CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

204410Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 204410
Submission date: 10/19/12
Drug: macitentan
Applicant: Actelion
Indication: pulmonary arterial hypertension

Reviewing Division: Division of Cardiovascular and Renal Products

Discussion:
The pharmacology/toxicology reviewer and supervisor concluded that macitentan can be approved from the pharmacology/toxicology perspective for the indication listed above.

Macitentan was tested in 2 year carcinogenicity studies in rats and mice using the oral gavage route of administration. Both studies were found to be acceptable by the executive carcinogenicity assessment committee. No drug-related neoplasms were noted in either study.

An appropriate Established Pharmacologic Class for macitentan is the existing class: “endothelin receptor antagonist”.

Macitentan was teratogenic in rabbits and rats, causing cardiovascular and mandibular arch fusion abnormalities at all doses tested. Doses of 150 and 450 mg/kg/day were assessed in rats and doses of 2.5, 12.5 and 25 mg/kg/day were assessed in rabbits. These doses produced exposures in rats and rabbits that provide a large multiple compared to the exposure in humans at the recommended dose of 10 mg. However, no NOAEL was identified and the adverse findings are consistent with this pharmacologic class.

Conclusions:
The pharmacology/toxicology reviewer conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that this NDA may be approved for the above indication. Contraindicating use of macitentan in pregnant women appears appropriate given the effects observed on fetal development. It appears that no additional nonclinical studies are needed at this time. I have provided comments on labeling to the division separately.

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/s/

PAUL C BROWN
09/24/2013

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 204410
Supporting document/s:	SDN 000
Applicant's letter date:	10/19/12
CDER stamp date:	10/19/12
Product:	Opsumit®, (macitentan)
Indication:	Pulmonary Arterial Hypertension
Applicant:	Actelion
Review Division:	DCRP
Reviewer:	William T. Link, Ph.D.
Supervisor/Team Leader:	Albert DeFelice, Ph.D.
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Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Macitentan (ACT-064992) is an orally active dual endothelin (ET) ETA and ETB receptor antagonist devoid of partial agonist activity and characterized by high receptor affinity and a slow receptor dissociation rate. *In vitro*, macitentan selectively inhibits the binding of ET-1 to both ETA and ETB receptors, with nanomolar potency, as well as the effects mediated by these receptors in functional assays in cells and isolated organs. In the latter, macitentan has an ETA/ETB inhibitory potency ratio of 50:1 i.e., is somewhat selective for the A sub-type. *In vivo*, macitentan increases ET-1 plasma concentrations.

Macitentan has one active circulating metabolite, ACT-132577, which is also a dual ETA/ETB receptor antagonist. In functional *in vitro* assays (inhibition of contraction of isolated rat aorta and trachea), ACT-132577 is, on average, only 8-fold less potent than macitentan on the ETA receptor and 2-fold less potent on ETB. Accordingly, ACT-132577 may contribute to the overall pharmacological effect of macitentan.

1.2 Brief Discussion of Nonclinical Findings

Pharmacology

Macitentan showed dose-dependent efficacy in rat models of systemic and pulmonary hypertension. In rats with systemic hypertension, single oral administration of macitentan dose-dependently decreased mean arterial blood pressure (MAP) without affecting heart rate (HR). Repeated oral administration of macitentan caused a sustained decrease in blood pressure that was not associated with tachyphylaxis, rebound effect after cessation of treatment, or an increase in HR.

In rats with pulmonary hypertension (PH), single oral administration of macitentan caused a sustained, dose-dependent decrease in mean pulmonary arterial pressure (mPAP) without affecting systemic blood pressure. Four weeks of macitentan treatment in rats with monocrotaline-induced PH dose-dependently inhibited pulmonary vascular remodeling and development of right ventricular hypertrophy, and prolonged survival. In rats with bleomycin-induced PH associated with pulmonary fibrosis, macitentan was superior to bosentan in inhibiting development of right ventricular hypertrophy.

Safety Pharmacology

The effect of macitentan on the physiological function of major organ systems was investigated at pharmacological and supra-pharmacological doses in several species. In rats, macitentan had no effect on a battery of behavioral and physiological variables covering the main central and peripheral nervous system functions or on physiological function of the respiratory system at doses up to 100 mg/kg, the highest dose tested.

Macitentan and its circulating metabolite ACT-132577 had no relevant effect (< 20% inhibition) on hERG-mediated potassium currents up to a concentration of 10 µM, which

is approximately 2000-fold higher than the unbound plasma concentrations of these two compounds at therapeutic doses in humans. In the same assay, the positive control, terfenadine, displayed an IC_{50} of 26 nM.

In vivo, macitentan did not induce changes in ECG variables, including ventricular repolarization, in either guinea pigs or dogs. In dogs, a dose-related decrease in blood pressure was observed, which at 5 and 30 mg/kg was associated with a slight increase in heart rate, considered to represent a compensatory physiological response. Exposure, at these doses, was 9- to 40-fold the human exposure at 10 mg/day.

Pharmacokinetics

Absorption
Macitentan was absorbed slowly after oral dosing with a T_{max} of 6 h in the rat at exposures clinically relevant to humans and a T_{max} of 6–10 h in man at a dose of 10 mg whereas in the dog, peak plasma concentrations were reached 2 h post-dose. Oral bioavailability was about 30% in the rat and 80% in the dog. In both species, blood clearance is about 20% of the respective liver blood flow and macitentan is therefore classified as a low clearance drug. Based on *in vitro* data from Caco-2 cells, macitentan permeates rapidly through membranes and is not a substrate of human P-gp. Its oral absorption is therefore not expected to be affected by concomitantly given P-gp inhibitors.

Enterohepatic recirculation was investigated in animals, as conjugates of macitentan and ACT-132577 with glucose and/or glucuronic acid represent a major fraction of the metabolite pool in bile. These conjugates are typically cleaved again by the gut microflora, and the liberated aglycones then become available for possible enterohepatic recirculation. The extent of such recirculation was assessed in a tandem rat model and found to be absent for macitentan and negligible for ACT-132577. The metabolic pattern in man is different, in that macitentan itself does not undergo phase II conjugation. A glucose conjugate of ACT-132577 was observed in human urine. In line with the metabolic pattern, the plasma concentration vs time profile of macitentan in man indicates no relevant role of enterohepatic recirculation in its overall pharmacokinetic profile

Distribution
Binding of macitentan to plasma proteins was in excess of 99% in all animal species and man. Free fractions in rat, mouse, and man were in a narrow range of 0.4-0.6%, and in the dog were slightly higher, at 0.9%. The highest binding was observed in the rabbit with a free fraction of only 0.1%. Binding studies of macitentan with human serum albumin and α_1-acid glycoprotein indicated extensive binding to both proteins. Considering the relative abundance of the two proteins in blood, serum albumin represents the major binding component of macitentan in man. Plasma protein binding of ACT-132577 varied from 98.3–99.9% and was generally lower compared to parent macitentan. The highest binding was again observed in plasma of rabbit and man, with

free fractions of 0.1% and 0.5%, respectively. Binding of ACT-132577 in all other species was lower compared to macitentan.

Partitioning studies with macitentan and ACT-132577 yielded blood/plasma ratios of 0.53–0.73 and 0.54–0.69, respectively, indicating limited penetration of either compound into red blood cells.

In tissue distribution studies with ^{14}C-labeled macitentan in the rat, radioactivity was rapidly absorbed and widely distributed. Concentrations of radioactivity in the majority of tissues at early sampling times were below those in plasma, with only the liver and kidney containing higher levels. At later sampling time points, concentrations in more than half of the tissues were above those in plasma. The highest concentrations of radioactivity were found in the liver, kidney cortex, plasma, blood, and lung. The tissues containing the lowest radioactivity were the body of the lens, brain and spinal cord, white fat, testis, and seminal vesicles.

Prolonged retention of radioactivity was observed in organs involved in macitentan excretion, i.e., liver and kidney, in which quantifiable levels were present up to 28 days after dosing. Radioactivity in melanin-containing tissues was slightly higher in pigmented rats suggesting some minor binding to melanin.

Hepatic uptake of several endothelin receptor antagonists has been shown to be dependent on OATP transporters eventually leading to accumulation of drug in liver tissue. Uptake of macitentan and ACT-132577 was equally fast in OATP-overexpressing cells as in control cells without OATP. The hepatic disposition of macitentan therefore appears to be driven by passive diffusion rather than by transporter-mediated uptake.

Partitioning of macitentan and metabolites into milk was demonstrated in lactating rats. The time course of total radioactivity in milk followed the known pharmacokinetic profile of macitentan in the rat. Radioactivity in milk was generally below the respective values in plasma with ratios ranging from 0.32–0.57 in the first 24 h post-dose. Parallel to the decline of macitentan concentrations in plasma, ratios shifted in favor of milk at later time points, likely the consequence of the higher fat content of milk and retention of macitentan and metabolites in a more lipid-rich environment. Metabolic profiles recorded from milk and plasma were qualitatively similar showing that macitentan and its circulating metabolites partition equally well into milk.

Metabolism

In man, macitentan has two primary metabolic pathways, which are both catalyzed by P450 enzymes. Macitentan metabolism in rat and dog shows large similarities to the pattern observed in man. Both metabolites circulating in human plasma, i.e., the pharmacologically active M6 (ACT-132577) and the inactive acid M5 (ACT-373898), were also found in rat and dog. Both species are therefore adequate choices for the preclinical safety program of macitentan.

The primary metabolism of macitentan is catalyzed by P450 enzymes, mainly by CYP3A4 with a minor contribution of CYP2C19. The expected interaction with strong CYP3A4 inhibitors was confirmed in a clinical study with ketoconazole in which an about 2-fold increase in macitentan exposure was observed. The decrease in macitentan levels in the presence of the potent CYP3A4 inducer rifampicin is another reflection of the role of CYP3A4. The involvement of CYP2C19 in macitentan metabolism is small based on enzyme kinetic data with recombinant P450 enzymes. Concomitant inhibitors of CYP2C19 are therefore not expected to alter macitentan pharmacokinetics. Similarly, no change in macitentan levels is expected in individuals with a modified CYP2C19 phenotype.

Macitentan is a microsomal enzyme inducer. Dose-dependent changes in P450 expression levels were observed in liver samples obtained from toxicity studies in the mouse, rat, and dog. Similarly, macitentan and ACT-132577 up-regulate CYP3A4 expression in human hepatocytes *in vitro*. Both compounds are activators of the human pregnane X receptor (PXR), one of the major transcriptional regulators of P450 expression.

Excretion
The excretion of ^{14}C-macitentan was investigated in rats and dogs after single i.v. and oral dosing up to 3 mg/kg. In rats and dogs, elimination via the feces was the major route of excretion and was largely independent of sex and route of administration. After oral dosing and extensive metabolism, recoveries in feces were 67–81% in rats and 69% in dogs. Metabolic profiling studies in both species using bile-duct cannulated animals confirmed that after extensive metabolism fecal excretion is the major elimination route, whereby in dogs these experiments showed a more balanced excretion pattern for macitentan with a recovery of about 40% of absorbed radioactivity in urine. Within the 48- and 96-h collection periods, 83–107% and 73–86% of the administered radioactive doses were recovered in the rat and dog, respectively.

Toxicology

Rats, mice and dogs were used for general toxicity studies, rats and rabbits for the reproductive toxicity studies, and rats and mice for the carcinogenicity studies. Impurities and metabolites were toxicologically qualified in these studies and in the *in vitro* and *in vivo* genotoxicity studies.

A single dose of 2000 mg/kg ACT-064992 did not produce signs of acute toxicity in mice or rats. Body weight and histopathology were unaffected.

Repeat dose studies in mice, rats and dogs revealed a pattern of findings which are remarkably similar to findings with other members of this drug class. These were:

Liver
Increased liver weight and centrilobular hypertrophy was consistently seen in all studies, in all three species and in all dose groups. In the mouse, this was associated with areas

of focal necrosis in all dose groups. In the rat, focal necrosis was only seen in the 13 week study and only in the context of high morbidity and multi-organ hemorrhage at the highest dose which was clearly above an MTD and necessitated sacrifice of the whole group after two weeks. PT and aPTT were elevated 5 and 6-fold, respectively in this group. No focal necrosis was observed in the dog. Both the hypertrophy and focal necrosis showed evidence of recovery upon cessation of dosing. The centrilobular hypertrophy is considered an adaptive response related to metabolizing enzyme induction.

Testes
Dilation of seminiferous tubules was a morphological change seen with macitentan which can be attributed to drug treatment up to chronic treatment (rat and dog). The incidence of affected animals was always low and the severity of these changes was mostly minimal. In studies with treatment-free recovery periods, the changes were fully reversible within 8–13 weeks in rats, or 8–16 weeks in dogs. Reversible changes in sperm parameters (increase in abnormal sperm) without histological changes in the testes were seen in the 26-week rat toxicity study

RBC parameters
A dose-related, reversible decrease in hematocrit, red blood cell count, and hemoglobin concentration without reticulocytosis was observed in most rat and dog toxicity studies. The effects were more pronounced in dogs than in rats, where the changes were minimal and mostly within the range of historical control values. Available data suggest an absence of drug-induced hemolysis and bone marrow toxicity.

Cardiac (dog only)
The main drug-related effect was intimal thickening of coronary arteries in all dog studies from subacute (4-week) to chronic (39-week) treatment. A no-observed-effect level (NOEL) of 5 mg/kg/day was established in all studies and the severity was generally minimal. Intimal thickening is interpreted as a residual finding of a previous acute arteritis. After subacute (4-week) treatment, other findings in the heart included atrial fibrosis with chronic inflammation, epicarditis, and neovascularization. These individual findings are considered to be the more mature appearance of previous acute myocardial changes. There were no findings in rats and mice, and the dog is considered to be highly sensitive, with respect this response, to vasdilator drugs.

Thyroid (rat only)
Increased thyroid weight and thyroid follicular cell hypertrophy were observed in rat studies. The findings were not present in dogs and mice. Induction of hepatic microsomal enzymes can increase the hepatic disposition of thyroid hormones, resulting in an adaptive increase in thyroid-stimulating hormone that leads to morphological changes such as thyroid follicular cell hypertrophy

Genetic toxicology

There was no evidence for genotoxicity with ACT-064992 in a battery of *in vitro* tests that included the bacterial reverse mutation assay in *Salmonella typhimurium*, the mouse lymphoma assay, and the chromosome aberration test in human lymphocytes. For some tests, different drug substance batches were used; all *in vitro* tests were conducted with and without metabolic activation.

An additional Ames test, performed with the metabolite ACT-080803, also gave no evidence of a mutagenic effect.

The mutagenic potential of the metabolite ACT-373898 was assessed as well. ACT-373898 did not produce an increase in the number of revertants in any of the strains tested in the presence and absence of metabolic activation.

There was no evidence of chromosome damage or effects on the spindle apparatus in an *in vivo* micronucleus test in rat bone marrow.

Carcinogenicity

Carcinogenicity was assessed in two-year studies in mice and rats. Both studies were in accordance with GLP and had prior concurrence, with the Executive Carcinogenicity Assessment Committee (CAC), regarding the strain, number of animals employed, and doses evaluated. Both studies were considered negative by the Committee.

Mouse study
The test item did not increase the incidence of neoplastic lesions when tested up to a MTD. High morbidity/mortality in group 5 females (allocation A: 35/60, 400 mg/kg/day) led to early termination of this dose group in week 79. The cause of death could not be established in most of the animals. The remaining treatment groups did not show significant differences in mortality compared to controls.

AUC exposure ratios (relative to human AUC at the 10 mg dose) at Week 26 for the 100 mg/kg dose, for ACT-064992 (parent), ACT-132577 (active metabolite) and ACT-373898 (inactive metabolite) were 139x, 77x and 1x (females) and 75x, 58x and 0.4x (males), respectively..

Rat study
The test item did not increase the incidence of neoplastic lesions when tested up to a MTD. High morbidity/mortality in Group 4 and Group 5 females led to dose reductions to 25 mg/kg and 50 mg/kg in these Groups, respectively. The cause of death could not be established in most of the animals. The low dose group did not show significant differences in mortality compared to controls.

AUC exposure ratios (relative to human AUC at the 10 mg dose) at Week 26 for the 50 mg/kg dose, for ACT-064992 (parent), ACT-132577 (active metabolite) and ACT-

373898 (inactive metabolite) were 42x, 14.6x and 0.64x (females) and 8.3x, 10.6x and 0,08x (males), respectively.

Reproductive toxicology

Fertility
Male fertility evaluation was reviewed under IND 77258. Although fertility in the absolute sense was unaffected (all sired liters), increased incidence of early intrauterine death (250 mg/kg) and post-implantation loss (50 and 250 mg/kg) in the mated dams were statistically significant, suggesting an effect is transferred from the treated males to the untreated females. This could indicate that either the sperm is affected directly, or that the female is dosed via semen during copulation and this, in turn, affects implantation. It is not known if macitentan is secreted into semen, and the dose would be expected to be small, however, exposure to the uterus could be significant if macitentan is secreted. Testicular tubular atrophy was noted in the 50 and 250 mg/kg groups.

There was no effect of treatment on sex ratio, mean litter weight, mean placental weight or mean fetal weight. There was no effect of treatment on the incidence or inter-group distribution of fetal abnormalities. A NOAEL of 10 mg/kg was observed.

Female fertility evaluation was also reviewed under IND 77258, Administration of 10, 50 and 250 mg/kg/day of ACT-064992 to the female Wistar rat elicited slight reductions in food intake in the parental females. There were no adverse effects on fertility or early-embryonic development at any dose level investigated. The no-observed-effect-level for fertility and early embryonic development was 250 mg/kg/day.

Fetal development
Fetal developmental effects were evaluated in rats and rabbits. Both studies were reviewed under IND 77258. Serious malformations of the fetus are apparent at all doses.

In rats, treatment with ACT-064992 at dosages of 150 and 450 mg/kg/day during the organogenesis phase of gestation resulted in a specific disruption of embryo-fetal development, which affected all fetuses in these groups. All fetuses had a number of craniofacial abnormalities (collectively described as mandibular arch fusion abnormalities) and there was also a high incidence of cardiovascular abnormalities at both dosages. These findings are consistent with other members of this class of compounds. Based on the results of this study, it was concluded that the no-observed-adverse-effect-level (NOAEL) for maternal toxicity was 450 mg/kg/day, while a NOAEL for embryo-fetal development was not established and lies below 150 mg/kg/day.

ACT-064992 exhibited a teratogenic potential in rabbits from the low dose of 2.5 mg/kg/day upwards and is fetotoxic at the high dose of 25.0 mg/kg/day. All fetuses were affected at the 12.5 and 25 mg/kg doses. No marked effects on the dams were seen up to 25.0 mg/kg/day.

Pre- and Postnatal Development
For F0 dams, a NOAEL of 50 mg/kg/day was established regarding clinical signs and general condition. No NOAEL could be established for reproduction parameters in the F0- generation. Increased pup mortality and increased pre- and post implantation loss was seen at all dose levels.

Mating of F1 males and females resulted in increased pre-implantation loss in all dose groups. Post-implantation loss was also marginally decreased in the 'treated' groups, although exposure to macitentan only occurred during nursing.

1.3 Recommendations

1.3.1 Approvability

In the opinion of this reviewer, the application is approvable.

1.3.2 Additional Non Clinical Recommendations

The same conditions for use and the REMS, in place for other drugs in this class of compounds, are equally applicable to this compound. No new emergent issues are apparent.

1.3.3 Labeling

Suggested edits to the label have been made in the Divisional eRoom.

2 Drug Information

2.1 Drug

CAS Registry Number: 441798-33-0

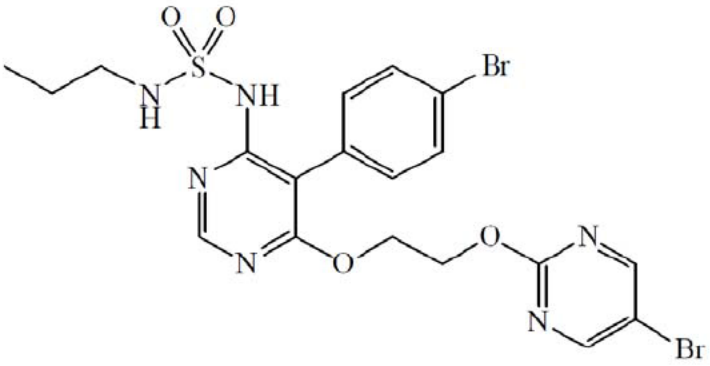
Generic Name: macitentan

Code Name: ACT-064992

Chemical Name: *N*-[5-(4-bromophenyl)-6-{2-[(5-bromopyrimidin-2-yl)oxy]ethoxy}pyrimidin-4-yl]-*N*'-propylsulfuric diamide

Molecular Formula/Molecular Weight: $C_{19}H_{20}Br_2N_6O_4S$ / 588.27 g/mol

Structure or Biochemical Description:



Pharmacologic Class: Endothelin receptor blocker

2.2 Relevant INDs, NDAs, BLAs and DMFs:

IND 77,258 - Pulmonary Arterial Hypertension; (b) (4)

2.3 Drug Formulation

Composition of macitentan 3 mg and 10 mg film-coated tablets used in clinical studies

Ingredients	Composition (mg)	
	3 mg	10 mg
Macitentan	3.00	10.00
Lactose monohydrate	(b) (4)	
Microcrystalline cellulose		
Sodium starch glycollate type A		
Povidone		
Magnesium Stearate		
Polysorbate 80		
(b) (4)		
Total	72.80	72.80

2.4 Comments on Novel Excipients

none

2.5 Comments on Impurities/Degradants of Concern

All impurities and degradants were adequately qualified in toxicology studies. QSAR analysis was performed on nine compounds, based on structural alerts, at the request of the CMC reviewer. No predictions for mutagenicity were revealed.

