	-3	27	26	-1	28	26	-2	27	24	-3
F 8	28	28	0	29	28	-1	26	25	-1	29	27	-2	26	23	-3
F 9	28	27	-1	28	27	-1	27	27	0	27	26	-1	27	24	-3
F 10	27	27	0	27	26	-1	28	27	-1	29	27	-2	29	25	-4
MEAN	27.2	26.8	-0.4	27.8	26.4	-1.4	27.2	26.4	-0.8	28.6	26.8	-1.8	27.6	24.2	-3.4
Sign.										*			**		
TOTAL															
MEAN	31.5	30.4	-1.1	30.8	30.1	-0.7	31.4	30.4	-1.0	31.7	29.5	-2.2	31.1	27.6	-3.5
Sign.											*		**		

Statistics on weight change computed by Mann-Whitney U test (two tailed probability) \* p < .05 \*\* p < .01 \*\*\* p < .001

Appears This Way  
 On Original

The sponsor noted that

However, with one male (animal No.5) and three females (animals Nos. 6, 7 and 10) of the 160 mg R067555/kg body weight dosage group and 48 hours sampling time, a slight increase (within the historical control value range of 0 to 5 micronucleated PCE/1000 PCE) in the number of micronucleated PCE was observed (table 15, page D 15). Furthermore PCE were observed containing nucleus fragments which were micronucleus a-typical for the shape and did not have the typical micronucleus colouration. The same nucleus fragments were found outside the cells.

With the same animals, many nucleated erythrocytes with lobulated nuclei and erythrocytes with pycnotic nuclei were observed. Bad nuclear extrusion was also frequently observed. These histological findings let us to conclude firstly, that the mice have been treated up to very bone marrow cytotoxic concentration levels, and secondly the slight increase in the number of micronucleated PCE observed with some animals can be secondarily related to errors in the process of erythrocyte enucleation or differentiation at this bone marrow-toxic dose level.

Appears This Way  
On Original

Department of toxicology

EXPERIMENT: 3892  
 Mutagenicity study  
 R067555 - OR - NICE - MICRONUCLEUS TEST

-----  
 | FULL SIZE STUDY |  
Summarized results : FEMALES

Time of sacrifice	Dosage group	Number of animals	Polychromatic erythrocytes		Normochromatic erythrocytes		Proportion of PCE to (PCE + NCE)		
			n examined	n micronucleated	n	n micronucleated	n examined	n	
+24 h	Negative Control	5	10000	8	0.08	1	5000	3147	62.94
+24 h	R067555	5	10000	14	0.14	5	5000	2688	53.76
+24 h	R067555	5	10000	11	0.11	9	5000	2576	51.52*
+24 h	R067555	5	10000	19	0.19	9	5000	2371	47.42**
+48 h	Negative Control	5	10000	12	0.12	5	5000	3009	60.18
+48 h	Positive Control (Cyclophosphamide)	5	10000	164	1.64**	32	5000	2097	41.94**
+48 h	R067555	5	10000	9	0.09	5	5000	2577	51.54
+48 h	R067555	5	10000	10	0.10	4	5000	2913	58.26
+48 h	R067555	5	10000	28	0.28	15	5000	2465	49.30

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

PCE : Polychromatic erythrocytes  
 NCE : Normochromatic erythrocytes

Appears This Way  
 On Original

EXPERIMENT: 3892  
 Mutagenicity study  
 R067555 - OR - MICE - MICRONUCLEUS TEST

Summarized results : MALES + FEMALES

Time of sacrifice	Dosage group	Number of animals	Polychromatic erythrocytes		Normochromatic erythrocytes		Proportion of PCE to (PCE + NCE)	
			n examined	n micronucleated	n examined	n micronucleated	n examined	n PCE
				% (1)			% (1)	
+24 h	Negative Control 0 mg / kg	10	20000	19   0.10	6	10000	5896   58.96	
+24 h	R067555 10 mg / kg	10	20000	24   0.12	12	10000	5201   52.01*	
+24 h	R067555 40 mg / kg	10	20000	25   0.13	15	10000	5271   52.71*	
+24 h	R067555 160 mg / kg	10	20000	32   0.16	18	10000	4849   48.49***	
+48 h	Negative Control 0 mg / kg	10	20000	29   0.15	14	10000	5615   56.15	
+48 h	Positive Control (Cyclophosphamide) 40 mg / kg	10	20000	318   1.59***	101	10000	3999   39.99***	
+48 h	R067555 10 mg / kg	10	20000	18   0.09	14	10000	5288   52.88	
+48 h	R067555 40 mg / kg	10	20000	26   0.12	11	10000	5250   52.50	
+48 h	R067555 160 mg / kg	10	20000	51   0.26	27	10000	4813   48.13*	

