APD50 was increased beyond the solvent effect in the d,l-sotalol-treated samples. Samples treated with nebivolol or propranolol showed a shortening of the APD50.

Isolated rabbit Purkinje fibers incubated with 4mM KCl Tyrode solution

Time (min)	0	15	30	45	60	65
Conc	0	0.1%DMSO				
Stim.rate (Hz)	1	1	1	1	1	0.2
APD50 (ms)solvent	156	163(4)	171(10)	183(17)	190(22)	200(28)
APD50: Nebivolol	162	169(4)	176(9)	173 (7)	110(-32)	89(-45)
APD50 :Propranolol	189	188	191(1)	165(-13)	74 (-61)	87(-54)
APD50:d,l- sotalol	159	151(-5)	163(3)	161(1)	213(34)	302(90)

Numbers in parentheses are percentage difference from baseline

Solvent alone caused a slight increase in APD90. Both d,l-sotalol and nebivolol caused further increases in APD90. Propranolol caused a decrease in this parameter.

Isolated rabbit Purkinje fibers incubated with 4mM KCl Tyrode solution

Time (min)	0	15	30	45	60	65
Conc	0	0.1%DMSO				
APD90 (ms) solvent	216	221(2)	228(6)	237(10)	242(12)	277(28)
APD90: nebivolol	234	245(5)	252(8)	259(11)	259	374(60)
APD90: propranolol	246	245	246	226(-8)	178(-28)	219(-11)
APD90: d,l-sotalol	208	210	218(5)	247(19)	333(60)	520(150)

Both solvent and all 3 drugs caused a decrease in Vmax up to 45 minutes. Both nebivolol and d,l-sotalol then began to increase the Vmax . Propranolol and the solvent continued to cause a decrease in Vmax.

Isolated rabbit Purkinje fibers incubated with 4mM KCl Tyrode solution

Time (min)	0	15	30	45	60	65
Conc	0	0.1%DMSO				
Vmax(V/s) solvent	556	543(-2)	486(-13)	442(-21)	405(-27)	412(-26)
Vmax: nebivolol	519	494(-5)	497(-4)	518	572(10)	547(5)
Vmax: propranolol	517	543(5)	499(-3)	485(-6)	401(-22)	478(-8)
Vmax: sotalol	400	390(-3)	365(-9)	450(12)	402	479(20)

Only d,l-sotalol produced EADs in this system under conditions of 0.2 Hz stimulation at 65 minutes incubation at a concentration of $1x10^{-4}$M. One out of 7 trials (1/7) showed an EAD. It

should be noted that if the positive control causes only 1 event out of 7 trials, there is small likelihood of seeing an event produced by the test article.

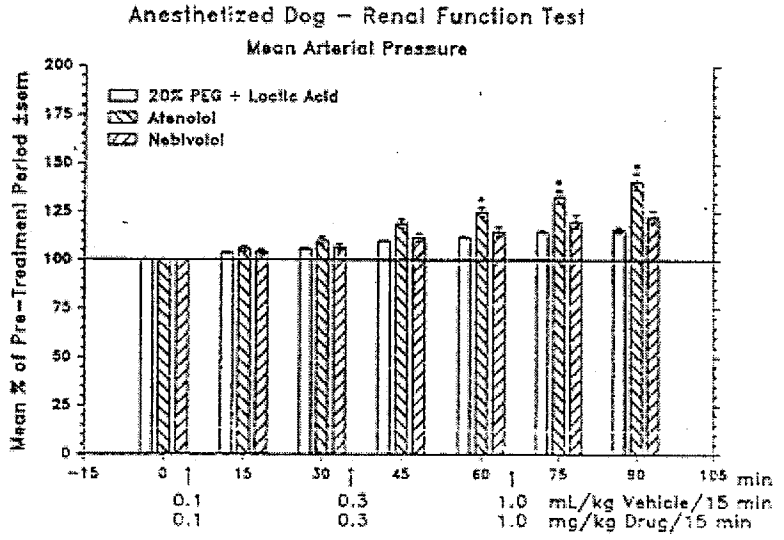
Renal effects:

N62264 Evaluation of nebivolol in the anesthetized dog renal function test
The effect of nebivolol on renal hemodynamics was evaluated in pentobarbital anesthetized dogs. The activity was compared to that of propranolol and atenolol. All compounds were tested at similar doses of 0.1, 0.3 and 1.0 mg/kg/15 min in 15 minute infusions of drug followed by a 15 minute rest period.

The pharmacological evaluation of nebivolol has shown cardioselective beta-adrenergic blocking activity (1,2,3). We have evaluated the effects of nebivolol, propranolol hydrochloride and atenolol on renal hemodynamics and function in the pentobarbital anesthetized dog. The hemodynamic parameters measured were diastolic, systolic and mean arterial blood pressure, heart rate, renal and femoral blood flow and calculated vascular resistances. Serum and urine sodium, potassium, chloride, uric acid, creatinine and osmolarity were determined and urine volume and pH was measured. The details of these experiments are the subject of this report.

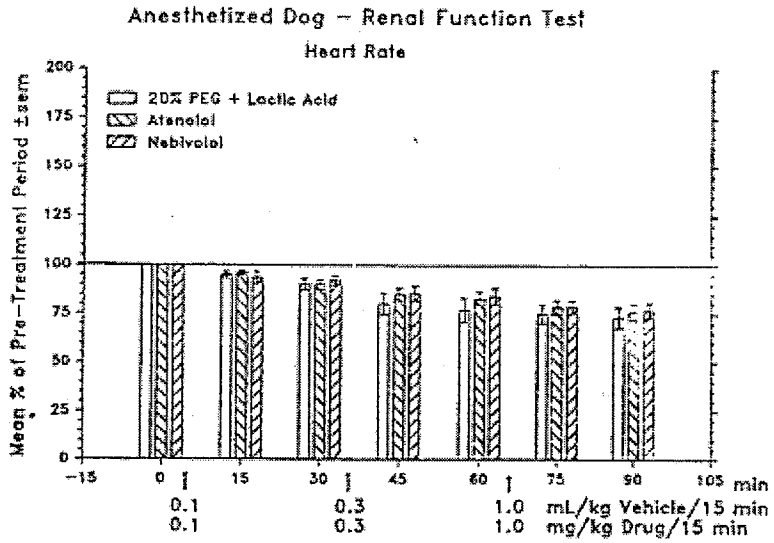
Nebivolol was initially studied using 20% PEG and lactic acid as a vehicle, however, this vehicle produced hemolysis and changes in renal function at the volumes administered. Data from the studies with nebivolol and atenolol in this vehicle are included in this report. Nebivolol was retested using 2% PEG plus 2% tartaric acid, a vehicle which we have shown does not produce significant changes in renal function and hemodynamics. Atenolol and propranolol hydrochloride were tested in saline for comparative data.

Female mongrels were used. Dogs were infused with 0.9% saline at the rate of 160 ml/mr. Creatinine was also infused at approximately 50 mg/kg/hr. The dogs were allowed to stabilize for at least three 15 minute control periods. Blood and urine samples were taken at the end of each of these periods. The vehicle was then infused for 15 minutes followed by a 15 minute rest period. The cycle of test compound and rest period



was repeated with increasing doses of compound. Pre and post-test doses of isoproterenol were administered to check for completeness of beta blockade.

The vehicle of 20% PEG and lactic acid itself caused some changes in heart rate, systolic and diastolic blood pressure. There were no obvious differences between nebivolol and atenolol at this point.



Vehicle and both drugs caused similar decreases in heart rate.

Vehicle, atenolol and nebivolol all caused increases in mean arterial pressure.

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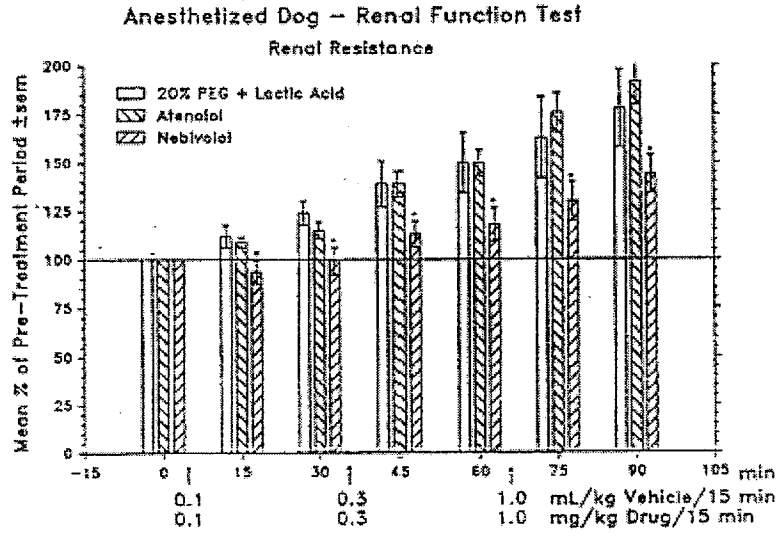
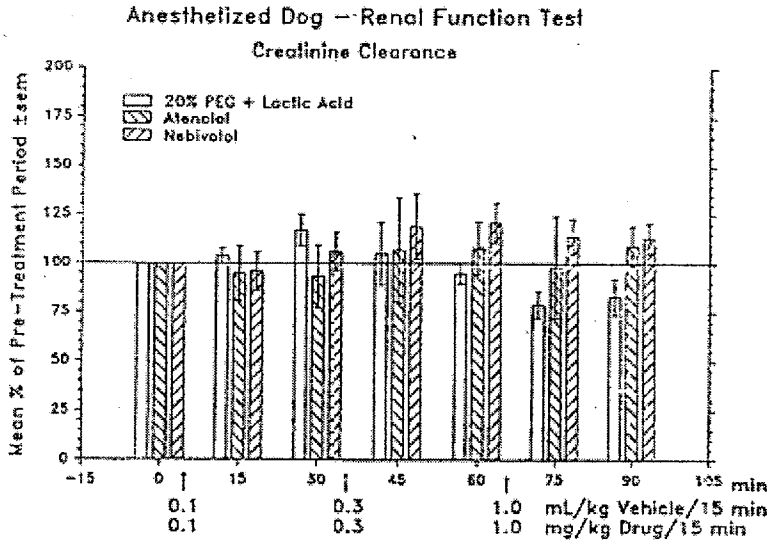
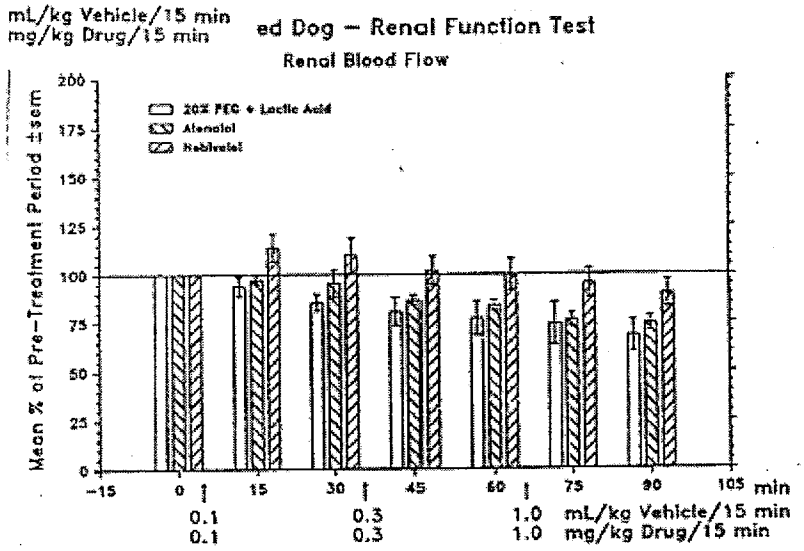


All 3 substances caused decreases in renal blood flow. Nebivolol appeared to cause less of a decrease than the other 2 chemicals.

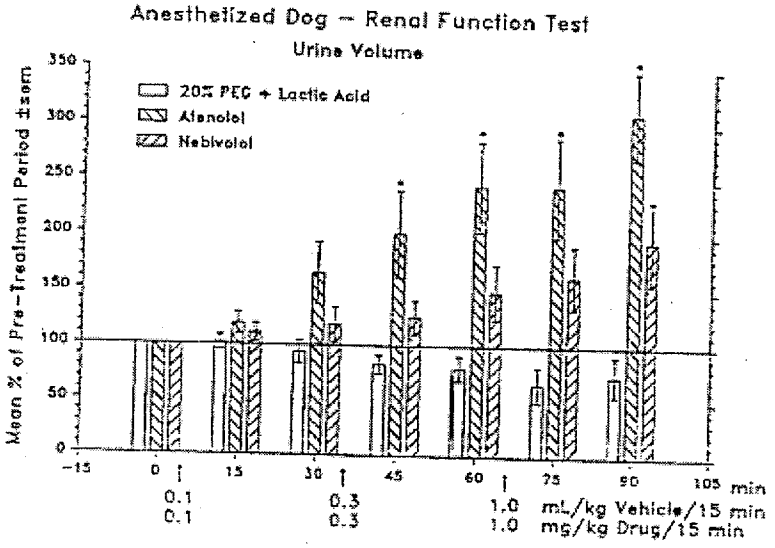


Over time, creatinine clearance decreased with the vehicle and appeared to increase slightly with atenolol and nebivolol.

Renal blood flow was decreased with all 3 substances. There was less of a decrease with nebivolol.



Urine volume was increased, to the greatest extent following atenolol.



The vehicle itself produced effects on the various parameters measured. There was no apparent difference between the 3 chemicals in effects on heart rate. All produce an increase in mean renal arterial pressure, with the greatest effect from atenolol. Creatinine clearance showed a slight decrease with vehicle and similar increases with atenolol and nebivolol. Urine volume showed a slight decrease with vehicle treatment and increases following atenolol or nebivolol. From the data presented, there do not appear to

be significant differences between atenolol and nebivolol in this test system.

Neurological effects:

CNS: *CPF:5668 Single oral dose safety pharmacology study in the rat: modified Irwin's test Feb 28, 2003*
Nebivolol was given by oral gavage to male Sprague-Dawley rats at single doses of 200, 400 or 800 mg base equivalent /kg body weight. The vehicle was 0.5% methocel. Mortality, behavioural observations, clinical signs and body weight were evaluated in the 7 days following the dose administration. Neurofunctional integrity and signs of neurotoxicity were assessed at 0.5, 2,4 and 24 hours after oral administration of drug. The rats were assessed again on day 7 for evidence of delayed neurotoxicity

Results

There was one unscheduled death in the HD group on day 3. The signs associated were poor condition, wet urogenital region, crusty nose, ptosis and hypothermia.

The 200 mg/kg dose did not cause obvious clinical signs. At 400 and 800 mg/kg, signs included body weight effects, ptosis (all animals: 5/5, 5/5) and nasal discharge (1/5 and 5/5). The HD group also had wet urogenital region.

Incidence of effects

	Dose mg/kg			
	0	200	400	800
Abnormal licking		2/5	2/5	3/5
Sedation*		3/5	2/5	4/5
Decreased vocalisation			"some"	"some"
Delayed visual placing response				
piloerection	0	1/5	3/5	5/5
Exertional tremors, 24 hours after dose	0	1/5	3/5	3/5

*By 4 hours after dosing, sedation was seen in all animals. This persisted until 24 hours at all doses.

Summary of further results

	Dose mg/kg (n=5 per group)			
	0	200	400	800
Spontaneous behaviour: Abnormal licking		2m at 0.5 hours	2m at 0.5 hrs	2m at 0.5 hrs 1 m at 2 hrs
Motor-affective responses sedation		3/5 at 2 hrs, 5/5 at 4 and 24 hours	5/5 at 2 and 4 hours, 4/5 at 24 hrs	4/5 at 2 hrs, 5/5 at 4 and 24 hrs
urinations				Slightly decreased @ 24hrs
defecations		Decreased @ 2 and 4 hrs	Decreased @ 2 hrs	Decrease at 0.5, 2,4 and 24 hrs
Delayed visual placing		@ 24 hrs	@ 24 hrs	Absent in 1, Delayed in others, delayed at day 7 in one
Corneal reflex slowed			4/5 @ 24hrs	4/4@24 hrs
Pinna reflex				Decreased @4 and 24 hrs
Righting reflex			↓1/5 at 24hrs	↓2/4@ 24hrs absent in 1
Narrowing of the palpebral fissure		Present at 2, 4 + 24 hours	Present in all rats at 2,4+24 hrs	Present in all rats at 2,4 +24 hrs. Eyes completely closed in 1 rat at 24 hrs.

Decreased pupil diameter was seen in all dosed groups up to 24 hours after dosing but not at 7 days.

Body temperature showed a decrease in all treatment groups. This decrease was present at 24 hours in the HD group..

	Dosage Group (mg / kg) Males			
Day	Vehicle	Low:500	Med.:400	High:800
-2	189 (4.2)	188 (3.6)	191 (3.6)	190 (3.6)
0	193 (2.3)	196 (3.7)	195 (4.1)	195 (2.9)
7	243 (2.9)	243 (5.9)	235 (5.8)	215 (5.6)

Standard error is shown between brackets

The MD and HD groups showed less weight gain than the control and LD groups

Mean body weight values per dosage group in g.

The sponsor summarized the results as follows:

Neurofunctional integrity of rats was slightly affected after a single oral dose of R067555 at 200 mg b.e./kg body weight, as evidenced by slight behavioural effects (abnormal licking, motor-affective and sensoro-motor responses), slight signs of neurological aberration (tremors at exertion) and slight autonomic impairment (eyes, decreased body temperature, piloerection). There were no signs of general toxicity recorded.

Dosing at 400 mg b.e./kg b.w. led to behavioural effects (abnormal licking, motor-affective and sensoro-motor responses). Neurological aberration was evidenced by impaired equilibrium and gait (delayed righting reflex) and signs of CNS excitation (tremors at exertion, clonic convulsions of the jaws). Autonomic impairment included a decreased pupil size, narrowing of the palpebral fissure, a decreased body temperature and piloerection. Signs of slight general toxicity included crusty eyes and chromodacryorrhea.

At 800 mg b.e./kg b.w. various neurotoxic signs were observed: besides abnormal licking, decreased motor-affective and sensoro-motor responses were noticed. Signs of neurological imbalance included an impaired righting reflex (equilibrium and gait), tremors at exertion and clonic convulsions of the jaws (CNS excitation). The decrease in pupil size, the narrowing or closure of the palpebral fissure, the decrease in body temperature, the development of piloerection and the decreased respiratory rate were considered signs of autonomic impairment. Crusty eyes were observed in several animals. Chromodacryorrhea was noticed in one male rat.

Peak effects were set at 4 hours post-dosing (ranging from 2 to 24 hours post-dosing).

Delayed neurotoxicity (day 7) was not considered an issue.

Some of the signs may be referable to exaggerated pharmacologic effect. Data to support this is not present in the report, but hypotension could explain the sedation evident as ptosis, hypothermia and delayed righting. However, grip strength did not appear to be affected as one would expect. There were also signs suggesting autonomic effects such as narrowing of the palpebral fissure, decreased respiratory rate, tremors at exertion and clonic convulsions of the jaws.

N62816 In vivo pharmacological profile of the selective beta1-adrenoceptor antagonist nebivolol in mice, rats and dogs. Sept 1988
Swiss mice, Wistar rats and Beagles were used.

Male mice were used for the following tests:

Hot plate- reaction time measured in 0.1 sec between contact with plate and a Licking or jumping response. Measurements were made before and 15, 30, 45 and 60 minutes after dosing. Criterion for drug effect was a reaction time of >20 seconds (occurring in <1% of control mice).

Pupil diameter: measured in 1 eye before and 15, 30, 45 and 60 minutes after Dosing. Miosis/mydriasis was defined as a diameter occurring in less than 2% of the control mice.

Traction test: mice were put on a grid and subjected to continuously increasing traction. Resistance to traction of the grasping mouse obtained in the second of two trials was read from a scale which was graduated in g of pulling force. The test was performed before and 15, 30, 45 and 60 minutes after dosing. Increases of >30 g were considered indicative of hypertonia. Decreases of >40 g were considered indicative for hypotonia.

Acetic acid-induced writhing: 75 minutes after dosing, 0.05 ml of acetic acid (1%) was injected intraperitoneally and the mice placed in individual chambers for observation. The number of writhing movements in 5 minutes was recorded. The drug was considered to have an inhibitory effect when the recorded number of writhing movements was less than 15.

Anticonvulsant activity after maximal electroshock: 85 minutes after dosing, the mice were tested for protection against corneally delivered electroshock. scoring was: 0=no inhibition of tonic or clonic convulsions, 1= inhibition of clonic convulsions of the hind-paws, 2= complete inhibition of tonic convulsions (fore and hind paws), 3= absence of both tonic and clonic convulsions.

Behavioral changes: Observations were made at 15, 30, 45 and 60 minutes after dosing for occurrence of straub tail on arched back, ptosis, exophthalmos, sedation, prostration, ataxia, hypnosis, chewing, excitation, tremors, convulsions, lacrimation, salivation, diarrhea and piloerection.

General screening test in rats: At least 3 female rats were used with 3 other rats serving as controls. Effects of the test article on behavior was evaluated on all-or-none criteria based on the frequency of results in a population (~2000) of control rats.

Palpebral opening was scored from 0-6: 0=eyes completely closed, 2=half open, 4= wide open, 6=exophthalmos. Scores 1,3 and 5 were intermediate values. Recordings were made before and 1,2 and 3 hours after dosing.

Pupil diameter of one eye was measured using a micrometer in gradations of 1/25mm. Measurements were made before and 1,2 and 3 hours after dosing. Pupil diameter <5 units was defined as miosis and diameter >20 units was called mydriasis.

Body temperature was measured using an esophageal probe and an electric thermometer. Measurements were made before and 1,2 and 3 hours after dosing. Hypothermia was defined as a decrease of 2◦C. An increase of 1 degree C was called hyperthermia.

Behavioral changes: animals were observed 1,2 and 3 hours after dosing for occurrence of sniffing, licking, rearing, preening, chewing, excitation, tremors, convulsions, lacrimation, salivation, diarrhea, piloerection, passivity, sedation, prostration, catalepsy, ataxia, hypnosis, pinna and cornea reflexes, muscle tone, dyspnea and cyanosis. Muscle tone was scored -3, -2, -1 or +1, +2,+3 according to the degree of tone decrease or increase.

Tail withdrawal test: 60 minutes after dosing, the rat's tail was dipped into a warm (50 C) water bath and the time of withdrawal of the tail was indicated in seconds. Withdrawal was considered to be inhibited when reaction time was >5 seconds.

Anti-diarrheal activity: 65 minutes after dosing, 1 ml of castor oil was given orally to the rats. Presence or absence of diarrhea 1 and 2 hours later was noted.

Anti-convulsant activity: 3 hours after dosing, pentylenetetrazole (80 mg/kg) was given intravenously. Scoring was: 0=no inhibition of tonic or clonic convulsions, 1= inhibition of clonic convulsions of the hind-paws, 2= complete inhibition of tonic convulsions (fore and hind paws), 3= absence of both tonic and clonic convulsions.

Tests related to specific neurotransmitters or mediators: apomorphine, tryptamine and norepinephrine antagonism in rats.

> Apomorphine was injected 30 minutes after dosing. Intensity of the stereotypical Behavior was scored 12 times, every 5 minutes over a 1 hour period following Injection of the apomorphine. The scoring was complicated and the sponsor's Description is shown below.

injection of apomorphine. The score system was pronounced (3), moderate (2), slight (1) and absent (0). An occurrence of less than 7 times score ≥ 2 (1.95% false positives in the control population) or less than 7 times score ≥ 1 (0.41% false positives) was considered as indicative for inhibition of agitation. An occurrence of more than 9 times score 3 (1.2% false positives) or more than 10 times score ≥ 2 (0.4% false positives) was considered to reflect potentiation of agitation. An occurrence of less than 4 times score 3 (0.28% false positives), less than 7 times score ≥ 2 (1.91% false positives), or less than 7 times score ≥ 1 (1.33% false positives) was considered as indicative for inhibition of stereotypy. An occurrence of more than 10 times score 3 (0.4% false positives), more than 10 times score ≥ 2 (0.1% false positives), or more than 12 times score ≥ 1(0.3% false positives) was considered to reflect potentiation of agitation. In addition, palpebral opening was scored according to a 6-point scale (0 = eyes closed; 4 = eyes open; 5 = exophtalmos; 1, 2, 3 = intermediate openings). More than 7 times score 0 (0.7% false positives) or 12 times score ≤ 1 (1.8% false positives) was considered to reflect inhibition of palpebral opening (palpebral ptosis). More than 5 times score 5 (1.3% false positives) or more than 10 times score ≥ 4 (1.4% false positives) was considered to reflect stimulation of palpebral opening (exophtalmos).

Tryptamine antagonism

Tryptamine (40 mg/kg, i.v.) was injected 60 min after the first (apomorphine) challenge. Several behavioral changes were observed within one min. Bilateral clonic seizures of the forepaws and coarse body tremors were scored as pronounced (3), moderate (2), slight (1) or absent (0). Scores less than 2 and less than 3 were considered indicative for antagonism of the tryptamine-induced bilateral clonic seizures and coarse body tremors, respectively. Score 3 at 5 min was considered to reflect potentiation of bilateral clonic seizures and coarse body tremors (0.0% false positives for both). In addition, palpebral opening was scored according to a 6-point scale (0 = eyes closed; 4 = eyes open; 5 = exophtalmos; 1, 2, 3 = intermediate openings). A score less than 4 occurred in 4.1% of the control rats and was considered to reflect antagonism of tryptamine-induced exophtalmos. The pupil diameter was measured (in 1/25 mm units)

and diameters > 68 (0.23% false positives) and < 21 (2.67% false positives) were considered to reflect mydriasis and miosis, respectively. Transient cyanosis, i.e. blueing of the ears was assessed 2 min after the injection of tryptamine. Significant hyperemia (red ears; 3.32% false positives) was considered indicative for reversal of cyanosis. Body temperature was measured; temperatures below 37.0° C (3.5% false positives) were considered to reflect hypothermia and temperatures above 39.5° C (3.7% false positives) were considered to reflect hyperthermia. Mortality occurred in 6.0% of the control rats and was considered to be drug-induced if occurring dose-dependently in pretreated animals.

Norepinephrine antagonism

Norepinephrine (1.25 mg/kg, i.v.) was injected 90 min after the first (apomorphine) challenge and mortality was recorded up to 60 min later. Survival for at least 15 min was selected as criterion for protection of the norepinephrine-induced lethality (0.80% false positives).