2.6 Proposed Clinical Population and Dosing Regimen

The application intends to support the use of macitentan at a dose of 10 mg once daily (o.d). for the long-term treatment of pulmonary arterial hypertension (PAH) [redacted] (b) (4)

2.7 Regulatory Background:

IND 77258 submitted to DCRP on 6/3/08.

3 Studies Submitted

3.1 Studies Reviewed

The majority of all GLP studies were reviewed under IND 77258. Reviewed herein are the studies on Peri and Postnatal Reproductive Toxicology and Carcinogenicity.

3.2 Studies Not Reviewed

Pharmacology studies (with the exception of Safety Pharmacology) and Pharmacokinetic studies were examined, but not formally reviewed.

3.3 Previous Reviews Referenced:

IND 77258 30 day Safety review, available in DARRTS

4 Pharmacology

4.1 PRIMARY PHARMACOLOGY

In vitro

Inhibition of ET-1 binding to ETA and ETB receptors [Study B-07.246]

The cDNAs for human ETA and ETB receptors were cloned, sequenced, and stably overexpressed in Chinese hamster ovary (CHO) cells. Binding assays with radio-iodinated human ET-1 (^{125}I-ET-1) were performed on microsomal membranes isolated from these recombinant cells. Macitentan inhibited the binding of ^{125}I-ET-1, with IC_{50} values of 0.49 ± 0.07 nM (n = 13) on the ETA receptor and 391 ± 49 nM (n = 14) on the ETB receptor.

Functional inhibition of ETA and ETB receptors

Inhibition of intracellular calcium increase in recombinant cells [Study B-12.108]

The ability of macitentan to inhibit the ET-1-induced increase in cytosolic Ca^{2+} was measured using a Ca^{2+}-sensitive fluorescent dye and recombinant CHO-K1 cells expressing human endothelin ETA or ETB receptors. First, IC_{50} values were determined from the peak fluorescence signals; these were transformed to Kb 2 values using the known EC50 of ET-1 and the ET-1 concentrations used in the assay (EC_{50}–EC_{70}) via the Cheng-Prusoff equation. In this functional assay, the geometric mean Kb values for macitentan were 0.81 nM on ETA (n = 8) and 128 nM on ETB (n = 5).

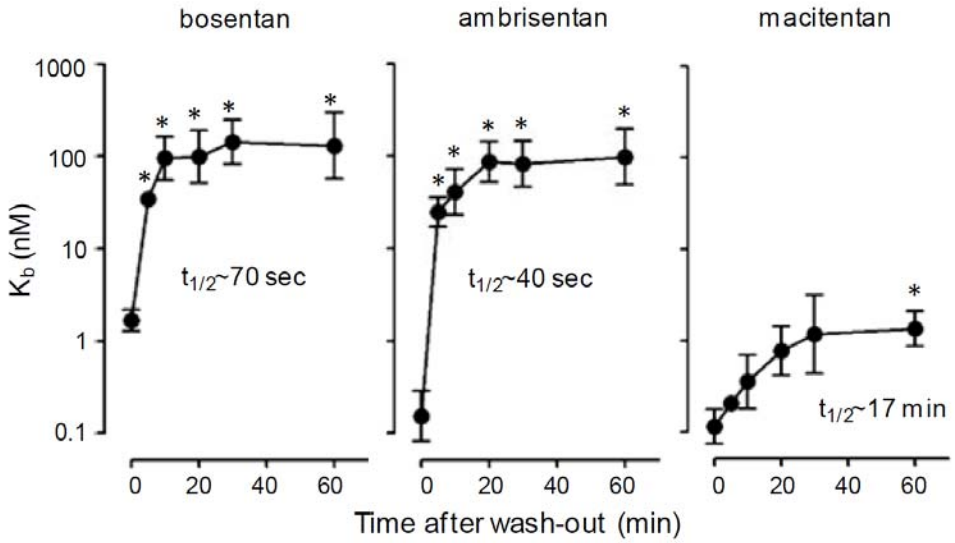
Inhibition of ET-1- induced contraction in isolated tissues [Study B-05.057]

Macitentan caused a parallel rightward shift in the concentration-response curves of ET-1-mediated contraction of isolated rat aorta denuded of endothelium (ETA receptor-mediated) and of sarafotoxin S6c-mediated contraction of rat trachea denuded of epithelium (ETB receptor-mediated). There was no significant change in the maximum response to ET-1 or sarafotoxin S6c. Macitentan behaved as a competitive antagonist at ET receptors, as Schild analyses yielded slopes that were not significantly different from unity. Calculated pA2 values for macitentan were 7.6 ± 0.2 (ETA receptor, n = 3) and 5.9 ± 0.2 (ETB receptor, n = 3). Thus, in these functional assays in isolated organs, macitentan has an ETA/ETB inhibitory potency ratio of 50:1. Macitentan did not exhibit agonist activity in isolated tissues.

Receptor dissociation kinetics in human pulmonary arterial smooth muscle cells [Study B-12.108]

Pulmonary arterial smooth muscle cells (PASMC) are crucial in the pathophysiology of PAH, as they are responsible for vessel constriction and contribute to remodeling. The inhibitory potency and dissociation kinetics of macitentan in human primary PASMC were determined using the Ca^{2+}-release assay, and compared with those of two other endothelin receptor antagonists (ERAs), ambrisentan and bosentan. In this functional assay, geometric mean Kb values (calculated from measured IC_{50} values) were 0.14 nM (n = 6) for macitentan, 0.12 nM (n = 6) for ambrisentan, and 1.1 nM (n = 6) for bosentan. To assess receptor occupancy half-life, PASMCs were incubated with dilution series of macitentan, ambrisentan, or bosentan for 120 min, and then washed to eliminate free compound from the culture medium. Residual antagonistic activity was measured at various times after wash-out. As shown below, PASMCs treated with bosentan and ambrisentan rapidly regained responsiveness to ET-1. Calculated receptor occupancy half-lives were 40 seconds for ambrisentan and 70 seconds for bosentan. In contrast, macitentan lost potency slowly over time and was still highly potent 60 min after washout, with a half-life of 17 min.

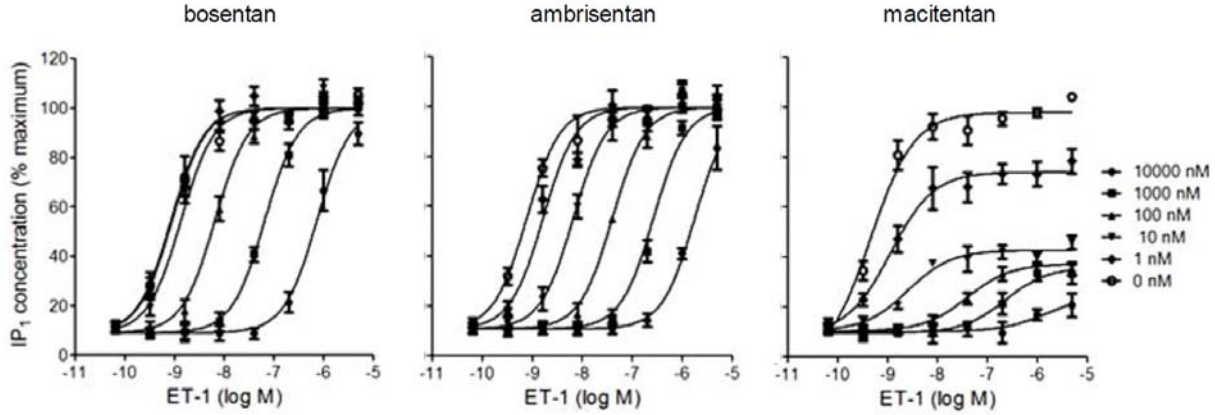
Receptor occupancy half-lives of bosentan, ambrisentan, and macitentan determined by Ca^{2+} release assays after antagonist wash-out in human PASMC



Kb values before and after washout were calculated from IC_{50} values using the Cheng-Prusoff equation. Data are presented as geometric mean ± SEM, n = 2–4. *, $p < 0.05$ vs Kb at t = 0 (one-way ANOVA with Dunnett´s post test).
Kb, calculated dissociation constant; PASMC, pulmonary arterial smooth muscle cells; t1/2, calculated receptor occupancy half-life.

To determine the mode of antagonism, all three antagonists were titrated against increasing concentrations of ET-1 in PASMCs, using accumulation of inositol-1-phosphate (IP1) as the read-out. As shown below, all three ERAs concentration-dependently blocked IP1 accumulation induced by 20-min stimulation with ET-1. Whereas ambrisentan and bosentan showed surmountable antagonism, reflected by a rightward shift of ET-1 concentration-response curves, macitentan displayed insurmountable antagonism with a depression of maximum response in addition to a rightward shift in the curves. When the ET-1 stimulation time was increased from 20 min to 90 min, macitentan behaved as a surmountable antagonist. In conclusion, macitentan is a competitive antagonist displaying slower receptor dissociation compared to bosentan and ambrisentan.

Effect of bosentan, ambrisentan, and macitentan on the concentration-response-curves of ET-1-induced IP1 accumulation in human PASMC



Human PASMCs were pre-incubated with dilution series of macitentan, ambrisentan, or bosentan for 120 min. Then, cells were stimulated with a dilution series of ET-1 for 20 min and accumulation of intracellular IP1 was measured. IP1 concentrations were normalized per experiment to the maximal response in absence of antagonist. Data are presented as arithmetic mean ± SEM, n = 4. ERA, endothelin receptor antagonist; ET-1, endothelin-1; IP1, inositol-1-phosphate; PASMC, pulmonary arterial smooth muscle cell.

In vitro pharmacology of metabolites

Two circulating macitentan metabolites have been identified in humans: ACT-132577 and ACT-373898. *In vitro* assays revealed that ACT-132577 is a dual ERA, whereas ACT-373898 is not active on ET receptors.

ACT-132577

The activity of ACT-132577 was measured with the same *in vitro* assays used for macitentan. On membranes from recombinant CHO cells, ACT-132577 inhibited binding to ETA and ETB receptors with IC_{50} values of 3.4 ± 0.20 nM (n = 4) and 987 ± 92 nM (n = 4) respectively. In recombinant CHO-K1 cell lines, ACT-132577 inhibited ET-1-induced release of intracellular Ca^{2+} with calculated Kb values of 5.5 nM on ETA (n = 5) and 319 nM on ETB (n = 4). As determined using intracellular Ca^{2+}-release assays in human PASMC, ACT-132577 did not display slow receptor dissociation as observed for macitentan. In rat isolated aortic rings and trachea denuded of epithelium, calculated pA2 values for ACT-132577 were 6.7 ± 0.2 (n = 3) for the ETA receptor and 5.5 ± 0.3 (n = 3) for the ETB receptor. Based on these data, ACT-132577 is a dual ET receptor antagonist with an ETA/ETB inhibitory potency ratio of 16:1. ACT-132-577 is approximately 8-fold less potent than macitentan on ETA and 2-fold less potent on ETB, and likely contributes to the overall pharmacological effect of macitentan *in vivo.*

ACT-373898

The antagonistic activity of ACT-373898 was measured using the intracellular Ca^{2+}-release assay and recombinant endothelin ETA and ETB receptors expressed in CHO-K1 cell lines. ACT-373898 displayed no measurable antagonistic activity at 10 ± M in these assays.

In vivo pharmacology

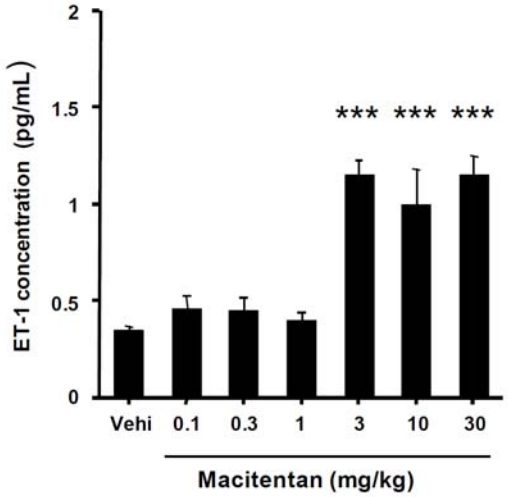
The pharmacodynamic effects of macitentan have been investigated in normal rats and in rat disease models in which ET-1 plays a pathological role. All blood pressure and HR data were collected using telemetry in conscious, freely moving animals unless otherwise noted.

Pharmacodynamic effects of macitentan in normal rats

Effect of macitentan on ET-1 plasma concentrations in normal rats

Binding of an ERA to ETB receptors causes an increase in ET-1 concentration, which can be used as a marker of pharmacological efficacy and potency on the ETB receptor. To confirm that macitentan functionally blocks ETB receptors *in vivo*, plasma ET-1 concentrations were measured by enzyme immunoassay after oral administration of macitentan to normal male Wistar rats (n = 5) [Study B-04.030]. At 6 h after oral administration, macitentan at a dose of 3 mg/kg induced a statistically significant, 2.5-fold increase in plasma ET-1 concentration as compared to baseline values, whereas no significant changes were observed at a dose of 0.3 mg/kg. This activity was confirmed in a second study [B-04.115], in which plasma ET-1 concentrations increased and reached a plateau at doses of macitentan ± 3 mg/kg [see Figure below], When compared with bosentan, macitentan was 10-fold more potent in increasing plasma ET-1 concentrations.

ET-1 plasma concentrations in conscious Wistar rats 6 h after administration of macitentan



Macitentan was administered by oral gavage in 7.5% gelatin solution. Data are presented as means SEM (n = 4).
*** p ≤ 0.001 compared to vehicle-treated rats (one-way ANOVA with Student-Neuman-Keuls test).
Vehi, vehicle; ET-1, endothelin-1.

Hemodynamic effects of macitentan in normotensive rats [Study B-12.447]

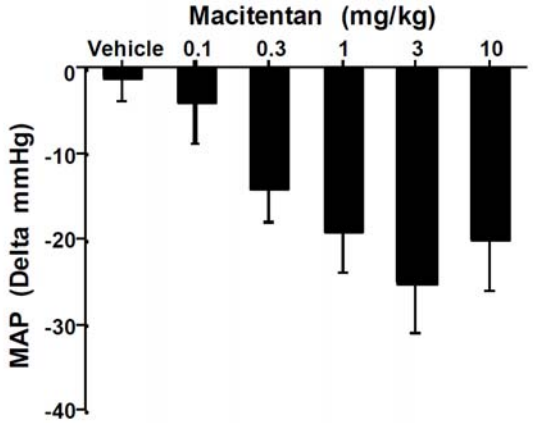
In normotensive conscious Wistar rats, a single oral administration of macitentan (10 and 30 mg/kg) had no effect on MAP or HR. In a study in rats implanted with a dual pressure transmitter to simultaneously measure systemic and pulmonary blood pressures, macitentan (10 mg/kg) had no effect on either MAP or mPAP. These data suggest that in the absence of upregulation of the ET system in the rat, macitentan does not have hemodynamic effects.

Effects of macitentan in rat models of systemic hypertension

Effect of single oral administration of macitentan on blood pressure and heart rate in hypertensive rats [Studies B-04.031, B-04.114]

The effect of a single oral administration of macitentan on HR and MAP was evaluated in conscious hypertensive rats equipped with telemetry [Study B-04.031]. Two rat models of hypertension were studied: Dahl salt-sensitive (Dahl-S) rats and deoxycorticosterone acetate (DOCA)-salt rats, both maintained on a 1% saline solution. Blood pressure and HR were measured continuously after administration of macitentan at doses of 0.1, 0.3, 1, 3, and 10 mg/kg in hypertensive Dahl-S rats (n = 5–6); and doses of 1, 3, and 10 mg/kg in hypertensive DOCA-salt rats (n = 7). In Dahl-S rats, macitentan decreased blood pressure dose-dependently, with a maximal decrease of 20 to 25 mmHg observed at doses of 1, 3, and 10 mg/kg [see Figure below].

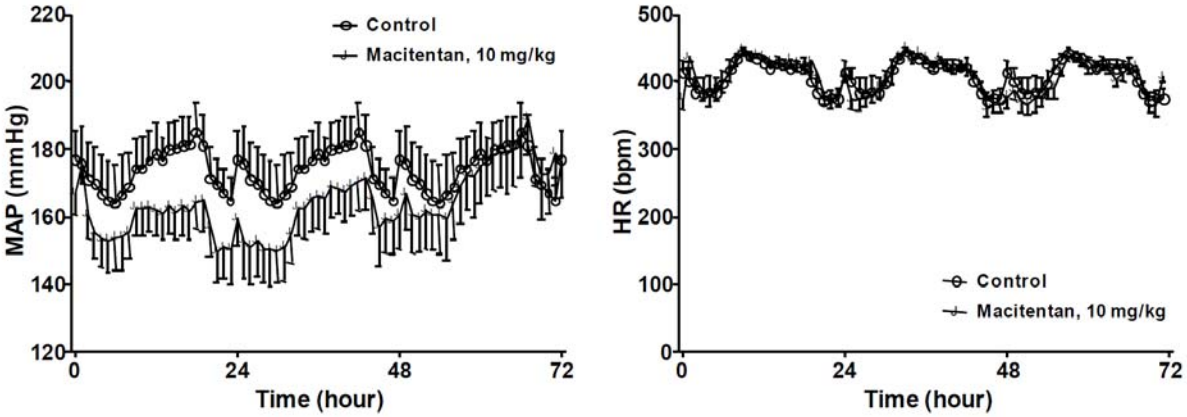
Dose-response relationship of the maximal effect of macitentan on mean arterial blood pressure in conscious hypertensive Dahl-S rats



Macitentan was administered by oral gavage in 5% Arabic gum. Results are expressed as means ± SEM, n = 6. MAP, mean arterial blood pressure. [B-04.031]

In hypertensive DOCA-salt rats, the maximal MAP decrease was 25 mmHg at 10 mg/kg No effect on HR was observed in either Dahl-S [Figure below] or DOCA-salt rats. The duration of action was at least 24 h at doses ≥ 3mg/kg in both studies.

Effect of a single oral administration of macitentan on mean arterial blood pressure and heart rate in conscious hypertensive Dahl-S rats



Macitentan (10 mg/kg) was administered by oral gavage in 5% Arabic gum. Results are expressed as means SEM, n = 6. bpm, beats per minute; HR, heart rate; MAP, mean arterial blood pressure. [B-04.031]

In another study in DOCA-salt hypertensive rats equipped with telemetry [B-04.114], the effect of a single oral administration of macitentan was compared with the effect of bosentan. Heart rate and arterial pressure were monitored up to 72 h after a single gavage of macitentan at doses of 0.3, 1, 3, 10, and 30 mg/kg (n = 6–9), bosentan at doses of 1, 3, 10, 30, 100, and 300 mg/kg (n = 6–9), or vehicle (n = 9). Both macitentan and bosentan dose-dependently decreased MAP, without affecting HR. For macitentan, a mean maximal reduction in MAP of 24 ± 4 mmHg was calculated at doses of 10 and 30 mg/kg (ED50 4, 1 mg/kg); for bosentan, the mean maximal reduction in MAP was 19 ± 3 mmHg at doses of 100 and 300 mg/kg (ED50, 10 mg/kg). Thus, in the DOCA-salt rat model of hypertension, macitentan is 10-fold more potent than bosentan and its maximal effect is larger. The duration of the blood pressure decrease with 10 mg/kg macitentan (40 h) was at least twice as long as with 100 mg/kg bosentan (20 h), at equally effective doses.

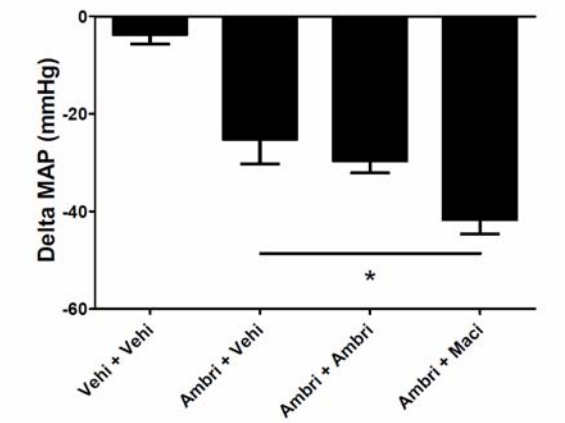
Effect of repeated oral administration of macitentan on blood pressure and heart rate in hypertensive rats [B-04.031]

Oral administration (5 days) of macitentan (1 mg/kg/day, n = 5) to conscious hypertensive Dahl-S rats maintained on a 1% saline solution and equipped with telemetry resulted in a sustained decrease in MAP of around 20 mmHg that was already achieved at Day 1 after administration. Repeated administration of macitentan had no effect on HR. At the end of treatment, blood pressure gradually returned to baseline values within 3 days. Repeated oral administration of macitentan is therefore not associated with tachyphylaxis or an increased effect over time, and its cessation does not result in a rebound effect.