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

TABLE 1: Mean (± S.D., n=3) nebiivolol plasma levels in mice after single oral administration of aqueous suspensions of nebiivolol hydrochloride (R067555) at 10, 40 or 160 mg (base-eq.) / kg in the micronucleus test.

Dose	Time (after dosing)	R067555-base (ng/ml)	
		Male mice	Female mice
10 mg (base-eq.) / kg	2 h	372 ± 119	258 ± 66
	4 h	176 ± 51	167 ± 73
40 mg (base-eq.) / kg	2 h	1227 ± 360	814 ± 68
	4 h	769 ± 304	988 ± 257
160 mg (base-eq.) / kg	2 h	2176 ± 378 <sup>1)</sup>	2252 ± 434 <sup>1)</sup>
	4 h	2699 ± 1100 <sup>1)</sup>	2705 ± 1040 <sup>1)</sup>

<sup>1)</sup> n=5.

The toxicokinetic study using the satellite animals showed detectable plasma levels at all dose levels.

APPEARS THIS WAY ON ORIGINAL

### 3.4.5. Carcinogenicity

#### Study title:

**Key study findings:** The carcinogenicity studies were reviewed under submission number 059. Results are briefly summarized here.

Adequacy of the carcinogenicity study and appropriateness of the test model: The Exec CAC assessed the studies as adequate.

Evaluation of tumor findings: The Exec CAC found the Leydig cell tumors in mice to be drug-related.

The mouse study used dietary administration to provide doses of 2.5, 10 and 40 mg/kg/day. The duration of the study was extended from 18 months to 20 to achieve a 50% mortality rate. Leydig cell tumors were present in the males : 2/50 (veh), 0/50 (LD), 1/50 (MD), 21/50 (HD). This was significant by the Exact Method and the Asymptotic method with the p value close to 0.

The rat study used dietary administration to provide doses of 0 (untreated control), vehicle control, 2.5, 10 and 40 mg/kg/day. The study duration was extended from 22 to a total of 25 months to achieve a 50% mortality rate. 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. The CDER statistician found no evidence of a carcinogenic effect of nebivolol in this species. The Leydig cell tumors in mice were determined to be drug-related.

#### Toxicokinetics:

Appears This Way  
On Original

**3.4.6. Reproductive and developmental toxicology** Some of these studies have been previously reviewed either by E. Barry or by E. Hausner. These reviews can be found with amendments 006, 016 and 097. Previously reviewed studies include:

- N69430 (Exp 1887) Oral male and female fertility study in Wistar rats
- N106655 (Exp 2774) Reproduction capacity study in Wistar rats (second generation reproduction study with one litter per generation).
- N92570 (Exp 2383) Peri- and postnatal reproduction study with a second generation evaluation in Wistar rats
- Exp 1888 Oral peri and postnatal study in Wistar rats

**A brief summary of key findings in studies previously reviewed will be provided here.**

**Fertility and Early Embryonic Development**

**Report N69430 (Study 1887) Oral male and female fertility study in Wistar rats.**

Doses of 10, 40 and 160 mg/kg were given orally via the diet. Body weight effects were seen in both sexes.