Apomorphine emesis in dogs

This test has been extensively described by Janssen et al. (1965b) and Niemegeers (1982). Apomorphine was subcutaneously administered to Beagle dogs in the dose of 0.31 mg/kg (4 times the ED_{95}). At 1 h before the apomorphine challenge, the dogs were pretreated with the test compound or solvent. Complete absence of emesis, for 1 h after the apomorphine challenge, was adopted as the criterion of antiemetic activity (never observed in controls, n > 1000).

Physostigmine lethality in rats

This test has been described by Niemegeers et al. (1982). At 1 h after administration of the test compound or solvent, the pupil diameter of the rats was measured in 1/25 mm units with a microscopic micrometer. Immediately thereafter, the rats were injected with physostigmine (1.0 mg/kg, i.v.) and survival time was recorded up to 120 min later. Mydriasis was considered to be present when the pupil diameter exceeded 20 units (found in 2.20% of > 500 control rats). Survival after physostigmine was found in 1.80% of the controls and was the criterion used for evaluation of physostigmine antagonism by the test compound.

Nicotine lethality in rats: Nicotine (10 mg/kg) was injected iv 1 hour after dosing. Survival of $\geq$2 hours was considered to show significant nicotine antagonism.

Reversal of antidiarrheal effect of clonidine in rats: Clonidine (0.04 mg/kg s.c.) and castor oil (1 ml, p.o.) were simultaneously administered at 0.5 hours after dosing. Diarrhea at 90 minutes after castor oil was considered to show clonidine antagonism.

Xylazine loss of righting reflex in rats: Xylazine 15 mg/kg iv was injected 1 hour after dosing. Absence of loss of righting was considered reflective of xylazine antagonism (alpha adrenergic agonist).

This test has been described by Janssen et al. (1988). Xylazine (15.0 mg/kg, i.v.) induced loss of the righting reflex in 95.8% of all control animals (n > 300). Xylazine was injected at 1 h after administration of the test compound or solvent. Absence of loss of righting was considered to reflect xylazine antagonism. The duration of the loss of righting reflex was almost never longer than 100 min in the control population (in only 1.3 % of the rats). Therefore, loss of righting reflex over a period longer than 100 min was considered to reflect xylazine potentiation.

Compound 48/80 lethality in rats

This test has been described by Niemegeers et al. (1978). Compound 48/80 is a synthetic mast cell activator. At 1 h after administration of the test compound or solvent, compound 48/80 (0.50 mg/kg, i.v.) was injected to produce anaphylactic shock by released mast cell mediators. Survival of rats at 240 min following the challenge occurred in 4.97% of the control rats (n = 1000) and was the criterion for protective activity.

compound 48/80-induced gastric ulcers in rats

This test has been extensively described by Awouters et al. (1985). Gastric lesions were induced by compound 48/80 (1.0 mg/kg, i.v.) in animals protected from lethality by the histamine H1 antagonist R 37 617 (10.0 mg.kg, s.c.) 1 h earlier. The animals were pretreated with test compound or solvent at 1 h before the compound 48/80 challenge. The animals were killed four hours after the injection of compound 48/80. The stomachs were removed and inspected for gastric lesions using a five-score system (0: absent; (+) traces of superficial erosion; + at least one distinct red area; ++ more and larger red areas; +++, red areas covering more than half the glandular tissue). Only 1.3% of the solvent-treated rats (n > 1000) was found to be completely free of gastric lesions (score 0) and 3.9% was found to have traces of erosion [score (+)]. Inhibition of gastric lesions was measured on the basis of scores (+) and (0). Five min after injection of compound 48/80, cyanosis of the ears was also assessed (0, 1, 2) and score 0 was considered indicative for reversal of cyanosis (0.4% in controls).

5-HTP potentiation in rats

The procedure and evaluation has been extensively described by Awouters and Niemegeers (1984). The rats were injected i.v. with L-5-hydroxytryptophan (5-HTP, 0.4 ml/100 g body weight) at 1 hr after s.c. pretreatment with test compound or solvent. The following phenomena were scored, counted or recorded: cyanosis of the ears (at 5 min); head twitches (between 2 and 10 min); forepaw trampling; shock-like behaviour (prostration and crawling, at 10 and 30 min); survival time (up to 120 min); and gastric mucosal lesions (120 min). The scores were given according to the intensity scale 0 (absent or doubtful), 1 (present), 2 (pronounced) and 3 (maximal, only for forepaw trampling and tremors). The following criteria for potentiation were used: cyanosis (score > 0, 4.9% false positives), head twitches (number > 2 counts, 6.8% false positives), forepaw trampling (score > 0, never observed in controls), shock [prostration (score > 1, 1.9% false positives) or crawling (score > 1, 3.7% false positives)], and death (early death, never observed in controls).

Observation test in rats

Catalepsy and palpebral ptosis were assessed at hourly intervals over a period of 8 hours. Additional behavioral phenomena were recorded and body temperature was measured at time intervals of 1,2,4,6 and 8 hours.

Motor activity in rats: recorded by microprocessors over a period of 27 minutes at 1 hour after dosing.

Castor oil diarrhea in rats: One hour after giving drug to female rats, castor oil was given p.o. Presence/absence of diarrhea was recorded 1,2,3,4,6 and 8 hours after giving oil.

Conditioned food consumption in rats: Rats were trained to consume a fixed amount of food over a 6 hour period. Half an hour before the start of the test the animals received the test compound. Food consumption, fecal and urinary excretion were also measured.

Fecal and urinary excretion test in rats: a separate category for these observations was listed. It is not clear if this was entirely a separate test.

Tail withdrawal reaction test in rats: performed 1 hour after dosing, tail was placed in 55C water bath. Time to withdrawal was recorded. Was this test performed twice?

Acetic acid-induced writhing in rats: Rats were given intraperitoneal injections of acetic acid. Fifteen minutes after this, drug was administered. Writhes were counted between 45 and 60 minutes after dosing.

Ethanol induced gastric necrosis in rats: 1 hour after dosing rats were gavaged with 1.0 ml of absolute ethanol. One hour later rats were euthanized and the intensity of gastric necrosis was scored.

Stress-induced gastric ulcers in rats: 1 hour after dosing, rats were placed in triangular stainless steel cages 21 cm in height. The cages were fitted into frames in a large reservoir which was filled with tap water to a level of 16 cm. The rats spontaneously climbed to the water-free top of the cage, a continuous effort being required to preserve breathing. The procedure was considered to induce active stress. After a 4 hour stress period, the rats were euthanized, the stomach collected and analyzed grossly for ulcers.

KCN lethality in rats: Potassium cyanide was injected iv 1 hour after dosing. Time of subsequent survival was determined.

BaCl2 lethality in rats: 1 hour after dosing, rats were given an iv injection of BaCl2. Time of survival was determined.

Oubain lethality test in rats: Oubain was injected 1 hour after dosing. Survival time was determined.

Acute toxicity tests in mice, rats and dogs: LD50 values were determined over the 14 days following dosing. It is not stated if these tests were distinct from the other reports of acute toxicity. Doses were selected from the progression 0.01, 0.02, 0.04 -... -640,

1280, 2560 mg/kg. For intraperitoneal administration, aqueous suspensions of nebivolol were prepared under addition of 1%polysorbate 80. For intravenous injections, nebivolol was dissolved in 12% (for mice) or 15% (for rats and dogs) aqueous solutions of HP-β-CD. For all other tests 20% polyethylene glycol 400 with 1 equivalent lactic acid was used for the formulation.

Results

General screening test in mice:

Apomorphine, tryptamine and norepinephrine antagonism in rats: nebivolol caused stimulation of apomorphine-induced agitation and stereotypies (ED50 14 and 19 mg/kg) and stimulation of mortality after tryptamine (25 mg/kg). The sponsor cites unpublished and unavailable data to support that this is seen with other beta-blockers.

This truly appalling collection of studies is presented without raw data for assessment. Each test was not judged on the basis of data but rather on a comparison with percentage distribution of the results in an undescribed population of rats. Only a few situations were judged to have positive results. These are described below in the sponsor's own words.

Nebivolol produced stimulation of apomorphine-induced agitation and stereotypies (ED50's: 14.2 and 18.7 mg/kg, respectively), and stimulation of mortality after tryptamine (24.7 mg/kg). Such effects have been previously also observed following other beta-adrenoceptor antagonists (Niemegeers and Awouters, unpublished results). The stimulatory effects on the apomorphine-induced abnormal behaviour seem to be aspecific and not due to stimulation of the central dopaminergic system as no evidence of such stimulation was obtained from other tests (e.g. in the general screening tests; see above). Nebivolol was virtually devoid of effects characteristic for central anti-dopaminergics (antagonism of apomorphine-induced agitation and stereotypies), alpha-adrenoceptor antagonists (inhibition of palpebral opening, protection from norepinephrine lethality) or agonists (stimulation of palpebral opening), serotonin S_2-antagonists (inhibition of tryptamine-induced coarse body tremors and bilateral clonic seizures, reversal of tryptamine-induced cyanosis), monoamino oxidase inhibitors (stimulation of tryptamine-induced coarse body tremors and bilateral clonic seizures), anticholinergics (mydriasis), or cholinomimetics (miosis). No consistent effects on body temperature were observed.

Other: ***N64811Local anesthetic properties of dl-nebivolol and its enantiomers. Comparison with atenolol, propranolol and lidocaine.***
Male, albino guinea pigs free of conjunctival and corneal disease were used. Six to 11 eyes were tested for concentration used. Concentrations of 0.63, 1.25, 2.5, 5 and 10 mg/ml were used of the following: lidocaine (R1125), propranolol (R9035), d-propranolol (R9035), atenolol (R38773), nebivolol (R67555), d-nebivolol (RR67138) and l-nebivolol (R67145).Hydroxypropyl-beta-cyclodextrin was used as vehicle when water solubility was poor. The corneal reflex was tested immediately before and at 1,2,3,4,5,10, 15, 20, 25, 30, 45, 60, 75, 90, 105 and 120 minutes after instillation of drug. Response was recorded as the number of times the animal fails to blink as a result of the stimulus.

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Table 1

R-number	Solvent[1]	pH	EC_{50}	(lower-upper limit)
lidocain	aq	6.85 ± 0.15	11.0	(6.68 - 29.1)
lidocain	cd	7.15 ± 0.15	2.36	(1.73 - 3.31)
nebivolol	aq	4.65 ± 0.15	0.57	(0.51 - 0.61)
nebivolol	cd	5.80 ± 0.20	1.14	(0.75 - 1.61)
d-nebivolol	cd	5.80 ± 0.20	2.23	(1.62 - 3.12)
l-nebivolol	cd	5.95 ± 0.05	3.75	(2.05 - 8.71)
propranolol	aq	5.70 ± 0.30	0.86	(0.64 - 1.01)
propranolol	cd	5.90 ± 0.30	1.59	(1.05 - 2.42)
d-propranolol	aq	5.60 ± 0.80	0.90	(0.68 - 1.10)
d-propranolol	cd	5.55 ± 0.75	1.96	(1.46 - 2.75)
atenolol	aq	6.10 ± 0.20	> 10	
atenolol	cd	6.25 ± 0.15	> 10	

1 solvent coded as aq : aqueous solution
cd : hydroxypropyl-beta-cyclodextrin

0=absence of corneal anesthesia
1-9=incomplete anesthesia
10= complete corneal anesthesia
Control animals received vehicle.

The sponsor noted that the addition of cyclodextrin caused a delay in onset of the anesthetic effect of R67555, R9035 and dR9035. The study indicated that nebivolol has local anesthetic properties that exceed lidocaine and are similar to propranolol. The d- and l- enantiomers showed similar properties and were reported to show a synergistic effect of at least 55% when tested as the racemic mixture (based on formulas stated in the report). Local anesthetics tend to work by decreasing sodium ion movement across the neuronal membrane. Anesthetic action of nebivolol would possibly be consistent with the Class I antiarrhythmic properties that nebivolol has been demonstrated to possess.

Pulmonary effects: see above

Renal effects: see above, N62264.

Gastrointestinal effects: not done

Abuse liability: not done

3.3 PHARMACOKINETICS/TOXICOKINETICS BRIEF SUMMARY

The original sponsor's studies detail low oral bioavailability (~10-27%), high protein binding (>97%), extensive metabolism and primarily fecal excretion (>60%, > 40% in rabbits) followed by the urinary route.

In tissue distribution studies in the rat, the radio-labeled drug was found in all tissues that were assayed. The highest levels of drug-derived radioactivity were reported in the lung, liver and adrenal glands. Radioactivity was also found in the central nervous system including the pineal gland, inner ear and eye and was also reported in the reproductive tract of the male (females not studied). A distribution study in pregnant Wistar rats examined a limited tissue list (plasma, placenta, uterus, fetal membranes, amniotic fluid, fetal blood, ovaries and mammary gland). Drug-derived radioactivity was found in all tissues examined in this study. The concentration of total radioactivity in the maternal ovaries (3.01±0.34 μg/g of wet tissue) was ~7.6X that in the plasma (0.393±0.128 μg/ml) at 1 hour after dosing when maximum values were shown for both plasma and ovaries.

A limited survey of distribution was also determined from the 1, 6 and 12 month dog studies. At the end of these studies, plasma, liver, kidney, lung, pancreas, brain, aorta, heart, muscle and fat were examined for nebivolol. In both studies the greatest accumulation of drug was in the lung. In the 1-month study the accumulation in the lung was ~124X the concentration in the plasma for rac-neb and for l-neb. The accumulation was 59x the plasma concentration for d-neb. In the 6-month study the ratio of tissue to plasma level for the lung was 168±50. In the 12 month study, the ratio of drug in lung compared to plasma was ~248±92.

The metabolites were described by HPLC and enantioselective antibodies in radio-immunoassays. A number of the reports are singularly lacking in the details necessary for independent interpretation. A number of the original pharmaco/toxicokinetic studies were also characterized by single blood samples on a given sampling day and enthusiastic extrapolation of data. The one report investigating pharmacokinetics in pregnant Sprague-Dawley rats was inadequate. The report only reported plasma levels of nebivolol with no characterization of levels of the enantiomers, conjugated species or hydroxyl phenols. The current sponsor repeated some pharmacokinetic studies using LC/MS/MS to characterize the metabolites. Blood levels in feeding studies were analyzed during both the light and dark cycles.

Reviewer's Summary of New PK Studies: Male animals

	Compound(s)	PK parameters			
		Tmax(hr)	Cmax ng/ml	AUC_{0-24} ng.hr/ml	$T_{1/2}$
Mouse, single oral dose 20/mg/kg	d,l-nebivolol	4	906	10700	3.11
	Total nebiv	4	1001	10705	3.02
	OH-phenols	4	98.2	854	8.66
Mouse,drug in diet, 14d 10mg/kg/day	d,l-nebivolol	18	203	2721	
	Total nebiv	20	187	2507	
	OH-phenols	2	21.7	344	
Rat single oral dose 20 mg/kg	d,l-nebivolol	1	502	2389	2.56
	Total nebiv	1	562	2595	2.44
	OH-phenols	2	225	1767	6.96
Rat drug in diet 10 mg/kg/day	d,l-nebivolol	18	44.4	592	
	Total nebiv	18	40.2	563	
	OH-phenols	20	41.2	696	
Dog, oral doses 14 day, 10 mg/kg/day	d,l-nebivolol	2	119	633	4.13
	Total nebiv	2	726	2390	3.75
	conjugated	2	607	1758	3.39
	OH-phenols	2.33	73	769	13.0

AUC_{0-24} for the dietary administration studies are the combination of the AUC_{0-12} light cycle and AUC_{0-12} dark cycle

Reviewer's Summary of New PK Studies: Female animals

	Compound(s)	PK parameters			
		Tmax(hr)	Cmax ng/ml	AUC_{0-24} ng.hr/ml	$T_{1/2}$
Mouse, single oral dose 20/mg/kg	d,l-nebivolol	1	884	7185	3.30
	Total nebiv	1	906	6880	3.20
	OH-phenols	2	116	1055	6.59
Mouse,drug in diet, 14d 10mg/kg/day	d,l-nebivolol	24	162	2095	
	Total nebiv	20	148	1894	
	OH-phenols	6	20.8	371	
Rat single oral dose 20 mg/kg	d,l-nebivolol	2	714	4911	3.26
	Total nebiv	2	816	5312	3.19
	OH-phenols	4	208	1760	7.05
Rat single oral dose 10 mg/kg 1987	Unchanged nebivolol	0.5-4	177	1101	
Rat drug in diet 10 mg/kg/day	d,l-nebivolol	24	135	1914	
	Total nebiv	24	124	1750	
	OH-phenols	16	35	627	
Pregnant rat 10mg/kg/day	nebivolol	2	317	2589	3.9

AUC_{0-24} for the dietary administration studies are the combination of the AUC_{0-12} light cycle and AUC_{0-12} dark cycle

Reviewer's Summary of PK parameters for the dog

	Compound(s)	PK parameters			
		Tmax(hr)	Cmax ng/ml	AUC_{0-24} ng.hr/ml	$T_{1/2}$
10 mg/kg/day day 1 (2003) male	d,l-nebivolol	2	24.7	170	4.69
	Total nebiv	2	217	768	4.44
	conjugated	2	192	597	4.06
	OH-phenols	9.33	17.2	212	17.8
10 mg/kg/day day 14 (2003) male	d,l-nebivolol	2	119	633	4.13
	Total nebiv	2	726	2390	3.75
	conjugated	2	607	1758	3.39
	OH-phenols	2.33	73	769	13.0

Reviewer's Summary of Dog PK parameters

	20 mg/kg/day m+f micro-xstalline (6 months)		80 mg/kg/day m+f micro-xstalline (6 months)		80 mg/kg/day β-CD (1month)			
	single	repeat	single	repeat	single	repeat		
Cmax (ng/ml)	14.6	29.4	38.1	76.4				
Tmax (h)	3.0	4.0	8.5	3.3				
AUC_{0-24} (ng.hr/ml)	123	290	294	700				
	2.5 mg/kg/day		10 mg/kg/day		40 mg/kg/day			
	single	repeat	single	repeat	Single	repeat		
Cmax (ng/ml)	14.5	21.1	67.6	112	186	640		
Tmax (h)	2.5	2.5	1.5	2.5	1.8	2.0		
AUC_{0-24} (ng.hr/ml)	76.1	99.0	285	683	1086	4276		
$T_{1/2}$ (h)	2.67	2.47	2.55	3.55	3.85	3.81		

Reviewer's summary of PK parameters in rabbits: single oral dose of 2.5 mg/kg

	Total radioactivity	Unchanged drug
Tmax(hrs)	2.0±0.0	0.8±0.3
Cmax(ng.eq/ml)	169±36	51.8±29.5
$AUC_{0-\infty}$ (μg(-eq)h/ml)	3.27	0.35±0.10
$T_{1/2}\beta$ (h)		5.9±0.4

Contributions of the enantiomers to the profile: N101244 Wistar rats given ^{14}C-racemate or radio-labeled isomer were compared. When the groups of major metabolites in the urine and feces were compared as a percentage of the administered dose, there was little difference between racemic, d- or l-nebivolol. Female rats showed lesser amounts of N-dealkylated metabolites and slightly more alicyclically mono-oxidized metabolites than did males treated with the corresponding compound. N109088 Dogs were given a single oral dose of 2.5 mg/kg ^{14}C-nebivolol or one of the isomers. There didn't appear to be a difference in the metabolic fate of either the racemate or the isomers.

The sponsor states that the original animal PK/TK studies were underestimates of exposure for the following reasons:

1. Because blood sampling extended through the working day, rather than having sampling performed at Cmax, the values underestimate actual plasma concentrations at steady-state (Css). For the rodent dietary studies, the areas under the plasma nebivolol concentration curves (AUC_{animal}) were estimated from the product of Css times 24hr, and are likely to be underestimations of the actual

2. According to the sponsor animal exposures are more representative of the extensive metabolizer humans than the poor metabolizers.

3. The older studies tended to measure unchanged nebivolol or unchanged nebivolol and certain metabolites (incomplete detection of metabolites) while measuring nebivolol plus metabolites would be more accurate.

The original studies also used HPLC and enantioselective RIA methods. LC/MS/MS is a preferable technique. The sponsor conducted a few PK studies using LC/MS/MS. These were single oral and 14-day repeat dose dietary administration studies(summarized above). While these are helpful, a certain caution must be used in extrapolating from 14 days to 24 months or even to 6 months.

Table 5.6-08 Metabolic Pattern of Nebivolol in Plasma Across Species (Mouse, Rat, Rabbit, Dog and Human)

Species (Strain)	Compound Dosed (Dose-mg/kg)	I *N*-dealkylated	II Combined di-OH	III Aromatic mono-OH	IV Alicyclic di-OH	V Alicyclic OH-keto	VI Alicyclic mono-OH or keto	Nebivolol	Reference
Mouse (Albino Swiss)	nebivolol HCl[a] (20)	-	Present	-	-	-	-	Present	TNEBI-0307
Rat (Wistar)[b]	^{14}C-*rac*-neb (2.5)	Present	<LOD	<LOD	<LOD	<LOD	Present	Present	N101244
	d-neb (1.25)	Present	<LOD	<LOD	<LOD	<LOD	Present	Present	
	l-neb (1.25)	Present	<LOD	<LOD	Present	Present	Present	Present	
	nebivolol HCl[a] (20)	-	Present	-	-	-	-	Present	TNEBI-0306
Rabbit (Cunistar MDL)[b]	^{14}C-*rac*-neb (2.5)	Present	As Gluc	As Gluc	<LOD	<LOD	Present	Present + Gluc	N106607
Dog (Beagle)[b]	^{14}C-*rac*-neb (2.5)	Present	<LOD	As Gluc	<LOD	<LOD	<LOD	<LOD	N109088
	d-neb (1.25)	Present	<LOD	As Gluc	<LOD	<LOD	<LOD	<LOD	
	l-neb (1.25)	Present	<LOD	As Gluc	<LOD	<LOD	<LOD	<LOD	
	nebivolol HCl[a] (10)	-	Present	-	-	-	-	Present + Gluc	TNEBI-0310
Human	^{14}C-*rac*-neb (15mg)								NEBI-0136; NEBI-0142
	PM	Present	<1ng-eq./mL	<1ng-eq./mL	<1ng-eq./mL for both	<1ng-eq./mL for both	<1ng-eq./mL	Present + Gluc	
	EM	Present	Present + Gluc	As Gluc			Present as Gluc mono-OH keto	Present + Gluc	

a: Plasma from mice, rats and dogs were analyzed for Group II metabolites, total nebivolol (conjugated + non-conjugated) and UD using LC/MS methods specific for these analytes.
b: The limit of detection was expressed as a percentage of the sample radioactivity and was calculated from the detection limit for ^{14}C-nebivolol (200 dpm) and the amount of radioactivity injected.

3.3.3 Absorption

N92692 Pharmacokinetics and absolute bioavailability of nebivolol in the male Wistar rat after single intravenous (1.25 mg/kg) and oral (10 mg/kg) administration.(1987-1992).
Plasma kinetics and bioavailability of nebivolol (batch A0301) were studied in fasted male Wistar rats after single iv administration (1.25 mg/kg, 11 groups of 3 rats) and single oral administration (10 mg/kg, 9 groups of 3 rats). Drug was dissolved in aqueous 5% HP-β-CD and 20% PEG-400. Groups of 3 rats were decapitated at 0.133, 0.25, 0.5, 1,2,4,6,8,26,24 and 32 hours after IV administration and at 0.5, 1,2,4,6,8,16,24 and 32 hours after gavage. Concentrations of unchanged nebivolol were detected in plasma samples using HPLC methods with fluorescence detection and a detection limit of 1.0 ng/ml.

After oral administration 1 rat showed Cmax at 2 hours, 1 at 4 hours and 1 at 1 hour.
The sponsor's summary is reproduced below.

Parameter	IV	PO
B[h^{-1}]	0.45	0.31
$T_{1/2\alpha}$ [h]	0.098	
$T_{1/2\beta}$ [h]	1.5	2.2
Vc [l/kg]	1.70	
Vdss[l/kg]	5.17	
Cl[l/h/kg]	2.65	
$AUC_{0-\infty}$ [ng.hr/ml]	471	1101
Cmax ng/ml		177
Tmax hours		0.5 -4
F %		29

A limitation of the study is that only parent drug was analyzed.

N106508 Absorption and plasma levels of nebivolol in the female rabbit after single oral administration at 2.5 mg/kg.(1992-1994)

Absorption and plasma levels of nebivolol (the sum of unchanged d- and l-nebivolol, the respective hydroxylated metabolites and blood and plasma levels of total radioactivity) were studied in 4 female Cunistar-MDL rabbits after single oral administration of ^{14}C-nebivolol at 2.5 mg/kg. Blood was collected at 0.5, 1,2,4,8,24, 48 and 72 hours after dosing. Radioactivity was determined by liquid scintillation counting. Plasma samples were incubated with β-glucuronidase. Concentrations of unchanged drug were determined by HPLC analysis before and after enzymatic hydrolysis. Concentrations of either d- or l-nebivolol and their respective hydroxylated metabolites before and after enzymatic hydrolysis were estimated in pooled plasma samples by radioimmunoassay using enantioselective antibodies.

Maximum plasma concentrations of unchanged drug were reached within 1 hour while peak total radioactivity was reached at 2 hours. Total radioactivity was greater than unchanged drug from the first point of determination. This suggested extensive metabolism. On the basis of 4 rabbits, the sponsor notes that there is a higher contribution of l-nebivolol plus its hydroxylated metabolites than of d-nebivolol + hydroxylated metabolites to the total plasma radioactivity. This is opposite to reported findings in rats where levels of d-nebivolol and its hydroxylated metabolites were higher than l-nebivolol and its hydroxylated metabolites.

parameter	Total radioactivity	Unchanged drug
Tmax [hours]	2.0±0.00	0.8±0.3
Cmax [ng(eq)/ml]	169±36	51.8±29.5
$AUC_{0-\infty}$ [µg(-eq)h/ml]	3.27±0.74	0.35±0.10
$T_{1/2}\beta$ [hours]		5.9±0.4

The elimination half life of unchanged nebivolol was approximately 3 times that reported in the previous study for rats.

N104624Pharmacokinetics and relative bioavailability of nebivolol in beagle dogs after single oral administration of nebivolol hydrochloride (R067555) at 20 mg (base-eq)/kg as an aqueous hydroxypropyl-β-cyclodextrin solution and as a mixture of nebivolol hydrochloride with β-cyclodextrin (1989-1994).