Addition of macitentan to a maximally effective dose of bosentan or ambrisentan in hypertensive rats [B-12.110]

To investigate whether macitentan may be more effective than the ETA-selective ERA ambrisentan or the dual ETA/ETB ERA bosentan, a study was designed in which conscious hypertensive Dahl-S rats were given a single maximally effective dose of either ambrisentan or bosentan and then, at the time of maximal blood pressure decrease, a single administration of a maximally effective dose of macitentan. The maximally effective doses were selected based on hemodynamics (MAP) and ET receptor selectivity, which was determined by effect on plasma ET-1 concentrations. Thirty and 100 mg/kg were selected as the maximally effective doses of dual ERAs macitentan and bosentan, respectively. Thirty mg/kg was selected as the maximal ETA-selective dose of ambrisentan. Administration of ambrisentan 30 mg/kg on top of ambrisentan 30 mg/kg, or of bosentan 100 mg/kg on top of bosentan 100 mg/kg, had no additional effect on MAP. Macitentan 30 mg/kg, when given on top of ambrisentan 30 mg/kg, further decreased MAP by 17 mmHg ($p < 0.05$ vs vehicle) [Figure below]. Similarly, macitentan 30 mg/kg further decreased MAP by 19 mmHg when given on top of bosentan 100 mg/kg ($p < 0.01$ vs vehicle), Conversely, ambrisentan 30 mg/kg or bosentan 100 mg/kg given on top of macitentan 30 mg/kg failed to induce any additional MAP decrease.

Additive effect of macitentan on mean arterial blood pressure in conscious hypertensive Dahl-S rats treated with ambrisentan



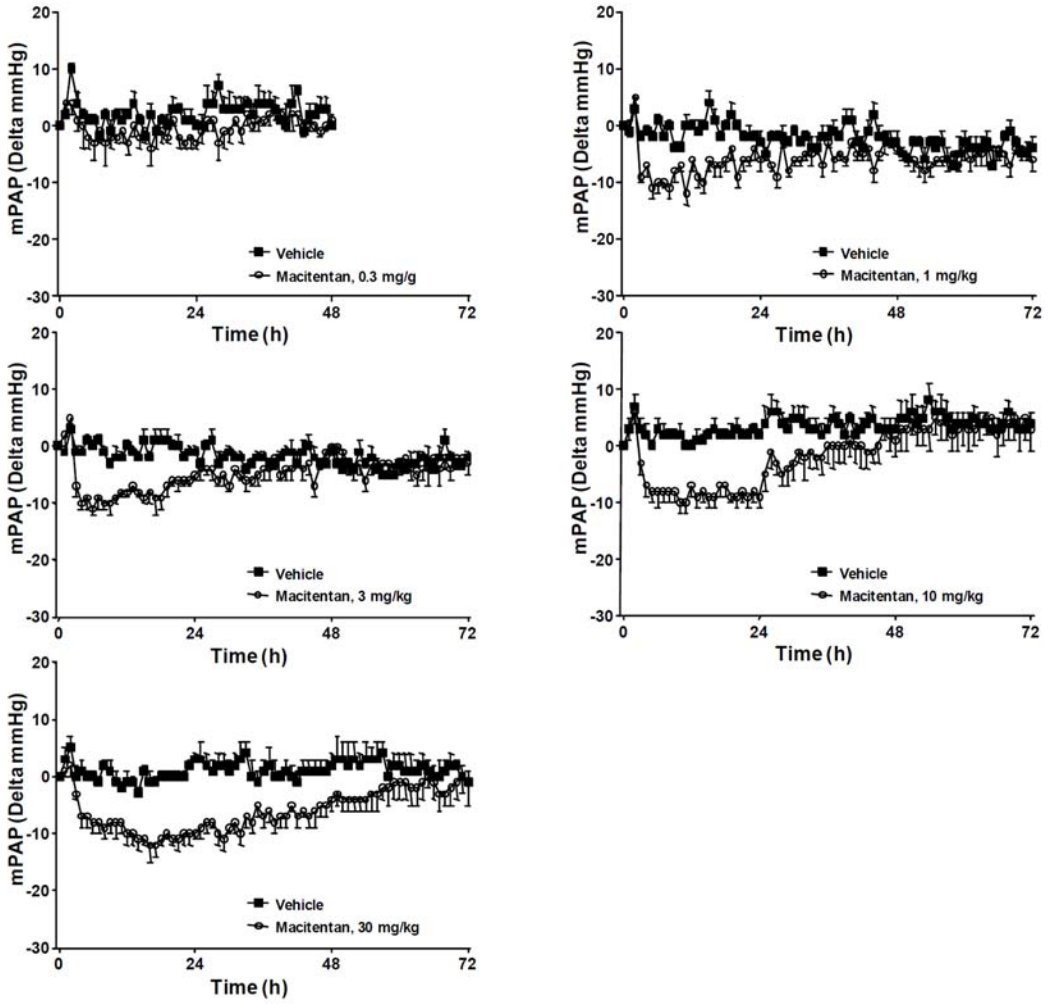
Additive effect of vehicle (Vehi), ambrisentan (Ambri, 30 mg/kg) or macitentan (Maci, 30 mg/kg) on top of ambrisentan 30 mg/kg on MAP in conscious Dahl-salt sensitive rats. Vehicle, ambrisentan, or macitentan were administered 6 h after single oral administration of vehicle or ambrisentan. MAP, mean arterial pressure. Data are presented as means ± SEM, n = 5–6. * $p < 0.05$, unpaired Student's t-test, ambrisentan + macitentan vs ambrisentan + vehicle. [B-12.110]

Effects of macitentan in rat models of pulmonary hypertension

Effect of a single oral administration of macitentan on mean pulmonary arterial pressure [B-12.111, B-12.109]

The effect of a single oral administration of macitentan on pulmonary arterial blood pressure and HR was evaluated in conscious Wistar rats with experimentally induced pulmonary hypertension (PH). Rats treated with monocrotaline develop PH accompanied by remodeling of pulmonary arteries and right ventricular hypertrophy. In rats with established PH due to monocrotaline treatment, oral administration of single doses of macitentan (0.3–30 mg/kg) dose-dependently decreased pulmonary arterial pressure without affecting HR [B-12.111]. At the maximally effective dose of 10 mg/kg macitentan, the maximal decrease in mPAP was 10 mmHg relative to vehicle-treated animals, and the effect lasted up to 48 hr [Figure below].

Effect of macitentan on mean pulmonary arterial pressure in monocrotaline-induced pulmonary hypertension in conscious Wistar rats



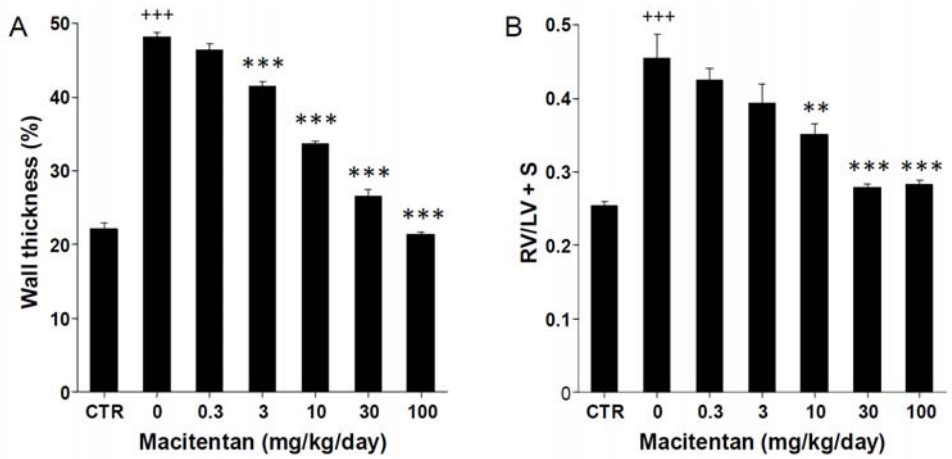
Vehicle: gelatin 7.5%, 5 mL/kg. mPAP, mean pulmonary arterial pressure. n = 5–12 per group. Data are presented as means ± SEM. [B-12.111]

The effect of a single oral administration of macitentan was also investigated in rats instilled with bleomycin [B-12.109]. While the bleomycin rat model has been primarily used to study pulmonary fibrosis, animals administered bleomycin also develop PH. Seven to nine days after a single intratracheal instillation of bleomycin (1.5 mg/kg), rats developed mild PH (mPAP ca. 30 vs 18 mmHg before bleomycin instillation) and were then treated orally with vehicle or macitentan (0.3–100 mg/kg). Similar to what was observed in monocrotaline-treated rats, macitentan dose-dependently decreased mPAP without increasing HR. The maximally effective dose of macitentan (10 mg/kg) decreased mPAP by 12 mmHg and the effect lasted up to 48 h. For comparison, oral administration of bosentan (3–600 mg/kg) caused a dose-dependent decrease in mPAP with a maximal effect of 8 mmHg observed at doses of 30 mg/kg and higher. The duration of bosentan action was less than 24 h at all doses.

Effect of chronic administration of macitentan on the development of right ventricular hypertrophy and survival in the rat model of monocrotaline-induced pulmonary hypertension [B-05.023]

The effect of macitentan (0, 0.3, 3, 10, 30, and 100 mg/kg/day, food admix, n = 15) was studied in the monocrotaline model of PH in Wistar rats and compared with the effect of bosentan (10, 30, 100, and 300 mg/kg/day, food admix, n = 15). Four weeks after a single monocrotaline injection (60 mg/kg, s.c.), mPAP measured under anesthesia had increased by 23 mmHg, a 2.4-fold increase relative to controls. Oral administration of macitentan for 4 weeks, beginning immediately after the monocrotaline injection, dose-dependently attenuated the monocrotaline-induced increase in mPAP. At the maximally effective dose of 30 mg/kg/day, the increase in mPAP was only 1.3-fold relative to controls ($p < 0.001$ vs vehicle). Monocrotaline causes pulmonary arterial hypertrophy, measured as an increase in medial wall thickness relative to external arterial diameter, as well as right ventricular hypertrophy, measured as an increase in the ratio of RV to LV+S. Repeated oral administration of macitentan dose-dependently inhibited the development of both pulmonary arterial and right ventricular hypertrophy at doses of 3 mg/kg and higher [Figure below]. In rats given 100 mg/kg/day, medial wall thickness was similar to values in controls, as was RV/LV+S in rats treated with 30 or 100 mg/kg/day (for both doses, $p < 0.001$ vs monocrotaline-treated rats administered vehicle). Bosentan treatment also reduced mPAP, medial wall thickness and RV/LV+S, but was 3- to 10-fold less potent than macitentan. Macitentan and bosentan had no effect on MAP or HR.

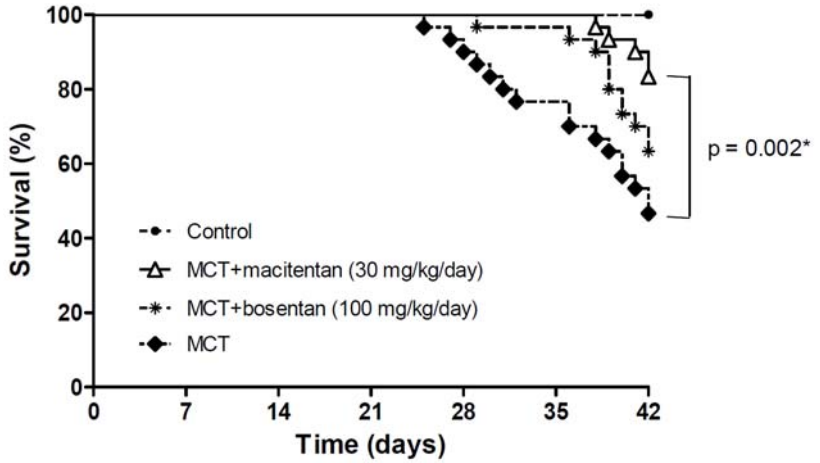
Effect of macitentan (4-week treatment) on pulmonary arterial wall thickness (A) and right ventricular hypertrophy (B) in monocrotaline-treated rats



CTR, control (n = 15); MCT, monocrotaline (n = 15). Wall thickness, %, ratio of pulmonary arterial total medial thickness to external diameter; RV, right ventricular weight; LV+S, left ventricular weight plus septal weight. Results are expressed as means ± SEM. +++, $p < 0.001$ vs control; **, $p < 0.01$, ***, $p < 0.001$ vs untreated MCT rats (oneway ANOVA with Student-Neuman-Keuls test). [B-05.023]

To determine the effect of chronic treatment on survival, rats were given a single monocrotaline injection (60 mg/kg, s.c.), and then treated with vehicle, macitentan at 30 mg/kg/day (food admix), or bosentan at 100 mg/kg/day (food admix). The study was terminated when 50% of vehicle-treated monocrotaline rats had died, after 42 days of treatment. Macitentan significantly increased the survival rate to 83%, ($p = 0.002$ vs control monocrotaline-treated rats), whereas the survival rate of rats receiving 100 mg/kg/day bosentan was 63%, not significantly different from the vehicle-treated monocrotaline group [Figure below].

Effect of macitentan and bosentan on survival of monocrotaline-treated rats

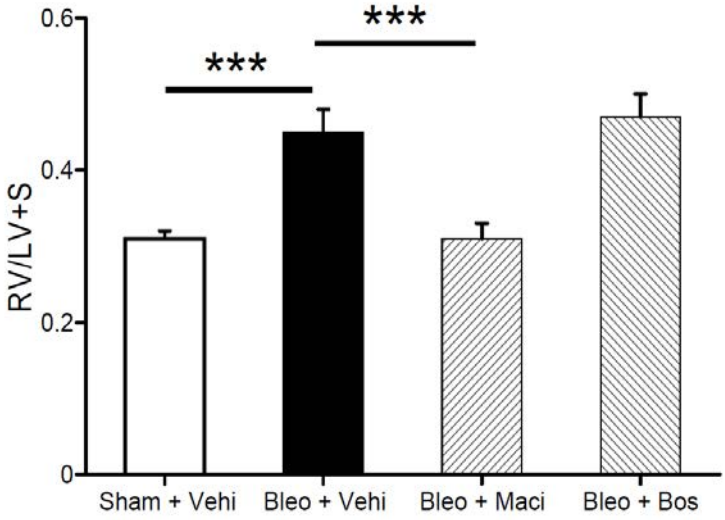


n = 10 for control rats, n = 30 for MCT (monocrotaline)-treated groups. The experiment was terminated when 50% of the untreated MCT rats had died, at 42 days. *Log-rank test, MCT plus macitentan vs MCT alone. [B-05.023]

Effect of chronic administration of macitentan on the development of right ventricular hypertrophy in the rat model of bleomycin-induced pulmonary hypertension and fibrosis [B-08.687, B-11.294]

The effect of orally administered macitentan (0, 0.3, 3, 30, and 100 mg/kg/day, gavage, n = 8–12) was evaluated in the rat model of PH and pulmonary fibrosis [B-08.687]. Eighteen days after a single intratracheal administration of bleomycin (1.5 mg/kg), right ventricular hypertrophy, measured as an increase in the ratio of right ventricular weight (RV) to left ventricular plus septal weight (LV+S) was determined. Oral administration of macitentan for 19 days, starting 1 day before bleomycin instillation, dose-dependently prevented development of right ventricular hypertrophy, with a significant effect observed at 100 mg/kg/day. Macitentan at 30 and 100 mg/kg/day significantly attenuated the bleomycin-induced increase in lung hydroxyproline content, a marker of collagen deposition. The effect of macitentan on the development of right ventricular hypertrophy was compared with the effect of bosentan in rats with bleomycin-induced PH [B-11.294]. Three-week treatment with macitentan (100 mg/kg/day) starting 1 day before instillation of bleomycin significantly decreased the development of right ventricular hypertrophy whereas bosentan (300 mg/kg/day) had no consistent effect [Figure below].

Effect of macitentan or bosentan (3-week treatments) on right ventricular hypertrophy in bleomycin-instilled rats



Wistar rats were instilled with saline and treated with gelatin 7.5% (Sham + Vehi, n=28) or instilled with bleomycin and treated with gelatin 7.5% (Bleo + Vehi, n=27), macitentan at a dose of 100 mg/kg/day (Bleo + Maci, n=18), or bosentan at a dose of 300 mg/kg/day (Bleo + Bos, n=16). RV, right ventricular weight; LV+S, left ventricular weight plus septal weight. Vehi, vehicle. ***, $p < 0.001$ (one-way ANOVA with Student-Neuman-Keuls test). [B-11.294]

4.2 SECONDARY PHARMACOLOGY

Selectivity screen [B-03.044]

To assess its interaction with other receptor systems and enzymes, macitentan was screened at a concentration of 10 µM in a panel of 63 radioligand binding assays. In none of these assays was more than 50% inhibition of radioligand binding observed in the presence of macitentan. Thus, macitentan can be considered selective for the human ETA and ETB receptors.

No other secondary pharmacodynamic studies have been conducted.

4.3 SAFETY PHARMACOLOGY

All Safety pharmacology studies were reviewed under IND 77,258. Investigations were performed *in vitro* or *in vivo* following oral administration of macitentan at pharmacological and supra-pharmacological doses. Macitentan had no apparent effect on physiological function of the central nervous or respiratory systems. Macitentan had no significant effect on cardiac repolarization, *in vitro* or *in vivo*.

Central nervous system

Modified Irwin screen test [T-04.051]

The modified Irwin screen test in the rat was performed to evaluate the effect of macitentan on a battery of behavioral and physiological (reflex measurement) variables covering the main central and peripheral nervous system functions. The evaluation was performed in a blinded fashion. Four groups of male Sprague Dawley rats (6 rats/group) received a single oral administration (by gavage) of either 0 (vehicle), 1, 10, or 100 mg/kg macitentan. A fifth group of rats (n = 6) was not dosed (naïve rats). Rats were assessed for behavioral changes up to 24 h after dosing. Macitentan had no relevant effect on spontaneous activity, excitability, or sensorimotor, autonomic, neuromuscular, or physiological functions in the rat at any dose. Macitentan also had no effect on body temperature. Thus, the administration of a single oral dose of macitentan at 1, 10, or 100 mg/kg does not modify behavioral or physiological nervous system function in the rat modified Irwin test.

Respiratory system

Respiratory function evaluated by whole body plethysmography [T-04.050]

The effects of macitentan on the respiratory system were investigated by whole-body plethysmography in conscious, unrestrained male Wistar rats. Five groups of eight rats received a single oral administration of either 0 (vehicle), 1, 10, or 100 mg/kg macitentan, or a positive control (morphine, 5 mg/kg intraperitoneally). Changes in

respiratory variables (respiratory rate, tidal volume, inspiratory time, expiratory time, peak inspiratory flow, peak expiratory flow, and relaxation time) were continuously recorded up to 240 min after administration. Macitentan did not affect measures of respiratory function at any of the doses tested. The positive control morphine increased respiratory rate and markedly shortened the inspiratory time, with consequent large increases in relaxation time and peak inspiratory flow. Macitentan did not induce relevant changes in respiratory function in rats.

Cardiovascular system

The effects of macitentan on cardiac repolarization were tested in anesthetized guinea pigs and in conscious Beagle dogs implanted with telemetry equipment. The studies in telemetered dogs also allowed the evaluation of the effects of macitentan on blood pressure and HR. Additionally, an *in vitro* hERG channel assay was performed with macitentan and ACT-132577.

hERG channel assay [T-03.019, T-04.106]

Potassium currents were measured by the whole-cell patch-clamp technique in hERG transfected CHO cells, before and after application of macitentan at a concentration of 10 µM (5880 ng/mL) [T-03.019]. Macitentan reduced the current ~ 18% at this concentration. This effect was reversible upon washout. The positive control terfenadine completely inhibited the potassium current, with an IC_{50} of 0.026 µM.

The effect of the metabolite ACT-132577 on potassium currents through hERG channels expressed in HEK293 cells was investigated using the patch clamp technique [T-04.106]. At concentrations up to 10 µM (5460 ng/mL), ACT-132577 had no effect on repolarizing currents through hERG K+ channels. At higher concentrations, ACT-132577 blocked both inward and outward currents slightly (IC_{20} ~ 18 µM, IC_{50} ~ 71 µM).

In healthy subjects administered 10 mg macitentan/day, mean Cmax values on Day 10 were 371 ng/mL (macitentan) and 802 ng/mL (ACT-132577) [D-06.044]. Due to high plasma protein binding of 99.6% and 99.5% for macitentan and ACT-132577, respectively, the free concentrations at Cmax were 1.5 ng/mL of macitentan and 4 ng/mL of ACT-132577. Overall, macitentan and its circulating metabolite ACT-132577 caused < 20% inhibition of hERG-mediated potassium currents up to a concentration of 10 µM, approximately 2000-fold higher than the unbound plasma concentrations of these two compounds at therapeutic doses.

Effects on electrocardiogram intervals in guinea pigs [B-04.032]

The effects of macitentan on ECG intervals and HR were studied in anesthetized guinea pigs. Following baseline measurements, guinea pigs randomly received an i.v. bolus injection of either macitentan (10 mg/kg, n = 6) or dofetilide (0.08 mg/kg, n = 4), a class III antiarrhythmic, as positive control. Macitentan did not alter HR or ECG intervals. In contrast, dofetilide decreased HR by 12% and increased QT by 20%, RR by 15%, and

QTc by 12%. Overall, an i.v. bolus injection of macitentan did not affect ECG intervals in guinea pigs.