Appears This Way  
On Original

In pregnant females, treated for minimum 14 days before being exposed to equivalently dosed males and further treated till mating occurred and during the first 8 days of pregnancy, the average body weight and weight gain were as follows:

The average body wei  
the pre-cohabitation pe:

Dosage group  
mg/100 g food  
(mg/kg body weight/day)

0  
10  
40  
160

\*\*\*p≤0.001

<u>Dosage group</u> <u>mg/100 g food</u> <u>(mg/kg body weight/day)</u>	<u>Body weight (g)</u>		
	<u>Pre-cohabitation period</u>		
	<u>1st day</u>	<u>last day</u>	<u>gain</u>
0	190.5	260.1	69.6
10	190.6	254.0	63.5
40	190.0	246.5***	56.5***
160	189.6	211.2***	21.6***

275

\*\*\*p≤0.001

<u>Dosage group</u> <u>mg/100 g food</u> <u>(mg/kg body weight/day)</u>	<u>Body weight (g)</u>		
	<u>Pregnancy period</u>		
	<u>day 1</u>	<u>day 9</u>	<u>day 22</u>
0	264.2	308.6	453.0
10	261.0	300.1	443.2
40	252.5**	282.2***	439.4
160	201.0	205.0	356.0 (one pregnant female)

\*\*  $p \leq 0.01$

\*\*\*  $p \leq 0.001$

The HD of 160 mg/kg resulted in ptosis (sedation) and some unscheduled mortalities. Males at this dose failed to copulate, possibly due to the sedation. Despite pronounced maternal and paternal toxicity, there were essentially no effects on litter size or corpora lutea.

Maternal toxicity as evidenced by decrease in weight gain compared to the control was seen at all dosages. At the HD, only 1 female was successfully mated and became pregnant. The HD males failed to copulate, presumably due to sedation as evidenced by ptosis. Two of the unscheduled male mortalities had soft or small testes. There was no histopathological information provided. There were also no sperm parameters provided. Given the sperm reserves of the rat, it is not clear from the data presented that there is no effect upon male fertility. The toxicity at the HD was so severe that there were insufficient numbers of females for evaluation. The weight loss seen at the MD suggests that 40 mg/kg could have been the HD and might be a NOEL. Female cyclicity was presented as only a cohabitation-mating interval median value (p.5386).

The median interval was within 4 days (one estrus cycle) for all groups.

Appears This Way  
On Original

Treatment - Males : 60 days before cohabitation and during cohabitation  
 - Females : 14 days before cohabitation, during cohabitation and pregnancy up to organogenesis (day 8)

		Control	10 mg	40 mg	160 mg
Number dead-sacr. males / total number of males	(1)	0/24	0/24	0/24	3/24
Number dead-sacr. females / total number of females	(1)	0/24	0/24	0/24	2/24
Pregnancy index	(1)	22/24	22/24	21/24	1/23***
Copulation index	(1)	22/24	23/24	23/24	1/23***
Fertility index	(1)	22/22	22/23	21/23	1/ 1
Precohabitation period					
- treated males					
Body weight : day 0	(2)	281.2	279.6	279.9	282.0
: day 60	(2)	532.8	527.0	514.3	395.0***
Weight change : precohabitation period	(2)	251.6	247.4	234.5	113.0***
Food consumption : precohabitation period	(2)	2264.3	2049.6**	2139.4	1939.7*
- treated females					
Body weight : day 0	(2)	190.5	190.6	190.0	189.6
: day 14	(2)	260.1	254.0	246.5***	211.2***
Weight change : precohabitation period	(2)	69.6	63.5	56.5***	21.6***
Food consumption : precohabitation period	(2)	401.4	404.5	383.8	408.1
Pregnancy period					
- Female rat data					
Cohabitation - mating interval (median)	(2)	3.0	3.5	3.0	2.0
Body weight : day 1 of pregnancy	(2)	264.2	261.0	252.5**	201.0
: day 9 of pregnancy	(2)	308.6	300.1	282.2***	205.0
: day 22 of pregnancy	(2)	453.0	443.2	439.4	356.0
Weight gravid uterus	(2)	100.3	90.9*	99.5	77.2
Weight change : day 22 - day 1 - gravid uterus	(2)	88.4	91.4	87.5	77.8
Food consumption : day 1 - day 8	(2)	265.5	263.7	258.3	448.0
: day 9 - day 21	(2)	486.5	483.7	487.0	604.0
- Litter rat data					
Number of live foeti	(2)	13.7	12.5*	13.5	12.0
dead foeti	(2)	0.00	0.00	0.00	0.00
Mean litter size	(2)	13.7	12.5*	13.5	12.0
Number of resorbed foeti	(2)	0.64	0.59	0.76	0.00
Number of implantations	(2)	14.4	13.1*	14.3	12.0
Number of corpora lutea	(2)	15.3	15.3	15.5	14.0
Mean weight of foeti at caesarean section	(2)	5.4	5.4	5.4	4.6
Number of abnormal foeti (sum)	(2)	1	1	0	0