The absorption, plasma kinetics and relative bioavailability of nebivolol in Beagles were compared after oral administration of 2 different formulations. In a 2-phase cross-over study, nebivolol was given at 20 mg/kg as an aqueous 12% HP-β-CD solution and as a mixture with β-CD given in a gelatin capsule. Blood samples were collected at 0, 0.5, 1,2,4,6,8, 24, 32 and 48 hours after dosing. Two dogs were given each formulation then after a 1 week washout period re-dosed with the other formulation.

Cmax was reached with the solution at 1 hour and at 2 hours with the gelatin capsules. The sponsor's summary is reproduced below.

Parameter	Solution formulation	Capsule formulation
Cmax (ng/ml)	127±31	99.4±24.0
Tmax (h)	1±0	2±0
$T_{1/2}$ (h)	5.31±1.60	6.15±0.96
AUC_{0-24} (ng.hr/ml)	733±87	748±184
$AUC_{0-\infty}$ (ng.h/ml)	762±86	797±194
F% relative		107±36

Calculating relative bioavailability from the two formulations is questionable. However, it appears that plasma level exposure to unchanged parent drug is similar between the two formulations based upon AUC determinations.

N109030 Comparative bioavailability of nebivolol in the beagle dog after oral administration of capsule formulations of nebivolol hydrochloride (R067555) at 20 mg(base eq)/kg provided as a mixture with β-cyclodextrin, as a microfine or as a microcrystalline powder (1992-1994).

The bioavailability and plasma levels of unchanged nebivolol, l-nebivolol and the hydroxylated metabolites and d-nebivolol and its hydroxylated metabolites were compared in the Beagle after dosing with 3 different formulations. Formulation I was a mixture of nebivolol with β-CD, Formulation II was a microfine powder and Formulation III a microcrystalline powder. There was no β-CD in formulations II and III. Four male Beagles were used in a 3 phase cross-over design with 2 weeks between sessions. Blood samples were collected at 0, 1,2,4,8,12,24, 48, 72 and 96 hours after dosing. Plasma concentrations of parent drug were determined by HPLC. The limit of quantification was 2.5 ng/ml. Plasma concentrations of each enantiomer and the hydroxylated metabolites were determined by radioimmunoassay using enantioselective antibodies.

Formulations I and II produced higher plasma levels of unchanged nebivolol compared to formulation III. The microfine formulation gave greater exposure than the other two formulations based on AUC comparisons. The sponsor' s numbers are shown in the table below.

PK parameters for unchanged nebivolol

parameter	Formulation I	Formulation II	FormulationIII
Cmax (ng/ml)	104±26	92.9±77.5	25.2±6.0
Tmax (h)	2.5±1.0	4.5±2.5	3.5±1.0
AUC (ng.hr/ml)	960±266	1144±611	249±93.9
F% relative to Fomulation II	96.4±33.6	100	27.7±21.4

PK parameters of l-nebivolol and the hydroxylated metabolites are shown below.

parameter	Formulation I B-CD	Formulation II microfine	FormulationIII Microcrystalline
Cmax (ng/ml)	697±191	428±241	145±45
Tmax (h)	2.5±1.0	4.5±2.5	3.0±1.2
AUC_{0-24} (ng.hr/ml)	4246±1074	3714±1664	1159±468

PK parameters of d-nebivolol and the hydroxylated metabolites are shown below.

parameter	Formulation I B-CD	Formulation II microfine	FormulationIII Microcrystalline
Cmax (ng/ml)	1683±440	1158±591	466±134
Tmax (h)	3.0±1.2	4.5±2.5	2.5±1.0
AUC_{0-24} (ng.hr/ml)	10081±1798	9243±3827	2994±1218

Based upon the very small sample size used in this study the sponsor drew inferences regarding the relative exposure to d- or l- forms of nebivolol and the related metabolites. That is, exposure to l-nebivolol and hydroxylated metabolites or d- nebivolol and hydroxylated metabolites was higher for formulation I than formulation II.

N106735 Pharmacokinetics and absolute bioavailability of nebivolol in the Beagle dog after single intravenous (0.32mg base-eq/kg) and oral administration (5 mg base-eq/kg) of aqueous solutions of nebivolol hydrochloride (R067555) (1987-1994)

2 male and 2 female dogs were first given the iv dose (0.32 mg/kg aqueous solution) and after a one week rest were given the oral dose of 5 mg/kg. Both the IV and the oral formulation contained PEG400 and HP-β-CD. Blood was collected at 0, 5,10, 15, 20, 30 and 45 minutes, 1,1.5, 2,3,4,6,8,12,24,32, 48 and 56 hours after iv administration. Following oral dosing, blood samples were collected 0, 15, 30, 45 minutes and 1, 1.5, 2,3,4,6,8,12,24, 32, 48 and 56 hours. Nebivolol plasma samples were determined by ——— HPLC.

Plasma concentrations of unchanged drug showed F% of 10% with the oral formulation and a slightly greater AUC compared to the iv formulation. The sponsor's numbers are shown below.

parameter	Intravenous (0.32 mg base-eq/kg)	Oral (5 mg base-eq/kg)
Cmax ng/ml		64.3±13.7
Tmax		0.8±0.2
$T_{1/2}$ (h)	4.21±0.44	3.95±1.22
Cl ml/kg/hr	1690±373	
Vd_{β} l/kg	10.3±3.2	
$AUC_{0-\infty}$ ng.hr/ml	201±34	305±93
F_{abs} (%)		10.2±3.68

A large volume of distribution was reported following IV administration. AUC was greater for oral versus IV administration. As may be seen, oral bioavailability was low. The sponsor stated that there were no sex-related differences in plasma concentrations after either IV or oral administration in dogs.

3.3.4 Distribution

N109029 Absorption and tissue distribution of SRRR and RSSS-nebivolol in the male Wistar rat after single oral administration at 1.25 mg/kg of either SRRR- or RSSS-nebivolol. 1990-1994

Single oral doses of ^{14}C-d-nebivolol and ^{14}C-l-nebivolol dissolved in distilled water were given to 7 groups of male Wistar rats (n=4 except for 1 group with n=3). Animals were orally gavaged to give a dose of 1.25 mg/kg. For each phase of the study, groups of 4 rats were decapitated at 1, 2, 4, 8, 24, 48 and 96 hours after dosing. Blood was collected with heparin. For each animal, the following organs were collected and weighed: brain, pituitary, lacrimal glands, thyroid, heart, lung, liver, kidney, adrenals, stomach, small intestine (tissue and contents separately), large intestine (tissue and contents separately), testicle, muscle, skin and fur, auricular pinna and peri-renal fat. For each rat, total radioactivity was measured in plasma, blood and the various tissues.

Concentrations of unchanged d- or l- nebivolol were determined before and after enzymatic hydrolysis with β-glucuronidase. Levels of unchanged drug were determined by HPLC. Concentrations of either d-nebivolol or l-nebivolol plus hydroxylated metabolites before and after β-glucuronidase treatment were estimated in plasma samples by RIA using enantioselective antibodies.

Results

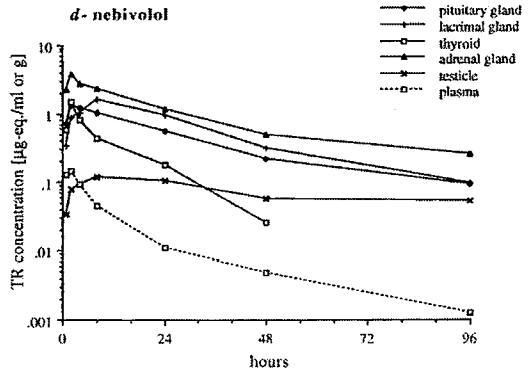
***d*- nebivolol**

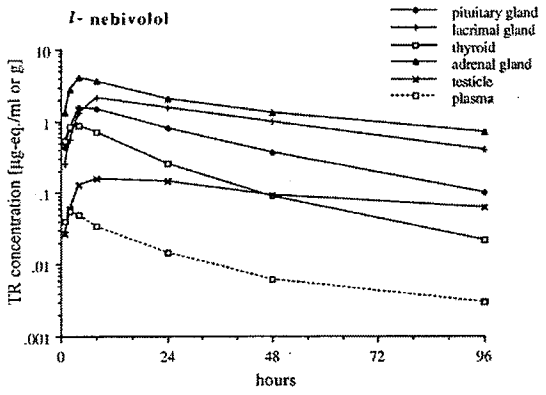
Parameters [units]	TR	UD
T_{max} [h]	1 - 2	1 - 2
C_{max} [ng(-eq.)/ml]	140 ± 45	48.0 ± 19.8
$t_{1/2,\beta}$ [h]		1.6
$AUC_{0-\infty}$ [µg(-eq.)h/ml]	1.56	0.208

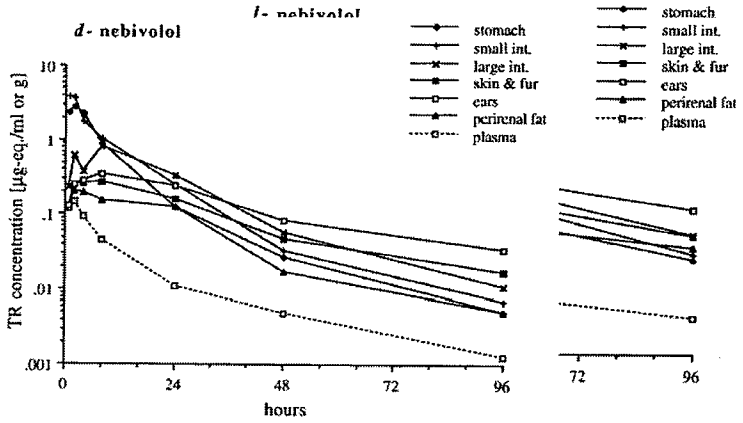
***l*- nebivolol**

Parameters [units]	TR	UD
T_{max} [h]	2 - 4	1 - 2
C_{max} [ng(-eq.)/ml]	52.4 ± 11.0	11.4 ± 7.6
$t_{1/2,\beta}$ [h]		3.1
$AUC_{0-\infty}$ [µg(-eq.)h/ml]	1.39	0.050

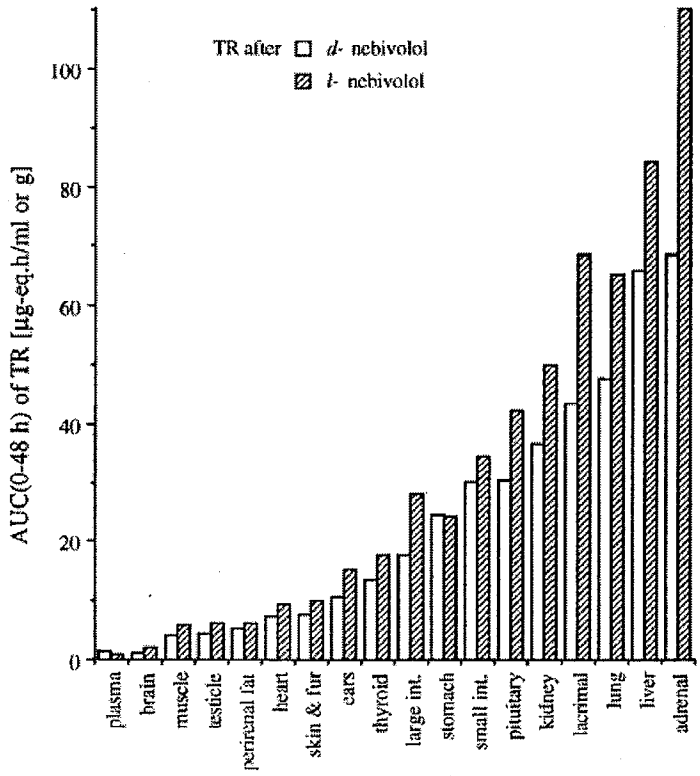
Tissue distribution of the enantiomers showed no major differences. Drug-associated radioactivity was found in all tissues analyzed. The sponsor's graphs are shown below.







Area under the curve for the total radioactivity was greater for l-nebivolol than d-nebivolol.



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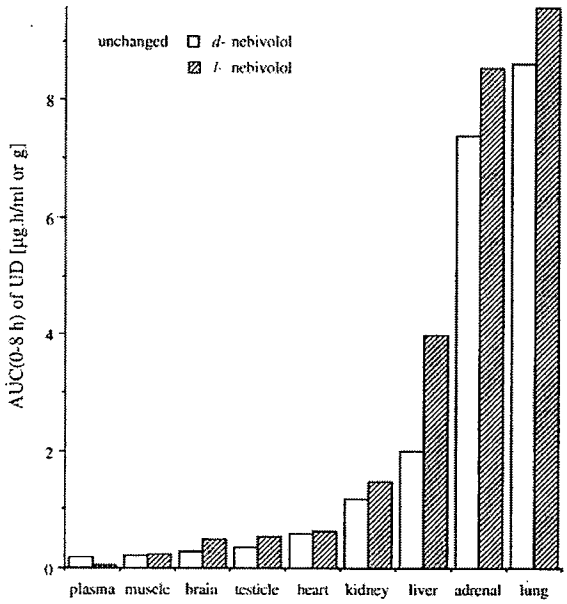


After dosing with ^{14}C-d-nebivolol maximum plasma concentrations of both TR and UD were observed at 1-2 hours after dosing. After dosing with the l-enantiomer, peak TR levels were reported for 2-4 hours and Cmax for UD was 1-2 hours after dosing.

Cmax for d-nebivolol was reported as 48±20 ng/ml and for l-nebivolol 11±8 ng/ml (n=8 for each compound). The ratio of unchanged drug /total radioactivity for both enantiomers was reported as <0.4 within 1 hour after gavage. This suggests extensive and fairly quick metabolism. AUC for unchanged drug was 4 times higher after administration of d-nebivolol compared to l-nebivolol. However, the difference between 0.208 and 0.050 µg.eq.hr/ml given the analytical techniques is of

questionable significance. Both unchanged drug and total radioactivity are accumulated to the greatest extent in the adrenal gland and lung.

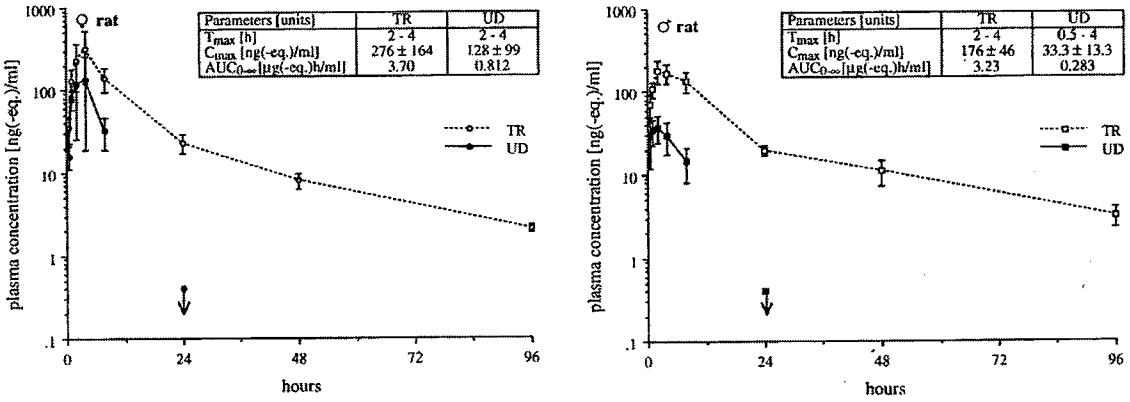
N99400Absorption and tissue distribution of nebivolol in the Wistar rat after single oral administration at 2.5 mg/kg. 1990-1993

^{14}C-rac-nebivolol, 10.2 mCi/mmole was dissolved in dH_2O. Eight groups of rats, 4/sex/group were given oral doses of 2.5 mg/kg. Groups of 4 male and 4 female rats were decapitated at 0.5, 1,2,4,8,24,48 and 96 hours after administration. Samples of blood were collected with heparin. For each animal, brain, pituitary, eyeballs, lacrimal glands, lymph nodes, salivary glands, thyroid, thymus, heart, lung, liver, kidney, adrenal, pancreas, spleen, esophagus, stomach, small and large intestine (tissue and contents separately), urinary bladder, muscle, skin & fur, auricular pinna, peri-renal and subcutaneous fat, brown fat, bone marrow, bone, trachea, testicle, seminal vesicle, epididymus, prostate, ovaries, vagina and uterus were collected. For each rat, concentrations of total radioactivity were measured in plasma, blood and in the various tissues. For a selection of tissues, the radioactivity was measured in pooled samples of homogenates from the four rats of the same time interval.

Concentrations of nebivolol as the sum of unchanged d- and l-nebivolol (UD) were determined before and after enzymatic hydrolysis with β-glucuronidase. Concentrations before hydrolysis were determined in individual samples. Levels after hydrolysis were determined in samples pooled per time interval and sex. Concentrations of the enantiomers and the respective hydroxylated metabolites were determined using RIAs with enantioselective antibodies.

Results

Unchanged drug disappeared rapidly from the plasma. There was less variability in the measurements from the male rats, giving the appearance that there was a sex-related difference. In both sexes Tmax was between 2-4 hours. The Cmax for both total radioactivity and unchanged drug was greater in the females although there was no significant difference in the AUC values. Average peak levels in the females were approximately 130 ng/ml compared to ~30 ng/ml in males.



Both enantiomers and the respective hydroxylated metabolites for which analysis was performed were present in both sexes out to 96 hours.

Table 5 : Plasma concentrations of TR and UD (mean, n=4) and of the separate enantiomers of nebivolol plus their hydroxylated metabolites, as well as their levels after hydrolysis with ß-glucuronidase (*E. coli*) (in pools, n=4) as a function of time in male Wistar rats after single oral administration of ^{14}C-nebivolol at 2.5 mg/kg.

	Plasma concentration: ng(-eq.)/ml										
	TR	UD		RSSS + HO-RSSS (RIA A)		SRRR + SRRR-OH (RIA B)		SRRR + HO-SRRR (RIA C)		RSSS + RSSS-OH (RIA D)	
Time [h]		before hydr.	after hydr.	before hydr.	after hydr.	before hydr.	after hydr.	before hydr.	after hydr.	before hydr.	after hydr.
0.5	70.2	30.5	31.2	5.4	7.1	39.6	37.0	57.4	45.0	7.2	6.0
1	108	34.5	32.6	15.7	11.4	46.6	43.8	79.3	63.6	11.0	9.6
2	181	37.8	39.0	24.6	15.0	64.8	57.8	109	97.4	16.7	11.7
4	170	30.4	29.7	22.2	13.3	61.5	52.5	99.9	83.8	15.3	10.3
8	134	14.6	14.5	17.5	10.5	36.6	29.2	66.0	55.3	10.9	7.9
24	19.9	≤ 0.40	≤ 0.50	2.0	1.5	5.4	4.2	10.1	8.6	2.1	1.2
48	11.0	≤ 0.40	≤ 0.50	≤ 1.0	≤ 1.0	1.9	1.8	2.8	2.5	≤ 1.0	≤ 1.0
96	3.2	≤ 0.40	≤ 0.50	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0
$AUC_{0-\infty}$ [ng(-eq.)h/ml]	3231	283	286	324	204	859	720	1483	1256	229	157

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Table 6 : Plasma concentrations of TR and UD (mean, n=4) and of the separate enantiomers of nebivolol plus their hydroxylated metabolites, as well as their levels after hydrolysis with ß-glucuronidase (*E. coli*) (in pools, n=4) as a function of time in female Wistar rats after single oral administration of ^{14}C-nebivolol at 2.5 mg/kg.

	Plasma concentration: ng(-eq.)/ml										
	TR	UD		RSSS + HO-RSSS (RIA A)		SRRR + SRRR-OH (RIA B)		SRRR + HO-SRRR (RIA C)		RSSS + RSSS-OH (RIA D)	
Time [h]		before hydr.	after hydr.	before hydr.	after hydr.	before hydr.	after hydr.	before hydr.	after hydr.	before hydr.	after hydr.
0.5	34.8	16.0	17.8	5.0	3.8	19.8	18.0	27.4	23.8	4.5	2.8
1	131	77.6	92.9	15.3	13.6	108	86.3	137	117	12.5	9.4
2	232	119	121	23.0	21.3	150	145	241	211	18.2	15.3
4	319	137	167	53.0	28.6	226	216	377	327	31.2	19.6
8	142	32.7	32.0	21.8	16.1	64.0	58.0	137	111	15.4	11.2
24	23.1	≤ 0.40	≤ 0.50	2.7	2.3	12.9	10.8	18.1	14.0	3.1	2.1
48	8.0	≤ 0.40	≤ 0.50	≤ 1.0	≤ 1.0	1.6	1.2	3.8	4.5	≤ 1.0	≤ 1.0
96	2.1	≤ 0.40	≤ 0.50	≤ 1.0	≤ 1.0	1.2	≤ 1.0	2.2	1.8	≤ 1.0	≤ 1.0
$AUC_{0-\infty}$ [ng(-eq.)h/ml]	3705	812	903	468	328	2006	1762	3582	3035	342	239

As may be seen above, there was a greater contribution of the SRRR enantiomer and its hydroxylated metabolites to the female profile than to the male.

Distribution of radioactivity- total radioactivity was found in all tissues that were examined in both sexes. Tissue concentrations reached maximum levels by 4 hours except in certain cases. In both sexes, eyeballs, lacrimal gland and large intestine reached peak levels at 8 hours. In males, peak levels were reached at 8 hours in the following tissues: pituitary, testicles, epididymus, prostate, urinary bladder and muscle. At 96 hours after a single oral dose detectable levels were found in all tissues in both sexes except for brain, heart and urinary bladder in males. However, the percent of total dose at that time point ranged from a high in the liver of 0.3% males, 0.9% females to ≤0.08% for the other tissues. The highest percent of the dose was found in the stomach, intestinal contents and liver. Further analysis of certain tissues was carried out.

Table 12 : Concentrations of total radioactivity (TR) and of unchanged drug (UD) as a function of time in a selection of tissues, pooled (n=4) proportionally per time interval, of male Wistar rats after single oral administration of ^{14}C-nebivolol at 2.5 mg/kg.

	Concentration: µg(-eq.)/g of wet tissue											
Tissue	Brain		Heart		Lung		Liver		Kidney		Adrenal gland	
Time [h]	TR	UD (+ TR)	TR	UD (+ TR)	TR	UD (+ TR)	TR	UD (+ TR)	TR	UD (+ TR)	TR	UD (+ TR)
0.5	0.027	0.0269 (1.0)	0.185	0.0979(0.53)	0.956	0.680 (0.71)	2.69	1.01 (0.37)	0.508	0.256 (0.50)	0.699	0.475 (0.68)
1	0.086	0.0676 (0.79)	0.493	0.143 (0.29)	2.68	1.78 (0.66)	5.44	0.915 (0.17)	1.67	0.413 (0.25)	2.08	1.05 (0.51)
2	0.143	0.0970 (0.68)	1.12	0.188 (0.17)	4.83	2.69 (0.56)	9.32	0.525 (0.056)	3.90	0.357 (0.092)	4.73	1.34 (0.28)
4	0.199	0.153 (0.77)	1.30	0.129 (0.099)	7.85	3.87 (0.49)	6.73	0.246 (0.037)	4.54	0.306 (0.067)	6.18	1.20 (0.19)
8	0.142	0.0725 (0.51)	1.04	0.101 (0.097)	7.21	2.21 (0.31)	5.95	0.174 (0.029)	4.40	0.209 (0.048)	5.13	0.603 (0.12)
24	0.030	0.0021 (0.070)	0.169	≤ 0.0014	1.26	0.0145 (0.011)	1.30	0.0080 (0.006)	0.868	0.0024 (0.003)	1.91	0.0829 (0.043)
48	0.011	≤ 0.0010	0.038	≤ 0.0014	0.39	0.0021 (0.005)	0.58	0.0014 (0.002)	0.313	≤ 0.0010	0.991	0.0337 (0.034)
96	≤ 0.005	≤ 0.0010	≤ 0.006	≤ 0.0013	0.038	≤ 0.0010	0.142	≤ 0.0010	0.074	≤ 0.0010	0.424	≤ 0.029

	Concentration: µg(-eq.)/g of wet tissue							
Tissue	Stomach		Small intestine		Large intestine		Testicle	
Time [h]	TR	UD (+ TR)	TR	UD (+ TR)	TR	UD (+ TR)	TR	UD (+ TR)
0.5	5.12	4.34 (0.85)	4.62	5.16 (1.1)	0.101	0.0730 (0.72)	0.012	0.0138 (1.2)
1	3.64	3.05 (0.84)	6.42	5.55 (0.86)	0.266	0.121 (0.45)	0.050	0.0463 (0.93)
2	4.46	3.13 (0.70)	7.26	5.79 (0.80)	0.598	0.160 (0.27)	0.107	0.0735 (0.69)
4	2.53	1.52 (0.60)	4.28	2.13 (0.50)	1.61	0.309 (0.19)	0.204	0.137 (0.67)
8	1.71	0.350 (0.20)	3.01	0.825 (0.27)	2.97	0.333 (0.11)	0.332	0.226 (0.68)
24	0.327	0.0018 (0.006)	0.577	0.0055 (0.010)	0.922	0.0053 (0.006)	0.218	0.0720 (0.33)
48	0.099	≤ 0.0010	0.161	0.0014 (0.009)	0.305	≤ 0.0010	0.152	0.0255 (0.17)
96	0.009	≤ 0.0010	0.017	≤ 0.0010[1)]	0.034	≤ 0.0010	0.108	0.0063 (0.058)

[1)] peaks interfering with peak of interest.

In both male and female rats highest tissue concentrations of total radioactivity were found in adrenal gland, lacrimal gland, pituitary gland, lung, liver and kidney. In these tissues, the AUC_{0-96} values were 30-70 times those in the plasma. AUC_{0-96} values of 5-8 times the plasma values were reported for the male reproductive organs, heart, bone, eyes, skin and fur.

N92609 Tissue distribution of nebivolol in the male Wistar rat after single oral administration at 10 mg/kg as studied by whole body autoradiography,1987-1992

Aqueous solutions of ^{14}C-nebivolol were combined with HP-β-CD. Formulation A (labeled in the SR or RS part) had a final specific activity of 5.5µCi/mg base and formulation B (labeled in the RR or SS part) had a final specific activity of 5.4 µCi/mg base. Six male Wistar rats were given oral doses of 10mg/kg, 3 rats per formulation. Animals were housed individually until euthanasia. At 1, 4 and 24 hours after dosing, 1 rat from each dosage preparation was euthanized and processed for whole body autoradiography (WBA). Quantitation was not performed.