Effects on electrocardiogram intervals and arterial blood pressure in conscious dogs [T-04.060, T-07.142]

The effects of a single oral administration of macitentan on arterial blood pressure, HR, ECG variables, and body temperature were examined in conscious, freely moving Beagle dogs equipped with telemetry [T-04.060]. Three male and three female dogs received 0, 1, 5, and 25 mg/kg macitentan (oral, capsule) in a crossover design with a minimum washout period of 7 days between dosing. Measurements were recorded for at least 2 h before and for 24 h after administration. Macitentan at 1, 5, and 25 mg/kg induced a statistically significant decrease in systolic, diastolic, and mean arterial blood pressure. The blood pressure decrease was maximal 3 h after treatment and in the range of 10 to 16 mmHg, independent of dose. Treatment with macitentan had no effect on HR, atrioventricular and intraventricular conduction velocities, RR and PR intervals, or the duration of the QRS complex at any dose. There was no effect on either QT or QTc intervals, indicating that the administration of macitentan at oral doses up to 25 mg/kg has no effect on ventricular repolarization.

A second telemetry study in Beagle dogs was performed in order to investigate the effect of macitentan on blood pressure, heart rate, and ECG parameters at lower dose levels [T-07.142]. Three male and three female dogs received 0, 0.1, 0.3, 1.0, 5.0, and 30 mg/kg macitentan (oral, capsule) in a crossover design with a washout period of 7 days between dosing. Measurements were recorded for 24 h before and for 48 h following administration. Macitentan induced dose-dependent decreases in systolic, diastolic, and mean arterial blood pressure that attained statistical significance at and above 0.3 mg/kg. The maximum decrease in MAP was 17 mmHg at 5 and 30 mg/kg. At these doses, exposure was 9- to 40-fold the human exposure at 10 mg/day. Heart rate expressed as mean bpm was only slightly increased at 5 and 30 mg/kg. When HR was evaluated as area under the HR vs time curve, the increase reached statistical significance at these dose levels. Treatment with macitentan had no effect on cardiac conduction times (PR, PQ, and QT interval duration with and without corrections and duration of the QRS complex) at any dose.

PHARMACODYNAMIC DRUG INTERACTIONS

Combination of macitentan and phosphodiesterase 5 inhibitor

The acute hemodynamic effect of combined treatment with macitentan and a phosphodiesterase 5 inhibitor was investigated in conscious Dahl-S and spontaneously hypertensive rats. Blood pressure was monitored by telemetry for up to 72 h after oral administration of 0.3 mg/kg macitentan and either 10 mg/kg tadalafil or 30 mg/kg sildenafil. The blood pressure-lowering effect of treatment was expressed as the area between curves (ABC, blood pressure vs time) before and after treatment, measured in the same animals. The ABC after combined treatment was greater than the sum of the

effects of each treatment given alone, indicating a synergistic increase in the duration of the blood pressure-lowering effect.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Pharmacokinetic profile of macitentan in the rat and dog

Methodology

The single-dose pharmacokinetic profile of macitentan was characterized in Wistar rats (n=4–5) and Beagle dogs (n=3) [B-04.023]. Intravenous doses were administered in the range of 0.1–3 mg/kg, while oral pharmacokinetics were assessed over a range of 0.3–30 mg/kg. Intravenous doses for rat experiments were formulated as TRIS-buffered aqueous solutions (50 mM, pH 8.3) containing 45% PEG400, whereas formulations for the dog contained 25% PEG400, 25% N-methylpyrrolidone and 50% aqueous TRIS buffer. Oral formulations in both species were suspensions of (b) (4) or (b) (4) material in 7.5% modified gelatin or solutions in PEG400. Additional experiments were performed to evaluate the effects of food and sex. Enterohepatic recycling was assessed in a bile duct-cannulated tandem rat model [section 3.1.5]. A summary of the main pharmacokinetic parameters is provided in the following Tables [Report B-04.023].

Absorption and bioavailability

In the rat, macitentan was absorbed slowly with a median Tmax of 6 h at doses up to 10 mg/kg. Oral absorption was further delayed at 30 mg/kg. In the dog, oral absorption was faster and independent of dose, with a median Tmax of 2 h. Oral bioavailability in the rat was about 30% at doses up to 3 mg/kg and then increased up to 89% at 30 mg/kg. The increase in exposure with increasing dose may be the result of saturation of clearance pathways. In the dog, oral bioavailability was around 80% at all doses. Clearance in the dog is about 20% of liver blood flow and thus oral bioavailability seems to be limited by clearance. Additional experiments in the rat and dog were also performed comparing a macitentan solution in PEG400 to suspensions of (b) (4) or (b) (4) macitentan in gelatin. In the rat, oral exposure was increased by 2.5-fold with the solution and by 2.1-fold with (b) (4) study material, both compared to (b) (4) material. A similar 2.6-fold increase in exposure was observed in the dog when macitentan was formulated as a PEG400 solution and compared to a suspension. All these data point to dissolution/solubility as key determinants in the oral absorption of macitentan.

Dose proportionality

After intravenous dosing, macitentan exhibited linear pharmacokinetics in the rat and dog over the entire dose range investigated. After oral dosing to the rat, exposure to macitentan deviated from linearity at 10 mg/kg and above, resulting in increases in oral

bioavailability from 30% at 1 and 3 mg/kg to 89% at 30 mg/kg. Saturation of metabolic clearance may account for the observed non-proportionality in the rat. In contrast, exposure to macitentan after oral dosing to the dog increased in a dose-proportional fashion.

Effect of food

The effect of food on the pharmacokinetic properties of macitentan was investigated in the rat and dog at a dose of 3 mg/kg formulated as suspension in gelatin. A comparison between fasted and fed rats indicated a more rapid absorption of macitentan in the fed state. Differences in the dissolution properties in the presence of food and changes in the gastric emptying time might contribute to the effect. A suspension of macitentan was administered to dogs in the fed or fasted state. While mean exposure to macitentan in the dog was only about 15% higher in the fed state, the variability in exposure was significantly higher in the presence of food.

Sex differences

Exposure to macitentan was 4- to 5-fold higher in female as compared to male rats after intravenous and oral dosing. This most likely reflects the lower metabolic turnover in female rats as also observed *in vitro* with liver microsomes and hepatocytes [B-04.022, B-04.093]. Sexual dimorphism in terms of metabolic competence is a well-documented phenomenon in the rat and has been observed with a variety of drugs. However, this observation in the rat has generally no relevance for man. No such sex difference was evident in the dog as judged from the toxicokinetic data of the 4-week toxicity study [T-04.049].

Enterohepatic recirculation

Conjugates, of macitentan and ACT-132577 with glucose and glucuronic acid, represent a major fraction of the overall metabolite pool in rat bile. These conjugates are typically cleaved again by the gut microflora, and the liberated aglycones, macitentan and ACT-132577, then become available for possible enterohepatic recirculation. The extent of such recirculation was investigated using the tandem rat model [B-05.130]. Enterohepatic recirculation of parent macitentan was not observed in the rat and only contributed to a minor extent to the pharmacokinetics of ACT-132577. Its overall role in the pharmacokinetic profile of macitentan is therefore considered negligible.

Pharmacokinetic parameters of macitentan in Wistar rats and Beagle dogs after a single intravenous administration

		Rat [1]		Dog [2]	
Parameter	Dose (mg/kg)	Mean	Range	Mean	Range
$AUC_{0\text{-inf}}$	0.1	274	159–333	319	291–335
[ng·h/mL]	0.3	611	557–664	908	710–1040
	0.3 (female)	2990	1470–4950		
	1	2680	1660–3590	3170	2570–3500
	3	10900	4570–24900	10800	10300–11100
CL	0.1	6.5	5.0–10	5.2	5.0–5.7
[mL/min·kg]	0.3	8.2	7.5–9.0	5.7	4.8–7.0
	0.3 (female)	2.0	1.0-3.4		
	1	6.7	4.6–10	5.4	4.8–6.5
	3	6.7	2.0-11	4.6	4.5–4.9
V_{ss}	0.1	1.6	1.1–2.2	1.2	0.9–1.5
[L/kg]	0.3	1.2	1.1–1.6	1.1	0.9–1.3
	0.3 (female)	1.4	1.0–2.0		
	1	1.8	1.1–3.3	0.9	0.7–1.1
	3	1.6	0.9-2.0	0.7	0.6–0.8
$T_{1/2}$	0.1	3.8	1.7–5.8	3.9	3.0–4.4
[h]	0.3	1.9	1.7–2.3	4.3	3.3–5.2
	0.3 (female)	8.9	6.6–12		
	1	5.1	3.6–7.7	4.1	3.8–4.5
	3	3.7	3.2-5.0	3.5	3.5–3.6

[1] n = 5; [2] n = 3. All experiments in male animals if not otherwise stated.

Pharmacokinetic parameters of macitentan in male Wistar rats and Beagle dogs after a single oral administration

Parameter	Dose (mg/kg)	Rat [1]			Dog [2]		
		Feeding state	Mean[4]	Range	Feeding state	Mean[4]	Range
$AUC_{0\text{-}inf}$ [3] [ng·h/mL]	0.3				fed	779	519–1020
	1	fed	1050	666–1770	fed	2370	1590–2940
	3	fed [7]	3240	1040–6200	fed	8770	5260–11600
	3	fed (f) [6]	18400	11000–28600	fasted [7]	6170	5880–6310
	3	fed [2]	3800	1280-6360	fed [7]	6990	3570–12200
	10	fed	18500	10900–29700	fed	27500	20100–32100
	30	fed	97400	82500–139000	fed		
C_{max} [ng/mL]	0.3				fed	137	70.2–179
	1	fed	175	55.7–293	fed	408	194–523
	3	fed	383	75.7–725	fed	1450	525–2050
	3	fed (f) [6]	851	625–1230	fasted [7]	855	737–987
	3	fed	531	80.8-1170	fed [7]	1020	671–1630
	10	fed	1670	1140–2140	fed	4590	2180–7240
	30	fed	7300	5550–8960	fed		
T_{max} [4] [h]	0.3				fed	2.0	2.0–3.0
	1	fed	6.0	4.0–8.0	fed	2.0	2.0–3.0
	3	fed	6.0	6.0–8.0	fed	2.0	1.0–3.0
	3	fed (f) [6]	8.0	8.0–8.0	fasted [7]	4.0	2.0–4.0
	3	fed	8.0	0.25-8.0	fed [7]	2.0	2.0–2.0
	10	fed	6.0	4.0–8.0	fed	2.0	1.0–3.0
	30	fed	16	8.0–24	fed		
F [5] [%]	0.3				fed	88	53–100
	1	fed	29	–	fed	77	46–100
	3	fed [7]	30	–	fed	81	51–100
	3	fed (f) [6]	62	–	fasted [7,8]	39	36–42
	3	fed [2]	35	–	fed [7,8]	44	24–79
	10	fed	51	–	fed	76	59–87
	30	fed	89	–	fed		

[1] n=5, macitentan formulated as suspension in modified gelatin unless stated otherwise; [2] n=3, macitentan formulated as solution in PEG400 unless stated otherwise; [3] $AUC_{0\text{-}last}$ is given for the 1 mg/kg dose; [4] median is given for T_{max}; [5] calculated using intravenous AUC at 3 mg/kg, except for female rats where the 0.3 mg/kg dose was used; [6] (f)=female, all other experiments were performed with males; [7] formulated as a suspension in modified gelatin; [8] relative bioavailability compared to PEG400 solution.

Multiple-dose pharmacokinetics

The pharmacokinetics of macitentan after multiple oral dosing were assessed as part of the toxicokinetic monitoring of the two 4-week toxicity studies in the rat and dog [T-04.043, T-04.049]. In mice, multiple oral dosing was assessed as part of the 13-week toxicity studies in the CD-1 and B6C3F1 strains [T-07.208, T-08.388]. Macitentan exposures in male animals are summarized in the Table below. The corresponding accumulation factors were calculated with reference to the first day of dosing.

Pharmacokinetic parameters of macitentan in male rats, dogs, and mice after multiple oral administrations

Species	Dose (mg/kg)	$AUC_{0-24\ h}$ (µg·h/mL)			Accumulation factor	
		Day 1	Day 7	Day 26/27	Day 1 – Day 7	Day 1 – Day 26/27
Rat	50	213	122	114	0.57	0.53
	150	382	183	200	0.48	0.52
	450	595	343	327	0.58	0.55
	1500	979	443	365	0.45	0.37
Dog	5	17.5	11.7	14.5	0.67	0.83
	50	134	46.5	39.1	0.35	0.29
	500	692	148	-- [(1)]	0.21	--

Species (strain)	Dose (mg/kg)	$AUC_{0-24\ h}$ (µg·h/mL)			Accumulation factor	
		Day 1	Week 3	Week 13	Day 1-Week 3	Day 1-Week 13
Mouse	5	13.5	23.7	19.7	1.8	1.5
(CD-1)	20	62.1	68.2	75.5	1.1	1.2
	75	122	159	224	1.3	1.8
Mouse	10	84.4	--	66.0	--	0.8
(B6C3F1)	50	357	--	307	--	0.9
	400	1120	--	653	--	0.6
	1500	1290	--	1090	--	0.9

[(1)] no data available on day 26 due to a reduction of dose to 250 mg/kg/day. Values are means of 6 rats/sex/dose, 3 dogs/sex/dose, or 4–6 mice/sex/dose/time point. Macitentan was formulated as a suspension in modified gelatin or methylcellulose for the studies in rat and mouse. In the dog study, macitentan was given in capsules.

After multiple dosing, no accumulation but rather a decrease in macitentan plasma concentrations was observed in rat and dog on study days 7 and 26/27 as compared to day 1. Exposure to macitentan was reduced by about 50% at all doses in the rat after 7 days of administration and stabilized thereafter. In the dog, the magnitude of decrease was dose-dependent, with a 33% reduction at the lowest dose of 5 mg/kg and a 79% reduction at 500 mg/kg. Mechanistic studies have identified macitentan as a microsomal enzyme inducer in both species and the decrease in plasma concentrations can thus be explained by auto-induction of macitentan metabolism. Macitentan did not exhibit auto-induction in the mouse. Exposure in the two 13-week toxicity studies in CD-1 and B6C3F1 mice revealed no indication of time-dependent changes in macitentan exposure upon repeated dosing. While a 1.1- to 1.8-fold accumulation was observed in CD-1 mice after 13 weeks of treatment at daily doses up to 75 mg/kg, the corresponding accumulation factors ranged from 0.6-0.9 in the B6C3F1 mice. In neither mouse strain was the extent of accumulation dependent on dose. Despite the absence of macitentan auto-induction, up-regulation of some P450 enzymes was also seen in the mouse [Reports T-04.043, T-04.049, T-07.208, and T-08.388].

Pharmacokinetics of ACT-132577 and ACT-080803

Methodology:

The pharmacokinetic properties of the pharmacologically active metabolite ACT-132577 were investigated in the rat at intravenous and oral doses of 0.5 and 3 mg/kg, respectively [B-05.130]. For the hydrolysis product ACT-080803, pharmacokinetic data were generated after oral dosing at 3 mg/kg. Serial blood samples of 0.25 mL each were taken from male Wistar rats (n=6) over a period of 56 h. Analysis of ACT-132577 and ACT-080803 in plasma was performed using LC-MS/MS.

Results

ACT-132577 exhibited a systemic plasma clearance of 1.3 mL/(min·kg) and a volume of distribution of 1.0 L/kg, indicating good distribution into tissues. The apparent terminal half-life was 8.7 h. Oral absorption was slow and peak plasma concentrations were reached after 8 h. Oral bioavailability was 41%. The longer plasma half-life of metabolite ACT-132577 is likely the result of its significantly lower plasma clearance. ACT-080803 was observed in feces of dog and rat as a result of non-enzymatic hydrolysis of macitentan and ACT-132577 in the gut. Its potential for intestinal absorption was investigated in the rat [B-05.130]. Plasma concentrations in this experiment were below the quantification limit at all time points, suggesting that ACT-080803 is not re-absorbed from the gut [Report B-05.130].

Assessment of intestinal permeability *in vitro*

Methodology

The transmembrane permeability of macitentan was investigated *in vitro* using the Caco-2 model and ^{14}C-labeled macitentan (ACT-064992B) at concentrations of 1 and 10 µM [B-05.040]. Caco-2 cells were cultured at passage number 31 and transport experiments were performed on day 27 post-seeding. The integrity of cell monolayers was checked by two independent methods, i.e., measurement of transepithelial electrical resistance and functional measurements using radiolabeled D-mannitol as a low permeability marker. ACT-064992B or ^{3}H-digoxin at single concentrations of 10 µM and 1 µM, respectively, were added to either the apical or basolateral compartment. After approximately 3–4 h of incubation at 37 °C, aliquots were taken from both compartments and total radioactivity quantified using liquid scintillation counting. To assess the effect of a known P-gp inhibitor on the permeability of macitentan, additional experiments were performed with verapamil at a single concentration of 20 µM. The results of these experiments are summarized below.

Results

Macitentan exhibited apparent permeability coefficients (Papp) of 11–13 x 10-6 cm/s in the A–B transport direction at the two investigated concentrations of 1 and 10 µM, indicating good permeation through the Caco-2 cell monolayer. The Papp values in the opposite B–A direction were 21–22 x 10-6 cm/s, indicating an about 2-fold difference in flux rates. Addition of 20 µM verapamil as a P-gp inhibitor had no effect on the A–B transport rates, ruling out a role of this efflux pump in the intestinal absorption of macitentan. The observed differences in Papp values might originate from the pH gradient between the two compartments as macitentan is a weak acid with a pKa of 6.2. Based on these *in vitro* data, it is not expected that the pharmacokinetic profile of macitentan in man is limited by its permeation in the gastro-intestinal tract.

Apparent permeability coefficients, Papp, of ^{14}C-radiolabeled ACT-064992B in the absence or presence of verapamil

Transport direction	ACT-064992B [µM]	Verapamil [µM]	P_{app} (1) [10^{-6} cm/s]	Recovery [%]
A–B (2)	1	--	11 ± 1	82
B–A	1	--	21 ± 0	90
A–B	1	20	9 ± 1	75
A–B	10	--	13 ± 0	81
B–A	10	--	22 ± 0	87
A–B	10	20	13 ± 0	76

(1) mean ± SD (n = 3); (2) A = apical compartment, B = basolateral compartment

Distribution

Volume of distribution

The volume of distribution of macitentan as a general measure for the extent of tissue distribution was determined in the rat and dog after intravenous administration [B-04.023]. Results are summarized in the following Table. The volume of distribution at steady state (Vss) was 1.2–1.8 L/kg in the rat, i.e., in excess of total body water volume. In the dog, Vss was a function of dose and decreased from 1.2 L/kg at 0.1 mg/kg to 0.7 L/kg at 3 mg/kg, pointing to changes in the distribution process at higher doses.

Tissue distribution

Methodology

The tissue distribution of ^{14}C-radiolabeled ACT-064992B was initially investigated in a pilot study with male albino and pigmented rats at an oral dose of 3 mg/kg using quantitative whole-body autoradiography [B-05.086]. One albino and one pigmented animal were assessed per time point. The time course of radioactivity in the different

organs was monitored over a period of 7 days. Using the data of the pilot study, a second study was conducted with an extended sampling period of 28 days, using one pigmented or two albino animals per time point [B-09.442]. The lower limit of quantification was 0.012 µg equiv/g.

Results

In the pilot study, the extent of absorption and subsequent distribution of radioactivity into tissues was largely similar between albino and pigmented rats. Radioactivity was well absorbed and widely distributed into most tissues. The highest radioactivity in all tissues was present at the first sampling point at 8 h and declined steadily thereafter. At 7 days after dosing, half of the organs and tissues, including blood, still contained quantifiable levels of radioactivity. Liver, kidney cortex, lung, and myocardium were among the organs with the highest exposures. Radioactivity levels in the uveal tract were only slightly higher in pigmented animals, indicating a minor degree of binding of drug-related material to melanin. Similar results were observed in the main study, as radioactivity was rapidly absorbed and widely distributed. Concentrations of radioactivity in the majority of tissues at early sampling times were below that in plasma, with only the liver and kidney (principally the cortex) containing higher levels. At later sampling times, concentrations in over half of the tissues were above that in plasma. At most sampling times, the highest concentrations of radioactivity were found in the liver, kidney cortex, plasma, blood, and lung. The tissues containing the lowest levels of radioactivity were the body of the lens, brain and spinal cord, white fat, testis, and seminal vesicles. Quantifiable levels of radioactivity were present in many tissues at 7 days, with levels in the kidney and liver remaining above this limit at 14 days and in the kidney cortex at 28 days. There was no obvious difference in tissue distribution between albino and pigmented animals although tissue half-lives were slightly longer in pigmented animals. Radioactivity in melanin-containing tissues was slightly above that in albino animals, suggesting some minor binding to melanin.