(1) Significance computed by Chi Square Test (two tailed) : \* p < .05 \*\* p < .01 \*\*\* p < .001

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

Appears This Way  
 On Original

*NI06655 (Study # 2774) Reproduction capacity study in Wistar rats*

Doses of 1.25, 5 and 20 mg/kg/day in a vehicle of hydroxypropyl- $\beta$ -cyclodextrin were orally gavaged to Wistar rats. There were no apparent body weight effects on either sex in the pre-mating period. During pregnancy, there were no dose-related effects on maternal body weight. Dose related effects on fetal weight and survival were apparent with no NOEL identified. These effects also occurred in the absence of maternal toxicity.

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

M: male, F: female

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

Survival of pups was recorded on days 4, 14 and 21 of age. The survival rate per litter is given in Tables 23, 25, 27 and 29 and averaged per dosage group in Table 21.

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

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

The method of assessing developmental effects was very insensitive. Instead of reporting the days at which developmental milestones were achieved, the sponsor waited until pre-specified

times, usually long after the natural time of occurrence and noted how many animals had achieved the milestone.

There were no survivors of the HD dams. The second generation showed strikingly decreased fertility.

$$\text{fertility index} = \frac{\text{number of pregnant animals}}{\text{number of animals with successful copulation}} \times 100$$

Dosage group mg/kg body weight/day	Fertility rate	Fertility index
0	11/12	92
1.25	11/12	92
5	2/5	40
20	-	-

Average weight of the gravid uterus was also decreased in the F1 generation with no NOEL identified.

**Weight of gravid uterus**

The weight of the gravid uterus per group of dams is given in Table 36. Individual data are given in Tables 37, 38 and 39.

Dosage group mg/kg body weight/day	Weight of gravid uterus (g)
0	100.5
1.25	93.0
5	90.1
20	-

The weight of the gravid uterus was comparable between groups.

Appears This Way  
On Original

Appears This Way  
On Original

Number of implantations and corpora lutea were decreased compared to control.

Number of implantations  
The number of implantations  
presented in Tables 37

Dosage group mg/kg body weight/c
0
1.25
5
20

Values were compared

	Vehicle	1.25 mg	5 mg
<b>Adult rat data</b>			
Number of dosed females	12	12	5
Number of dead females (1)	0/12	0/12	0/5
Fertility rate (1)	11/12	11/12	2/5
Body weight day 1 (2)	315.3	301.2	325.5
Body weight day 22 (2)	473.9	456.1	470.5
Weight gravid uterus (2)	100.5	93.0	90.1
Weight change of pregn. females (2)	58.2	61.9	55.0
Food consumption (d 1 - d 21) (2)	724.3	676.6	643.5
<b>Litter data</b>			
Number of live foeti / female (2)	13.9	13.2	12.0
Number of dead foeti / female (2)	0.09	0.09	0.00
Mean litter size (2)	14.0	13.3	12.0
Number of resorptions / female (2)	1.00	0.18 *	0.50
Number of implantations / female (2)	15.0	13.5	12.5
Number of corpora lutea / female (2)	19.3	16.2	16.0
Weight of live foeti (caes.del.) (2)	5.4	5.3	5.4
Sex ratio (%) (2)	42.1	60.2 **	45.8
Number of malformed pups (2)	0	0	0

In  
the  
FO

(1) Significance computed by Chi Square Test (two tailed) : \* p < .05 \*\* p < .01 \*\*\* p < .001  
 (2) Significance computed by Mann-Whitney U Test (two tailed) : \* p < .05 \*\* p < .01 \*\*\* p < .001

dams, no NOEL was identified for decreased pup birth weight and the decrease in pup survival. In the second generation, number of corpora lutea, number of implantations and number of live fetuses were decreased in the offspring of LD and MD-treated females. There were no survivors of the HD dams. It may be concluded from the data that nebivolol had effects upon fertility of drug-treated offspring with no NOEL identified. No developmental effects were demonstrated. However, the method of analysis, to check only at day 21 or 42 is insensitive. The developmental landmark data is inconclusive.