The sponsor presented 3 autoradiograms. One hour after dosing, maximum concentrations of radioactivity were reported in the gastrointestinal contents of the stomach and small intestine. Radioactivity was also reported for the lung, liver, kidney and adrenal, lacrimal gland, salivary gland, preputial gland, pancreas and pituitary. Other organs reported to show radioactivity were heart, , lymph nodes, bone marrow, inner ear and eye, central nervous system, seminal vesicles and testicles.

At 4 and 24 hours after dosing radioactivity was still present in the tissues listed above. Tissue levels at 24 hours were described as lower than at 4 hours.

The distribution of radioactivity after oral administration of the 2 different formulations was described as similar.

N106696 absorption and tissue distribution of nebivolol-related radioactivity in the male Wistar rat after single oral administration of ^{14}C-nebivolol at 10 mg/kg. 1987- 1994

Table 2: Mean (± S.D., n=3) blood and plasma concentrations of total radioactivity (TR) and AUCs calculated from mean plasma-level data in fasted male Wistar rats after single oral administration of ^{14}C-nebivolol at 10 mg/kg.

	Concentration of TR [µg-eq./ml]		Concentration-ratio
Time [h]	Blood	Plasma	Blood/Plasma
1	0.978 ± 0.085	1.08 ± 0.130	0.91 ± 0.07
4	0.725 ± 0.038	0.739 ± 0.043	0.98 ± 0.08
8	0.618 ± 0.172	0.663 ± 0.185	0.93 ± 0.03
24	0.095 ± 0.002	0.101 ± 0.004	0.94 ± 0.06
48	0.037 ± 0.006	0.035[1]	1.04[2]
$AUC_{0-\infty}$ [µg-eq.h/ml]	-	14.3	-

[1] median value
[2] mean, n=2

Aqueous ^{14}C-nebivolol was prepared with HP-β-CD and PEG400. Five groups of 3 male Wistar rats, fasted, were given single oral doses of 10 mg/kg. Groups of 3 rats were decapitated at 1,4, 8, 24 and 48 hours after dosing. For each animal, blood and the following tissues were collected: brain, liver, lung, kidney, muscle, abdominal fat and heart. Total radioactivity was measured in blood, plasma and the tissues.

Plasma concentration for total radioactivity reached a maximum at 1 hour (1.08±0.13 µg.eq/ml) with an $AUC_{0-\infty}$ of 14.3µg.eq.hr/ml. The ratio of blood and plasma concentrations of radioactivity were close to 1.

This is in comparison to non-fasted rats in previous studies where peak plasma levels after 2.5 mg/kg were reported at 2-4 hours. The $AUC_{0-\infty}$ for the non-fasted rats was 3.23 µg-eq.hr/ml following a single oral dose of 2.5 mg/kg. There appears to be linear increase in the $AUC_{0-\infty}$ between this dose and the dose of 10 mg/kg used in the present study despite the difference in fasting and fed states.

Total radioactivity in the tissues reached maximum levels within 8 hours after dosing. The highest levels of radioactivity were reported for the lung, liver and kidney. In lung and liver, tissue levels were ~30 times those of the plasma. Concentrations in the heart averaged ~ 5 times plasma values. Tissue levels showed a progressive decline over the sampling period.

Table 3: Concentrations of total radioactivity (TR) (mean ± S.D., n=3) as a function of time in plasma and various tissues of the fasted male Wistar rat after a single oral administration of ^{14}C-nebivolol at 10 mg/kg.

	Concentration of TR [µg-eq./ml or g of wet tissue]				
Time [h]	1	4	8	24	48
Group code	A	B	C	D	E
Tissue					
Plasma	1.08 ± 0.13	0.739 ± 0.043	0.663 ± 0.185	0.101 ± 0.004	0.035[1)]
Brain	0.530 ± 0.124	0.562 ± 0.056	0.453 ± 0.087	0.110 ± 0.015	≤ 0.04
Heart	1.72 ± 0.48	3.02 ± 1.14	3.74 ± 0.74	0.693 ± 0.222	≤ 0.06
Lung	20.8 ± 3.0	22.1 ± 5.9	23.6 ± 3.5	3.70 ± 0.40	0.768 ± 0.145
Liver	39.7 ± 8.1	21.9 ± 1.0	13.2 ± 0.8	2.43 ± 0.28	1.00 ± 0.20
Kidney	13.6 ± 2.7	11.6 ± 0.8	9.70 ± 1.04	1.90 ± 0.12	0.584 ± 0.123
Muscle	1.07 ± 0.09	1.75 ± 0.52	2.38 ± 0.22	0.290 ± 0.050	≤ 0.04
Fat	0.736 ± 0.268	0.953 ± 0.171	1.09 ± 0.15	0.178 ± 0.027	≤ 0.06

[1)] median value

N119533 Tissue distribution of nebivolol in the male pigmented Dunning rat after single oral administration at 5 mg/kg, as studied by whole-body autoradiography 1990-1996

^{14}C-Nebivolol was dissolved in water and given orally to 3 male pigmented (spotted) Dunning rats at a dose of 5 mg/kg. At 4, 24 and 96 hours after dosing the rats were euthanized and processed for whole-body autoradiography. Quantification of total radioactivity (parent drug plus metabolites) was performed with a bio-imaging analyzer system. Standards were processed along with the body sections and linear regression curves were calculated for calibration curves.
Results
General distribution of radioactivity was similar to that seen in previous studies in the albino rats. Concentrations in general were higher at 4 hours than at 24 hours. The highest levels at 4 hours were found in the GI contents and lungs. High levels were reported in the liver, kidney, urinary bladder, adrenal and other glandular tissues. Lower concentrations were reported in the testicle, cerebral ventricles, pituitary and pineal. At 24 hours after dosing relatively high concentrations were still seen in the lung, lymph nodes, some glandular tissue and GI contents. By 96 hours,

TABLE 1: Concentrations of total radioactivity (TR) as a function of time as determined by radioluminograpghy in various tissues of the male pigmented Dunning rat after single oral administration of ^{14}C-nebivolol at 5 mg/kg.

Time (h) / Tissue	Concentrations of TR: µg-eq./g tissue		
	4	24	96
adrenal gland	5.72	-1)	-
bone marrow	2.11	0.67	≤ 0.32)
brown fat	2.08	0.65	-
cerebrospinal fluid	-	1.10	-
caecum contents	31.5	-	≤ 0.3
eye (choroid)	7.35	14.6	9.07
eye (ciliary body)	11.2	35.6	27.0
eye (iris)	3.46	4.72	3.31
inner ear	-	1.51	0.78
heart	2.52	-	-
kidney	7.05	1.62	≤ 0.3
lacrimal gland	2.56	3.72	0.91
large int. contents	13.6	4.26	-
large int. tissue	2.98	-	-
liver	11.3	2.08	≤ 0.3
lung	9.49	4.66	0.67
lymp nodes	2.48	-	0.67
meninges	1.22	1.16	0.46
muscle	0.81	≤ 0.3	-
pancreas	6.86	1.49	-
parotid	2.37	0.85	-
prostate	-	0.79	-
pituitary gland	3.58	-	-
preputial gland	3.26	-	-
salivary gland	3.70	1.73	-
seminal vesicle (cortex)	1.57	1.16	-
skin (pigmented)	2.06	1.44	0.41
spleen	-	1.05	≤ 0.3
small int. tissue	13.2	-	-
small int. contents	113	2.50	-
stomach tissue	22.6	-	-
stomach contents	96.9	9.16	-
thymus	1.68	0.78	-
ventricle (of brain)	3.24	1.42	-

1) no quantification

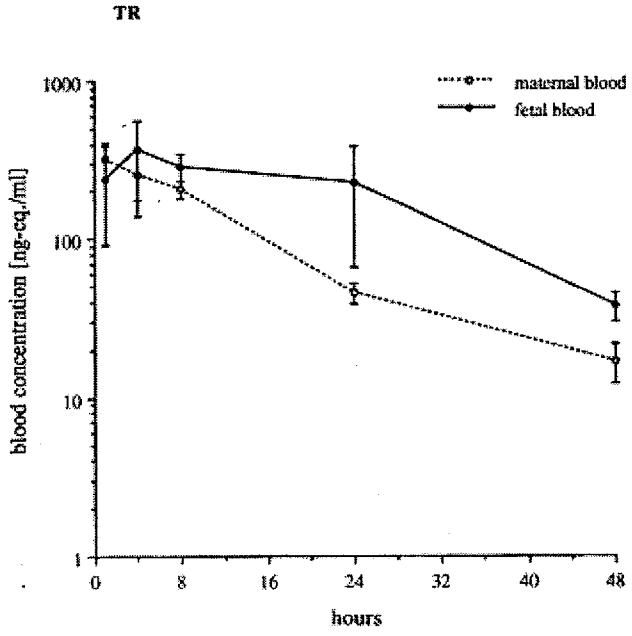
2) ≤ the limit of quantification

radioactivity was still detectable in the lungs, lacrimal glands and lymph follicles of the lymph nodes and spleen. Low levels of radioactivity were observed in GI content and liver.

In the pigmented rat, there were high levels of radioactivity in the melanin containing portions of the eye: choroid, retinal pigment epithelium, iris and ciliary body. Other areas with high levels of radioactivity were hair follicles, pigmented areas of skin, meninges of the CNS. Detectable levels of radioactivity were also reported for the inner ear.
It may be seen in the sponsor's table that radioactivity in the ciliary body equals radioactivity in the liver.

N99399 Placental transfer of nebivolol in the Wistar rat after single oral administration at 2.5 mg/kg.1990-1993

^{14}C-Nebivolol dissolved in water was used. Five groups of 3 pregnant Wistar rats were dosed orally with 2.5 mg/kg of drug on GD18. Groups of 3 rats were decapitated at 1,4,8,24 and 48 hours after administration. Blood was collected with heparin. The following tissues were collected: placenta, uterus, fetuses, fetal membranes, amniotic fluid, fetal blood, ovaries and mammary gland. For each rat concentrations of TR were measured in the various samples. In addition, radioactivity was measured in proportionally pooled samples of the homogenates from the 3 rats of each time interval for a comparison with levels of unchanged nebivolol which were also determined in these pooled samples. The quantification limit for unchanged drug was 1.0 ng/ml.



From approximately 4 hours after dosing, the levels of total radioactivity

in fetal plasma exceeded the levels in maternal plasma.

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Table 2 : Mean (± S.D., n=3) blood and plasma concentrations of total radioactivity (TR) and/or unchanged drug (UD), and AUCs calculated from mean plasma level data in the pregnant Wistar rat after single oral administration of ^{14}C-nebivolol at 2.5 mg/kg at the 18th day of gestation.

	Concentration: ng(-eq.)/ml			Concentration - ratio	
	Blood	Plasma		Blood/Plasma	Plasma
Time [h]	TR	TR	UD	TR	UD/TR
1	322 ± 81	393 ± 128	223 ± 104	0.84 ± 0.08	0.55 ± 0.10
4	257 ± 117	253 ± 150	80.4 ± 77.0	1.07 ± 0.13	0.28 ± 0.11
8	205 ± 25	192 ± 39	35.1 ± 11.3	1.09 ± 0.17	0.18 ± 0.02
24	45.7 ± 6.8	33.9 ± 6.4	≤ 1.0	1.37 ± 0.22	≤ 0.03
48	16.6 ± 4.9	11.0 ± 1.4	≤ 1.0	1.49 ± 0.23	-
AUC $_{0-\infty}$ [ng(-eq.)h/ml]		4636	932		0.20

Maximum plasma concentrations of drug-associated radioactivity were found in the dams at 1 hour and in the fetuses at 4 hours (tables 2 and 3). Fetal membranes on the other hand showed highest levels at 24 hours after dosing.

The concentration of total radioactivity in the ovaries exceeded that in the plasma.

Table 3 : Concentrations of total radioactivity (TR) (mean ± S.D., n=3) in various body fluids and tissues of the pregnant Wistar rat after single oral administration of ^{14}C-nebivolol at 2.5 mg/kg at the 18th day of gestation.

	Concentration: µg-eq./ml or g of wet tissue				
Time [h] / Tissue	1	4	8	24	48
Maternal plasma	0.393 ± 0.128	0.253 ± 0.150	0.192 ± 0.039	0.034 ± 0.006	0.011 ± 0.001
Maternal blood	0.322 ± 0.081	0.257 ± 0.117	0.205 ± 0.025	0.046 ± 0.007	0.017 ± 0.005
Fetal blood	0.238 ± 0.146	0.369 ± 0.192	0.288 ± 0.058	0.228 ± 0.161	0.038 ± 0.008
Amniotic fluid	0.039 ± 0.008	0.059 ± 0.033	0.122 ± 0.020	0.048 ± 0.004	0.022 ± 0.004
Ovaries	3.01 ± 0.34	2.47 ± 0.63	2.23 ± 0.21	1.22 ± 0.11	0.566 ± 0.149
Fetal membrane	0.772 ± 0.148	3.44 ± 0.62	7.03 ± 0.48	7.70 ± 1.03	4.13 ± 0.48
Uterus	0.324 ± 0.058	0.650 ± 0.212	0.786 ± 0.140	0.362 ± 0.101	0.230 ± 0.044
Placenta	0.658 ± 0.136	1.19 ± 0.03	1.38 ± 0.22	0.758 ± 0.131	0.362 ± 0.042
Fetus	0.139 ± 0.002	0.282 ± 0.009	0.334 ± 0.043	0.183 ± 0.004	0.088 ± 0.008
Mammary gland	0.755 ± 0.120	1.37 ± 0.16	1.48 ± 0.14	0.714 ± 0.129	0.337 ± 0.058

Something not answered in this study is distribution within the fetus and the rate of clearance from the fetus. Since blood values of total radioactivity exceeded those of the dam at almost all points of determination, it is of interest to know where the drug distributed and for how long.

N92862 Placental transfer of nebivolol in the Wistar rat after single oral administration at 5 mg/kg as studied by whole body autoradiography. 1990-1993

^{14}C-Nebivolol dissolved in water was used. Three pregnant Wistar rats were dosed with 5 mg base equivalents/kg on GD18. One rat was euthanized at each time point of 2, 8 and 24 hours after dosing. The rats were then processed for whole body autoradiography.

Results as presented in the textual summary: The distribution of radioactivity in maternal tissues was similar to that in male rats after oral administration of ^{14}C-nebivolol at 10 mg/kg (5). At 2 h after administration, maximum concentrations were observed in the gastrointestinal contents of stomach and small intestine. Highest tissue concentrations, at 2 h after administration, were reached in lung, liver, kidney (more in the cortex than in the medulla), and in adrenal gland. Marked levels were observed in pituitary gland, pineal body, lacrimal glands, salivary glands, preputial gland, pancreas, thyroid and spleen. Concentrations in intestinal tissues, brown fat (hibernating gland), bone marrow, heart muscle and lymph nodes, in the inner ear and in some parts of the eye (e.g. the choroid) were marked as well. Low levels were seen in blood and in the central nervous system. Concentrations in procreative tissues were not very marked. However, concentrations in mammary gland and placenta were higher than those in maternal muscle. In placenta, levels were slightly higher in the maternal part (decidua basalis) than in the fetal part. Levels in the fetal membrane were more conspicuous but not extremely high either. In fetuses, levels were very low. Radioactivity in some organs (e.g. liver) could faintly be discerned.

At 8 h after administration, radioactivity levels in intestinal contents were highest in the distal part of the gut . Tissue levels were similar to slightly higher than at 2 h after gavage. Just as in the quantitative placental transfer study, 8 h may be considered as peak time for many tissues. Highest tissue concentrations occurred in lung, somewhat lower concentrations in liver, kidney (most in the inner part of the cortex), glandular and lymphatic tissues. Concentrations in the fetal

membrane were higher than at 2 h after dosing. Levels in mammary gland, placenta and fetuses however, were just as concentrations in most tissues, similar to slightly higher than at 2 h after administration.

At 24 h after administration, radioactivity levels in intestinal contents were only marked in large intestine (caecum and rectum). In tissues, levels were much lower than at 8 h after administration. Highest levels were observed in kidney (cortical part) and in glandular tissues: mainly in lacrimal gland, but also in adrenal gland, pituitary gland, preputial gland and to a lesser extent in other glandular tissues. Further, radioactivity was observed in the inner ear and in the lymph follicles of spleen and lymph nodes. Concentrations in fetal membranes were marked as well. Levels in mammary gland, placenta and fetuses at 24 h were considerably lower than at 8 h after dosing.

Sample autoradiograms were presented. The accuracy of the sponsor's statements could not be confirmed.

N109063 Plasma levels and transition into the milk of nebivolol in the lactating Wistar rat and plasma levels in suckling pups after single oral administration at 2.5 mg/kg and after repeated oral administration at 2.5 mg/kg/day for 8 days. 1992-1994

Seven groups of 3 lactating Wistar rats with 8 or 9 pups were used. Dams were given oral doses of aqueous ^{14}C-nebivolol at a dose of 2.5 mg base.eq/kg. Maternal blood samples were collected with heparin at 1,4,8 and 24 hours after single doses and at 0(pre-dose), and 1 hour after the 4th dose and 6th dose and at 0,1,4,8,24, 48 and 72 hours after the eighth dose. Milk samples were obtained at corresponding time intervals with a special milking device. Pups were removed from the mothers about 16 hours prior to sampling. Pup blood samples with heparin were collected at 1, 4 and 24 hours after a single dose and at 1,4, 24 and 48 hours after the 8th dose by collecting from 1 pup from each of 3 dams at each time interval. Pups were removed for approximately 16 hours prior to milk sampling.
Concentrations of total radioactivity were measured in individual samples of maternal plasma, milk and pup plasma. Concentrations of unchanged drug (d- and l-nebivolol) were determined in pooled samples.

Results
Plasma concentrations of unchanged drug in the pups were at or below limits of quantification at most time points.

Table 4 : Mean maternal (dam) plasma, milk and pup plasma concentrations and AUC-values ($AUC_{0-\infty}$ after single dosing and $AUC_{0-24\,h}$ after repeated dosing) of total radioactivity (TR; mean ± S.D., n=3) and of nebivolol (UD; pools, n=3) in the lactating Wistar rat after single oral administration of ^{14}C-nebivolol at 2.5 mg/kg and after repeated oral administration of ^{14}C-nebivolol at 2.5 mg/kg/day.

		concentration : ng(-eq.)/ml						concentration ratio						
		TR			UD			UD/TR			TR		UD	
dose no.	time [h]	dams plasma	milk	pups plasma	dams plasma	milk	pups plasma	dams plasma	milk	pups plasma	milk / plasma	pups pl. / dams pl.	milk / plasma	pups pl. / dams pl.
1	1	242 ± 35	100 ± 38	≤ 4	142	83.7	≤ 3.4	0.59	0.84		0.41±0.11	≤ 0.02	0.59	≤ 0.02
	4	305 ± 88	360 ±224	≤ 4	117	190	≤ 6.6	0.38	0.53		1.25±0.59	≤ 0.01	1.62	≤ 0.06
	8	254 ± 50	173 ± 38	-	86.4	49.8	-	0.34	0.29	-	0.69±0.16	-	0.58	-
	24	48 ± 10	117 ± 27	11 ± 3	(1.7)[3]	2.3	≤ 6.6	0.04	0.02	≤ 0.6	3.06±0.78	0.23	1.35	≤ 4
$AUC_{0-\infty}$ [µg(-eq.)h/ml]		4.94	6.73	-	1.58	1.36	-	0.32	0.20	-	1.36	-	0.86	-
4	0	70[1]	100[1]	-	NS	1.2[1]	-	-	0.01[1]	-	1.56[1]	-	-	-
	1	360 ±119	321 ±190	-	164	73.0[1]	-	0.46	0.33[1]	-	0.93±0.65	-	0.45	-
6	0	92 ± 23	507 ±106	-	6.4	11.1	-	0.07	0.02	-	5.57±0.24	-	1.73	-
	1	313 ±155	556[1]	-	141	97.7[2]	-	0.45	0.25[2]	-	1.84[1]	-	0.69	-
8	0	98 ± 12	168 ± 30	-	3.9	2.3	-	0.04	0.01	-	1.73±0.39	-	0.59	-
	1	280 ±165	187 ± 98	36 ± 7	105	39.9	≤ 2.0	0.38	0.21	≤ 0.06	0.77±0.29	0.13	0.38	≤ 0.02
	4	523 ± 60	450 ± 82	43 ± 10	262	151	≤ 2.0	0.50	0.34	≤ 0.05	0.86±0.06	0.08	0.58	≤ 0.008
	8	242 ± 6	417[2]	-	59.5	64.1[2]	-	0.25	0.15[2]	-	1.76[2]	-	1.08	-
	24	77 ± 37	96 ± 17	33 ± 1	3.2	2.1	≤ 2.0	0.04	0.02	≤ 0.06	1.43±0.61	0.43	0.66	≤ 0.6
	48	39 ± 2	84 ± 68	25 ± 4	≤ 2.0	≤ 1.0	≤ 2.0	≤ 0.05	≤ 0.01	≤ 0.08	2.14±1.69	0.64	-	-
	72	22 ± 4	16 ± 8	-	≤ 2.0	≤ 1.0	-	-	-	-	0.71±0.08	-	-	-
$AUC_{0-24\,h}$ [µg(-eq.)h/ml]		5.48	6.97	0.91	1.75	1.27	≤ 0.05	0.32	0.18	≤ 0.05	1.27	0.17	0.72	≤ 0.03

[1] n=2
[2] n=1
[3] Value estimated by extrapolation below the quantification limit of 2.0 ng/ml.
NS: no sample (not enough plasma remained to determine UD).

It can be concluded that oral dosing of nebivolol will result in detectable levels of drug in the dam's milk which can in turn be detected in pup plasma. The sponsor carries the interpretation slightly further.

After a daily dose of nebivolol at 2.5 mg/kg/day, the $AUC_{0-24\,h}$ - value of steady-state milk levels was 1.27 µg.h/ml for unchanged nebivolol and 6.97 µg-eq.h/ml for TR (Table 4). The average steady-state level (C_{ss}) in milk, as calculated from these data, is 52.9 ng/ml for UD and 290 ng-eq./ml for TR. Assuming that a 35-g pup consumes 5 ml milk per day (9), the predicted ingestion is 7.6 µg/kg/day for UD and 41 µg/kg/day for TR. This corresponds to only 0.30 % of the dam dose in mg per kg body weight for UD and to 1.7 % of the dam dose for TR. For a 35-g pup, the total amount consumed is 3800 times lower than the dose to the 0.40-kg dam for unchanged nebivolol (0.27 µg *versus* 1.0 mg), and 700 times lower than the dose to the dam for TR (1.5 µg *versus* 1.0 mg).

Plasma concentrations of UD in pups were all below the limit of quantification. Plasma concentrations of TR in pups were below the limit of quantification at 1 and 4 h after single oral administration, but amounted to 11 ng-eq./ml at 24 h after the first dose. After the 8th maternal dose, maximum concentrations of TR in pup plasma were observed at 4 h after administration, just as in dams, but levels of TR in pups increased and decreased more slowly. Peak plasma levels in pups amounted to 43 ng-eq./ml. A comparison of $AUC_{0-24\ h}$ -values after repeated administration revealed pup to dam plasma ratios of 0.17 for TR and ≤ 0.03 for UD.

Table 5 : Concentrations of total radioactivity (TR), unchanged drug (UD) in some pooled tissues and in amniotic fluid (mean ± S.D., n=3) of the pregnant Wistar rat after single oral administration of ^{14}C-nebivolol at 2.5 mg/kg at the 18th day of gestation.

	Concentration: ng(-eq.)/ml or g of wet tissue									
	Uterus		Placenta		Fetus		Mammary gland		Amniotic fluid	
Time [h]	TR	UD	TR	UD	TR	UD	TR	UD	TR	UD
1	332	182	660	404	131	90.3	751	473	38.6 ± 7.5	25.3 ± 5.6
4	646	181	1187	367	284	95.7	1402	265	58.9 ± 33.1	19.6 ± 17.5
8	784	120	1407	237	335	51.8	1495	111	123 ± 20	15.6 ± 4.2
24	368	8.7	771	13.4	194	2.2	720	3.8	47.9 ± 3.8	≤ 1.0
48	246	2.1	382	1.4	87.8	≤ 1.0	342	≤ 1.0	22.4 ± 3.9	≤ 1.0

3.3.5

Metabolism

N104625 Study on the possible induction and/or inhibition of drug-metabolizing enzymes in the liver of male and female SPF Wistar rats, after oral administration of nebivolol hydrochloride at doses of 5, 20 and 80 mg (base-equivalents)/kg/day7 for 12 months. 1990-1994

The present report describes a study on the possible induction and/or inhibition potential of nebivolol towards drug-metabolizing enzymes in the liver of male and female SPF Wistar rats. Liver parts of male and female Wistar rats obtained from a 12 month chronic oral toxicity study (Exp 1964) were used in the present study. Nebivolol /β-CD was present in the food to provide doses of 5,20 and 80 mg/kg/day. Control and vehicle control groups were included. Liver and plasma samples were collected at the scheduled end of the study for 4/rats/sex/group. Hepatic microsomes were prepared. Aniline hydroxylase (CYP2E1) activity was determined by measuring formation of p-aminophenol. N-demethylation of N-ethylmorphine (EM) (CYP3A1, CYP3A2) was determined by measuring formation of formaldehyde. The O-deethylation of 7-

ethoxyresorufin (7-ER) (CYP1A1, CYP1A2) was determined fluorometrically as was the O-dealkylation of 7-pentoxyresorufin (CYP2B). Microsomal hydroxylation of lauric acid (CYP4A1) was also determined. Hepatic microsomes from Phenobarbital-treated rats were analyzed for the each of the different enzymes listed above as a control process. Ethanol, dexamethasone, clofibrate and 3-methylcholantrene-induced microsomes were also processed.
Results: Minimal induction or inhibition of enzymatic activity was seen.
Results for male Wistar rats are shown below.