Plasma protein binding and partitioning into red blood cells

Methodology

Plasma protein binding

Binding of macitentan and ACT-132577 to plasma proteins was determined for rat, mouse, rabbit, dog, and man using equilibrium dialysis and the ^{14}C-radiolabeled analogues ACT-064992B and ACT-132577B in a concentration range from of 0.1 to 300 µg/mL [B-04.025, B-07.077]. Equilibrium dialysis was performed at 37 °C against 0.02 M phosphate-buffered saline (pH 7.4) for 6 h. Aliquots taken from both donor and buffer compartments at the 300 µg/mL concentration were pooled by species and subjected to radio-HPLC to determine the nature of the free fraction. For metabolite ACT-373898, plasma protein binding was determined using rapid equilibrium dialysis and LC-MS/MS [B-12.256]. Two independent sets of experiments were performed with plasma of rat, mouse, dog, and man at the two concentrations of 1 and 10 µM.

Binding to human serum albumin and α1-acid glycoprotein

Solutions of human serum albumin at a concentration of 0.6 mM and α1-acid glycoprotein at a concentration of 20 µM were prepared in 0.02 M phosphate-buffered saline []. Aliquots were fortified with ACT-064992B at concentrations of 0.1, 0.3, 1, 3, and 10 µg/mL, and the free fraction was determined by equilibrium dialysis and liquid scintillation counting.

Partitioning into red blood cells

Fresh whole-blood samples were prepared containing ACT-064992B and ACT-132577B at target concentrations of 0.5 and 100 µg/mL [B-04.025, B-07.077]. Duplicate blood samples from each species were incubated at 37 °C for 2 h. Following incubation, samples were taken to measure total radioactivity in whole blood, to determine packed cell volume, and to prepare plasma. Duplicate aliquots of blood and plasma samples were analyzed by liquid scintillation counting. Aliquots, from the experiments using 100 µg/mL ACT-064992B or ACT-132577B, were also subjected to radio-HPLC to determine the chemical nature of the radioactivity.

Results

The binding of macitentan and ACT-132577 to plasma proteins and their blood-to-plasma partitioning were determined *in vitro* for concentrations from 0.1 to 300 µg/mL with plasma of rat, mouse, rabbit, dog, and man. For ACT-373898, plasma protein binding was determined for rat, mouse, dog, and man at concentrations of 1 and 10 µM. An overview of the plasma protein binding results for all three compounds is given in the Table below.

Plasma protein binding of macitentan and its metabolites ACT-132577 and ACT-373898

	Fraction unbound (%)		
Species	macitentan	ACT-132577	ACT-373898
Mouse	0.4	1.0	5.6
Rat	0.6	1.3	3.7
Rabbit	0.1	0.1	ND
Dog	0.9	1.7	9.0
Man	0.4	0.5	1.5

ND: not determined

Plasma protein binding of macitentan was > 99% in all species. Free fractions differed by 9-fold between rabbit and dog, whereas in rat, mouse, and man they were all in the range of 0.4–0.6%. Plasma protein binding of metabolite ACT-132577 varied from 98.3–99.9%. Similar to macitentan, the highest binding was observed in plasma of rabbit and man, with free fractions of 0.1% and 0.5%, respectively. Binding of ACT-132577 in all other species was lower compared to macitentan. Plasma protein binding of ACT-

373898 was the same at the two concentrations of 1 µM and 10 µM. The free fraction ranged from 3.7% in the rat to 9.0% in the dog and was 1.5% in man.

Binding studies of macitentan with human serum albumin and α1-acid glycoprotein indicated binding to both plasma proteins with mean values of 98.9% and 91.6%, respectively. Saturation of macitentan binding to α 1-acid glycoprotein was evident at concentrations above 3000 ng/mL. Considering the relative abundance of the two proteins in blood, i.e., 600 µM vs 20 µM, serum albumin represents the major binding component of macitentan in man.

Partitioning studies were performed at concentrations 0.5 and 100 µg/mL of ACT-064992B or ACT-132577B in blood. The mean blood/plasma ratios were in the range of 0.53–0.73 for macitentan, and 0.54–0.69 for ACT-132577, indicating limited partitioning of either compound into red blood cells.

Distribution into milk

Methodology: A single ACT-064992B dose of 3 mg/kg nominally containing 8 MBq/kg was administered to female Wistar rats (n=3) on approximately day 10 post-partum. A control animal (n=1) received vehicle only [B-11.837]. To induce milk production, each dam received subcutaneous oxytocin. Milk samples were collected from the control animal and each of three dosed rats at 1, 4, 8, 24, 48, and 96 h after dosing. Milk and plasma samples were collected in triplicate and the number and nature of metabolites determined in milk and plasma using HPLC with radiodetection. Technical controls included stability assessments of ACT-064992B in rat milk and plasma under the conditions of sample work-up.

Results: The time course of total radioactivity in milk and plasma is illustrated in Table 6. Radioactivity was detectable in both sample types over the entire collection period. Peak concentrations of total radioactivity in milk and plasma were observed at 4 h post-dose, in line with the pharmacokinetic profile of macitentan in the rat. Concentrations of total radioactivity in milk were generally below those in plasma, with milk to plasma ratios ranging from 0.32–0.57 in the first 24 h post-dose. Thereafter, milk/plasma ratios appeared to shift in favor of milk, reaching a maximum of 2.0 after 96 h.

Table 6 Total radioactivity in milk and plasma of lactating Wistar rats

Time (h)	Total radioactivity (ng equiv/g) (1)		Ratio
	Milk	Plasma	Milk : plasma
1	530 ± 362	1490 ± 597	0.32 ± 0.14
4	1300 ± 17	2810 ± 227	0.47 ± 0.04
8	1230 ±162	2230 ± 497	0.57 ± 0.17
24	273 ± 100	598 ± 106	0.46 ± 0.14
48	71.8 ± 22.5	78.4 ± 32.9	0.95 ± 0.13
96 (2)	10.3	5.10	2.0

(1) data are means ± SD (n=3); (2) n=2 only, as one animal received an incorrect dose

Metabolic profiles recorded from milk and plasma were qualitatively similar. Macitentan and ACT-132577 were observed consistently in both sample types, together with the hydrolysis product ACT-080803. Small amounts of metabolites M4, M16, M18, and M19 were observed at 24 h and 48 h post-dose only. M4 was present as a minor component in plasma of female rats, whereas it was absent in male animals [B-05.001]. Metabolites M16, M18, and M19 have not been observed in non-lactating animals. Changes in the expression levels of drug-metabolizing enzymes during pregnancy and lactation are described in the literature and might contribute to the observed differences. Control experiments have identified ACT-080803 in plasma as a likely artifact of sample work-up.

METABOLISM

Systemic plasma clearance

Plasma clearance was determined after intravenous dosing in the rat and dog in a dose range from 0.1–3 mg/kg [B-04.023]. In general, exposure after intravenous administration in male rats and dogs was linear within the dose range tested. Dose-corrected AUC values for 0.1 mg/kg and 3 mg/kg in the rat were 1.0 and 1.4, respectively, while the ratios were around unity at both doses in the dog. Plasma clearance ranged from 6.5–8.2 mL/(min·kg) in the rat, and 4.6-5.7 mL/(min·kg) in the dog. Considering the respective blood/plasma ratios, blood clearance is approximately 20% of the liver blood flow in both species. For ACT-132577, plasma clearance in the rat at a dose of 0.5 mg/kg was 1.3 mL/(min·kg), i.e., considerably lower compared to macitentan and likely contributing to the longer plasma half-life of the metabolite.

Metabolism of macitentan *in vitro*

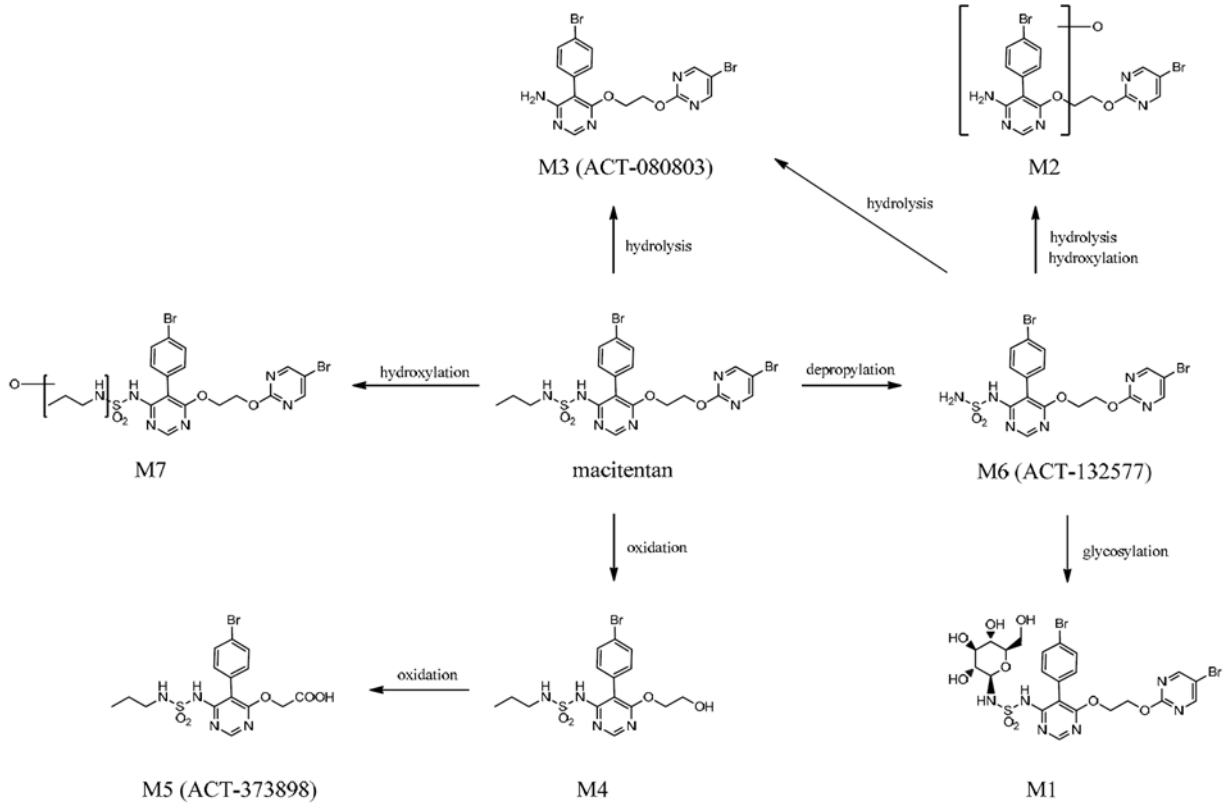
Methodology: The *in vitro* metabolism of macitentan has been characterized with liver microsomes, hepatocytes, and liver S9 fractions of all species used in the non-clinical safety assessment and man [B-04.022, B-04.093, B-04.099]. The ^{14}C-radiolabeled ACT-064992B was incubated with the different liver preparations at a single concentration of 10 µM, and metabolic profiles were recorded using HPLC coupled to radiodetection. Metabolite structures were identified with LC-MS/MS technology and the use of chemical reference compounds [B-07.372]. Experiments with rat and human liver S9 fractions were performed in support of the genotoxicity assessment of macitentan.

Results: The proposed metabolic scheme of macitentan with human liver preparations is depicted in the Figure below. Macitentan undergoes two types of biotransformation reactions. Hydroxylation of the propyl chain at the carbon atom adjacent to the sulfamide function followed by non-enzymatic hydrolysis of the resulting hemi-aminal leads to the pharmacologically active metabolite M6 (ACT-132577), whereas hydroxylation at one of the distal carbon atoms – the exact site of hydroxylation is unknown – yields M7.

Oxidative cleavage of the ethylene glycol linker, catalyzed by microsomal proteins, leads to the alcohol M4. In hepatocytes, M4 is further oxidized to the corresponding acid M5 (ACT-373898). Moreover, M6 undergoes conjugation with glucose in hepatocytes to yield M1. Both macitentan and M6 undergo non-enzymatic hydrolysis of the sulfamide to the aminopyrimidine M3 (ACT-080803). Another metabolite, M2, is the product of combined microsomal oxidation and hydrolysis.

The chemical nature of all above metabolites was elucidated with the help of LC-MS/MS technology and with authentic chemical references for metabolites M3, M5, and M6. The *in vitro* metabolic pattern of macitentan was well conserved across species. In microsomal incubations, formation of M3 and M6 represented the major metabolic pathways. M6 was also the major product in experiments with liver cells. All human metabolites were present in experiments with liver preparations of animals. In light of the similarities in metabolic patterns, the choice of dog as non-rodent species for non-clinical safety assessment is justified. Incubations of macitentan with rat and human liver S9 fractions were performed in support of the *in vitro* genotoxicity assessment with metabolic activation. Metabolites M3 and M6 were consistently formed with liver preparations from both rat and human, whereas small amounts of M7 were formed only with rat liver S9.

Proposed metabolic pathways of macitentan in human liver preparations



Metabolism in rat and dog

Methodology: The metabolism of macitentan in the rat [B-05.001] and dog [B-07.182] has been characterized after intravenous and oral dosing of ^{14}C-labeled ACT-064992B to bile duct-cannulated animals and collection of plasma, urine, bile, and feces. Plasma samples in the rat were collected from non-cannulated satellite animals after implantation of a jugular vein catheter. Bile duct-cannulated rats (n=2 per sex and route) and dogs (n=2 males per route) received intravenous and oral ACT-064992B doses of 0.6 mg/kg and 3 mg/kg, respectively, corresponding to radioactive doses of 50 µCi/kg and 10 µCi/kg. Formulations were identical to those used in the main pharmacokinetic program. Animals were placed singly in metabolic cages and excreta were collected at pre-defined intervals over 48 h for the rat and 96 h for the dog. Satellite rats (generally n=2) received the same total and radioactive ACT-064992B dose, and plasma was collected over a period of 32 h. Metabolic profiles were recorded using HPLC coupled to radiodetection. Metabolite structure elucidation was performed using LC-MS/MS methodology [B-07.372].

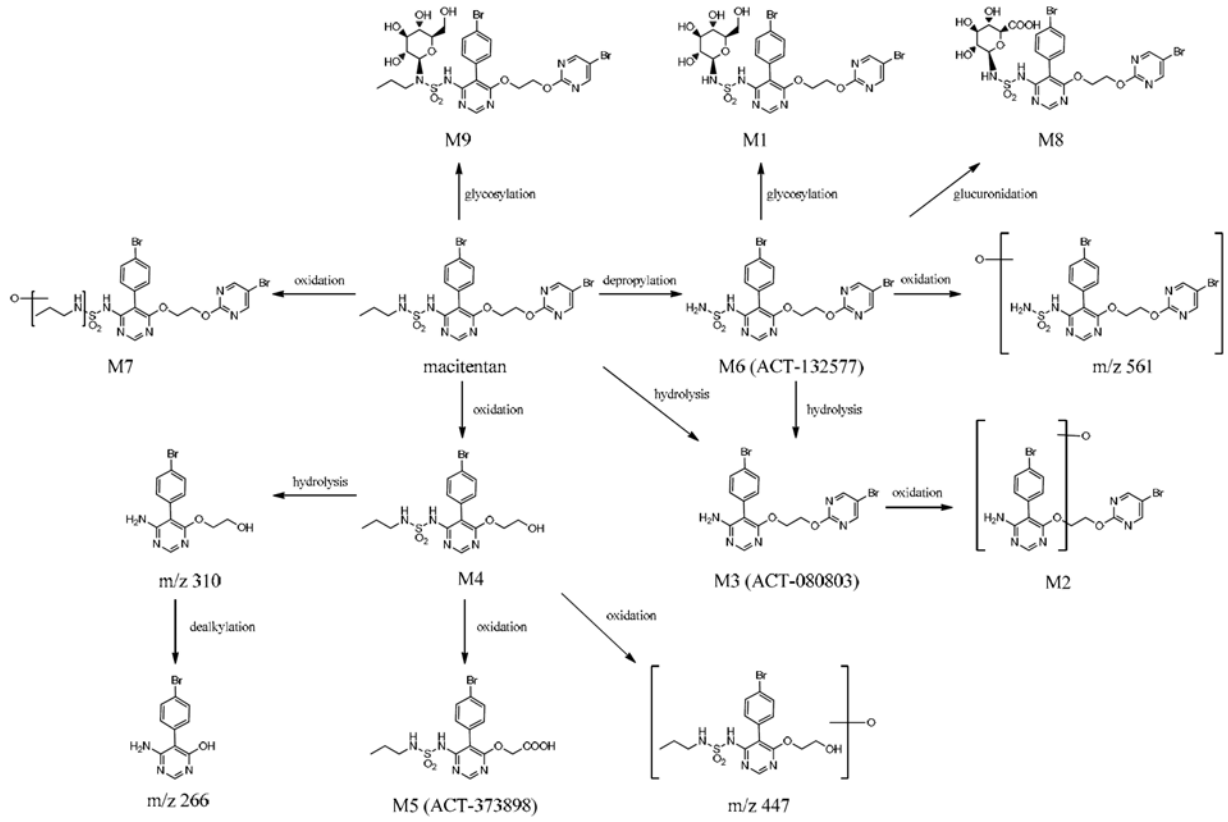
Results: In the rat, macitentan underwent extensive metabolism prior to excretion and unchanged drug was found in neither urine nor bile. The proposed metabolic pathways of macitentan in the rat are depicted in the Figure below. The pathways outlined are intended to illustrate the chemical interrelationship between the various metabolites, but without claiming knowledge of the exact sequence of metabolic events. Macitentan is metabolized via three independent pathways, i.e. 1) oxidative depropylation of the sulfamide function to form M6, 2) oxidative cleavage of the ethylene glycol linker to yield the alcohol M4, and 3) conjugation with glucose to give M9. Hydrolysis of both macitentan and M6 to form M3 is another pathway, but the result of a chemical rather than an enzyme-mediated process. M6 is subsequently conjugated with glucose or glucuronic acid to form metabolites M1 and M8, or oxidized to yield metabolite m/z 561. The alcohol M4 undergoes further oxidation to the corresponding acid M5 and hydroxylation to metabolite m/z 447. Further metabolic reactions include the hydrolysis of M4 to metabolite m/z 310 and cleavage of the ethylene glycol chain to yield metabolite m/z 266.

The active metabolite M6 and the conjugates M1, M8, and M9 were found as major products in rat bile together with a few other metabolites of unknown chemical identity, including M10, M11, and M12. The conjugates are subsequently cleaved to their corresponding aglycones, macitentan and M6, by the microflora in rat intestine. Finally, both aglycones are hydrolyzed to give the aminopyrimidine M3, the major product found in rat feces. Enterohepatic recirculation of macitentan and M6 after bacterial cleavage of the conjugates was investigated in the rat and shown to be of little importance. All other metabolites mentioned in the above section were excreted in rat urine together with a number of other products of unknown chemical nature. Unchanged macitentan and M6 were detected in feces samples after intravenous dosing, indicating direct secretion of both entities into the gastro-intestinal lumen. There were no obvious differences in metabolic patterns between male and female rats, with the exception of M14, identified only in the bile of female animals. Metabolic patterns did also not differ significantly

between oral and intravenous dosing. Metabolism is a prerequisite for excretion also in the dog as unchanged macitentan was virtually absent in bile or urine. Based on available structural information, the metabolic pathways of macitentan in the dog are largely similar to those in the rat. Major differences are the absence of metabolites secondary to the alcohol M4, whereas metabolite M2 was specific to the dog. Several other metabolites were present in dog urine and bile whose chemical nature remains unknown.

As in the rat, biliary elimination was the main excretion pathway also in the dog. The phase I metabolites M2, M4, M6 as well as the conjugates M1, M8, and M9 were found in dog bile. The latter conjugates undergo enzymatic cleavage by the gut microflora, liberating the aglycones, macitentan and M6, which subsequently hydrolyze to M3. Similar to the rat, in the dog direct secretion of macitentan and M6 from blood into the gastro-intestinal lumen was observed after intravenous dosing. Four metabolites, i.e., M16, M18, M19, and M20, were found in dog urine.

Proposed metabolic pathways of macitentan in the rat and dog



Circulating metabolites

Analysis for metabolite M5/ACT-373898 in rat, mouse, and dog plasma samples from various toxicology studies was performed with formally validated LC-MS/MS methods.

Results: In the studies with radiolabeled ACT-064992B in the rat [B-05.001] and dog [B-07.182], M6 was the only metabolite observed in all blood samples of both species. In blood of female rats, M4 was identified as an additional but minor component. M4 was more prevalent in blood of pregnant rats [B-11.837]. Metabolite M5 was observed in small amounts in human plasma alongside with the pharmacologically active M6. In the low-dose rat and dog studies performed with radiolabeled ACT-064992B, M5 was not observed. Its presence in plasma of rat, mouse and dog has, however, been verified with LC-MS/MS technology in samples from several toxicity studies.

Identification of metabolizing enzymes

Methodology: The human P450 enzymes catalyzing the oxidative depropylation of macitentan to M6 have been identified using two independent approaches, i.e., incubation of radiolabeled ACT-064992B with human liver microsomes in the presence of P450-specific chemical inhibitors, and incubation of ACT-064992B with recombinant P450 enzymes expressed in baculovirus-infected Sf9 cells [B-06.219]. Prior to the determination of enzyme kinetic parameters, initial rate conditions were identified with respect to microsomal enzyme concentrations and incubation time such that M6 formation was 10–25%. The rat and dog P450 enzymes involved in M6 formation were identified by incubation of ACT-064992B with recombinant P450 enzymes of both species at a single concentration of 10 µM.