Appears This Way  
On Original

### Embryofetal development

*Study title: N51171 -- Embryotoxicity and teratogenicity study in Sprague-Dawley rats (Segment II). Exp N86-02*

**Key study findings:** There is no detail of what was found in the examination of the fetuses. There is no indication of maternal toxicity. It can't be said from the material presented that there is no teratological potential associated with this drug. The study itself may have been adequate, but the reporting is not.

**Study no.:** N51171

**Conducting laboratory and location:** Research department, Janssen Laboratories, Aubervilliers, France

**Date of study initiation:** January 21, 1986

**GLP compliance:** statement included

**QA reports:** yes ( ) no ( )

**Drug, lot #, and % purity:** R67555, lot V8510-201, purity —  
Vehicle of Tween 80, —

#### Methods

R67555 was given orally to female Sprague-Dawley rats at doses of 0, 1.25, 5 and 20 mg/kg/day from GD6 through GD16.

Parameters studies:

- Dams: mortality
- Clinical signs
- Body weight
- Food consumption
- Pregnancy

weight of gravid uterus  
gross necropsy findings

Litter: litter size and number of live, dead and resorbed fetuses  
Weight of live pups at cesarean section  
Abnormalities  
Salewski technique for litters with 0, 1,2 or 3 fetuses

### Results

Four females died during the study, 3 from unknown causes.

1 control day 18 (pregnant)

1 LD day 16 (not pregnant)

1 HD day 12 (not pregnant)

1 HD day 15 (pregnant) gavage accident

Average body weight gain was non-significantly decreased in the treated groups compared to the control group.

Dosage groups	Body weight on day 17	Body weight on day 22	Body weight gain
Control group	308.7	371.1	45.6
R 67555 1.25 mg/kg	299.1	364.4	38.7
R 67555 5 mg/kg	300.3	363.5	41.0
R 67555 20 mg/kg	296.9	355.9	35.5

Food consumption was decreased in the drug-treated groups even before dosing began and remained significantly lower than the control group during the period of drug administration.

Appears This Way  
On Original

Dosage groups	Food consumption		
	day 1 through 5	day 6 through 16	day 17 through 21
Control	155.4	317.4	149.6
R 67555 1.25 mg/kg	140.5**	289.8**	142.8
R 67555 5 mg/kg	139.4**	288.7**	145.7
R 67555 20 mg/kg	145.6*	287.9**	144.8

Appears This Way  
On Original

The sponsor's summary table of pregnancy data was poorly readable and is re-produced here for greater ease of reading.

	Treatment from day 6 to day 16 after mating			
	Dosage groups: R67555 mg/kg/day			
	control	1.25	5	20
Adult rat data				
# of dosed females	24	24	24	24
# of dead females	1/24	1/24	0/24	2/24
# of pregnant females	20/24	21/24	21/24	21/24
Body weight of preg. Fem. d. 17	308.7	299.1	300.3	296.9

Body weight of preg. Fem. d. 22	371.1	364.4	363.5	355.9
Weight change of preg females	45.6	38.7	41.0	35.5
Food consumption (d1-d5)	155.4	140.5**	139.4**	145.6*
Food consumption (d 6-d16)	317.4	289.8**	288.7**	287.9**
Food consumption (d 17-d21)	149.6	142.8	145.7	144.8
Litter data				
# living pups /female	12.4	13.0	12.0	12.7
# dead pups/female	0	0.1	0	0
Mean litter size	12.4	13.1	12.0	12.7
#resorptions/female	0.5	0.5	0.3	0.3
#implantations/female	12.8	13.6	12.4	12.0
???? of living pups (???del.)	5.2	5.1	5.2	4.9**
abnormalities	0/235	0/275	1/253	0/254

\*p<0.05, \*\*p<0.01 Chi Square and Mann Whitney U test

This is the extent of data found in this 30 page teratology study. There is no detail of what was found in the examination of the fetuses. There is no indication of maternal toxicity. It can't be said from the material presented that there is no teratological potential associated with this drug.