Parameter	Control	Placebo	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
Serum concentrations					
ng/ml	-	-	9.8 ± 3.1 (n=10)	83.8 ± 25.3 (n=10)	840 ± 337 (n=9)
Relative liver weight					
g liver/100 g body weight	3.17 ± 0.14	3.33 ± 0.08	3.35 ± 0.45	3.67 ± 0.67	3.25 ± 0.13
Microsomal protein					
mg/g liver	21.0 ± 1.0	29.9 ± 2.5***	23.3 ± 3.7*	19.5 ± 1.7***	21.7 ± 3.7**
Cytochrome P-450					
maximum (nm)	449.9 ± 0.3	450.1 ± 0.5	449.7 ± 0.4	449.6 ± 0.4	450.0 ± 0.2
nmol/mg protein	0.697 ± 0.020	0.594 ± 0.069*	0.657 ± 0.021	0.598 ± 0.059	0.712 ± 0.117
Aniline hydroxylase					
nmol/mg protein.min	0.54 ± 0.14	0.44 ± 0.12	0.44 ± 0.14	0.38 ± 0.08	0.66 ± 0.05*
nmol/nmol P-450.min	0.78 ± 0.21	0.73 ± 0.13	0.66 ± 0.19	0.64 ± 0.13	0.95 ± 0.14
N-Ethylmorphine *N*-demethylase					
nmol/mg protein.min	8.70 ± 2.15	7.94 ± 0.82	7.46 ± 2.19	7.09 ± 2.01	6.17 ± 2.68
nmol/nmol P-450.min	12.5 ± 3.2	13.4 ± 0.9	11.3 ± 3.2	11.8 ± 2.9	9.3 ± 5.4
7-Ethoxyresorufin *O*-deethylase					
nmol/mg protein.min	0.33 ± 0.11	0.42 ± 0.05	0.42 ± 0.15	0.47 ± 0.16	0.69 ± 0.24
nmol/nmol P-450.min	0.48 ± 0.16	0.71 ± 0.14	0.64 ± 0.21	0.79 ± 0.24	0.96 ± 0.25
7-Pentoxyresorufin *O*-dealkylase					
nmol/mg protein.min	0.037 ± 0.010	0.031 ± 0.007	0.040 ± 0.015	0.029 ± 0.011	0.039 ± 0.007
nmol/nmol P-450.min	0.053 ± 0.015	0.053 ± 0.014	0.060 ± 0.022	0.049 ± 0.018	0.056 ± 0.020
Lauric acid hydroxylation					
nmol/mg protein.min	1.40 ± 0.38	1.61 ± 0.28	1.31 ± 0.19	1.28 ± 0.20	1.76 ± 0.30
nmol/nmol P-450.min	2.02 ± 0.55	2.71 ± 0.41	1.99 ± 0.24*	2.13 ± 0.22*	2.49 ± 0.29

*P≤0.05, **P≤0.01, ***P≤0.001 (Statistical significance levels for the placebo rats were calculated with respect to the control rats, for the nebivolol-treated rats with respect to the placebo rats).

Table 3 : Serum concentrations, liver microsomal protein and cytochrome P-450 contents, and hepatic monooxygenase activities for five groups of four female SPF Wistar rats after daily dosing of nebivolol hydrochloride at doses of 5, 20 and 80 mg (base-equivalents)/kg for twelve months. The drug was administered orally as a β-cyclodextrin/drug mixture in normal rat food. Control rats received only standard rat diet whereas the placebo group received β-cyclodextrin-containing food without nebivolol. The values represent the mean ± S.D. for each group.

Parameter	Control	Placebo	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
Serum concentrations					
ng/ml	-	-	12.1 ± 4.6 (n=9)	192 ± 54 (n=9)	931 ± 317 (n=9)
Relative liver weight					
g liver/100 g body weight	3.60 ± 0.57	3.14 ± 0.30	3.38 ± 0.35	3.88 ± 0.87	3.87 ± 0.42*
Microsomal protein					
mg/g liver	17.1 ± 0.9	19.3 ± 1.8	15.7 ± 2.6	18.1 ± 3.5	16.9 ± 1.8
Cytochrome P-450					
maximum (nm)	448.9 ± 0.2	449.9 ± 0.2***	449.6 ± 0.5	449.5 ± 0.4	449.7 ± 0.7
nmol/mg protein	0.631 ± 0.048	0.692 ± 0.077	0.663 ± 0.106	0.690 ± 0.175	0.763 ± 0.038
Aniline hydroxylase					
nmol/mg protein.min	0.50 ± 0.02	0.44 ± 0.02**	0.49 ± 0.08	0.52 ± 0.13	0.44 ± 0.05
nmol/nmol P-450.min	0.79 ± 0.09	0.64 ± 0.07*	0.74 ± 0.10	0.75 ± 0.04*	0.58 ± 0.09
N-Ethylmorphine *N*-demethylase					
nmol/mg protein.min	2.08 ± 0.64	2.00 ± 0.14	1.69 ± 0.26	2.36 ± 0.41	1.96 ± 0.52
nmol/nmol P-450.min	3.32 ± 1.11	2.90 ± 0.24	2.57 ± 0.36	3.50 ± 0.53	2.58 ± 0.75
7-Ethoxyresorufin *O*-deethylase					
nmol/mg protein.min	0.83 ± 0.20	0.77 ± 0.09	0.67 ± 0.03	0.67 ± 0.10	0.48 ± 0.09**
nmol/nmol P-450.min	1.33 ± 0.37	1.11 ± 0.04	1.04 ± 0.20	1.00 ± 0.20	0.63 ± 0.12***
7-Pentoxyresorufin *O*-dealkylase					
nmol/mg protein.min	0.016 ± 0.004	0.017 ± 0.010	0.015 ± 0.022	0.018 ± 0.012	0.022 ± 0.007
nmol/nmol P-450.min	0.026 ± 0.007	0.025 ± 0.015	0.024 ± 0.038	0.025 ± 0.014	0.029 ± 0.010
Lauric acid hydroxylation					
nmol/mg protein.min	1.10 ± 0.07	1.11 ± 0.23	1.11 ± 0.23	1.11 ± 0.46	1.11 ± 0.26
nmol/nmol P-450.min	1.76 ± 0.24	1.62 ± 0.44	1.67 ± 0.15	1.56 ± 0.26	1.47 ± 0.37

*P≤0.05, **P≤0.01, ***P≤0.001 (Statistical significance levels for the placebo rats were calculated with respect to the control rats, for the nebivolol-treated rats with respect to the placebo rats).

N104626Studies on the possible induction and/or inhibition of drug-metabolizing enzymes in the liver of male and female Beagle dogs, after oral administration of nebivolol hydrochloride at doses of 2.5, 10 and 40 mg(base-equivalents)/kg/day for twelve months.1990-1994

Liver samples were obtained from a 12-month chronic oral toxicity study in which the drug was given in gelatin capsules. A vehicle group received β-CD containing capsules. For each dose level, 0, vehicle, 2.5, 10 and 40 mg/kg, liver samples were collected from 2 male and 2 female dogs. Microsomes were prepared. Enzymatic activity measured was:
Aniline hydroxylase, N-demethylation of N-ethylmorphine, 7-ethoxyresorufin, 7-pentoxyresorufin O-dealkylation and lauric acid hydroxylation.

Parameter	Control	Placebo	2.5 mg/kg/day	10 mg/kg/day	40 mg/kg/day
Plasma level ng/ml	-	-	21.1 ± 6.2	112 ± 41	640 ± 391
Liver concentrations mg/g liver	-	-	8.5 ± 6.0	40.0 ± 29.6	62.4 ± 26.6 (n=3)
Relative liver weight g liver/100 g body weight	2.23 ± 0.23	2.29 ± 0.19	2.09 ± 0.12	2.41 ± 0.27	2.44 ± 0.38
Microsomal protein mg/g liver	15.8 ± 3.2	16.0 ± 5.7	18.9 ± 3.3	19.2 ± 3.6	22.5 ± 3.8
Cytochrome P-450 maximum (nm)	450.4 ± 0.4	450.0 ± 0.6	450.0 ± 0.4	450.0 ± 0.7	450.3 ± 0.4
nmol/mg protein	0.784± 0.036	0.844± 0.088	0.761± 0.081	0.789± 0.124	0.713± 0.080
Aniline hydroxylase nmol/mg protein.min	0.37 ± 0.09	0.42 ± 0.08	0.31 ± 0.06	0.37 ± 0.06	0.35 ± 0.08
nmol/nmol P-450.min	0.47 ± 0.10	0.50 ± 0.07	0.41 ± 0.08	0.47 ± 0.07	0.48 ± 0.06
N-Ethylmorphine *N*-demethylase nmol/mg protein.min	1.77 ± 0.42	2.19 ± 0.63	2.06 ± 0.45	2.13 ± 0.59	1.72 ± 0.07
nmol/nmol P-450.min	2.26 ± 0.51	2.56 ± 0.53	2.69 ± 0.46	2.66 ± 0.38	2.42 ± 0.18
7-Ethoxyresorufin *O*-deethylase nmol/mg protein.min	2.44 ± 0.93	2.38 ± 0.51	1.49 ± 0.56	2.25 ± 0.60	1.99 ± 0.16
nmol/nmol P-450.min	3.09 ± 1.15	2.80 ± 0.40	1.98 ± 0.80	2.95 ± 1.17	2.80 ± 0.13
7-Pentoxyresorufin *O*-dealkylase nmol/mg protein.min	0.47 ± 0.18	0.43 ± 0.09	0.36 ± 0.11	0.45 ± 0.11	0.49 ± 0.21
nmol/nmol P-450.min	0.60 ± 0.23	0.51 ± 0.08	0.47 ± 0.12	0.57 ± 0.08	0.68 ± 0.22
Lauric acid hydroxylation nmol/mg protein.min	1.52 ± 0.12	1.72 ± 0.43	1.40 ± 0.23	1.49 ± 0.23	1.24 ± 0.35
nmol/nmol P-450.min	1.94 ± 0.13	2.04 ± 0.46	1.83 ± 0.16	1.90 ± 0.22	1.71 ± 0.30

Results
The data presented doesn't indicate any significant differences between groups.

N109064 The in-vitro metabolism of nebivolol and its enantiomers in cultures of human hepatocytes1992-1994

Pieces of human liver were obtained from a kidney transplant donor and processed into suspension cultures in multi-well plates. Viability of 3 suspension preparations was 91%, 68% and 45%. The 91% viability suspension was used to make the primary cultures. The remaining cultures were pooled for a final viability of 57%.

Suspension cultures: ^{14}C-labelled nebivolol and ^{14}C-labelled enantiomers were used. An aliquot of ^{14}C-nebivolol, ^{14}C-d-nebivolol or ^{14}C-l-nebivolol was added to give final concentrations in the incubate of 5µM or 10µM. To some incubates, quinidine was added at a final concentration of 1 or 5µM. Incubations were carried out at 37°C for 29 and 120 minute.

Primary cell cultures were established in multi-well plates. After 6 hours and 45 minutes, non-adherent cells were removed and serum-free media containing ^{14}C-nebivolol or its ^{14}C-labelled enantiomers at a final concentration of 5µM or 10µM was added per well. To some incubates, quinidine was added at a final concentration of 1 or 5µM. After 24 hours, total homogenates were prepared by homogenization of the cells. The homogenates of 2 wells (10-µM incubates, incubates without cells and incubates with quinidine) or 4 wells (5-µM incubates) were pooled. Controls containing 5 µM ^{14}C-nebivolol or its ^{14}C-labelled enantiomers, but no hepatocytes, were incubated together with the cultures containing viable hepatocytes.

Protein concentrations were determined in aliquots of fresh cell suspensions and of homogenates obtained by scraping the individual wells at the end of the adhesion period and after the different culture periods.

CYP450 content was determined in fresh suspensions and in the scrapings from primary cell cultures.

Ethoxycoumarin O-deethylase activity was determined after incubation of ethoxycoumarin with suspensions of isolated hepatocytes. Samples were enzymatically treated as described for scoparone. Metabolism of scoparone was determined after incubation with hepatocytes in suspension culture and in primary cell culture. After stopping the reactions in both culture types with acetate buffer, the samples were divided and one aliquot was treated with β-glucuronidase/arylsulphatase. Scoparone is O-demethylated by several forms of CYP450.

Results

Recovery of radioactivity in the supernatants for suspensions and for primary cultures was

	suspension	primary culture
^{14}C-Nebivolol:	91±6%	95 ±11%
^{14}C-d-nebivolol	88±5	95±5
^{14}C-l-nebivolol	87±3	96±6

Metabolism of nebivolol was analyzed by HPLC. Metabolites were characterized by comparison with reference compounds. The sponsor states that

> After incubation of **nebivolol** with human hepatocytes, metabolite M9 (1.0 % of the sample radioactivity at 10 µM after 2 h) was the main one in suspension culture, metabolite M6 the main one in primary cell culture (9.4 % of the sample radioactivity at 10 µM after 24 h). Other major metabolites or metabolite fractions were M5, M6/M7 and

However, the sponsor's tabulated results shows that the majority of species found was unchanged parent drug.

The sponsor also noted that after incubation of d-nebivolol with human hepatocytes metabolites

Table 2: Mass balance of nebivolol (UD) and its major metabolites after incubation of ^{14}C-nebivolol with human hepatocytes suspension culture (SK) or in primary cell culture (PCK). The effect of co-incubation of 1 or 5 µM quinidine in the incubation medium, is also shown. Mass balances were determined by reversed phase radio-HPLC. The figures represent the percentage of the sample radioactivity.

Incubation conditions	Nebivolol concentration (µM)	Time (h)	Metabolite of metabolite fraction (% of injected)											
			M1	M2	M3	M4	M5	M6/M7	M8	M9	M10/M11	M12	UD	Sum
SK	5	2	0.6	ND[1]	ND	ND	0.9	0.6	ND	1.8	0.6	0.4	99.3	104.2
	10	2	0.6	ND	0.1	0.3	0.7	0.4	0.2	1.0	0.4	0.3	100.8	104.8
	10 + 1 µM quinidine	2	0.2	ND	ND	ND	0.2	0.1	ND	0.4	ND	ND	99.6	100.4
	10 + 5 µM quinidine	2	0.4	ND	ND	0.1	ND	0.4	0.2	ND	ND	ND	102.2	103.3
PCK	5	0	ND	ND	ND	ND	ND	ND	ND	ND	1.4	ND	96.9	98.3
	5	8	0.9	1.3	ND	ND	1.3	4.6	2.2	4.3	3.9	2.2	78.3	99.0
	5	24	1.9	0.7	1.7	1.1	3.0	13.3	5.4	5.4	5.6	3.3	55.1	96.5
	10	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.6	101.3	101.9
	10	8	1.1	0.7	0.9	0.6	1.4	3.5	2.0	3.1	4.2	1.6	83.7	102.8
	10	24	1.7	1.1	1.7	0.7	1.8	9.4	5.7	3.7	5.8	2.8	66.2	100.6
	10 + 1 µM quinidine	24	1.3	0.8	1.1	ND	1.1	9.2	4.6	ND	3.3	1.9	71.0	94.3
	10 + 5 µM quinidine	24	1.1	0.9	ND	ND	0.6	9.7	5.0	1.2	4.3	1.4	77.8	102.0

[1]ND: not detected.

Table 3: Mass balance of *d*-nebivolol (UD) and its major metabolites after incubation of ^{14}C-*d*-nebivolol with human hepatocytes suspension culture (SK) or in primary cell culture (PCK). The effect of the co-incubation of 1 or 5 µM quinidine in the incubation medium, is also shown. Mass balances were determined by reversed phase radio-HPLC. The figures represent the percentage of the sample radioactivity.

Incubation conditions	*d*-Nebivolol concentration (µM)	Time (h)	Metabolite or metabolite fraction (% of injected)									
			M1	M2	M4	M5	M6	M8	M10	M12	UD	Sum
SK	5	2	ND[1]	ND	0.3	0.9	0.8	0.3	1.2	1.0	97.8	102.3
	10	2	0.2	ND	0.1	T[2]	0.7	0.3	0.8	0.7	101.4	104.2
	10 + 1 µM quinidine	2	ND	ND	ND	ND	0.6	0.3	ND	0.3	100.0	101.2
	10 + 5 µM quinidine	2	T	ND	ND	ND	0.6	T	T	T	103.8	104.4
PCK	5	0	ND	ND	ND	ND	ND	ND	ND	ND	100.6	100.6
	5	8	1.1	1.0	ND	3.1	7.0	2.8	4.5	4.0	74.6	98.1
	5	24	1.7	0.9	1.9	2.3	18.6	6.3	7.3	7.2	45.2	91.4
	10	0	ND	ND	ND	ND	ND	ND	ND	1.0	101.0	102.0
	10	8	1.3	0.8	ND	1.3	5.1	2.5	4.9	3.2	80.3	99.4
	10	24	2.2	0.9	1.0	1.4	13.4	7.0	8.5	5.0	56.4	95.8
	10 + 1 µM quinidine	24	2.3	1.1	0.8	1.3	9.9	4.6	5.7	4.5	72.3	102.5
	10 + 5 µM quinidine	24	1.8	1.0	T	0.9	10.7	5.6	4.6	2.1	76.4	103.1

[1]ND: not detected.
[2]T: traces (<0.1 %).

M5, M6, M10 and M12 were main metabolites in suspension culture and M6 the main metabolite in primary cell culture. Again, the majority of drug appears to be the unchanged parent.

After incubation of l-nebivolol with human hepatocytes, the sponsor felt that M9 was the predominant metabolite. Again, unchanged parent was the major species present.

Table 4: Mass balance of *l*-nebivolol (UD) and its major metabolites after incubation of ^{14}C-*l*-nebivolol with human hepatocytes suspension culture (SK) or in primary cell culture (PCK). The effect of co-incubation of 1 or 5 µM quinidine in the incubation medium, is also shown. Mass balances were determined by reversed phase radio-HPLC. The figures represent the percentage of the sample radioactivity.

Incubation conditions	*l*-Nebivolol concentration (µM)	Time (h)	Metabolite or metabolite fraction (% of injected)										
			M1	M3	M4	M5	M7	M8	M9	M10/M11	M12	UD	Sum
SK	5	2	T[1]	0.6	0.4	1.0	ND[2]	ND	1.6	T[1]	ND	95.6	99.2
	10	2	0.3	0.1	0.4	0.6	ND	ND	1.2	ND	ND	100.3	102.3
	10 + 1 µM quinidine	2	0.5	ND	ND	ND	ND	ND	0.5	ND	ND	99.4	100.4
	10 + 5 µM quinidine	2	0.6	0.4	T	0.1	ND	ND	0.4	ND	ND	104.9	106.4
PCK	5	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	102.2	102.2
	5	8	0.7	2.1	1.5	2.3	1.0	0.8	7.4	2.9	ND	76.1	94.8
	5	24	1.6	2.9	2.9	4.4	3.1	1.8	11.8	5.0	ND	56.3	89.8
	10	0	ND	ND	ND	ND	ND	ND	ND	0.7	0.5	97.6	98.8
	10	8	1.3	2.1	1.1	1.6	1.4	1.1	4.4	3.4	0.7	82.4	99.5
	10	24	1.8	1.4	1.9	2.8	2.9	2.4	8.5	5.8	0.9	68.1	96.5
	10 + 1 µM quinidine	24	1.7	2.2	1.3	2.0	3.4	2.3	5.1	4.7	0.8	77.8	101.3
	10 + 5 µM quinidine	24	1.4	1.0	0.6	0.9	2.6	2.1	3.3	4.4	0.8	82.4	94.5

[1]T: traces (< 0.1 %).

The sponsor further states that:

> The interpretation of radiochromatograms such as those obtained in the present study has been discussed previously (17,18,19). Most metabolites could not accurately be characterized by co-chromatography with the available reference compounds due to the extremely high number of possible positional isomers and stereoisomers. The identification of the metabolites in the present study was based on their HPLC-retention times, relative to the HPLC-retention times for metabolites identified in previous *in-vivo* studies by mass spectrometry of the isolated and purified metabolites. Depending on the elution order of the available reference compounds, metabolites were characterised as *N*-dealkylated, aromatic-hydroxylated or alicyclic-hydroxylated metabolites. Further information on the identity of the metabolites was obtained from their UV-diode array spectra, in comparison with the spectra obtained in previous *in-vivo* studies for isolated and purified metabolites (17,18,19).

The sponsor also states that M6, M7, M8, M15 and M16 were glucuronides. The sponsor also feels that the main metabolic pathway for d-nebivolol was glucuronidation of the unchanged drug to metabolites M6 and M8 while the main metabolic pathway of l-nebivolol was alicyclic oxidation to produce M9, M10 and M11.

Towards the end of the text of the report, the sponsor states that the use of quinidine was, in essence, to simulate the poor metabolizer phenotype.

monohydroxy- metabolites, occurred. Quinidine is a well-known potent inhibitor of CYP2D6-mediated oxidations (20), which can switch the metabolic capacity of an extensive metabolizer of debrisoquine into a poor metabolizer (21). From the results of the present study which used hepatocytes of an extensive metabolizer of debrisoquine (table 1), it can be concluded that aromatic hydroxylation, and to a lesser extent alicyclic oxidation, of the benzopyran moieties will be impaired in poor metabolizers of debrisoquine, whereas oxidative *N*-dealkylation seems to be unaffected by the debrisoquine-type genetic polymorphism. Oxidation at the aromatic part of the benzopyran moieties of nebivolol were predicted to be affected by CYP2D6 by a molecular modelling technique for substrates of this isozyme (22) based on the distance between the site of aromatic hydroxylation and the basic nitrogen which varied between 6.2 and 8.2 Å. On the other hand, the model predicted that alicyclic oxidation of the

The results presented here cause the reviewer some hesitation for several reasons. 1) in vivo results typically show very little unchanged parent drug and a predominance of metabolites. The in vitro results shown here show the opposite. 2) The sponsor specifically states that the radiochromatograms were difficult to interpret and relied on elution times relative to reference compounds. While this may be a valid method, application of LC/MS technology would have been very beneficial. 3) The supposition that addition of quinidine should mimic the poor metabolizer phenotype seems somewhat paradoxical since the amount of unchanged parent drug was unchanged with the addition of quinidine.

3.3.6 Excretion

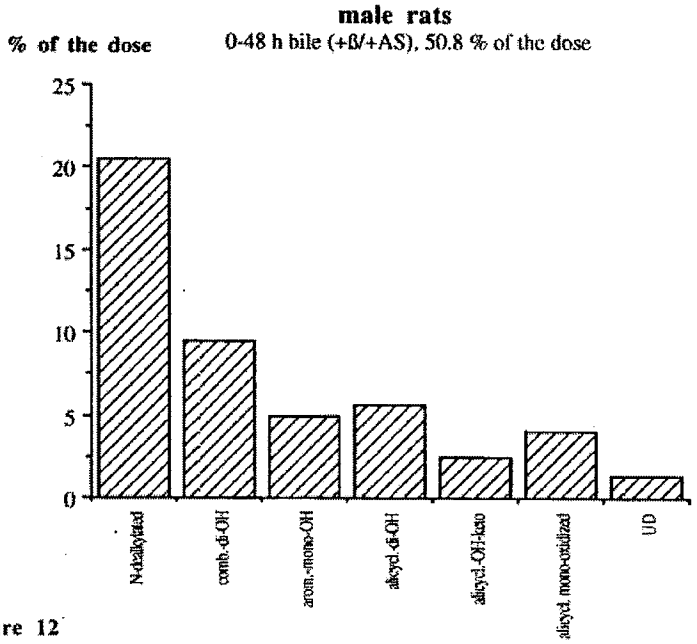
N106518 ***Biliary excretion and enterohepatic circulation of nebivolol after a single oral dose in rats. 1990-1994***

Four male Wistar rats with bile duct cannulas were given a single oral dose of racemic nebivolol to provide a dose of 2.5 mg/kg. The radioactive dose was 563 kBq or 15 µCi per rat. Two male donor rats were given oral doses of 2.5 mg/kg racemic nebivolol. The radioactive dose for these rats was 16 µCi. The acceptor rats received a direct continuous intraduodenal infusion of the bile from an orally dosed donor rat. Acceptor rats were placed in cages 10-15cm lower than the donor

rats to facilitate bile flow in the catheter between the donor and acceptor animals. Bile from simply cannulated rats was collected in 30 minute intervals up to 2 hours after dosing and in 1 hour intervals up to 48 hours after dosing. Bile of the acceptor rats was collected from the catheter in the proximal part of the bile duct in 30 minute intervals up to 3 hours (1 rat) or 3 hours(2^{nd} rat), in 1 hour intervals up to 48 hours after dosing of the donor rats. At 48 hours after dosing the donor and acceptor rats were disconnected and the bile excreted by the donor rats was collected in 30 minute intervals from 48 to 50 hours after dosing to verify a normal bile flow from the donor rat. Bile collected prior to dosing (blank bile) was spiked with an aliquot of drug formulation and processed along with the samples to detect possible artifacts. Liquid scintillation counting was used for determination of radioactivity levels. Samples were also subjected to enzymatic hydrolysis with β-glucuronidase/arylsulphatase

Results

Biliary excretion of nebivolol metabolites was fairly rapid with 42 ±11% of administered radioactivity excreted within the first 24 hours with the maximum excretion rate reported between 1 and 3 hours. About 50% of the administered radioactivity was excreted in the 48 hours after dosing. The acceptor rats (n=2) excreted by 48 hours approximately 10% of the radioactivity that was orally administered to the donor rats.



ure 12

The predominant species identified was N-dealkylated metabolites. Unchanged drug was present in the lowest amounts. A variety of hydroxylated metabolites were found in varying amounts.

N109036 The metabolism and excretion of nebivolol after a single oral dose of 10 mg/kg in rats. Comparison of two different forms of ^{14}C-labelled nebivolol and identification of metabolites. 1987-1994.

The present report focused on the metabolic consequences of the different positions of the radiolabel, and no detailed mass balance of nebivolol and its metabolites was given. The identification of metabolites will also be described. The metabolic stability of both radio-labeled molecules was sufficient to perform metabolism studies.

^{14}C-Nebivolol hydrochloride, (±)-([2R*[1S*,5S*(S*)]])-α,α'-[iminobis-(methylene)]bis [6-fluoro-3,4-dihydro-2*H*-1-benzopyran-2-methanol] hydrochloride was specifically labelled on the methylene group at the R*S*-side (coded A-nebivolol) or at the S*S*-side of the molecule (coded B-nebivolol) (Figure 1) (5). ^{14}C-Nebivolol (A) hydrochloride, batch No. 510, showed a specific activity of 174 MBq/mmole (4.7 mCi/mmole) or 428 kBq/mg nebivolol base (11.6 µCi/mg nebivolol base), and ^{14}C-nebivolol (B) hydrochloride, batch No. 505, showed a specific activity of 160 MBq/mmole (4.3 mCi/mmole) or 392 kBq/mg nebivolol base (10.6 µCi/mg nebivolol base). The radiochemical purity of both batches was ——— HPLC). Unlabelled nebivolol (batch No. A0301) was used for the dilution of the radiolabelled drugs.