Results: Using human liver microsomes, only ketoconazole had a clear inhibitory effect, indicating a role of CYP3A in M6 formation. As ketoconazole did not completely inhibit M6 formation, additional experiments with recombinant human P450 enzymes were performed. From a comprehensive panel of recombinant P450 enzymes, only CYP3A4 and CYP2C19 catalyzed M6 formation.

Based on enzyme kinetic data, CYP3A4 is the major contributor to M6 formation, whereas the role of CYP2C19 is negligible. Macitentan showed saturable kinetics with human liver microsomes as well as with recombinant P450 enzymes. Km and Vmax values obtained with human liver microsomes were 27 µM and 591 pmol/(min·mg). The corresponding values were 71 µM and 44 pmol/(min·pmol P450) for recombinant CYP3A4, and 58 µM and 0.4 pmol/(min·pmol P450) for recombinant CYP2C19. Intrinsic clearance Clint was calculated for both enzymes as a measure of the efficiency of the catalytic process. For concentrations up to 100 µM, CYP3A4 is responsible for about 99% of total intrinsic clearance, while CYP2C19 accounts for only 1%.

Experiments have also been performed with recombinant rat and dog P450 enzymes. Similar to the picture in man, M6 formation was catalyzed by CYP3a1 and CYP 3a2 in the rat. Among the members of the CYP 2c subfamily, CYP 2c11 was the major enzyme involved in M6 catalysis, with minor contributions of CYP 2c6 and CYP 2c12. No turnover was observed upon incubation with CYP 2b1. Among the dog P450 enzymes, only CYP 3a12 catalyzed formation of M6, whereas macitentan was not a substrate of CYP 2c21.

Mechanisms of auto-induction in animals

Methods: Changes in the expression levels of cytochrome P450 enzymes involved in macitentan metabolism were studied in the rat and dog to assess whether the decrease in plasma exposure after multiple dosing can be explained by auto-induction [B-07.261]. For this purpose, microsomes were prepared from liver samples of each dose and sex of the 4-week toxicity studies in both species [T-04.043, T-04.049]. Changes in the expression levels of a battery of hepatic P450 enzymes were determined on an mRNA level for each microsomal preparation. In addition, enzyme kinetic parameters of M6 formation were determined using macitentan concentrations from 1–100 µM. Initial rate conditions with respect to microsomal protein concentration and incubation time were determined individually for each batch. Samples were analyzed with LC-MS/MS for the rat, and with HPLC coupled to radiodetection for the dog. Though auto-induction was not consistently observed in all mouse studies, liver samples from the 13-week toxicity study in B6C3F1 mice [T-08.388] were also analyzed for changes in mRNA levels of selected P450 enzymes [B-12.113].

Results: Two complementary approaches have been used to investigate the induction of P450 enzymes in the rat and dog; i.e., biochemically by determination of changes in enzyme kinetic parameters of M6 formation as the major metabolic pathway in both species, and on a molecular biology level by changes in the mRNA expression of selected CYP proteins. M6 formation followed Michaelis-Menten kinetics in both species and sexes. Sex differences in the enzyme kinetics were evident in the rat. In male rats, Km was in the range of 1.2–7.4 µM with no obvious difference between dose groups. Vmax was increased over control in all dose groups but without any relation to dose. In female rats, Km was significantly lower in liver samples of all macitentan-treated groups, with an apparent plateau being reached already at the lowest dose of 50 mg/kg. Moreover, there was a consistent increase in Vmax with dose. No plateau was reached at the highest dose of 1500 mg/kg.

Enzyme kinetic parameters of M6 formation with liver microsomes from the 4-week toxicity studies in the rat and dog

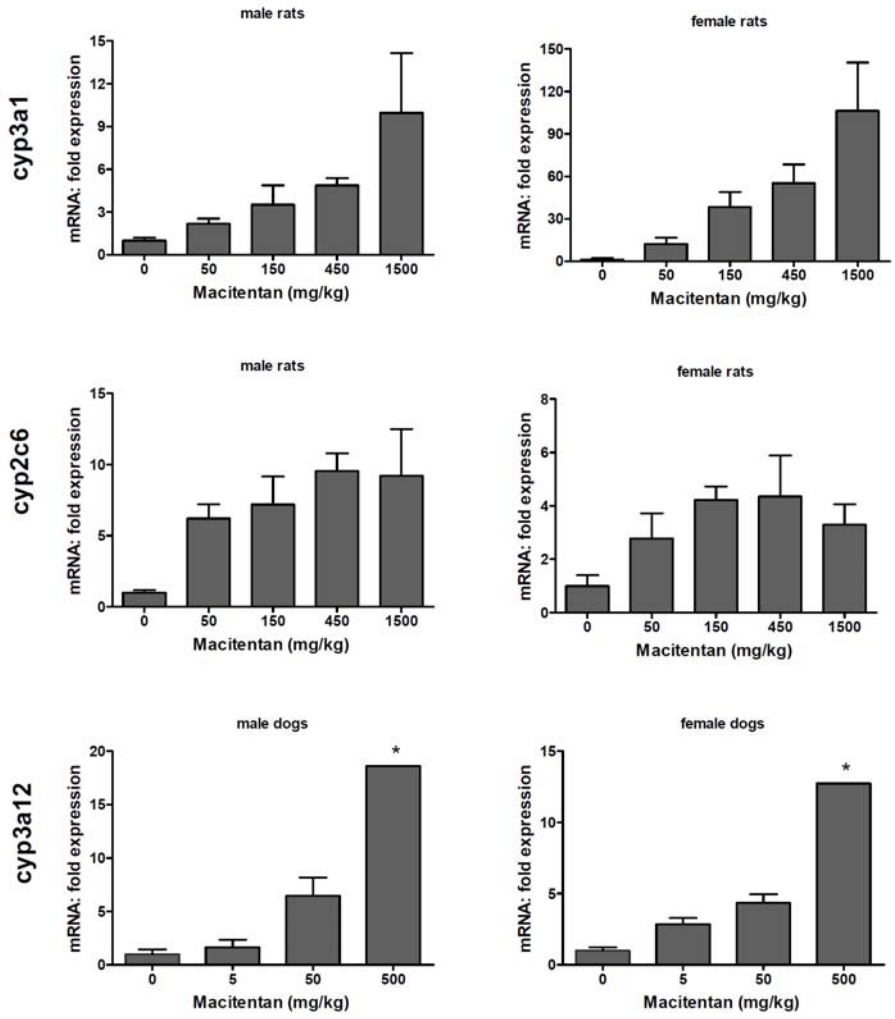
Species (sex)	Dose [mg/kg]	K_m [μM]	V_{max} [pmol/min·mg protein]
Rat (male)	0	4.9 ± 0.8	332 ± 14
	50	7.4 ± 2.6	709 ± 67
	150	3.3 ± 1.0	543 ± 33
	450	1.2 ± 0.8	405 ± 36
	1500	2.4 ± 0.7	522 ± 29
Rat (female)	0	20 ± 4	124 ± 9
	50	3.5 ± 1.5	166 ± 16
	150	1.7 ± 0.8	195 ± 15
	450	2.1 ± 0.6	345 ± 18
	1500	3.8 ± 1.0	478 ± 27
Dog (male)	0	6.7 ± 1.2	304 ± 14
	5	8.6 ± 0.9	517 ± 15
	50	10 ± 2	998 ± 51
	500	5.3 ± 1.0	1011 ± 46
Dog (female)	0	4.5 ± 1.0	682 ± 33
	5	6.2 ± 0.5	1028 ± 23
	50	7.2 ± 1.0	1730 ± 67
	500	6.7 ± 2.2	1250 ± 107

K_m = Michaelis-Menten constant, V_{max} = maximum velocity of an enzyme-catalyzed reaction. All data are means ± SD (n = 3) 4-week toxicity studies: T-04.043, T-04.049.

The changes in the expression of rat CYP 3a1 and CYP 2c6 mRNA with increasing doses are illustrated below. In male and female animals, there was a dose-dependent increase in CYP 3a1 mRNA which did not reach a plateau at the highest dose of 1500 mg/kg, and which was more pronounced in female rats. mRNA levels of CYP 2c6 also increased in both sexes in a dose-dependent manner and reached plateaus at 450 mg/kg and 150 mg/kg in male and female rats, respectively. In contrast, CYP 2c11 was not induced at any macitentan dose. No such sex differences were observed in the dog. Km values in both sexes were consistently between 4.5–10 μM without any dose dependence. In contrast, Vmax increased in a dose-dependent fashion with the exception of the 500 mg/kg dose in the female dog. Maximum increases in Vmax were 3.3-fold in males and 2.5-fold in females.

The changes in dog CYP 3a12 mRNA levels with increasing macitentan dose are depicted in the same Figure While increases in mRNA did not exceed 2- or 3-fold at the lowest dose of 5 mg/kg, hepatic expression of CYP 3a12 increased by 18- and 13-fold in male and female dogs, respectively, at the highest daily dose of 500 mg/kg.

CYP 3a1, CYP 2c6, and CYP 3a12 mRNA levels in liver samples from the 4-week toxicity studies in the rat and dog



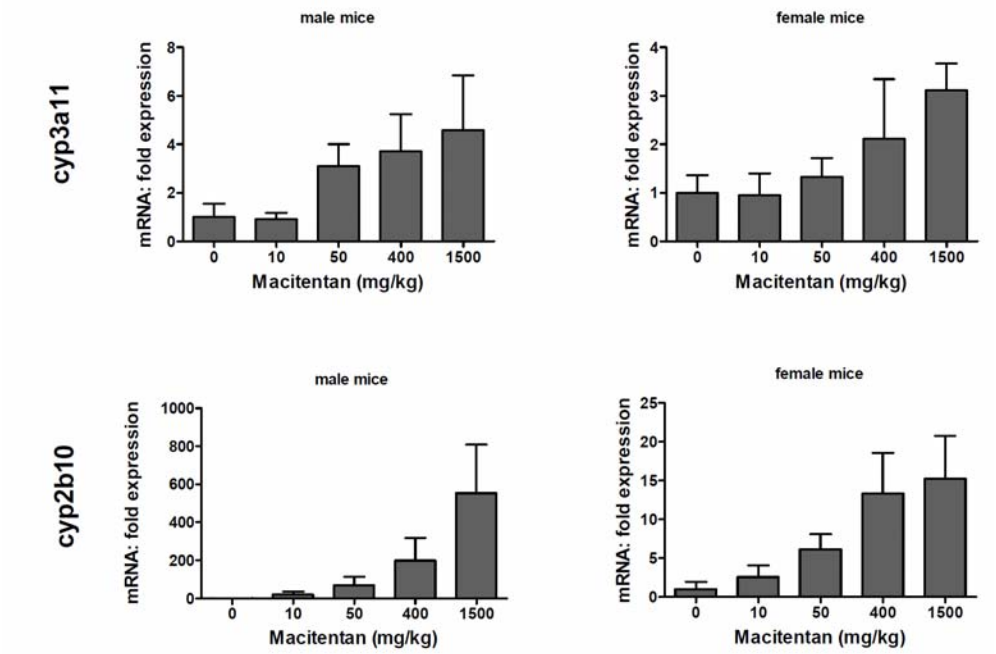
Data are means + SD (n=5–6 for rats and 2–3 for dogs). [B-07.261]

Up-regulation of selected P450 enzymes at an mRNA level was also investigated in liver samples of the 13-week toxicity study in B6C3F1 mice to see whether the microsomal enzyme-inducing properties of macitentan were conserved across species. The changes in CYP 2b10 and CYP 3a11 expression with dose are depicted below.

Significant upregulation of CYP 2b10 mRNA was observed over the entire dose range from 10 to 1500 mg/kg. As in the rat, sex differences were observed. Induction of CYP 2b10 was more pronounced in male mice and no plateau was reached at the highest dose of 1500 mg/kg. In female mice, CYP 2b10 up-regulation was weaker and appeared to plateau at 400 mg/kg. Changes in CYP 3a11 mRNA followed a similar trend but overall were less important. No change in CYP 1a1 or CYP 1a2 expression was observed in this study. In conclusion, macitentan is a microsomal enzyme inducer in all species used in the preclinical safety program based on the observed changes in

mRNA of a number of CYP proteins. As some of these induced P450 enzymes are also involved in the metabolism of macitentan in the rat and dog, auto-induction is a likely explanation for the observed time-dependent changes in macitentan exposure in both species. These changes were not consistently seen in the various mouse toxicity studies despite up-regulation of selected P450 enzymes.

Hepatic CYP 2b10 and CYP 3a11 mRNA levels in samples from the 13-week toxicity study in B6C3F1 mice



Data are means + SD (n = 4). [B-12.113]

EXCRETION

Methodology: The time course and completeness of excretion of ACT-064992B were investigated in male and female Wistar rats [B-05.034] and in male Beagle dogs [B-09.441]. Four male and female rats each received a single intravenous or oral dose of 0.5 mg/kg and 3 mg/kg, respectively, each containing a radioactive dose of 50 µCi/kg. Feces and urine were collected up to 216 h and expired air was collected up to 48 h. Four male dogs were used for single intravenous or oral administrations at 0.6 mg/kg and 3 mg/kg, respectively, containing a radioactive dose of 200 µCi/animal. Urine and feces were collected up to 336 h and total radioactivity was quantified using liquid scintillation counting.

Results: In rats and dogs, biliary elimination was the major route of excretion. In rats, recoveries in feces were 67–81% of the administered dose; whereas urinary excretion was 13–25%. Excretion patterns were largely independent of sex and route of administration. Excretion was complete at the end of the 9-day collection period, with recoveries ranging from 94–97%. In dogs, the major route of excretion was also via the feces after oral and intravenous dosing, with recoveries of 69% and 56%, respectively.

On average, 17% and 31% of the dose were recovered from urine. At the end of the 336-h collection period, 89% and 88% of the administered dose were recovered from both excreta. Information on the excretion pathways of macitentan after intravenous and oral dosing was also generated as part of the two metabolic profiling studies in the rat and dog using bile duct-cannulated animals. Following extensive metabolism, the major excretion pathway of macitentan in the rat was via the bile, accounting for 72–74% of the orally absorbed radioactivity, whereas renal excretion represented 26–28%.

A more balanced picture was observed in the dog. Irrespective of the dosing route, about 60% of the absorbed radioactivity was recovered from bile and about 40% was detected in urine. Radioactivity was detected in rat and dog feces after intravenous dosing, indicating direct secretion of drug-related material into the gastrointestinal lumen. Within the 48- and 96-h collection periods, 83–107% and 73–86% of the administered radioactive doses were recovered in the rat and dog, respectively.

PHARMACOKINETIC DRUG INTERACTIONS

Inhibition of cytochrome P450 enzymes

Methodology: The potential of macitentan, its active metabolite ACT-132577, and their common hydrolysis product ACT-080803 to elicit P450-mediated drug–drug interactions was studied *in vitro,* using either human liver microsomes and P450 isoform-specific marker substrates, or recombinant P450 enzymes expressed in baculovirus-infected Sf9 cells [B-04.024, B-05.130, B-12.103]. For the determination of IC_{50} values, all marker substrates were used at a single concentration around their Km value. Macitentan, ACT-132577, and ACT-080803 concentrations up to 100 µM were used. Metabolite formation was quantified with HPLC coupled either to radio-detection or mass spectrometry. For macitentan and ACT-132577, time-dependent inhibition was assessed for CYP2C9, CYP2D6 and CYP3A4 activity using human liver microsomes and the same P450 markers as for the competitive inhibition experiments. Assessment of time-dependent inhibition consisted of two independent experiments in which the effect of macitentan and ACT-132577 on P450 activity was assessed with and without a pre-incubation period.

Results: The results of the competitive inhibition experiments with macitentan and ACT-132577 and the derived IC_{50} and Ki values are summarized in below.

IC_{50} and Ki values of macitentan and ACT-132577 on human cytochrome P450 enzymes

P450 enzyme	Assay	macitentan IC_{50} (μM)	ACT-132577 IC_{50} (μM)
CYP1A2	phenacetin-O-deethylation	> 50	> 50
CYP2A6	coumarin 7-hydroxylation	> 50	> 100
CYP2B6	(S)-mephenytoin N-demethylation	> 50	> 100
CYP2C8	paclitaxel 6α-hydroxylation	21	23
CYP2C9	diclofenac 4′-hydroxylation	5.6 (5.0)*	31 (11)*
CYP2C19	(S)-mephenytoin 4′-hydroxylation	> 50	15
CYP2D6	dextromethorphan O-demethylation	> 50	> 100
CYP2E1	chlorzoxazone 6-hydroxylation	> 50	> 100
CYP3A4	midazolam 1'-hydroxylation	37	7.3
CYP3A4	testosterone 6β-hydroxylation	24	11 (6.3)*

* K_i value in parentheses

Macitentan had no relevant inhibitory effect on most of the human P450 enzyme tested including CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, and CYP2E1 [B-04.024]. Macitentan inhibited CYP3A4 with IC_{50} values of 24–37 μM and CYP2C8 with an IC_{50} of 21 μM. The strongest inhibition was observed on CYP2C9 with an IC_{50} value of 5.6 μM. Enzyme kinetic analysis revealed a Ki value of 5.0 μM and mixed-type inhibition as the mode of action.

The pattern of ACT-132577 was largely similar to that of macitentan [B-05.130]. No relevant inhibition was observed on CYP1A2, CYP2A6, CYP2B6, CYP2D6, and CYP2E1 activity. ACT-132577 inhibited CYP2C8 to a similar extent as parent macitentan whereas its effect on CYP2C19 and CYP3A4 was more pronounced. IC_{50} values were 15 μM for CYP2C19 and 7.3–11 μM for CYP3A4. A Ki of 6.3 μM was determined for the latter P450 enzyme using testosterone as a substrate.

ACT-080803 did not have an inhibitory effect on the activities of CYP1A2, CYP2A6, CYP2B6, CYP2D6, and CYP2E1 up to the highest concentrations of 50 μM or 100 μM, respectively. ACT-080803 showed the strongest inhibition on CYP2C19 activity with an IC_{50} of 3.7 μM, and weaker inhibition on CYP2C9, with an IC_{50} value of 17 μM. [B-05.130]. In both CYP3A4 assays, ACT-080803 had only a weak inhibitory effect with IC_{50} values of 47 and 50 μM, respectively. CYP3A4 is the major P450 isoform present in human intestine, and ACT-080803 has been detected in human feces.

Time-dependent inhibition of CYP2C9, CYP2D6, and CYP3A4 was investigated for macitentan and ACT-132577 by comparing the IC_{50} values obtained with and without a pre-incubation period [B-12.103]. This so-called P450 shift is a marker for the extent of change in enzyme activity during the pre-incubation period.

Macitentan and ACT-132577 exhibited no time-dependent inhibition of CYP2C9, CYP2D6, and CYP3A4 activities. Results for both compounds together with the positive controls tienilic acid, paroxetine, and mibefradil are presented in below. Based on these

in vitro data the potential of either macitentan or ACT-132577 to elicit drug-drug interactions with concomitantly administered cytochrome P450 substrates is considered low.

The effect of pre-incubation with macitentan and ACT-132577 on CYP2C9, CYP2D6, and CYP3A4 activities

	IC_{50} without pre-incubation (μM)	IC_{50} with pre-incubation (μM)	IC_{50} shift
	CYP2C9		
macitentan	30	29	1
ACT-132577	69	73	0.9
tienilic acid	5.8	0.09	64
	CYP2D6		
macitentan	>100	>100	ND [(1)]
ACT-132577	>100	>100	ND
paroxetine	4.4	0.15	29
	CYP3A4		
macitentan	>100	100	ND
ACT-132577	61	50	1.2
mibefradil	13	0.07	190

[(1)] ND = not determined due to absence of inhibition

Induction of cytochrome P450 enzymes

Methodology: Activation of the human pregnane X receptor (PXR) by macitentan and ACT-132577 was investigated in a reporter gene assay. In addition, changes in CYP1A2, CYP2C9, and CYP3A4 activity and/or mRNA expression were assessed in human hepatocytes using rifampicin and omeprazole as positive controls [B-05.107]. In the reporter gene assay, transiently transfected CV-1 cells carrying the carrier plasmid, the expression vector for PXR, the luciferase reporter CYP3A4-pGL3, and an expression vector of β-galactosidase were incubated with macitentan and ACT-132577 concentrations up to 30 μM. Luciferase and β-galactosidase activities were determined after a pre-defined incubation period. For CYP3A4 activity and mRNA measurements, three different batches of long-term cultured human hepatocytes, each from a different donor, were used. Hepatocytes were treated with macitentan and ACT-132577 concentrations up to 10 μM for up to 89 h. Thereafter, nifedipine, diclofenac, and phenacetin were used to quantify changes in CYP3A4, CYP2C9, and CYP1A2 activity using LC-MS/MS.

Results: Macitentan is a microsomal enzyme inducer in preclinical animal species, resulting in auto-induction after multiple dosing. To assess the relevance of this observation for man, the potential of macitentan and ACT-132577 to induce changes in CYP3A4, CYP2C9, and CYP1A2 expression was investigated. PXR activation was studied to identify the underlying biomolecular mechanism.