**Study title: N74513 -- Embryotoxicity and teratogenicity study in Sprague-Dawley rats (Segment II)**

**Key study findings:** Split center of the thoracic vertebrae was increased over control levels at MD (no maternal tox) and at HD( in the presence of maternal toxicity). The number of rudimentary sternums was increased at all dose levels, significantly so at the MD and HD. Ureteral dilatation was significantly increased at both MD and HD. The values also exceeded the historical ranges.

**Study no.: Exp 2111/88-17**

**Conducting laboratory and location:** Research Department, Janssen Laboratories, Aubervilliers, France

**Date of study initiation:**

**GLP compliance:** statement included

**QA reports:** yes ( ) no ( )

**Drug, lot #, and % purity:** R67555 in hydroxylpropyl- $\beta$ -cyclodextrin batch PFA081, purity not listed

**Methods**

In a preliminary conducted study (exp. No 2060/88-14), R 67555 was administered orally by gavage as a solution at 40, 80 and 160 mg/kg. The dose of 40 mg/kg induced moderate maternal toxicity and embryotoxicity. Eighty and 160 mg/kg were two toxic doses in pregnant females and resulted in a high mortality rate, indicating that dose level selection was not appropriate for the purpose of the study.

Doses of 0, 2.5, 10 and 40 mg/kg were given orally from GD6-GD16 to female Sprague-Dawley rats.

**Observations:**

Dams: mortality  
Clinical signs  
Body weight  
Food consumption  
Pregnancy

Litters: litter size and number of live, dead and resorbed fetuses  
Weight of live pups at caesarean section  
abnormalities

**Results**

Two HD females died of gavage accidents.

Body weight changes are summarized in the sponsor's table below.

Appears This Way  
On Original

Dosage groups	Body weight		Body weight gain (uterus weight subtracted)
	day 17	day 22	
Control group	340.6 ± 4.6 (17)	418.3 ± 9.0 (17)	53.6 ± 3.2 (17)
R 67555 2.5 mg/kg	337.4 ± 4.6 (21)	401.6 ± 8.6 (21)	52.3 ± 3.0 (21)
R 67555 10 mg/kg	344.3 ± 4.4 (21)	422.8 ± 6.1 (21)	56.0 ± 2.6 (21)
R 67555 40mg/kg	332.1 ± 4.7 (19)	384.9 ± 11.3* (18)	51.4 ± 3.5 (18)

The figure in parentheses ( ) indicates the actual number of females per group.

\*p < 0.05 by Mann-Whitney U test as compared to the respective controls.

Body weight was comparable between groups except at 40 mg/kg which showed a slight decrease on day 17 (statistically non-significant) and on day 22 (p < 0.05), as well as a slightly decreased weight gain (statistically non-significant).

Food consumption was slightly lower in the drug-treated groups before dosing began and was significantly lower in the HD group during the period of drug administration. Also noted in the HD group is a significant decrease in the weight of the gravid uterus.

While there was no reported increase in dead feti per female, there was a decrease in live pups per female and corresponding decrease in mean litter size at the HD. The weight of the live fetuses was also decreased at the HD. This is summarized in the sponsor's table below.