Aqueous solutions of ^{14}C-nebivolol (A) or (B) were prepared by dissolving one of the radio-labeled batches together with unlabeled drug in propylene glycol to a concentration of 5 mg/ml. The final formulation contained 0.2ml propylene glycol per ml and 2% HP-β-CD. Five fasted male Wistar rats per group were given oral doses of 10 mg/kg (~14µCi per rat). The rats were housed individually in metabolism cages. Urine was collected from 0-4, 4-8, 8-24, 24-48, 48-72 and 72-96 hours. Feces were collected from 0-24, 24-48, 48-72 and 72-96 hours after dosing. The radioactivity in the various samples was measured by liquid scintillation counting. A metabolite profile of nebivolol in urine and methanolic extracts of feces was made by reversed phase HPLC. Samples were analyzed ± hydrolysis with β-glucuronidase/arylsulphatase. Although mass spectroscopy was not mentioned in the methods section, it was described in one of the appendices and results were presented.

Results

Both forms of radio-labeled material were excreted rapidly, primarily in the feces. By 24 hours 85% of the radioactivity had been excreted. Some 25% was recovered in the urine in the first 24 hours. There was no difference in the excretion of the 2 differently labeled forms of nebivolol. This is summarized in the reviewer's table below.

matrix	time	^{14}C-A-nebivolol	^{14}C-B-nebivolol
urine	0-24	24.88±2.17	25.89±3.06

Urine	0-96	27.93±2.11	28.82±3.18
feces	0-24	60.33±2.30	56.84±3.67
feces	0-96	68.45±2.51	64.92±3.49

No major differences in the urinary metabolite profile were reported for dosing with ^{14}C-A- vs ^{14}C-B- nebivolol. There were minor differences in the N-dealkyated metabolites. The differences in retention times was due to separation of diastereomeric N-dealkylated metabolites of nebivolol, indicating that N-dealkylation took place at both sides of the secondary amine.

The fecal metabolite profile was very similar after A- and B-nebivolol administration. In the methanolic extracts, unchanged drug was found as well as most of the urinary metabolites. It was stated that few N-dealkylated metabolites were present. Overall, within the variability inherent in the techniques, the metabolite profiles were not significantly different after administration of the two forms of nebivolol. Nebivolol, the N-dealkylated metabolites, the alicyclic-dihydroxy-metabolites, the alicyclic-hydroxy-keto and the alicyclically mono-oxidized metabolites were present in similar amounts following the dosing with two different radio-labeled forms of nebivolol.

N101244 The metabolism and excretion of nebivolol and its enantiomers after a single oral dose in rats. 1990-1994

^{14}C-nebivolol (A configuration) and radio-labeled enantiomers were used in aqueous solution. Wistar rats, 5/sex received a dose of the racemic nebivolol formulation at a dose of 2.5 mg/kg (16 μCi per rat). Five male Wistar rats received the d-nebivolol formulation at a dose of 1.25 mg/kg. Five other male Wistars were given l-nebivolol at a rate of 1.25 mg/kg. The animals were housed in separate metabolism cages. Urine was collected from 0-4, 4-8, 8-24, 24-48, 48-72 and 72-96 hours after dosing. Feces were collected from 0-24, 24-48, 48-72 and 72-96 hours after dosing. Urine and feces were collected from untreated rats and spiked with drug formulation to determine the presence of interfering factors. Individual and overall urine pools were examined per sex and per drug. Overall pools of plasma samples were prepared per time point, per sex and per drug by mixing constant fractions of the individual samples of all or of some rats.

A mass balance of nebivolol and it major metabolites in urine, methanolic extracts of feces and in plasma was made based upon recovery of the radioactivity in samples and pools of urine, fecal extracts and plasma pools as well as on the areas of the radioactivity peaks obtained after reverse phase HPLC of appropriate aliquots of these samples.

Urine samples were also analyzed ± enzymatic hydrolysis.

Results

After a single oral dose of racemic nebivolol the radioactivity was excreted primarily in the feces. Approximately 72-77% of the administered dose was excreted by 24 hours. In 96 hours, 16-19% of the administered dose was excreted.

Summary of urine and feces excretion of drug

sample	Male 2.5 mg/kg Rac-nebivolol	Female 2.5 mg/kg rac-nebivolol	Male 1.25 mg/kg d-nebivolol	Male 1.25 mg/kg l-nebivolol
Urine 0-24	15.81±1.25	12.65±2.09	21.19±1.08	18.82± 4.02
Urine 0-96	18.59±1.07	16.16±2.25	24.96±1.08	24.09±4.90
Feces 0-24	61.58±2.92	59.00±1.07	62.12±4.17	55.47±9.34
Feces 0-96	73.60±1.20	75.74±2.07	77.11±2.33	76.44±6.42

No major sulphate or glucuronide conjugates were detected in the urine samples.

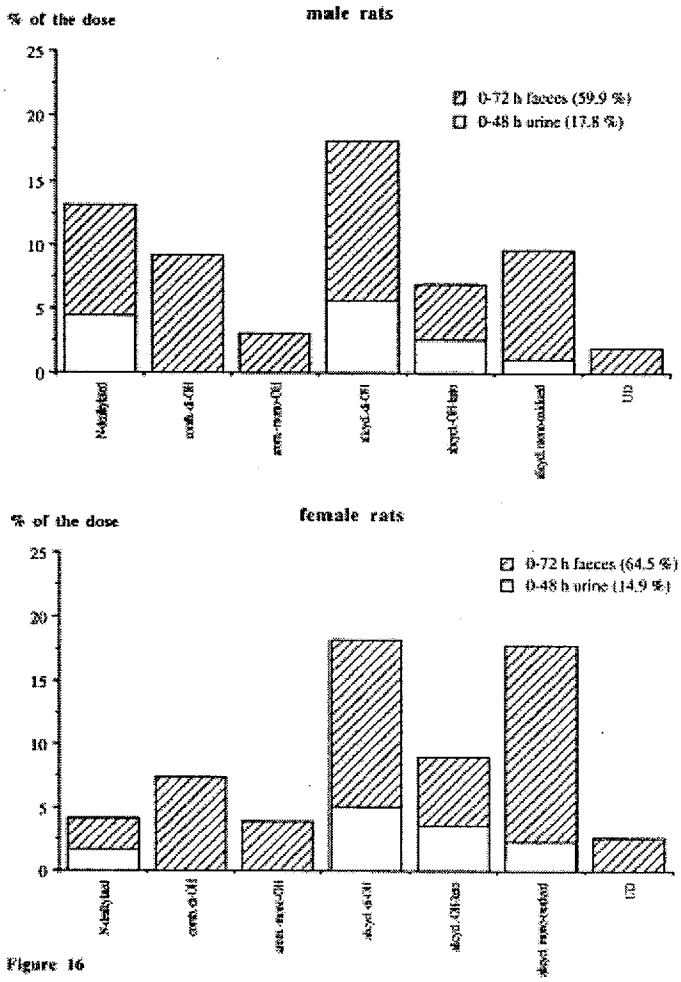


Figure 16

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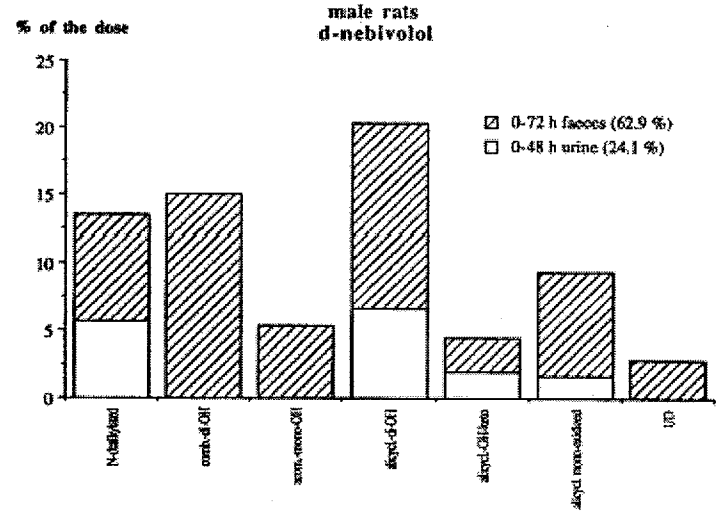


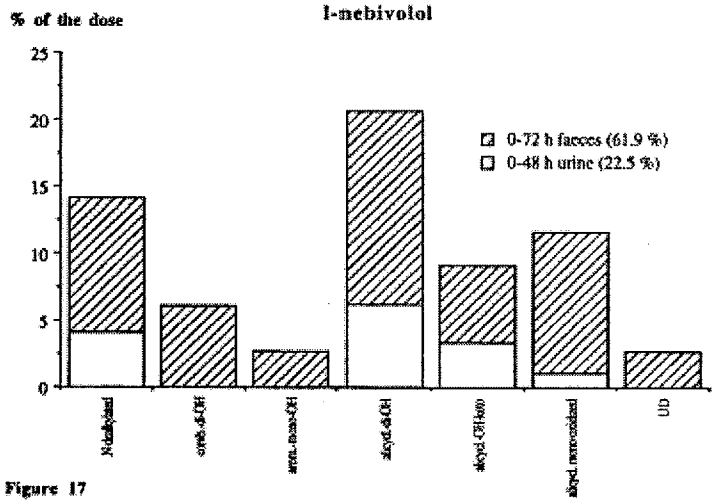


Figure 17

Overall, when groups of major metabolites in the urine and feces are compared as a percentage of the administered dose, there is very little difference between racemic, d- or l- nebivolol. Female rats did show lesser amounts of N-dealkylated metabolites and slightly more alicyclically mono-oxidized metabolites than did males treated with either racemic mixture, d- or l- nebivolol.

Metabolites as % of the administered dose

Nature of the metabolites	Female rats	Male rats		
	Rac-nebivolol (urine + feces)	Rac-nebivolol (urine + feces)	d-nebivolol (urine + feces)	l-nebivolol (urine + feces)
Unchanged drug	In feces only ~3%			
N-dealkylated	4.1	13.2	13.5	14.1
Alicyclically mono-oxidized	17.8	9.7	9.4	11.6

Numbers from table 13.

The main radioactive species found in plasma were parent drug, alicyclically mono-oxidized metabolites N, O, and S and the acid metabolite R80371 (the only N-dealkylated metabolite in the plasma). After administration of l-nebivolol, alicyclic-dihydroxy and alicyclic-hydroxy-keto-metabolites were also found in the plasma. Given the small sample sizes and the variability inherent in the process, there do not appear to be any significant differences in metabolism between racemic nebivolol, the enantiomers and male and female rats.

N106607 The excretion and metabolism of nebivolol in the rabbit after a single oral dose of 2.5 mg/kg. 1992- 1994

Four female Cunistar-MDL albino rabbits received a single oral dose of 2.5 mg ^{14}C-nebivolol/kg (the lowest dose used in the Segment II reproductive study in rabbits). The drug was formulated in HP-β-CD. The rabbits were housed individually. Urine and feces were collected daily from the time of dosing up to 3 days after dosing. The urine production of one rabbit was reported as irregular and as a result, the urine from that rabbit was collected from 0-48h and 48-144 hours after dosing. Plasma samples were collected from the same female rabbits for an absorption study and they were pooled proportionally per time interval (unexplained in methods section).

Radioactivity in the samples was determined by liquid scintillation counting. Overall pools of the 0-24h, 24-48h and 48-72h urine and pools of the fecal extracts were prepared from rabbits 1-3. Samples from Rabbit 4 (with irregular urine output) were not included in the overall pools because reportedly no urine was produced during the 0-24 and 48-72 hour periods and excretion into the feces was markedly lower than in the other 3 rabbits (29 vs 43% of the dose). Plasma pools were prepared by mixing constant volumes of the individual plasma samples of the 4 rabbits. Mass balance was determined by HPLC. Metabolites were identified by co-chromatography techniques. Enzymatic hydrolysis of samples was performed with β-glucuronidase/arylsulphtase. Rabbit samples were also reported as co-injected with samples obtained from excretion and metabolism studies in rat, dog and human.

Results
The excretion of total radioactivity is summarized below. At the end of the collection period there is still approximately 20% of the administered radioactivity unaccounted for.
This differs from rodents where excretion is essentially complete within 24 hours.

Recovery of radioactivity as % of administered dose

sample	0-24	0-72
urine	24.33±3.10	36.30±4.49
feces	21.58±2.43	42.87±4.67
sum		80.10±3.77

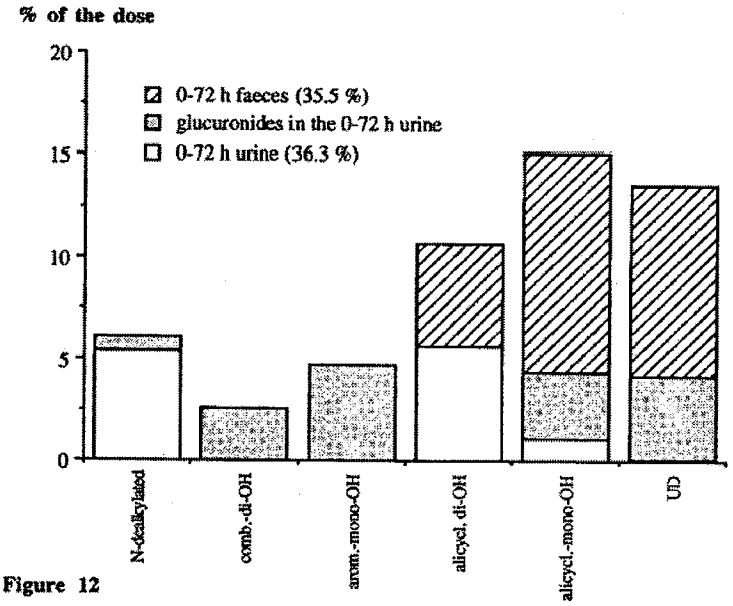


Figure 12

Nebivolol was extensively metabolized in the rabbits. In urine, nebivolol and ~ 7 of the hydroxylated metabolites were excreted as glucuronides. Unchanged drug was detected in trace amounts (<0.05%) in the urine. The sum of unchanged drug found in the feces was ~9% of the administered radioactivity. Alicyclic-monohydroxy-metabolites were the major metabolites of nebivolol in feces, accounting for ~11% of administered radioactivity.
The major metabolic pathways were reported to be aromatic monohydroxylation, dihydroxylation and oxidative N-dealkylation to the acid metabolite (R080371) and the diol metabolite (R080289). In urine, nebivolol and most of the hydroxylated metabolites were excreted as glucuronides. Unchanged drug was present at 0.1% of the dose and in feces was present at 9.4% (0-72 hours).

In the 0.5-h plasma, UD and the acid metabolite (R080371) were the only detectable compounds. Later on, the relative abundance of UD decreased, but the acid metabolite remained the main plasma metabolite. Alicyclic-monohydroxy- metabolites and glucuronic acid conjugates of combined-dihydroxy-, of aromatic-monohydroxy- metabolites and of UD were also detected in plasma from 2 h post dose-on. No alicyclic-dihydroxy-metabolites could be detected in plasma.

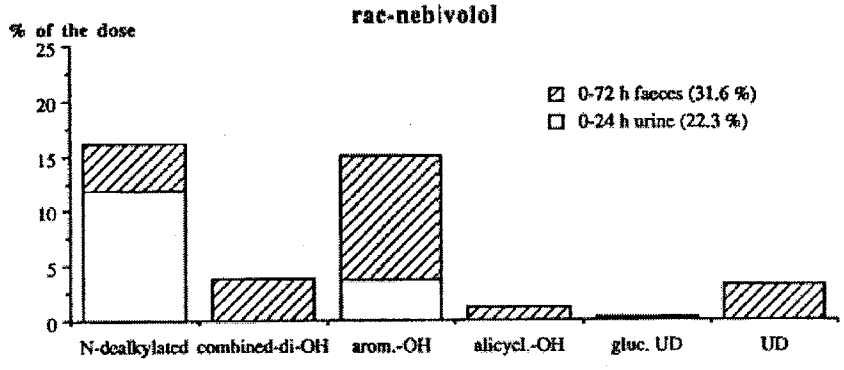
N109088 Absorption, metabolism and excretion of nebivolol in the dog after a single oral dose of 2.5 mg/kg of rac-nebivolol or 1.25 mg/kg of the separate enantiomers. 1990-1994.

Three male Beagles received a single oral dose of 2.5 mg ^{14}C-nebivolol/kg in the first phase of the study. In a second and third phase of the study the same dogs received a single oral dose of either the radio-labeled enantiomers at 1.25 mg/kg. Between the 3 phases, wash-out periods of four weeks were included. The drug was formulated in PEG400 and delivered by gavage. During each of the 3 phases blood samples were collected before dosing and at 0.5, 1,1.5, 2, 4, 6, 8, 24, 32, 48, 72 and 96 hours after dosing. Urine was collected from 0-4h, 4-8h, 8-24 h and "further on daily for up to 168 hours after dosing." Feces were collected daily for up to 7 days (or 168 hours) after dose administration. Blood and plasma levels of total radioactivity were determined by liquid scintillation counting. Hydroxy metabolites in plasma were determined before and after enzymatic deglucuronidation using enantio-selective immunoassays.

Results

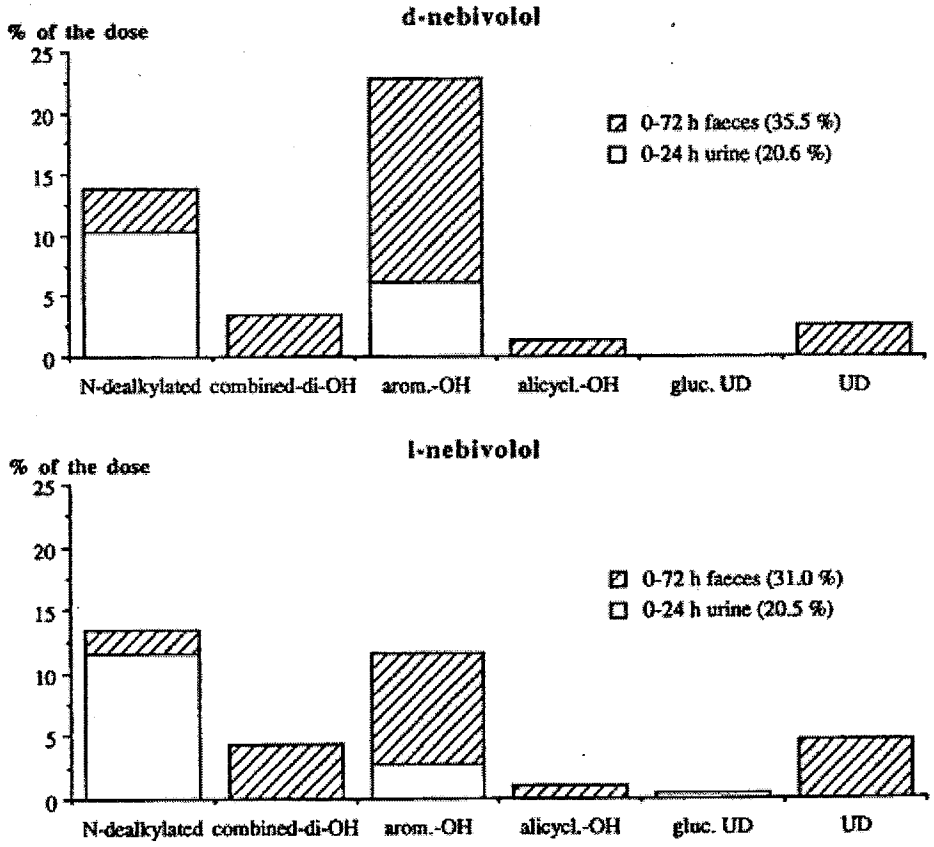
Recovery of radioactivity from the fecal extracts was low and reported as 33-56%. The sponsor felt that after supplementary extraction steps were performed, "... the mass balance of UD and the various metabolites determined in the original methanolic extracts obviously represents an underestimation with respect to total fecal radioactivity. However, an estimation of the mass balance in the extracts of fecal residues was not possible due to poor chromatographic resolution of the radioactivity peaks...(p. 17)." The analysis of the results should be read keeping the recovery levels in mind.

After receiving either racemic nebivolol or one of the enantiomers, radioactivity was reported as predominantly excreted in the feces. Seven days after a single oral dose, 16-35% of the administered radioactivity was excreted in the urine



and 61-72% in the feces. There did not seem to be a difference in excretion route or rate between the separate enantiomers.

Total radioactivity excreted in urine and feces was essentially the same for the racemic mix and the enantiomer: 24±1% - 27±10% in the urine from 0-168 hours and 66±5% - 70±1% in the feces from 0-168 hours.



Page 15, the sponsor states with reference to the urine that "A lot of conjugates were detected, mainly glucuronides." This finding is not apparent in the sponsor's graphical summary. From the above information there do not appear to be significant differences in the metabolism between racemate and the enantiomers. What is somewhat unclear is the information in the sponsor's tables 14-16 which indicate substantial glucronidation or sulfation.

Table 14: Pharmacokinetic parameters after a single oral dose of 2.5 mg ^{14}C-*rac*-nebivolol/kg to three male dogs.

TR: [2R*[1S*,5S*(S*)]]-nebivolol and metabolites

Parameters		dog No. 10432	dog No.10433	dog No.10434	mean ± S.D.
C_{max}	ng-eq./ml	2,456	1,606	2,368	2,143 ± 467
T_{max}	h	1.5	1.0	1.0	1.2 ± 0.3
β^a	h^{-1}	0.01403	0.01543	0.01697	0.01548 ± 0.00147
$t_{1/2}{}^a$	h	49.4	44.9	40.8	45.0 ± 4.3
AUC_{0-96h}	µg-eq..h/ml	21.2	16.5	20.6	19.4 ± 2.6
$AUC_{0-\infty}$	µg-eq..h/ml	22.9	17.6	21.7	20.7 ± 2.8

a: calculated from 48 h to 96 h after dosing.

UD: [2R*[1S*,5S*(S*)]]-nebivolol

Parameters		individual samples				after enzymatic hydrolysis[a]
		dog No. 10432	dog No. 10433	dog No. 10434	mean ± S.D.	pooled samples
C_{max}	ng/ml	28.6	12.6	20.3	20.5 ± 8.0	338
T_{max}	h	0.5	1.0	0.5	0.7 ± 0.3	1.0
β^c	h^{-1}	0.09468	0.2397	0.2446	0.1930 ± 0.0852	0.1445
$t_{1/2}{}^c$	h	7.3	2.9	2.8	4.3 ± 2.6	4.8
$AUC_{0-8\ h}$	ng.h/ml	77.6	40.3	50.7	56.2 ± 19.2	864
$AUC_{0-t}{}^b$	ng.h/ml	108.2	40.3	50.7	66.4 ± 36.6	1,083
$AUC_{0-\infty}$	ng.h/ml	115.5	49.3	57.7	74.2 ± 36.0	1,102
UD/TR AUC ratio		0.005	0.003	0.003	0.004 ± 0.001	0.053

a: enzymatic hydrolysis with ß-glucuronidase from *E. coli*.

b: AUC_{0-24h} for dog No. 10432 and for the pooled samples, and AUC_{0-8h} for dogs No. 10433 and 10434.

c: calculated from 8 h to 24 h after dosing for dog No. 10432, 1 h to 8 h for dog No. 10433, from 4 h to 8 h for dog No. 10434, and from 6 to 24 h for the hydrolysed pooled samples.

Total radioactivity summary

	Racemic nebivolol	d-nebivolol	l-nebivolol
Cmax ng-eq/ml	2143±467	1016±35	1017±165
Tmax h	1.2±0.3	1.0±0.0	1.5±0.0
$T_{1/2}$	45±4.3	16.9±7.1	79.6±7.0
$AUC_{0-\infty}$	20.7±2.8	7.3±0.8	10.1±1.2
Unchanged Drug			
Cmax ng-eq/ml	20.5±8.0	9.7±4.1	10.4±2.8
Tmax h	0.7±0.3	0.5±0.0	0.7±0.3
$T_{1/2}$	4.3±2.6	3.7±0.5	2.6±0.3
$AUC_{0-\infty}$	74.2±36.0	30.4±11.2	36.2±6.8

Protein Binding

N80722 The plasma protein binding and distribution in blood of rac-nebivolol and of its two enantiomers in rats, dogs and humans.

Human blood was collected from healthy volunteers. Blood was also used from Wistar rats and Beagles.
Tritiated racemic nebivolol, ^{3}H-d-nebivolol, ^{3}H-l-nebivolol was mixed with unlabelled rac, d- or l-nebivolol to achieve desired concentrations. To examine the concentration dependence of plasma protein binding, ^{3}H-d-nebivolol and ^{3}H-l-nebivolol in ethanol were prepared at 10, 5, 1.25, 0.25, 0.05 and 0.02 µg/ml for human plasma and 50, 10, 1, 0.1µg/ml for rat and dog plasma. For concentrations above 0.1 µg/ml the radio-labeled drugs were diluted with the appropriate unradio-labeled drug. In another experiment, pH dependence was examined in human plasma.

Ghost cells(RBC without Hb) were prepared from human, dog and rat samples. Radio-labeled enantiomers were added at 1 ng/ml.

To study the possible interaction between the two enantiomers solutions of the tritiated enantiomers were prepared. Five µl of a solution of a radio-labeled enantiomer and 5 µl of a solution of the unlabelled other enantiomer were added to 1 ml of blank plasma or blood.

Plasma from 5 humans, 5 pooled rat samples and 5 beagles was fortified with 3H-d-nebivolol at 1 ng/ml and a "fraction of these samples " unlabeled l-nebivolol was added at 1 ng/ml. Albumin concentrations were determined with a colorimetric method, α1-acid glycoprotein concentrations with an immunoturbidimetric method. Purified proteins of albumin, α1-acid glycoprotein, α-globulin, α1-globulin, β-globulin and γ-globulin.

Results

Protein binding of the enantiomers was graphically presented as being pH dependent over the tested range of pH= 6.8 to 8.2. The percent bound drug changed from 93.5%(l-neb) and 94.5%(d-neb) at pH 6.6 to 99% for both enantiomers at pH 8.2.