Macitentan and ACT-132577 activated human PXR with EC50 values of 1.1-1.2 μM and 7.2–8.7 μM, respectively [Table below]. In human hepatocytes, both compounds elicited

concentration-dependent increases in CYP3A4 mRNA and enzyme activity [Figure below Table].

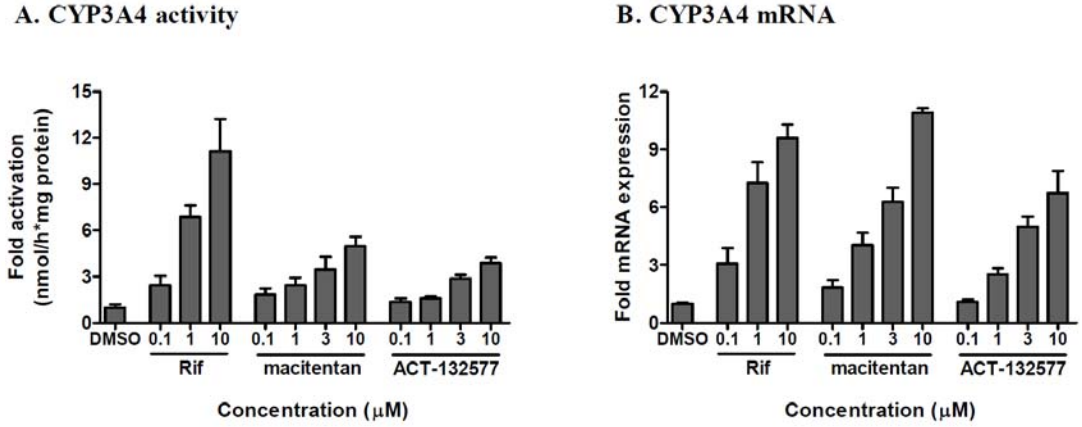
CYP3A4 activity was increased by up to 5-fold and CYP3A4 mRNA was increased by up to 11-fold at the highest concentration of 10 µM. Neither macitentan nor ACT-132577 elicited relevant changes in CYP1A2 and CYP2C9 activity up to 10 µM. The observed up-regulation of CYP3A4 expression is not considered to alter pharmacokinetics of concomitant CYP3A4 substrates in clinical use. Major reasons are the proposed macitentan clinical dose of 10 mg and the high degree of plasma protein binding. Indeed, no effect on the urinary 6β-hydroxycortisol/cortisol ratio was observed in the multiple-ascending dose study at the highest dose of 30 mg [D-06.044].

Activation of human PXR by macitentan and ACT-132577

Compound	EC_{50} (µM)	E_{max}
macitentan (1)	1.1-1.2	5.8
ACT-132577 (1)	7.2-8.7	6.0-6.5
rifampicin (2)	0.5-0.6	4.6-4.8

(1) n = 2; (2) n = 3. EC_{50} = concentration that induces 50% of the maximal response; E_{max} = maximum observed effect; PXR = pregnane X receptor.

Up-regulation of CYP3A4 activity (panel A) and mRNA (panel B) by macitentan and ACT-132577



Data represent means + SD (n=3 replicates from one donor). DMSO, dimethylsulfoxide. Rif = rifampicin. [B-05.107]

Interaction with transport proteins

Inhibition of the multidrug resistance protein P-gp/MDR-1

Methodology: The effect of macitentan on the activity of the human multidrug resistance protein P-gp (MDR-1, ABCB1) was investigated in the Caco-2 model using tritiated digoxin and rhodamine123 as substrates [B-05.040]. Caco-2 cells were cultured at passage number 31 and transport experiments were performed on day 27 post-seeding.

The integrity of cell monolayers was checked by two independent methods, i.e., measurement of transepithelial electrical resistance and functional measurements using radiolabeled D-mannitol as a low permeability marker. In the apical compartment, a HBSS buffer with a pH 6.5 was used, while the basolateral compartment contained HBSS buffer at pH 7.4. Rhodamine123 or ^{3}H-digoxin at single concentrations of 10 µM and 1 µM, respectively, were added to either the apical or basolateral compartment. Macitentan as a potential P-gp inhibitor was used up to 100 µM. After 3–4 h of incubation, aliquots were taken from both compartments and quantified by either liquid scintillation counting or colorimetrically with a spectrophotometer. Verapamil at 20 µM was used as a reference inhibitor for P-gp activity. Recoveries in these experiments were in excess of 73%.

Results: Results of these transport experiments are summarized in the Table below. No effect of macitentan on the apparent permeability coefficients (Papp) of either digoxin or rhodamine123 was observed up to 100 µM, indicating that macitentan is unlikely to be an inhibitor of P-gp-mediated efflux. Drug-drug interactions with compounds whose pharmacokinetics are dependent on P-gp activity are therefore not expected in therapeutic use.

Effect of macitentan on the apparent permeability coefficients, Papp, of ^{3}H-digoxin and rhodamine123 in the Caco-2 model

Transport direction	macitentan [µM]	P_{app} [10^{-6} cm/s] digoxin (1)	rhodamine123 (2)
A–B (3)	0	5–6	3
B–A	0	25	16
A–B (4)	0	19	13
A–B	3	4	ND (5)
A–B	10	5	3
A–B	30	4	3
A–B	100	5–6	3

(1) at a ^{3}H-digoxin concentration of 1 µM; (2) at a rhodamine123 concentration of 10 µM; (3) A = apical compartment; B = basolateral compartment; (4) plus verapamil at 20 µM; (5) ND = not determined.

Interaction with OATP transporters

Methodology: To study potential interactions of macitentan and ACT-132577 with the two major hepatic organic anion-transporting polypeptides (OATP), transport and inhibition studies were performed in OATP1B1- and OATP1B3-overexpressing Chinese hamster ovary (CHO) cells [B-10.642]. Wild-type CHO cells were used as controls for passive diffusion. Uptake experiments were performed for both OATP transporters using macitentan and ACT-132577 concentration ranges of 0.01–100 µM and 0.01–300 µM, respectively. At the end of the uptake experiment, cells were extensively washed to eliminate non-specific binding. Total radioactivity in cell lysates was quantified using liquid scintillation counting.

Prior to the main transport experiments, the time dependence of cellular uptake was determined individually for each OATP transporter and compound in order to optimize experimental conditions. The potential of macitentan and ACT-132577 to inhibit OATP activity was investigated using 1 µM atorvastatin and 5 µM estrone-3-sulfate as model substrates for OATP1B1 and for OATP1B3, respectively. The inhibition experiments were performed with the macitentan and ACT-132577 concentration ranges described above. Cellular uptakes were normalized for total protein content and incubation time.

Results: Rapid uptake of macitentan and ACT-132577 was observed in wild-type and OATP-overexpressing cells. The absence of a marked difference in uptake rates between wild-type and OATP-overexpressing cells suggests that hepatic uptake is mostly driven by passive diffusion and not dependent on OATP transport. The potential of macitentan and ACT-132577 to inhibit OATP1B1 and OATP1B3 transporters was investigated at concentration up to 100 µM and 300 µM, respectively. Macitentan inhibited OATP1B1 uptake of atorvastatin with an IC_{50} of 6.9 µM, whereas OATP1B3 uptake of estrone-3-sulfate was inhibited with an IC_{50} of 14 µM. The respective IC_{50} values for ACT-132577 were 21 µM and 56 µM.

Unlike other endothelin receptor antagonists, macitentan hepatic uptake is driven by passive diffusion and thus insensitive to concomitant OATP inhibitors such as HIV protease inhibitors and calcineurin inhibitors. This view is confirmed by the virtually unchanged macitentan pharmacokinetics in the presence of cyclosporine A [D-10.542]. Moreover, macitentan is not expected to interact with OATP substrates, such as most statins, as its inhibitory potential is too low in light of the proposed macitentan clinical dose of 10 mg and its high plasma protein binding.

Effects on bile salt transport

Methodology: The effect of macitentan and ACT-132577 on hepatic bile salt transport was investigated on the level of basolateral bile salt uptake from blood into hepatocytes mediated by the sodium-dependent taurocholate co-transporting polypeptide (NTCP), and the canalicular export out of hepatocytes into bile mediated by the bile salt export pump (BSEP). Inhibition experiments were performed with both human transporters, overexpressed in either CHO cells (NTCP) or Sf9 membrane vesicles (BSEP) [B-05.044]. Taurocholate at 5 µM was used as a prototypical bile salt for both transporters together with macitentan or ACT-132577 at concentrations up to 100 µM. Total radioactivity in CHO cells and Sf9 vesicles was quantified using liquid scintillation counting. NTCP transport rates were calculated as the difference in sodium vs choline buffer. BSEP transport rates were calculated as the difference in the absence and presence of ATP.

Results: Macitentan and ACT-132577 inhibited human NTCP-mediated uptake of taurocholate with IC_{50} values of 19 µM and 14 µM, respectively. Human BSEP-mediated efflux of taurocholate was also inhibited by both compounds. The respective IC_{50} values for macitentan and ACT-132577 were 18 µM and 50 µM. In light of the macitentan concentrations achieved at a dose of 10 mg [D-06.044], the high plasma protein

binding, and the fact that the hepatic uptake of macitentan is driven by passive diffusion rather than by active OATP transport, it is unlikely that macitentan will interfere with bile salt trafficking in man via interaction with NTCP or BSEP.

5.2 TOXICOKINETICS

Toxicokinetics are discussed in the context of the toxicology studies below.

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose toxicity study in the OF1 mouse

ACT-064992 was given orally (by gavage) to OF1 mice, first at dose levels of 1000 or 2000 mg/kg (1/sex/dose) in a pilot toxicity study, then at a dose of 2000 mg/kg (5 males and 5 females) [T-04.053]. In the pilot study, clinical signs were recorded 0.25, 1, 2, and 4 h after ACT-064992 administration and mice were observed daily for 7 days.

There was no mortality at 1000 or 2000 mg/kg; however, ptosis and/or slightly subdued behavior were noted 1 h after administration in both male and female mice receiving the 2000 mg/kg dose. The dose level of 2000 mg/kg was selected for the subsequent study. Clinical signs were recorded 0.25, 1, 2, and 4 h after ACT-064992 administration and mice were observed daily for 14 days, after which they were sacrificed and necropsied.

The ptosis and/or slightly subdued behavior observed earlier were not observed in this study, nor was there any effect of treatment on body weight. There were no macroscopic findings at necropsy or any clinical signs indicative of an acute toxic effect. Overall, a single dose of 2000 mg/kg ACT-064992 did not produce signs of acute toxicity in mice.

Single-dose toxicity studies in the rat

In the first study [T-03.039], Wistar rats (3 males and 3 females) were given ACT-064992 orally (by gavage) at a dose of 1000 mg/kg. Clinical signs were recorded 0.25, 1, 2, 4, and 24 h after ACT-064992 administration and necropsies were performed after the last observation on Day 1 There were no unscheduled deaths, clinical signs, or macroscopic abnormalities.

In the second study [T-04.052], Wistar rats (5 males and 5 females) were given ACT-064992 orally (by gavage) at a dose of 2000 mg/kg. Clinical signs were recorded 0.25, 1, 2, and 4 h after ACT-064992 administration and animals were then observed once daily for 14 days. At the end of the 14-day observation period, the rats were sacrificed and necropsied.

There were no deaths and no clinical signs noted in any animal either after dosing or throughout the 14-day observation period. ACT-064992 at a dose level of 2000 mg/kg had no effect on body weight and no macroscopic findings at necropsy were reported. Overall, a single dose of 2000 mg/kg ACT-064992 did not produce signs of acute toxicity in rats.

6.2 Repeat-Dose Toxicity

Repeat dose toxicology was evaluated in mice, rats and dogs. All major studies were previously reviewed under IND 77258. Major findings are summarized below.

Mouse
Study [T-05.155]:
A 13-week oral (gavage) toxicity study was conducted in CD-1 mice (10/sex/group) with macitentan (batch 4) at 0 (vehicle), 75, 300, and 900 mg/kg/day. Additional groups of satellite animals (12/sex/group) were included for toxicokinetic determinations and for assessment of reversibility of selected findings after a 6-week treatment-free period.

There were no deaths attributed to treatment during the treatment period. However, some animals given 300 or 900 mg/kg/day were in a poor clinical condition during the second half of the treatment period, and one high-dose satellite female was found dead on Day 95 (4 days after treatment was stopped). Clinical signs included occasional breathing difficulties in males given 900 mg/kg/day and females given 300 mg/kg/day, sometimes associated with piloerection, coldness, unsteady gait, or subdued behavior. At the end of the treatment period, some animals at 300 or 900 mg/kg/day had a distended abdomen or were thin. During the recovery period, clinical signs disappeared except for distended abdomen which was still present in two males previously treated with 900 mg/kg/day.

There were slight changes in RBC count, RBC distribution width, corpuscular volume, and/or corpuscular hemoglobin in mice given 300 or 900 mg/kg/day. WBC counts were high in some male animals given 300 or 900 mg/kg/day. Serum AST and ALT levels were higher than controls in all treated groups, and albumin, cholesterol, and/or urea concentrations decreased with increasing dose. Chloride concentration was lower than in controls in both sexes given 300 or 900 mg/kg/day, in females sometimes in association with other electrolyte changes. High individual globulin values were observed with a dose-related increased incidence, correlating with reduced albumin and lower albumin/globulin ratio. At the end of the recovery period, mean liver enzyme activities (AST, ALT, or γ-glutamyl transferase [GGT]) returned to normal.

Necropsy at the end of the 3-month treatment period revealed dose-related incidences of pale or raised areas in the liver, with adhesions between lobes or surrounding tissues, and enlargement of the spleen. The mean liver and spleen weights were higher than in controls in all treated groups. After the 6-week recovery period, organ weights were similar in control and treated groups. Microscopically, the following findings were observed in the liver at all dose levels: centrilobular hepatocellular hypertrophy, focal necrosis without relationship to the lobular liver structure, surrounded by a demarcation zone of neutrophils and macrophages, pigmented macrophage aggregates, and widespread associated acute/chronic inflammation, whereas major parts of liver tissue appeared normal. Reactive simple hyperplasia was seen in bile ducts at 300 and 900 mg/kg/day, and in the gallbladder of two males treated at 900 mg/kg/day. After the

recovery period, bile duct hyperplasia and focal liver necrosis were largely reversible. Inflammation and pigment deposits were not reversible. A NOAEL was not established.

Since no NOAEL was established in the previous 13-week mouse study, a follow-up study was performed exploring dose levels at and below 75 mg/kg/day [T-07.208]. This study was an oral (gavage) toxicity study in CD-1 mice (15/sex/group), in which macitentan (batch 4) was given at 0 (vehicle), 5, 20, and 75 mg/kg/day. Additional groups of satellite animals (18/sex/group) were included for toxicokinetic determinations.

No mortality or clinical signs related to treatment with macitentan were recorded. There was no influence on body weight or food consumption.

The following changes in hematology variables were detected: decrease in hemoglobin and hematocrit in males at 20 mg/kg/day and 75 mg/kg/day, and increase in red cell volume distribution width, whereas RBC count was unaffected. No changes in hematology variables were recorded in either sex at 5 mg/kg/day or in females at 20 mg/kg/day.

Liver weights were increased in both sexes at 75 mg/kg/day. No effects on organ weights were recorded at 5 and 20 mg/kg/day.

Macitentan induced macroscopic and microscopic findings in the liver in all treatment groups. Macroscopically,aberrant liver foci were noted at all dose levels. Microscopic findings consisted of focal necrosis, focal organizing necrosis, granulocytic infiltration, and the presence of pigmented macrophages. The appearance of these lesions was similar to those reported in the first 13-week toxicity study in CD-1 mice. A NOAEL could not be established in this study.

Study [T-08.388]
Macitentan was administered daily by oral gavage to B6C3F1 mice of both sexes (15/sex/group) at dose levels of 0 (vehicle), 10, 50, 400, and 1500 mg/kg/day for 13 weeks . Additional groups of satellite animals (12/sex/group) were included for toxicokinetic determinations.

There was no influence on body weight, food consumption, or ophthalmoscopic and hematological variables.

The systemic exposure was comparable with the exposure in CD-1 mice at similar dose levels.

The absolute and relative liver weights were increased in both sexes at 400 and 1500 mg/kg/day. In females, relative liver weight was also increased at 50 mg/kg/day.

No test item-related macroscopic changes were observed. In the liver, centrilobular hepatocellular hypertrophy was observed in females at 1500 mg/kg/day and low

incidences of focal necrosis in males at 400 and 1500 mg/kg/day and in females at 50, 400, and 1500 mg/kg/day. The histological appearance of the focal necroses was similar to that observed in CD-1 mice but with fewer and smaller necrotic areas. The incidence of necrosis was low and not clearly related to dose level. A NOAEL was established at 10 mg/kg/day.

Rat
Study T-04.043

This 4-week oral (gavage) toxicity study was conducted with macitentan in Sprague Dawley rats (10/sex/dose level) at dose levels of 0 (vehicle), 50, 150, 450, and 1500 mg/kg/day.. An 8-week recovery group (5/sex/dose level), as well as an additional group of satellite animals (6/sex/dose level except control group) for toxicokinetics, were included in the study.

There was no mortality that was considered related to treatment with macitentan and there were no adverse clinical signs. There were no changes in body weight, and only slight and transient decreases in food consumption in females at ≥ 150 mg/kg/day.

After 4 weeks of treatment, there was a slight decrease in RBC count, hemoglobin, and hematocrit and a slight increase in platelet counts in treated males of all dose groups and in females at 1500 mg/kg/day. No effect on reticulocytes and no indication of bone marrow effects were reported.

At necropsy following the 4-week treatment period, mean absolute and relative liver weights were dose-dependently increased in both sexes at all doses. Absolute mean thyroid gland weight was increased in males in all treated groups. At the end of the recovery period, liver weights had returned to normal, except for a slight increase still apparent at the high dose. Thyroid gland weights returned to normal.

At the end of the 4-week treatment period, histopathological examination showed dose-dependent, minimal to marked centrilobular hepatocellular hypertrophy in most rats from all treated groups. Hepatocellular vacuolation occurred at slightly higher incidences and severity in treated groups. In the thyroid gland of most of these rats, there was minimal to moderate follicular cell hypertrophy associated with an apparent increase in the number of follicles. Minimal to moderate atrophy of seminiferous tubules in testes was noted in one male each in control, 50, and 150 mg/kg/day groups and in two males at 450 mg/kg/day.

All of the histopathological changes observed after 4 weeks of treatment were reversible except for hepatocellular hypertrophy that was observed at a minimal degree in one male previously treated at 1500 mg/kg/day. Hepatocellular hypertrophy and thyroid follicular cell hypertrophy are considered to be related to microsomal enzyme induction, and as adaptive and not adverse effects.

The NOAEL was considered to be the high dose of 1500 mg/kg/day.

Study T-04.076

This 13-week oral (gavage) toxicity study was conducted in Wistar rats (15/sex/group) with macitentan at dose levels of 0 (vehicle), 10, 50, 250, and 1500 mg/kg/day.. A 13-week treatment-free recovery period (6/sex/group) was included in the study. Additional groups of satellite animals (6/sex/group) were included for toxicokinetic determinations.

Six males treated with 1500 mg/kg/day were found dead or killed moribund between Day 6 and Day 11. Four rats died because of hemorrhage and in two, the cause of demise was treatment-related poor condition. Because of the severity of clinical signs and/or poor condition, all remaining males and females in the 1500-mg/kg/day group were terminated in Week 2. At necropsy, in males treated with 1500 mg/kg/day, PT values were approximately 5-fold and aPTT values approximately 6-fold higher than normal.

Large liver and increased relative liver weight were recorded for most rats, and in one male the thyroid was enlarged. Red or dark discolorations were noted in various organs of a few rats. Microscopically, centrilobular hepatocellular hypertrophy, periportal vacuolation, and focal necrosis in the liver and follicular cell hypertrophy in the thyroid were noted. In several males, notably decedents, there were hemorrhages in various organs and, in a few males, epididymitis and dilation of testicular tubules and rete testis. Hemorrhage was noted in the thymus of a few males and one female. A low-grade focal nephropathy was seen in several animals.

In the remaining treatment groups, clinical signs were generally unremarkable. Body weights were similar between treatment groups at the end of treatment. There were no effects on food consumption.

There were no effects of treatment on immunotoxicological markers at necropsy.

In Weeks 4 and 13, males treated at 250 mg/kg/day had significantly prolonged aPTTs and slightly elevated platelet counts and plateletcrit. In females, slightly reduced PTs were observed at 250 mg/kg/day in Weeks 4 and 13 and slightly reduced fibrinogen at all dose levels in Week 13. Slight increases in red cell- and hemoglobin-distribution width, decreased hematocrit, and increased WBC counts were observed mainly at 250 mg/kg/day, and were inconsistent between males and females. No effect on RBC count, reticulocytes, or bone marrow was noted.

After 13 weeks of treatment, liver weight was significantly increased in all remaining treatment groups, correlating with macroscopically large liver in few high-dose animals. Heart and thyroid/parathyroid weights were significantly increased in both sexes at 250 mg/kg/day. In addition, in the 50- and 250-mg/kg/day groups kidney weight was increased in males and the relative thymus weight was increased in females. With the

exception of heart weight, which remained significantly increased in females treated with 250 mg/kg/day, all organ weights had returned to normal after the recovery period.