Appears This Way  
On Original

Treatment : from day 8 through day 16 of pregnancy

	Control	2.5 mg	10 mg	40 mg
Adult rat data				
Number of dosed females	24	24	24	24
Number of dead females (1)	0/24	0/24	0/24	2/24
Number of pregnant females (1)	17/24	21/24	21/24	21/24
Body weight day 1 (2)	256.1	260.3	257.4	261.2
Body weight day 5 (2)	274.1	279.3	276.8	278.5
Body weight day 17 (2)	340.6	337.4	344.3	332.1
Body weight day 22 (2)	418.3	401.6	422.8	384.9 *
Weight gravid uterus	108.8	89.0	109.3	71.5 **
Weight change of pregn. females (2)	53.8	52.3	56.0	51.4
Food consumption (d 1 - d 5) (2)	150.5	146.7	146.9	142.6
Food consumption (d 6 - d16) (2)	349.2	333.7	336.0	300.7 ***
Food consumption (d17 - d21) (2)	182.2	170.3	180.9	162.1 *
Litter data				
Number of live foeti / female (2)	14.1	11.3	14.3	9.3 **
Number of dead foeti / female (2)	0.00	0.00	0.00	0.00
Mean litter size (2)	14.1	11.3	14.3	9.3 **
Number of resorptions / female (2)	0.35	0.46	0.33	3.21 **
Number of implantations / female (2)	14.5	11.8	14.6	12.5
Number of corpora lutea / female (2)	15.8	14.8	15.7	14.8
Weight of live foeti (cass.del.) (2)	5.8	5.9	5.7	4.8 ***
Sex ratio (X) (2)	45.0	52.8	51.0	44.4

(1) Significance computed by Chi Square Test (two tailed) : \* p < .05 \*\* p < .01 \*\*\* p < .001  
 (2) Significance computed by Mann-Whitney U Test (two tailed) : \* p < .05 \*\* p < .01 \*\*\* p < .001

Appears This Way  
 On Original

The results of the fetal examinations are summarized in the sponsor's table below.

Observation	Dosage group ( mg / kg )			
	Control	2.5	10	40
A. Affecting the whole body	0	0	0	0
B. Affecting parts, regions or organs				
1. Cranium and contents	0	0	0	0
2. Spine and spinal cord	0	0	0	0
3. Face and sense organs	0	0	0	0
4. Neck	0	0	0	0
5. Thorax and contents				
- split center of the thoracic vertebra(e)	41	30	52	100 **
- rudimentary 14th pair of ribs	1	0	0	0
- one rudimentary 13th rib	0	0	1	2
- missing vertebra(e)	0	0	0	1
- reduced ossification of the thoracic vertebra(e)	0	0	0	1
- sternum bone(s): poorly ossified	4	4	5	2
- dumbbell-shaped sternum bone(s)	5	13	8	6
- asymmetrical dumbbell-shaped sternum bone(s)	1	4	3	5
- rudimentary sternum bone(s)	1	5	11 *	19 **
- asymmetrical sternum bone(s)	5	4	5	11
- sternum bone(s): cleaved	0	1	0	1
- sternum bone(s): missing	0	1	1	2
6. Abdomen and contents				
- celoschisis	0	0	1	0
7. Pelvis and contents				
- ectopic kidney	0	0	0	1
8. Urogenital system				
- ureter(s) torsion	13	12	21	19
- ureter(s) dilatation	3	2	21 **	42 ***
- ectopic testes	0	1	0	3
- ureterohydronephrosis	0	0	1	0
9. Extremities				
- talipes	5	5	5	0 *
- twisted tail	1	0	0	0

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

Appears This Way  
On Original

Appears This Way  
On Original

Anomalies were reported for the LD and MD group with no apparent maternal toxicity. The historical incidences of these anomalies are shown below in the sponsor's tables.

Appears This Way  
On Original

HISTORICAL CONTROL DATA OF RAT FETAL OBSERVATIONS Experiment No.										
Observations	88-01	88-05	88-07	88-09	88-10	88-15	89-01	89-03	89-07	89-08
<b>A. Affecting the whole body</b>										
- hydrops										1
- meiomeilus									1	
<b>B. Affecting parts, regions or organs</b>										
<b>1. Cranium and contents</b>										
<b>2. Spine and spinal cord</b>										
<b>3. Face and sense organs</b>										
- microblepharia					1					
<b>4. Neck</b>										
<b>5. Thorax and contents</b>										
- split center of the thoracic vertebra(e)	10	8	6	8	15	1	15	27	12	7
- missing 12th rib(s)									1	
- rudimentary 13th rib(s)	1						1	1		
- one rudimentary 14th rib	1				1			3		2
- rudimentary 14th pair of ribs						1				4
- wavy rib(s)				1	1					
- sternum bone(s)										
asymmetrical						5	2	5		1
dumbbell-shaped						8	4	5	2	10
rudimentary						3	2	4	8	20
missing							1		4	
incomplete ossification							1			
cleaved							2			
<b>6. Abdomen and contents</b>										
- celoschisis		1								
<b>7. Pelvis and contents</b>										
- lumbar vertebra : displaced						1				
<b>8. Urogenital system</b>										
- ureter(s) dilated or twisted	4				12	15	4	29	10	
- ectopic ovaries									1	
<b>9. Extremities</b>										
- missing phalanges								19		6
- talipes	1				1				39	1