The greatest difference in protein binding of the enantiomers was seen in the rat. The d-neb showed ~2% greater protein binding than l-neb. The sponsor's presentation is shown below. The percentage of bound drug appeared to be independent of concentration over the ranges tested.

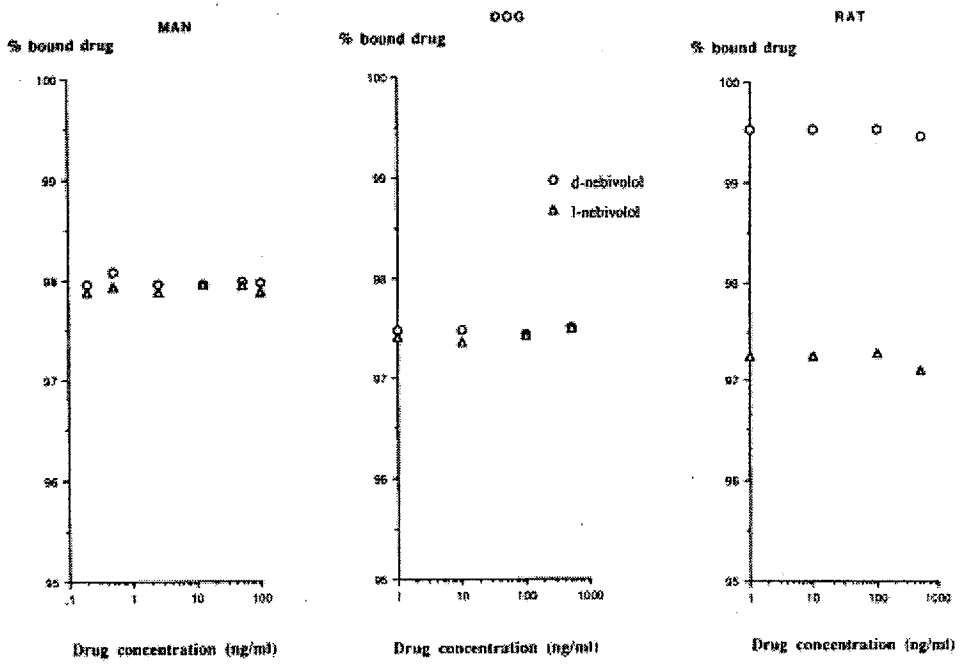


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Table 3: Plasma protein binding of total radioactivity (TR = unchanged nebivolol + metabolites) in male subjects, male and female rats and male dogs after single oral administration of ^{14}C-nebivolol or its ^{14}C-labelled enantiomers.

Species	Dose	Sample	% bound radioactivity*
Man (m)	15 mg ^{14}C-R067555 (R067555/FK1006)	1 - 4 h (EM)	88.5
		1 - 4 h (PM)	96.4
Rat (f)	2.5 mg ^{14}C-R067555/kg (R067555/FK1021)	1 h	92.9
		4 h	89.3
		8 h	88.6
Rat (m)	2.5 mg ^{14}C-R067555/kg (R067555/FK1021)	1 h	86.0
		4 h	79.9
		8 h	78.6
Rat (m)	1.25 mg ^{14}C-R067138/kg (R067555/FK1067)	1 h	92.3
		4 h	85.9
		8 h	81.4
Rat (m)	1.25 mg ^{14}C-R067145/kg (R067555/FK1067)	1 h	87.7
		4 h	74.5
		8 h	74.1
Dog (m)	2.5 mg ^{14}C-R067555/kg (R067555/FK1127)	1 h	84.3
		4 h	89.1
		24 h	88.9
	1.25 mg ^{14}C-R067138/kg (R067555/FK1127)	1 h	79.8
		4 h	87.7
		24 h	87.8
	1.25 mg ^{14}C-R067145/kg (R067555/FK1127)	1 h	86.6
		4 h	87.7
		24 h	88.8

* For humans, individual data for an extensive (EM) and a poor metabolizer (PM) of debrisoquine (pool of the 1-h and 4-h plasma); for rats and dogs, data for pools.

The data for the range of drug concentrations tested was not shown.

Protein binding for plasma samples with 1 ng/ml drug (human samples) and 50 ng/ml (rat and dog samples) showed protein binding $\geq$97% across species. However, plasma protein binding of total radioactivity (unchanged nebivolol and metabolites) after a single oral dose of ^{14}C-nebivolol showed protein binding of 88% and 96% in human samples. Rat samples showed 1 hour post dose values of 86-92% protein binding while dogs showed values of 80% to 86%. The difference in protein binding values between in vitro and in vivo was not discussed.

Addition of α1-acid glycoprotein to human plasma changed the protein binding of rac-neb from 97.5±0.2 in unfortified plasma to 97.96±0.12%. To the reviewer, this does not seem to be an impressive change. The sponsor, on the other hand, found this highly significant ($p<0.0005$).

The binding curves for nebivolol (racemate and enantiomers) and the purified proteins was shown. The highest percent of bound drug was shown with human serum albumin. The concentration of the albumin used (0.1, 0.25, 0.5, 1.0, 2.0, 4.3, 6.0 w/v %) was higher than the concentrations used for the other proteins (0.25-2.0% for α globulins, 0.05-1.0% for α1-globulins). Perhaps this was done to reflect differences in plasma concentrations.

l-nebivolol and to 98.85 % for *rac*-nebivolol. The lower binding of *rac*-nebivolol compared with that of its enantiomers was most probably due to its lower radiochemical purity. As determined by radio-HPLC, the radiochemical purity of ^{3}H-*rac*-nebivolol in the stock solution (2.3.) was about 1 % lower than the radiochemical purity of the enantiomers. The binding to α_1-acid glycoprotein at 0.07 % (w/v, physiological concentration) amounted up to 69.73 % for *rac*-nebivolol, 74.17 % for d-nebivolol and 71.53 % for l-nebivolol (Figure 5).

Nevertheless, the binding of *rac*-nebivolol to human plasma fortified with α_1-acid glycoprotein to a final concentration of 0.2 % (w/v) was significantly increased compared with its binding in untreated human plasma (97.96 ± 0.12 % *versus* 97.50 ± 0.20 %, $n = 5$, $0.0001 < p < 0.0005$).

The sponsor concludes that the plasma protein binding of both enantiomers as well as their distribution in the blood was stereoselective in all three species examined. Differences between the two enantiomers were larger in the rat than in the dog and human.

N84300 An in vitro study on protein binding interactions of rac-nebivolol with other drugs in human plasma. 1990-1993

The following drugs were studied for their influence on the protein binding of ^{3}H-rac-nebivolol:warfarin, diazepam, tolbutamide,indomethacin, imipramine, sulfamethazine, digitoxin, diphenylhydantoin, hydrochlorthiazide, L-propranolol and enalapril. ^{3}H-rac-nebivolol(14.7 Ci/mmole), ^{14}C-hydrochlorthiazide, ^{14}C-warfarin sodium,^{14}C-diphenylhydantoin, ^{3}H-digoxin, ^{3}H-imipramine, ^{3}H-diazepam, ^{3}H-propranolol were used. Unlabelled rac-nebivolol was used to study its influence on the plasma protein binding of the other drugs. Human blood was obtained from volunteers.

In the first series of experiments, ^{3}H-nebibvolol was added to plasma from 5 humans to give a final concentration of 1 ng/ml. Ethanolic solutions of the various unlabelled other drugs were added to 1 ml aliquots of the plasma + ^{3}H-nebivolol. The final plasma concentrations of the added drugs was said to correspond to therapeutic plasma levels. The samples were then subjected to equilibrium dialysis. Samples were run in duplicate.

In a second series of experiments, the influence of nebivolol on the plasma protein binding of imiprimine (binds to α1-acid glycoprotein), diphenylhydantoin, warfarin, digitoxin, propranolol, hydrochlorthiazide and diazepam was studied. Aliquots of the radio-labeled drugs were added to

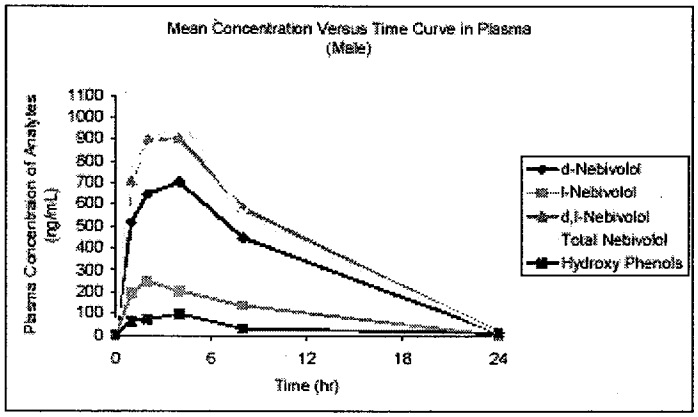
plasma to obtain concentrations corresponding to normal therapeutic levels. Unlabelled rac-nebivolol was added at a final concentration of 25ng/ml. Samples were subjected to equilibrium dialysis. Samples were run in duplicate.

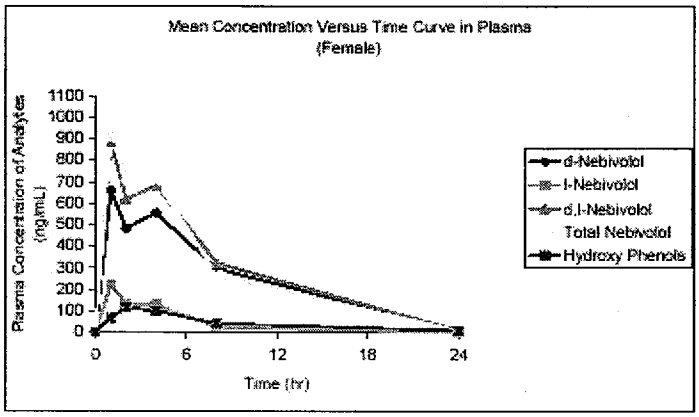
The fraction of unbound drug was calculated as the ratio of the unbound concentration to the total concentration.

Results
The protein binding of rac-nebivolol was approximately 97%, consistent with other studies. Diphenylhydantoin, sulfamethazine, indomethacin, warfarin, propranolol, hydrochlorthiazide, digitoxin and enalapril were reported to have no effect on the protein binding of nebivolol. Imipramine, diazepam and tolbutamide altered protein binding under the conditions used. In the presence of imipramine and diazepam, the free fraction of rac-nebivolol was increased by 2.8% ($p<0.01$) and 4.6% ($p<0.05$)respectively. In the presence of tolbutamide, the free fraction of nebivolol was increased by 9.4% ($p<0.05$). Significance was determined by a two-tailed Students t-test.

As nebivolol is highly protein bound, an increase in the free fraction of 9.4% could have significant clinical consequences.

3.3.7 Pharmacokinetics The sponsor conducted more recent PK studies reviewed below.





TNEB-I0307 Pharmacokinetics of d- and l- nebivolol and drug-related metabolites in the mouse following a single oral administration of nebivolol HCl (20 mg/kg). 2003

Adult male and female SPF albino Swiss mice (4/sex/timepoint)were given single oral doses of 20 mg/kg nebivolol. Blood was collected pre-dose, 1,2,4,8 and 24 hours after dosing. Samples were analyzed with LC/MS/MS to determine concentrations of the enantiomers and hydroxyl phenols. Samples were also incubated with β-glucuronidase for determination of total deconjugated nebivolol.

Results
The concentration of l-nebivolol seems to decrease somewhat faster than d-nebivolol. The sponsor's summary of PK parameters is shown below.

Pharmacokinetic Parameters of *d*-, *l*-, *d,l*-, and Total Nebivolol, and Hydroxy Phenols in Mouse Plasma

PK Parameters	Male Mouse				
	d-Nebivolol	*l*-Nebivolol	*d, l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	4	2	4	4	4
C_{max} (ng/mL)	703	248	906	1001	98.2
AUC_{0-24} (hr*ng/mL)	8176	2524	10700	10705	854
AUC_{0-inf} (hr*ng/mL)	8240	2524	10760	10758	989
$T_{1/2}$ (hr)	3.32	1.66	3.11	3.02	8.66
K_{el} (1/hr)	0.209	0.418	0.223	0.230	0.080
AUC_{0-inf} Ratio	0.77	0.23	1	1	0.09
PK Parameters	Female Mouse				
	d-Nebivolol	*l*-Nebivolol	*d, l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	1	1	1	1	2
C_{max} (ng/mL)	660	224	884	906	116
AUC_{0-24} (hr*ng/mL)	6110	1082	7185	6880	1055
AUC_{0-inf} (hr*ng/mL)	6162	1083	7237	6920	1149
$T_{1/2}$ (hr)	3.41	1.79	3.30	3.20	6.59
K_{el} (1/hr)	0.203	0.387	0.210	0.217	0.105
AUC_{0-inf} Ratio	0.85	0.15	1	0.96	0.16

AUC_{0-inf} ratio is relative to *d,l*-Nebivolol.
Total Nebivolol includes non-conjugated and conjugated Nebivolol.

Total deconjugated and conjugated nebivolol (total nebivolol) was approximately the same as the sum of d- and l-nebivolol, suggesting very little conjugation. The sponsor notes that d-nebivolol, l-nebivolol and total nebivolol were higher in males while hydroxyl phenols were higher in females. The standard deviations for the parameters are not provided. Given the expected level of variability in pharmacokinetic studies, the sponsor's suggestion of a sex-related difference is not entirely persuasive.

TNEBI-0309 Pharmacokinetics of d- and l-Nebivolol and drug-related metabolites in the mouse following a 14-day feeding study of nebivolol HCl at a target dose of 10 mg base-eq/kg/day.2003

Adult male and female SPF albino Swiss mice were fed a diet containing nebivolol at a level to provide 10 mg/kg/day. Blood was collected from 4 mice/sex/timepoint pre-dose (prior to start of the light cycle day 14) and 0.75, 2,4,6,8,10 and 12 hours after the start of the light cycle(group I) and 0.75, 2,4,6,8,10 and 12 hours after the start of the dark cycle (group II). Plasma samples were analyzed by LC/MS/MS to determine concentrations of the enantiomers as well as hydroxy

phenols. Plasma samples were also incubated with β-glucuronidase to determine total deconjugated nebivolol. The sum of d- and l-nebivolol was designated d,l-nebivolol. Total nebivolol refers to total de-conjugated and conjugated nebivolol. D,l-nebivolol was the sum of d- and l-nebivolol.

Results

There were several times when analysis showed that the concentration of nebivolol in the feed was outside the ±10% range of the target concentration.

Nebivolol Concentration in Mouse Feed

Batch No.	Assay time	Nominal concentration (mg/g)	Mean Assay concentration (mg/g)	% of Target
1	Pre-dose	0.073	0.072	98.2
	Post-dose		0.060	82.2
2	Pre-dose	0.058	0.056	96.6
	Post-dose		0.049	84.5
3	Pre-dose	0.070	0.061	87.1
	Post-dose		0.059	84.3

All Nebivolol concentrations were base-eq.

The calculated dose received was slightly greater during the dark cycle compared to the light cycle. Since more feeding occurs in the dark cycle, one would expect a greater difference.

	Light cycle mg/kg/day	Dark cycle mg/kg/day
Males	9.81±0.42	10.8±0.14
Females	10.1±0.53	12.3±0.40

The sponsor's summary of PK parameters is shown below.

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Day 14 Male Mice					
PK Parameters	*d*-Nebivolol	*l*-Nebivolol	*d,l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	18	18	18	20	2
C_{max} (ng/mL)	167	35.8	203	187	21.7
Css (ng/mL)	97.4	15.8	113	104	14.3
AUC_{0-24} (hr*ng/mL)	2337	380	2721	2507	344
AUC_{0-24} Ratio	0.86	0.14	1	0.92	0.13
Day 14 Female Mice					
PK Parameters	*d*-Nebivolol	*l*-Nebivolol	*d,l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	20	24	24	20	6
C_{max} (ng/mL)	139	22.7	162	148	20.8
Css (ng/mL)	76.3	10.9	87.3	78.9	15.5
AUC_{0-24} (hr*ng/mL)	1830	262	2095	1894	371
AUC_{0-24} Ratio	0.87	0.13	1	0.90	0.18
AUC_{0-24} Male/Female	1.28	1.45	1.30	1.32	0.93

AUC_{0-24} (hr*ng/mL) = AUC_{0-12} light cycle + AUC_{0-12} dark cycle
Css (ng/mL) = AUC_{0-24}/24
C_{max} was the highest concentration in the 24 hr period, T_{max} was the time associated with the highest concentration in the 24 hr period.

There do not appear to be substantive differences between the male and female profiles. The AUC_{0-24} values for hydroxyl phenols do not differ substantially between males and females. The AUC_{0-24} ratio reflects the light and dark cycle ratios. Values in the dark cycle were slightly higher for both sexes, not unexpected since dark cycle is when most feeding occurs. The AUC for total nebivolol was 25% higher for males than females. Is this a true metabolic difference or due to higher food consumption on the part of the males? There were substantial differences between light and dark cycle plasma values.

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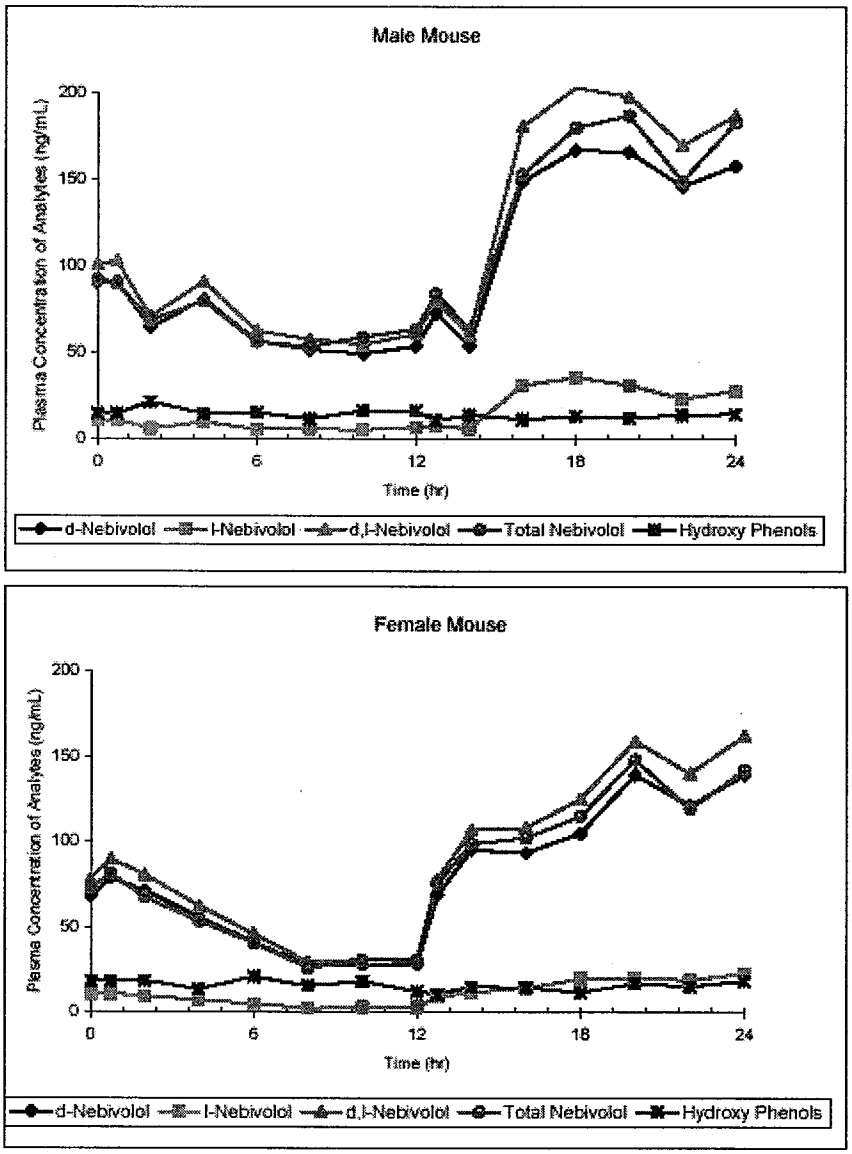
Mean PK parameters of Nebivolol and Metabolites in Male Mice Plasma					
	Light Cycle				
PK Parameters	*d*-Nebivolol	*l*-Nebivolol	*d,l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	0.75	0.75	0.75	0	2
C_{max} (ng/mL)	91.1	11.4	103	92.2	21.7
AUC_{0-12} (hr*ng/mL)	762	88.0	851	798	191
	Dark Cycle				
PK Parameters	*d*-Nebivolol	*l*-Nebivolol	*d,l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	18	18	18	20	24
C_{max} (ng/mL)	167	35.8	203	187	14.8
AUC_{0-12} (hr*ng/mL)	1575	292	1870	1709	153
Mean PK parameters of Nebivolol and Metabolites in Female Mice Plasma					
	Light Cycle				
PK Parameters	*d*-Nebivolol	*l*-Nebivolol	*d,l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	0.75	0.75	0.75	0.75	6
C_{max} (ng/mL)	78.9	10.8	89.8	81.2	20.8
AUC_{0-12} (hr*ng/mL)	551	65.9	617	548	203
	Dark Cycle				
PK Parameters	*d*-Nebivolol	*l*-Nebivolol	*d,l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	20	24	24	20	24
C_{max} (ng/mL)	139	22.7	162	148	18.3
AUC_{0-12} (hr*ng/mL)	1279	196	1478	1346	168

Mean Plasma Concentration (Linear Scale) of *d-, l-, d,l-* and Total Nebivolol and Hydroxy Phenols Versus Time Curve in Male and Female Mouse



The data indicates that the enantiomers are the predominant species in the blood with little conjugated material. The hydroxyl phenols constitute 14%(males) and 20%(females) of the AUC_{0-24}.

TNEBI0306 Pharmacokinetics of d- and l-nebivolol and drug-related metabolites in the rat following a single oral administration of nebivolol hydrochloride (20 mg/kg). 2003)
Adult Wistar rats (3/sex/timepoint) were given single oral doses of nebivolol formulated in carboxymethylcellulose sodium salt. Blood was collected pre-dose and 1,2,4,8 and 24 hours after dosing. Plasma samples were analyzed by LC/MS/MS to determine concentrations of d- and l-nebivolol as well as hydroxy-phenols. Samples were also incubated with β-glucuronidase and total deconjugated nebivolol concentration was determined. D,l-nebivolol was the sum of d- and l-nebivolol.

Results

The sponsor's Pk summary is shown below. Tmax for l-nebivolol and the hydroxyl phenols was reached at 2 hours in the males and at 1 hour for the other parameters. In females, Tmax was reached at 2 hours for all parameters except the hydroxyphenols which reached Tmax at 4 hours. Cmax and AUC for the enantiomers, racemic mixture and total nebivolol was slightly greater in females than in males, opposite to what was seen in the mice. The AUC_{0-24} was 51% higher in females than in males. $T_{1/2}$ was also slightly greater for the females than the males.

PK Parameters	Male Rat				
	d-Nebivolol	*l*-Nebivolol	*d, l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	1	2	1	1	2
C_{max} (ng/mL)	348	182	502	562	225
AUC_{0-24} (hr*ng/mL)	1530	858	2389	2595	1767
AUC_{0-inf} (hr*ng/mL)	1532	859	2392	2598	1941
$T_{1/2}$ (hr)	2.46	2.71	2.56	2.44	6.96
K_{el} (1/hr)	0.282	0.256	0.271	0.284	0.100
AUC_{0-inf} Ratio	0.64	0.36	1	1.09	0.81
PK Parameters	Female Rat				
	d-Nebivolol	*l*-Nebivolol	*d, l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	2	2	2	2	4
C_{max} (ng/mL)	494	219	714	816	208
AUC_{0-24} (hr*ng/mL)	3301	1614	4911	5312	1760
AUC_{0-inf} (hr*ng/mL)	3309	1638	4941	5340	1929
$T_{1/2}$ (hr)	2.77	3.98	3.26	3.19	7.05
K_{el} (1/hr)	0.250	0.174	0.213	0.217	0.098
AUC_{0-inf} Ratio	0.67	0.33	1	1.08	0.39

AUC_{0-inf} ratio is relative to *d,l*-Nebivolol.
Total Nebivolol includes non-conjugated and conjugated Nebivolol.

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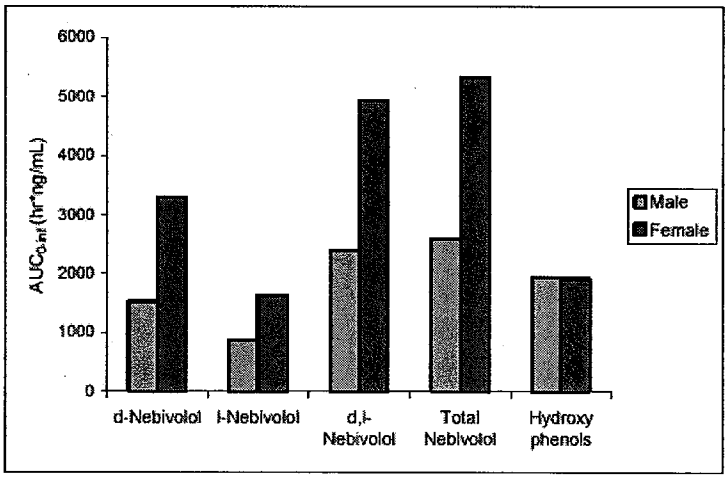
AUC_{0-inf} of *d*-, *l*-, *d,l*-, and Total Nebivolol, and Hydroxy Phenols in Male and Female Rats



Within either sex there was little difference between total nebivolol and d,l nebivolol. Of all parameters measured, there was no difference between the exposure to hydroxyphenols in males and females.

TNEBI 0308 Pharmacokinetics of d- and l- nebivolol and drug-related metabolites in the rat following a 14-day feeding study of nebivolol HCl at a target dose of 10 mg base.eq/kg/day.2003

Adult Wistar rats, 4rats/sex/timepoint were fed with a rodent diet containing Nebivolol at a concentration to provide a target dose of 10 mg base-eq/kg/day. Rats were euthanized pre-dose(prior to the beginning of the light cycle on Day 14) and 0.75, 2,4,6,8,10 and 12 hours after the start of the light cycle (Group I). Group II was euthanized at 0.75, 2,4,6,8, 10 and 12 hours after the start of the dark cycle. Plasma samples were analyzed with LC/MS/MS to determine concentrations of the enantiomers and hydroxyl phenols. Plasma samples were also treated with β-glucuronidase for determination of total nebivolol.