**Study title: N74514 Segment II Oral embryotoxicity and teratogenicity in Sprague-Dawley rats**

**Key study findings:** The findings of the previous study were repeated with respect to 1) an increased number of split thoracic vertebrae in all drug-treated groups 2) Increased number of rudimentary sternum in all drug-treated groups and 3) increased number with ureteral dilatation but not with the dose-response as seen in the other two effects.

**Study no.:** 2210/89-10

**Conducting laboratory and location:** Research Department, Janssen Laboratories, Aubervilliers, France

**Date of study initiation:**

**GLP compliance:** statement included

**QA reports:** yes ( ) no ( )

**Drug, lot #, and % purity:** R67555 in a vehicle of HPβCD lot # PFA101 (as noted in report and ZR067555PFA101 as noted on the certificate of analysis)% purity not apparent

**Methods**

This study was conducted at doses of 0, 2.5, 10 and 40 mg/kg to see if the results of the previous study could be repeated. Sprague-Dawley rats \_\_\_\_\_ were again used.

The day that sperm was located in the vagina was taken as Day 1 of gestation.

**Observations:**

- Dams:
  - mortality
  - Clinical signs
  - Body weight
  - Food consumption
  - Pregnancy rate
  - Gross necropsy
  - Weight of gravid uterus and number of corpora lutea
- Litter:
  - litter size and number of live, dead and resorbed fetuses
  - Weight of live pups at caesarean section
  - Sex of live pups
  - Salewski technique for litters with 0, 1,2 or 3 fetuses
  - Abnormalities

Appears This Way  
On Original

## Results

One HD female died prior to scheduled euthanasia. The sponsor's summary of pregnancy data is shown below. Maternal toxicity was not apparent in the MD or LD groups.

Treatment : from day 5 through day 16 of pregnancy

	Control	2.5 mg	10 mg	40 mg
Adult rat data				
Number of dosed females	24	24	24	24
Number of dead females (1)	0/24	0/24	0/24	1/24
Number of pregnant females (1)	22/24	23/24	21/24	18/24
Body weight day 1 (2)	233.1	232.8	230.2	237.4
Body weight day 6 (2)	259.2	258.0	254.1	260.5
Body weight day 17 (2)	313.6	325.7	320.0	310.8
Body weight day 22 (2)	365.9	384.9	383.3	358.2
Weight gravid uterus	66.6	88.7	79.0	63.9
Weight change of pregn. females (2)	68.2	73.3	74.1	57.0 *
Food consumption (d 1 - d 5) (2)	121.8	127.1	125.0	128.6
Food consumption (d 6 - d16) (2)	312.1	316.7	301.5	277.5 ***
Food consumption (d17 - d21) (2)	140.6	153.9 *	149.8	138.7
Litter data				
Number of live foeti / female (2)	8.7	11.7	10.6	8.6
Number of dead foeti / female (2)	0.00	0.00	0.00	0.00
Mean litter size (2)	8.7	11.7	10.6	8.6
Number of resorptions / female (2)	0.91	0.13 *	0.43	1.94
Number of implantations / female (2)	9.6	11.8	11.0	10.6
Number of corpora lutea / female (2)	13.5	15.3	14.8	14.4
Weight of live foeti (cases.del.) (2)	6.0	5.9	5.6 *	5.0 ***
Sex ratio (%) (2)	51.5	50.7	48.0	50.8

(1) Significance computed by Chi Square Test (two tailed) : \* p < .05 \*\* p < .01 \*\*\* p < .001

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

Appears This Way  
On Original