Results

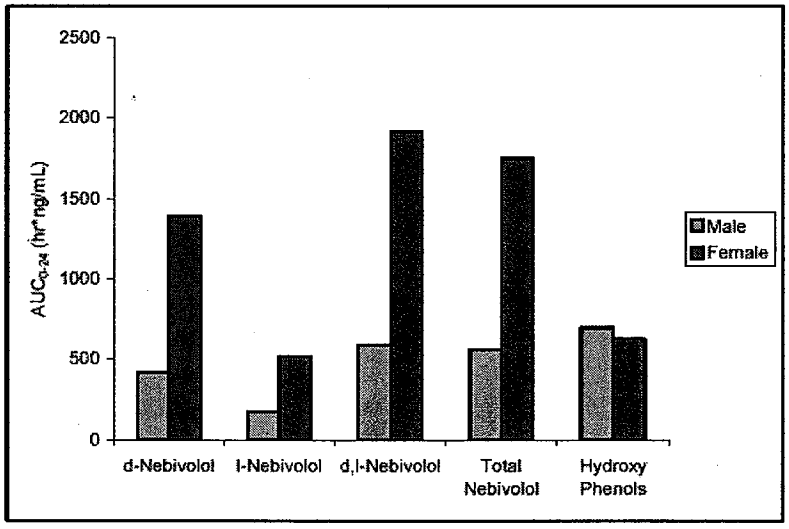
Tmax was achieved at 18 hours in the males and at 24 hours in females. Cmax in the females was approximately 3X greater for all categories except the hydroxyl phenols than in males. AUC_{0-24} was approximately 3x greater in the females than in the males for d,l-nebivolol and total nebivolol.

The sponsor's summary of results is shown below.

Day 14 Male Rat					
PK Parameters	*d*-Nebivolol	*l*-Nebivolol	*d,l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	18	18	18	18	20
C_{max} (ng/mL)	30.4	14	44.4	40.2	41.2
Css (ng/mL)	17.3	7.46	24.7	23.5	29.0
AUC_{0-24} (hr*ng/mL)	415	179	592	563	696
AUC_{0-24} Ratio	0.70	0.30	1	0.95	1.18
Day 14 Female Rat					
PK Parameters	*d*-Nebivolol	*l*-Nebivolol	*d,l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	24	24	24	24	16
C_{max} (ng/mL)	96.1	39	135	124	35
Css (ng/mL)	58.0	21.8	79.8	72.9	26.1
AUC_{0-24} (hr*ng/mL)	1393	522	1914	1750	627
AUC_{0-24} Ratio	0.73	0.27	1	0.91	0.33
AUC_{0-24} Male/Female	0.30	0.34	0.31	0.32	1.11

AUC_{0-24} (hr*ng/mL) = AUC_{0-12} light cycle + AUC_{0-12} dark cycle
Css (ng/mL) = AUC_{0-24}/24
C_{max} in the 24 hr period were the highest concentration, T_{max} was the time associated with the highest concentration in the 24 hr period.
For light and dark cycle data see Appendix F.
AUC_{0-24} Ratio was relative to *d,l*-Nebivolol.

AUC_{0-24} of *d*-, *l*-, *d,l*- and Total Nebivolol and Hydroxy Phenols in Rat Plasma



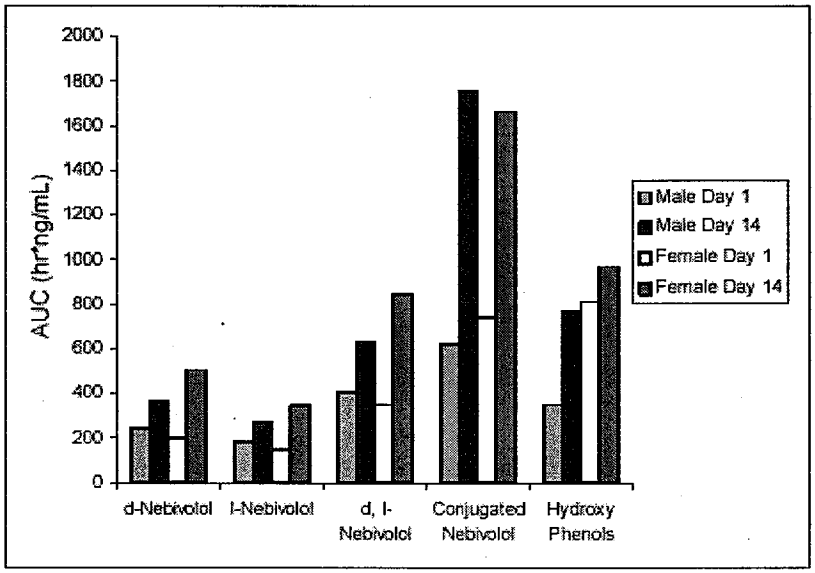
Consistent with the oral gavage study, there was little difference in the detected plasma levels of hydroxyl phenols between males and females.
When examined on day 14, there was little difference in the mean dose achieved in either males or females in the light cycle vs the dark cycle.

Day 14 Male Rat Light Cycle					
PK Parameters	*d*-Nebivolol	*l*-Nebivolol	*d,l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	0	0	0	0	0.75
C_{max} (ng/mL)	22.1	9.57	31.7	29.2	38.3
AUC_{0-12} (hr*ng/mL)	147	68.6	215	204	280
Day 14 Male Rat Dark Cycle					
PK Parameters	*d*-Nebivolol	*l*-Nebivolol	*d,l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	18	18	18	18	20
C_{max} (ng/mL)	30.4	14	44.4	40.2	41.2
AUC_{0-12} (hr*ng/mL)	268	110	377	359	416
Day 14 Female Rat Light Cycle					
PK Parameters	*d*-Nebivolol	*l*-Nebivolol	*d,l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	0.75	0.75	0.75	0.75	2
C_{max} (ng/mL)	80.5	34.6	115	100	32.7
AUC_{0-12} (hr*ng/mL)	609	242	851	755	291
Day 14 Female Rat Dark Cycle					
PK Parameters	*d*-Nebivolol	*l*-Nebivolol	*d,l*-Nebivolol	Total Nebivolol	Hydroxy Phenols
T_{max} (hr)	24	24	24	24	16
C_{max} (ng/mL)	96.1	39	135	124	35.0
AUC_{0-12} (hr*ng/mL)	784	280	1063	995	336

TNEBI0310 Pharmacokinetics of d- and l-nebivolol and drug-related metabolites in the dog following oral administration of nebivolol HCl (10mg/kg/day)2003

Adult Beagles, 3/sex,were given daily oral doses of nebivolol in gelatin capsules. Blood was collected with sodium heparin pre-dose and at 2,4, 8 and 24 hours after dosing on Day 1, at pre-dose and 1 hr post dose days 12 and 13 and at pre-dose and 0.5, 1,2,3,4,5,8,12,15 and 24 hours post-dose on Day 14. Plasma samples were analyzed by LC/MS/MS to determine the

Comparison of AUC for *d*-, *l*-, *d,l*-, and Conjugated Nebivolol and Hydroxy Phenols in Male and Female Dog Plasma



Day 1: AUC_{0-inf}
Day 14: AUC_{0-24}

concentrations of the enantiomers and hydroxy phenols. Plasma samples were also incubated with β-glucuronidase to determine total deconjugated nebivolol.

Results

In both males and females significant amounts of conjugated nebivolol were found. Accumulation of drug from day 1 to day 14 was also apparent. There was an increase in hydroxyl phenols from day 1 to day 14 in both sexes. The increase was greater in males than in females. The AUC_{0-24} in males increased by 3.6x in males from day 1 to 14 compared to an increase of 3X in females. The conjugated nebivolol was 78% of total nebivolol AUC_{0-24} in males on Day 1and 84% on Day 14. The sponsor's summary of PK is shown below.

Summary of Mean (SD) PK Parameters of *d*-, *l*-, *d,l*-, Total and Conjugated Nebivolol and Hydroxy Phenols in Male Dog Plasma (n=3)

Mean PK Parameters	Day 1 Male Dogs					
	d-Nebivolol	*l*-Nebivolol	*d, l*-Nebivolol	Total Nebivolol	Conjugated Nebivolol	Hydroxy Phenols
T_{max} (hr)	2 (0)	2 (0)	2 (0)	2 (0)	2 (0)	9.33 (12.7)
C_{max} (ng/mL)	13.9 (10.9)	10.8 (4.83)	24.7 (15.7)	217 (230)	192 (214)	17.2 (3.48)
AUC_{0-24} (hr*ng/mL)	94.8 (60.0)	75.9 (23.6)	170 (83.3)	768 (727)	597 (646)	212 (44.2)
AUC_{0-inf} (hr*ng/mL)	113 (NC)	81.7 (NC)	195 (NC)	986 (NC)	793 (NC)	346 (NC)
$T_{1/2}$ (hr)	4.23 (NC)	5.25 (NC)	4.69 (NC)	4.44 (NC)	4.06 (NC)	17.8 (NC)
K_{el} (1/hr)	0.174 (NC)	0.137 (NC)	0.155 (NC)	0.159 (NC)	0.171 (NC)	0.040 (NC)
AUC_{0-inf} Ratio	0.58	0.42	1	5.06	4.07	1.77
Mean PK Parameters	Day 14 Male Dogs					
	d-Nebivolol	*l*-Nebivolol	*d, l*-Nebivolol	Total Nebivolol	Conjugated Nebivolol	Hydroxy Phenols
T_{max} (hr)	2 (0)	2 (0)	2 (0)	2 (0)	2 (0)	2.33 (0.58)
C_{max} (ng/mL)	70.7 (32.3)	48.6 (11.1)	119 (43.2)	726 (322)	607 (279)	73.0 (6.89)
AUC_{0-24} (hr*ng/mL)	360 (199)	272 (111)	633 (310)	2390 (1336)	1758 (1041)	769 (135)
$T_{1/2}$ (hr)	3.95 (1.02)	4.51 (1.73)	4.13 (1.33)	3.75 (0.36)	3.39 (1.28)	13.0 (3.41)
K_{el} (1/hr)	0.183 (0.04)	0.168 (0.05)	0.179 (0.05)	0.186 (0.02)	0.225 (0.08)	0.056 (0.02)
AUC_{0-24} Ratio	0.57	0.43	1	3.78	2.78	1.21

Total Nebivolol includes non-conjugated and conjugated Nebivolols.
AUC_{0-24} or AUC_{0-inf} ratios were calculated relative to *d,l*-Nebivolol
NC: not calculated (n=2)

Conjugated nebivolol in females was 69% of total AUC0-24 on day 1 and 66% on day 14.

Mean PK Parameters	Day 1 Female Dogs					
	d-Nebivolol	*l*-Nebivolol	*d, l*-Nebivolol	Total Nebivolol	Conjugated Nebivolol	Hydroxy Phenols
T_{max} (hr)	2 (0)	2.67 (1.15)	2 (0)	2 (0)	2 (0)	6 (3.46)
C_{max} (ng/mL)	16.6 (7.35)	11.1 (4.42)	27.7 (11.8)	249 (56.9)	222 (49.1)	19.4 (9.12)
AUC_{0-24} (hr*ng/mL)	192 (88.7)	144 (62.0)	336 (151)	1068 (224)	733 (165)	328 (146)
AUC_{0-inf} (hr*ng/mL)	198 (90.3)	150 (64.1)	349 (155)	1086 (229)	739 (168)	NC
$T_{1/2}$ (hr)	4.92 (1.05)	5.15 (1.14)	5.08 (1.16)	4.32 (0.77)	4.17 (0.63)	NC
K_{el} (1/hr)	0.146 (0.03)	0.14 (0.04)	0.142 (0.04)	0.164 (0.03)	0.169 (0.03)	NC
AUC_{0-inf} Ratio	0.57	0.43	1	3.11	2.12	2.34
Mean PK Parameters	Day 14 Female Dogs					
	d-Nebivolol	*l*-Nebivolol	*d, l*-Nebivolol	Total Nebivolol	Conjugated Nebivolol	Hydroxy Phenols
T_{max} (hr)	4.5 (6.50)	4.5 (6.50)	4.5 (6.50)	5 (6.08)	5 (6.08)	5 (6.08)
C_{max} (ng/mL)	86.5 (53.5)	57.9 (35.1)	145 (88.3)	601 (253)	481 (201)	76.3 (10.7)
AUC_{0-24} (hr*ng/mL)	501 (123)	342 (68.1)	844 (191)	2507 (528)	1663 (493)	969 (271)
$T_{1/2}$ (hr)	4.84 (2.99)	5.90 (4.26)	5.26 (3.49)	5.87 (4.81)	9.47 (10.7)	10.8 (1.64)
K_{el} (1/hr)	0.176 (0.08)	0.156 (0.08)	0.168 (0.08)	0.172 (0.10)	0.163 (0.13)	0.066 (0.01)
AUC_{0-24} Ratio	0.59	0.41	1	2.97	1.97	1.15

Total Nebivolol includes non-conjugated and conjugated Nebivolols.

AUC_{0-24} or AUC_{0-inf} ratios were calculated relative to *d,l*-Nebivolol

NC: not calculated (n=1).

N109083 Comparative toxicokinetics of rac-, d- and l- nebivolol in the beagle dog in a one-month subchronic oral pilot toxicity study (Exp No. 2671) with R067555, R085547 and R085548 administered as a mixture with β-cyclodextrin at 80 mg(base-eq.)/kg/day(R067555, rac-nebivolol) or 40 mg (base-eq.)/kg/day (R085547, d-nebivolol and R085548, l-nebivolol). Tissue distribution at the end of the study. 1992-1994.

The toxicokinetics of rac-, d- and l- nebivolol were studied in Beagles in a one-month oral pilot toxicity study with each of the compounds given formulated as a co-precipitate with β-CD in gelatin capsules at 80 mg/kg/day (rac-nebivolol) or 40 mg/kg/day for each of the enantiomers. Tissue distribution was studied at necropsy (24 hours after the last dose). Details of the study can

be found in the specific review. Blood samples were collected at pre-dose, 1,2,4,8 and 24 hours after the first and last dose administration. Tissue samples collected for analysis were liver, brain, lung, heart, muscle, kidney, fat, pancreas, adrenal gland and spleen. Plasma and tissue were analyzed for unchanged drug
using an HPLC method. Concentrations of the enantiomers and the hydroxylated metabolites were determined by enantio-selective RIAs.

Results
The most striking results were the accumulation of drug in the tissues examined.

Table 3: Mean (n=2) tissue concentrations[1] (ng/g) and tissue to plasma concentration ratios of *rac*-, *d*- or *l*-nebivolol in the beagle dog at 24 h after the last dose administration of *rac*-nebivolol or its enantiomers as a mixture with ß-cyclodextrin in a one-month subchronic oral pilot toxicity experiment (Exp. No. 2671) of *rac*-nebivolol at 80 mg(base-eq.)/kg/day, and of R085547 (*d*-nebivolol) or R085548 (*l*-nebivolol) at 40 mg(base-eq.)/kg/day.

Tissue concentration (ng/g)	80 mg(base-eq.)/kg/day *rac*-nebivolol	40 mg(base-eq.)/kg/day *l*-nebivolol	40 mg(base-eq.)/kg/day *d*-nebivolol
Plasma	22	≤ 10	14
Fat	119	29.3	35.3
Heart	174	72.7	56.8
Muscle	221	62.6	39.1
Spleen	295	143	66.1
Kidney	360	150	96
Brain	461	174	131
Adrenal gland	624	308	210
Liver	189 [2]	125	40.8
Pancreas	897	332	329
Lung	3125	1256	828
T/P ratio			
Fat	5.16	- [3]	2.58
Heart	8.22	-	4.02
Muscle	10.3	-	2.85
Spleen	14.4	-	4.78
Kidney	16.5	-	7.12
Brain	21.1	-	9.89
Adrenal gland	28.8	-	15.7
Liver	7.26 [2]	-	3.09
Pancreas	44.0	-	22.7
Lung	143	-	62.8

[1] Tissue concentrations are expressed as base-equivalents.

[2] n=1; see Table A5.

[3] Parameter not determined; see Table A5.

The greatest accumulation was seen in the lung followed by pancreas and then the adrenal gland. There was a great amount of variability in the pharmacokinetic parameters as shown below. There were lower concentrations of l-nebivolol compared to d-nebivolol. The concentration of l-nebivolol decreased with repeated dosing.

Table 1: Mean (n=2) plasma concentrations[1] (ng/ml) and some pharmacokinetic parameters of *rac*-, *d*- and *l*-nebivolol in the beagle dog in a one-month subchronic pilot toxicity experiment (Exp. No. 2671) with oral administration of *rac*-nebivolol at 80 mg(base-eq.)/kg/day, and of R085547 (*d*-nebivolol) or R085548 (*l*-nebivolol) at 40 mg(base-eq.)/kg/day as mixtures with ß-cyclodextrin.

	80 mg(base-eq.)/kg/day *rac*-nebivolol				40 mg(base-eq.)/kg/day *d*-nebivolol		40 mg(base-eq.)/kg/day *l*-nebivolol	
	HPLC							
Time (h)	Single		Repeated		Single	Repeated	Single	Repeated
0	≤ 10		33		≤ 10	16 [2]	≤ 10	14
1	291		526		156	309	90	89
2	384		1047		478	624	223	108
4	441		596		366	505	169	91
8	293		338		135	187	54	44
24	13 [2]		22		≤ 10	14	≤ 10	≤ 10
C_{max} (ng/ml)	577		1047		543	624	223	110
T_{max} (h)	2.5		2.0		3.0	2.0	2.0	1.5
$t_{1/2}$ (h)	3.4		4.2		2.7	4.0	2.4	3.8
$AUC_{0-\infty}$ (ng.h/ml)	5044		7450 [3]		2782	4742 [3]	1230	616 [3]
	RIA after extraction							
	d	*l*	*d*	*l*	*d* [4]	*d* [4]	*l* [4]	*l* [4]
0	≤ 5.0	≤ 5.0	39.3	23.0	≤ 5.0	36.3	≤ 5.0	15.2
1	198	146	440	310	166	411	103	102
2	307	197	856	481	536	915	275	136
4	342	195	564	285	462	637	216	126
8	257	135	334	142	199	307	78.5	52.7
24	14.9	11.0	29.5	14.8	10.7	35.1	6.3	7.4
C_{max} (ng/ml)	412	257	865	481	613	915	275	136
T_{max} (h)	2.5	2.5	3	2	3	2	2	2
$t_{1/2}$ (h)	3.9	3.8	4.6	4.9	3.9	5.3	3.3	5.7
$AUC_{0-\infty}$ (ng.h/ml)	4455	2411	7011 [3]	3430 [3]	4486	7058 [3]	1860	1274 [3]

1) Plasma concentrations are expressed as base-equivalents.
2) Median value.
3) $AUC_{0\text{-}24\ h}$.
4) Plasma concentrations of the opposite enantiomer are not given because they could be explained exclusively to the cross-reactivity of the other enantiomer and its metabolites.

N108425Toxicokinetics of nebivolol in the Beagle dog in a six-month chronic oral toxicity study (Exp. No. 1896) on a microcrystalline powder of nebivolol hydrochloride (R067555) at 20 or 80 mg(base-eq.)/kg/day. 1987-1994

In the present study, the toxicokinetics of nebivolol were investigated in the Beagle after single and chronic oral administration of a microcrystalline powder of nebivolol hydrochloride in gelatin capsules at 20 or 80 mg/kg/day. Blood and tissue samples were collected from the first two male and female dogs from each drug-treated group. Blood samples were collected pre-dose and at 0.5, 1,2,4,6,8 and 24 hours after dosing on day 1 and day 84. Additional blood samples were collected before dosing on days 3,4,8,15,29, 64 and 127 of the experiment. At necropsy, day 192, 24 hours after the last dose, blood and liver, kidney, lung, pancreas, brain, aorta, heart, muscle and fat were also collected. Plasma and tissue were analyzed for nebivolol using HPLC methods. Tissue concentrations were determined for the 20 mg/kg dosed animals only.

Results
The increase in plasma exposure was non-linear with increased dose, with less than the expected exposure. There was also a tendency to some accumulation with repeated dosing seen at both doses (2.3X at 20 mg/kg and 1.5X at 80 mg/kg).

Time (h) after administration		20 mg/kg/day			80 mg/kg/day		
Single administration							
0			≤ 0.20			0.655[1]	
0.5			1.13[1]			1.17[1]	
1		6.06	±	2.75	5.29	±	3.63
2		13.0	±	5.6	13.7	±	6.6
4		12.2	±	5.5	36.7	±	31.6
6		8.12	±	3.85	23.8	±	22.8
8		5.95	±	1.70	23.4	±	17.8
24			0.45[1]		8.06	±	11.8
C_{max}	(ng/ml)	14.6	±	4.9	38.1	±	30.4
T_{max}	(h)	3.0	±	1.2	8.5	±	10.4
AUC_{0-24h}	(ng.h/ml)	123	±	40	294[2]	±	144
$AUC_{0-\infty}$	(ng.h/ml)	126	±	38	468[2]	±	401
Repeated administration							
0		4.96	±	7.90	3.56	±	1.55
0.5		7.29	±	9.76	5.50	±	3.85
1		9.44	±	9.00	15.5	±	9.2
2		18.0	±	9.9	47.2	±	31.0
4		29.4	±	18.4	73.0	±	54.7
6		25.6	±	18.7	52.1	±	35.1
8		13.1	±	6.6	36.9	±	18.6
24		2.92	±	3.61	4.00	±	1.32
C_{max}	(ng/ml)	29.4	±	18.4	76.4	±	50.1
T_{max}	(h)	4.0	±	0.0	3.3	±	1.5
AUC_{0-24h}	(ng.h/ml)	290	±	159	700	±	377

[1] Median.
[2] n=3.

Again, consistent with the previous study, the most striking finding was the accumulation of drug in the lung.

Table 3: Mean ± S.D. (n=4) plasma and tissue levels (ng/ml or ng/g) of nebivolol in the beagle dog at autopsy, i.e. 24 h after the last dose administration, of a six-month chronic oral toxicity study on a microcrystalline powder of nebivolol hydrochloride, weighed in gelatin capsules, at **20 mg (base-eq.)/kg/day**. The corresponding tissue to plasma concentration ratios are given as well.

Plasma or tissue	Plasma or tissue levels (ng/ml or ng/g)			Ratio tissue/plasma		
plasma	3.85	±	1.74			
fat	48.8	±	21.9	13.4	±	5.4
muscle	17.3	±	6.98	5.05	±	2.09
heart	36.1	±	21.2	9.90	±	3.50
aorta	32.6	±	11.6	9.61	±	3.61
brain	77.0	±	43.0	20.1	±	2.5
pancreas	194	±	114	51.4	±	13.2
lung	652	±	407	168	±	50
kidney	98.2	±	75.1	27.6	±	18.0
liver	70.1	±	52.5	18.3	±	8.7

109347 Toxicokinetics of nebivolol in Sprague-Dawley rats on day 16 of pregnancy in a segment II embryotoxicity and teratogenicity study (Exp. No. 3005) on nebivolol hydrochloride (R067555) at 2.5, 10 or 40 mg(base.eq)/kg/day. 1993-1994

Nebivolol in HP-β-CD was given in daily oral doses of 0, 2.5, 10 or 40 mg/kg to pregnant Sprague-Dawley rats (n=6 per group) from GD6-GD16. On GD16, blood samples were collected from the dosed animals at 1, 2,4, 8 and 24 hours after dosing (3 animals per group per timepoint). Plasma samples were analyzed for nebivolol by HPLC methods.

Results
The sponsor's summary is shown below. The AUC values showed slightly greater than expected plasma levels with increased dose.

Table 2: Plasma levels (mean ± S.D.; n = 3) of nebivolol and the corresponding pharmacokinetic parameters in female Sprague-Dawley rats on day 16 of pregnancy after daily dosing, from day 6 to day 16 of pregnancy, of aqueous (0.5 - 8 %) hydroxypropyl-β-cyclodextrin solutions of nebivolol hydrochloride at 2.5, 10 or 40 mg nebivolol/kg/day.

Time (h) after dosing	Plasma concentration (ng/ml)		
	Low 2.5 mg (base-eq.)/kg	*Medium* 10 mg (base-eq.)/kg	*High* 40 mg (base-eq.)/kg
1	44.3 ± 11.9	224 ± 45	826 ± 106
2	79.0 ± 6.8	317 ± 50	1146 ± 437
4	40.5 ± 16.5	194 ± 107	791 ± 264
8	23.1 ± 10.1	126 ± 23	765 ± 249
24	4.2[1]	6.0 ± 3.4	206 ± 96
C_{max} (ng/ml)	79.0	317	1146
T_{max} (h)	2	2	2
$t_{1/2}$ (h)	6.19[2]	3.87[3]	8.45[4]
AUC_{0-24h} (ng.h/ml)	549	2589	14216

1) Median; n=2.
2) $t_{1/2}$ calculated between 4 and 24 h.
3) $t_{1/2}$ calculated between 2 and 24 h.
4) $t_{1/2}$ calculated between 8 and 24 h.

The analysis is inadequate to determine whether or not there are metabolic differences between gravid and non-gravid Sprague-Dawley rats. Characterization of the enantiomers, conjugated forms and hydroxyl phenols are also necessary at a minimum.

N109346 Toxicokinetics of nebivolol in the Beagle dog in a twelve-month chronic oral toxicity study of nebivolol hydrochloride (R067555) provided as a mixture with β-cyclodextrin at 2.5, 10 or 40 mg(base-equivalents)/kg/day. Tissue distribution of nebivolol after chronic dosing. 1989 - 1994

Three groups of 4 Beagles (2/sex) were used. There were 4dogs/sex/group in the toxicology study. The nebivolol was mixed with β-CD in gelatin capsules to provide the specified dosages. Blood was collected before dosing, and 0.5, 1,2,4,6,8 and 24 hours after dosing on the first and last days of the study. Samples were also taken for determination of trough levels on days 3,4,8,15, 43, 57,120, 176, 239, 303 and 358. At necropsy, samples of liver, brain, heart, kidney, pancreas, spleen, lung, muscle and fat were collected. Plasma and tissue samples were analyzed by HPLC for nebivolol.

Results

The sponsor reports that there were no sex-related differences in PK parameters. Separate results for the sexes were not provided. The combined results are shown below.