After 13 weeks of treatment there was dose-dependent, minimal to moderate centrilobular hypertrophy in the liver at all remaining dose levels. In females, minimal to slight hepatocyte vacuolation was seen in the 50- and 250-mg/kg/day groups. There was no indication of hepatocellular necrosis. A dose-dependent increase in follicular cell hypertrophy was seen in the thyroid in most males at all dose levels and, to a lesser extent, in females. In the testes, 1 of 15 males at 250 mg/kg/day showed a slight bilateral dilation of the rete testis that was considered drug related. In addition, there was diffuse or multifocal tubular atrophy in two males at 250 mg/kg/day. At the end of the 13-week recovery period, microscopic findings in treated animals were generally comparable with those in the controls. Seminology did not show any drug-related effect.

The NOAEL is considered to be 10 mg/kg/day.

Study T-05.045

This 26-week oral (gavage) toxicity study was conducted in Wistar rats (15/sex/group) with macitentan at dose levels of 0 (vehicle), 10, 50, and 250 mg/kg/day.. A 9-week treatment-free recovery period (5/sex/group) was included in the study. Additional groups of satellite animals (9/sex/group) were included for toxicokinetic determinations.

The treatment was well tolerated without mortality, with only minor clinical signs, and with no adverse effects on body weights or food consumption.

There were no ophthalmologic findings.

Hematological examination at Weeks 13 and/or Week 26 revealed slight effects on RBC variables including decreased hemoglobin concentration in males at ≥ 50 mg/kg/day, decreased RBC in males at 250 mg/kg/day, reduced hematocrit, and higher hemoglobin distribution width in both sexes at 250 mg/kg/day, and higher mean cell hemoglobin concentration in females at 250 mg/kg/day. No effect on reticulocytes and no indication of bone marrow effects were noted. Slight effects on blood coagulation variables were seen in males with reduced platelet distribution width at ≥ 50 mg/kg/day, and higher platelet counts, higher platelet:blood volume ratios, and slightly shorter PT but longer aPTT at 250 mg/kg/day. In general, these changes were within historical background data except for longer aPTTs and higher platelet counts. The effects had disappeared at the end of the recovery period.

Males at ≥ 50 mg/kg/day had a significantly higher percentage of abnormal sperm after 26 weeks of treatment. After the recovery period, there were no differences between treatment groups in the proportion of abnormal sperm, indicating apparent reversal of effects.

Liver and heart weights were increased in males and females at ≥ 50 mg/kg/day. Additionally, males at ≥ 50 mg/kg/day showed higher prostate weights. At the end of the recovery period, organ weights were comparable between groups.

Macroscopically, large liver and thyroid were recorded for a small number of animals at 250 mg/kg/day. Microscopically, there was a dose-related increase in centrilobular hypertrophy in the liver of males and females at 10, 50, and 250 mg/kg/day and an increase in hepatocellular vacuolation in females at 250 mg/kg/day. Thyroid follicular cell hypertrophy was recorded for males at 50 mg/kg/day and males and females at 250 mg/kg/day. In the kidney, the incidence of hyaline/pigment droplets was marginally increased in males at ≥ 50 mg/kg/day and increased levels of pigment were recorded in the proximal tubules of females at 250 mg/kg/day. In the adrenals, there was a minor increase in the level of cortical vacuolation in males at 250 mg/kg/day. At the end of the recovery period there were no macroscopic findings related to macitentan. Microscopically, there was evidence of either complete or partial reversal of all treatment-related changes recorded at the end of the treatment phase.

The NOAEL is considered to be 10 mg/kg/day.

Dog

Study T-04.048

This 2-week oral (capsule) dose range-finding toxicity study was conducted in the Beagle dog (1/sex/group) with macitentan at dose levels of 0 (vehicle), 30, 100, 300, and 600 mg/kg/day []. Systemic exposure was assessed on treatment days 1, 8, and 14.

There was no mortality or adverse clinical signs. Reduced body weight and food consumption in the high-dose male was reported.

There was a marked reduction in the RBC count, hemoglobin concentration, and hematocrit in both sexes and a decreased platelet count in males of the 300- and 600-mg/kg/day groups.

Alkaline phosphatase was increased in males treated at 300 and 600 mg/kg/day and in all treated females.

Increased liver weight and hepatocellular hypertrophy were seen in all treated animals.

In the testes, minimal hypospermatogenesis and slight tubular dilation were noted in the male given 100 mg/kg/day. Slight testicular tubular atrophy and minimal focal arteritis in the testes was noted in the male given 600 mg/kg/day. There were no changes in the testes at 30 and 300 mg/kg/day.

Study T-04.049

This 4-week oral (capsule) toxicity study was conducted with macitentan in Beagle dogs (3/sex/group) at dose levels of 0 (vehicle), 5, 50, and 500/250 mg/kg/day . The 4-week treatment period was followed by an 8-week treatment-free period in two dogs/sex from the control and high-dose groups. Systemic exposure was assessed on treatment days 1, 7, and 28.

One male treated at 500 mg/kg/day was found dead on Day 13 following a period of anorexia. In high-dose dogs, anorexia, subdued behavior and/or reduced activity, and a weak condition (unable to stand) were noted. Because of these findings, the high dose was reduced from 500 to 250 mg/kg/day for the last 2 weeks of the treatment period.

At the high dose, decreased food consumption and body-weight loss occurred during the first 2 weeks, with some recovery after lowering the dose to 250 mg/kg/day.

There were no treatment-related changes in ophthalmologic findings or in cardiovascular variables (diastolic and systolic blood pressure, heart rate, or ECG findings) in any dose group.

In hematology, there was a dose-related reduction in the RBC variables (RBC count, hemoglobin, and hematocrit) in both sexes in the 50- and 500/250-mg/kg/day groups in Weeks 2 and 4. Reticulocytes slightly increased (statistically not significant) in high-dose animals in Week 4. Recovery was noted after the treatment-free period. In males, there was an increase in WBC count at the high dose, mainly due to a single dog found in poor condition in Week 2. There was also a decrease in platelet counts, mainly in individual animals of the high-dose group. No effects on bone marrow were noted.

Changes in clinical chemistry variables included increased alkaline phosphatase and decreased cholesterol and phospholipids in both sexes in the high-dose group in Weeks 2 and 4. Similar decreases in cholesterol and phospholipids were noted for both sexes at the mid dose. In addition, slight increases in lactate dehydrogenase (LDH) and creatine kinase (CK) concentrations were observed in Week 4 in high-dose males.

At the end of the treatment period, organ weight changes included higher absolute and relative liver weights in all treated groups compared with the control. At the end of the 8-week recovery period, there were no treatment-related organ weight changes.

Microscopic changes were observed in the heart, mainly in the right atrium and right circumflex artery, in the 50- and 500/250-mg/kg/day groups [Table below]. The changes consisted of arterial intimal thickening, atrial fibrosis with chronic inflammation, epicarditis, and neovascularization.

In the liver, centrilobular hepatocellular hypertrophy was noted in all treated groups.

In the testes, minimal tubular atrophy in 2/3 males treated with 50 mg/kg/day and tubular dilation in one male each of the mid- and high-dose groups, in the high-dose male accompanied by hypospermatogenesis, were noted. There were no heart or testicular findings at 5 mg/kg/day.

At the end of the 8-week recovery period, the cardiac lesions decreased markedly in extent and severity, indicating nearly complete reversibility. Hepatocellular hypertrophy was still present in animals previously treated with 500/250 mg/kg/day at a minimal level, indicating partial reversibility. In the testes, tubular degeneration was seen in one control and one treated animal, and therefore the testicular effects were considered reversible.

The NOAEL is considered to be 5 mg/kg/day.

Cardiac findings in the 4-week study in Beagle dogs

Main groups (sacrifice after 4 weeks of treatment)

Group	1 (control)		2 (5 mg/kg/d)		3 (50 mg/kg/d)		4 (500/250 mg/kg/d)	
Sex	Males	Females	Males	Females	Males	Females	Males	Females
Exposure [1]	–	–	161	175	1401	1270	2570	3213
Margin [2]	–	–	7.5	8.1	65	59	120	149
Arterial intimal thickening	0	0	0	0	1	3	1	2
Atrial fibrosis with chronic inflammation [3]	0	0	0	0	1	1	1	0
Epicarditis [3]	0	0	0	0	0	1	2	0
Neovascularization [3]	0	0	0	0	1	1	2	0

[1] AUC_{0-24} of macitentan + ACT-132577 in µg*h/mL at Week 4.
[2] Relative to human dose of 10 mg per day and based on steady-state AUC_{0-24} of macitentan plus ACT-132577 in study AC-055-102 [D-06.044].
[3] Incidence, i.e., number of affected dogs out of three dogs per sex and group.

Recovery groups (sacrifice after 8 weeks of recovery)

Group	1 (control)		2 (5 mg/kg/d)		3 (50 mg/kg/d)		4 (500/250 mg/kg/d)	
Sex	Males	Females	Males	Females	Males	Females	Males	Females
Arterial intimal thickening [1]	0	0	0	0	0	0	2	1
Epicarditis [1]	0	1	0	0	0	0	1	0

[1] Incidence, i.e., number of affected dogs out of two dogs per sex and group.

The sub-acute cardiac pathology recalls that seen with a variety of vasodilating agents in toxicity studies in this species. The sponsor identified hypotensive activity of this compound in a CV safety study at dosages/exposures well below those used in this toxicity study (T-04.060, T-07.142)

Study T-04.077

This 13-week oral (capsule) toxicity study of macitentan was conducted in Beagle dogs (4/sex/group) at dose levels of 0 (vehicle), 2, 5, 30, and 100 mg/kg/day []. The 13-week treatment period was followed by a 16-week recovery period (3 dogs/sex/group). The study included toxicokinetic evaluations.

There were no deaths or treatment-related clinical signs.

In dogs treated at 100 mg/kg/day, there was a slight decrease in body weight of males. The very slight, transient decreases in body-weight gain and food consumption in males and females were no longer evident at the end of the treatment period.

There were no findings in ophthalmologic or ECG variables, including heart rate.

Hematological evaluation showed reductions in hemoglobin, RBC count, and hematocrit in males at all dose levels and in females at 30 and 100 mg/kg/day. No effect on reticulocytes and no indication of bone marrow effects were noted.

Alkaline phosphatase was elevated in dogs treated at 30 and 100 mg/kg/day. Cholesterol and phospholipids were decreased in dogs treated at 30 and 100 mg/kg/day. Bile acids were unchanged. Following a 16-week treatment-free period, none of the findings seen during the dosing period and considered treatment related were present, suggesting complete reversibility.

There were no treatment-related changes in coagulation variables or in urinalysis.

An increase in liver weight was found in males and females treated at 30 and 100 mg/kg/day that correlated with macroscopically enlarged liver and the presence of centrilobular hepatocellular hypertrophy. Together with the increase in alkaline phosphatase, these findings are considered to reflect metabolic induction in the liver. Following completion of a 16-week treatment-free period, none of these findings were present, indicating their complete reversibility. The livers of dogs treated at 2 and 5 mg/kg/day were comparable to controls.

In the testes, treatment-related tubular dilation was present in males treated at 30 and 100 mg/kg/day, in high-dose males accompanied by tubular degeneration/ hypospermatogenesis and sperm stasis. In the absence of any degenerative changes and considering the background range for this finding, the minimal tubular dilation in a single animal treated at 5 mg/kg/day was not considered drug-related. No treatment-related findings were observed at the end of the 16-week treatment-free period, indicating complete reversal of the testicular findings seen at completion of the dosing period.

The incidence and the number of recordings of intimal thickening in the heart were increased in males and females treated at 30 and 100 mg/kg/day, whereas the 2- and 5- mg/kg/day groups were comparable with the controls [Table below]. Intimal thickening was recorded slightly more frequently in the right side of the heart and was characterized by minimal thickening of the intimal layer of the blood vessel wall. At the end of the 16-week treatment-free period, the number of recordings in dogs treated at 30 mg/kg/day was comparable with controls, suggesting partial reversal of this change.

Slight arteritis, characterized by periarterial and/or intramural inflammatory cell infiltration and fibrinoid necrosis, was recorded for the heart of individual control and treated dogs. These findings were considered to be part of the spontaneous background arteritis that occurs in animals of this strain and age.

In summary, organ toxicity and changes in hematology were observed at 30 and 100 mg/kg/day. Increased liver weight, correlating with hepatocellular hypertrophy and increased alkaline phosphatase, is considered a metabolic adaptation of the liver to drug treatment, and was seen at 30 and 100 mg/kg/day. The NOAEL was determined to be 5 mg/kg/day.

Cardiac findings in the 13-week study in Beagle dogs

Main groups (sacrifice after 13 weeks of treatment)

Group	1 (control)		2 (2 mg/kg/d)		3 (5 mg/kg/d)		4 (30 mg/kg/d)		5 (100 mg/kg/d)	
Sex	M	F	M	F	M	F	M	F	M	F
Exposure [1]	–	–	66	74	193	184	1021	877	2050	1681
Margin [2]	–	–	3.1	3.4	9.0	8.6	48	41	95	78
Arteritis [3]	1	0	0	0	0	1	0	1	0	1
Arterial intimal thickening [3,4]	1 (1)	0 (0)	0 (0)	2 (2)	1 (1)	1 (1)	3 (10)	3 (7)	3 (10)	3 (6)

[1] AUC_{0-24} of macitentan + ACT-132577 in µg·h/mL at Week 13.
[2] Relative to human dose of 10 mg per day and based on steady-state AUC_{0-24} of macitentan plus ACT-132577 in study AC-055-102 [D-06.044].
[3] Incidence, i.e., number of affected dogs out of four dogs per sex and group.
[4] Intimal thickening in all cardiac compartments; number of recordings in parenthesis.

Recovery groups (sacrifice after 16 weeks of recovery)

Group	1 (control)		2 (2 mg/kg/d)		3 (5 mg/kg/d)		4 (30 mg/kg/d)		5 (100 mg/kg/d)	
Sex	M	F	M	F	M	F	M	F	M	F
Arteritis [1]	1	0	0	0	0	1	0	0	1	0
Arterial intimal thickening [1,2]	2 (3)	1 (1)	2 (2)	0 (0)	2 (2)	2 (2)	2 (4)	3 (3)	3 (9)	3 (5)

[1] Incidence, i.e., number of affected dogs out of three dogs per sex and group.
[2] Intimal thickening in all cardiac compartments; number of recordings in parenthesis.

Again, the cardiac pathology is reminiscent of that encountered with vasodilators in this species. Exacerbation of idiopathic canine polyarteritis (BPS) is described in the literature.

Study T-05.027

This 39-week oral (capsule) toxicity study of macitentan was conducted in Beagle dogs (4/sex/group) at dose levels of 0 (vehicle), 5, 30, and 100/75 mg/kg/day . The 100-mg/kg/day dose was reduced to 75 mg/kg/day during Week 20 due to clinical signs (noisy breathing/rales). The treatment period was followed by a 16-week recovery period (4 dogs/sex/group). The study included toxicokinetic evaluations.

Four dogs were prematurely terminated on welfare grounds during the treatment period. These animals included two males (in Weeks 12 and 21) and one female (Week 21) receiving 100 mg/kg/day, all assigned to the recovery group, and one main group male receiving 30 mg/kg/day (Week 13). The main clinical signs and the histopathological lesions were identified in three of the four animals as being affected by idiopathic canine polyarteritis (Beagle pain syndrome). An additional male in the 100/75-mg/kg/day group displayed the majority of these clinical signs during the course of the study, and exhibited histopathological changes consistent with those noted for the prematurely sacrificed animals, but completed the treatment period. The high-dose male killed in Week 12 showed signs indicating acute infection and histopathological evidence of hemorrhagic bronchopneumonia. Therefore, the reason for premature termination on welfare grounds was clinical signs that can be attributed to idiopathic canine polyarteritis or pneumonia and poor general condition.

Epiphora was apparent in animals receiving 30 mg/kg/day or above, and, to a minimal extent, in animals at 5 mg/kg/day. This finding persisted to the end of the recovery period in animals previously treated at all dose levels. Reddening of the extremities, muzzle, pinna, and ventral abdominal region was apparent in animals at all dose levels, but regressed by Week 11. Noisy breathing was apparent from Week 14 in animals receiving 30 mg/kg/day or above and regressed following cessation of treatment. Watery nasal discharge was apparent in a few dogs at 30 or 100/75 mg/kg/day between Weeks 9 and 28.

Lower mean body-weight gain and some individual body weight losses were noted over the treatment period for males receiving 100/75 or 30 mg/kg/day.

Blood pressure measurements in Weeks 13 and 26 showed substantially reduced group mean arterial pressure (between -16 and -27 mmHg) 2 h after dosing in animals at 30 mg/kg/day and above.

Hematological evaluation after 39 weeks of treatment showed slight decreases in hematocrit, hemoglobin concentration, and RBC count in males and females at 100/75 mg/kg/day, and in males at 30 mg/kg/day. Elevated platelet counts were apparent at 100/75 and 30 mg/kg/day in both sexes, and a dose-related slight increase in platelet distribution width was noted in females. Full recovery was demonstrated following cessation of treatment, except for the platelet distribution width. There were no effects on WBC variables, except for clearly reduced numbers of WBC in all four of the animals which showed signs associated with idiopathic canine polyarteritis and were receiving

100/75 mg/kg/day. No effect on reticulocytes and no indication of bone marrow effects were noted.

Treatment-related blood chemistry findings comprised increased alkaline phosphatase in dogs receiving 100/75 mg/kg/day, with a greater effect apparent when these dogs were receiving 100 mg/kg/day than at 75 mg/kg/day. Recovery was apparent in males but not females. Serum bile acids were generally lower in treated groups than in control.

Cardiac arterial intimal thickening as the essential drug-related finding was observed at an increased incidence and severity in males and females receiving 30 or 100/75 mg/kg/day [Table below]. In most cases, intimal thickening was associated with breaks of the internal elastic lamina. Cardiac or multicentric arteritis/periarteritis of the type commonly observed in dogs presenting with idiopathic canine polyarteritis were observed at 30 mg/kg/day and above. It cannot be excluded that the treatment with the test item in this study non-specifically facilitated the development, or aggravated the course, of this spontaneous canine disease.

After a 16-week treatment-free period, one male of the low dose group, and one female each of mid and high dose groups showed arteritis.

Cardiac findings in the 39-week study in Beagle dogs

Main groups (sacrifice after 39 weeks of treatment)

Group	1 (control)		2 (5 mg/kg/d)		3 (30 mg/kg/d)		4 (100/75 mg/kg/d)	
Sex	Males	Females	Males	Females	Males	Females	Males	Females
Exposure[1]	–	–	124	84	488	363	973	1238
Safety margin[2]	–	–	5.8	3.9	22.7	16.9	45	58
Arteritis/periarteritis[3]	0/4	0/4	1/4	1/4	0/4	0/4	2/6	2/5
Arterial intimal thickening[3]	1/4	0/4	1/4	1/4	2/4	1/4	4/6	2/5

[1] AUC_{0-24} of macitentan + ACT-132577 in µg*h/mL at Week 39.
[2] Relative to human dose of 10 mg per day and based on steady-state AUC_{0-24} of macitentan plus ACT-132577 in study AC-055-102 [D-06.044].
[3] Incidence, i.e., number of affected animals/number of animals per sex and group.

Recovery groups (sacrifice after 16 weeks of recovery)

Group	1 (control)		2 (5 mg/kg/d)		3 (30 mg/kg/d)		4 (100/75 mg/kg/d)	
Sex	Males	Females	Males	Females	Males	Females	Males	Females
Arteritis/periarteritis[1]	0/4	0/4	1/4	0/4	0/4	1/4	0/2	1/3
Arterial intimal thickening[1]	1/4	0/4	1/4	0/4	0/4	1/4	0/2	1/3

[1] Incidence, i.e., number of affected animals/number of animals per sex and group.

Again, the cardiac vasculopathy is not unexpected.
In the liver, centrilobular hepatocyte hypertrophy and marginally increased brown hepatocyte pigment were observed in the 30- and 100/75-mg/kg/day groups, with a dose-dependent relationship. This correlated with macroscopically enlarged liver and increased liver weight. These findings were not apparent following the recovery period.

In the testes, an increased degree and incidence of hypospermatogenesis was observed in males receiving 100/75 mg/kg/day. Seminiferous tubular dilation was also noted in males receiving 30 mg/kg/day or above. Complete recovery was apparent.

In the spleen, a marginally increased degree of extramedullary hematopoiesis was apparent in females receiving 100/75 mg/kg/day. Complete recovery was apparent.

Due to the occurrence of only minor clinical signs without toxicological significance (e.g., epiphora) in the low-dose group, the NOAEL for the study was established at 5 mg/kg/day.

Toxicokinetics in repeat-dose studies

The systemic exposures to macitentan and its active metabolite, ACT-132577, were monitored in repeated-dose toxicity studies in mice (2- and 13-week studies), rats (4-, 13-, and 26-week studies), and dogs (4-, 13-, and 39-week studies), in the pilot toxicity study in rabbits, and in the embryo-fetal toxicity studies in rats and rabbits. The inactive metabolite ACT-373898 was monitored in 13-week mouse studies, in 4-, 13-, and 26-week rat studies, and in 13- and 39-week dog studies. Tabulated summaries of exposures at the beginning and the end of treatment in individual studies are provided below.

Summary of macitentan and ACT-132577 exposures in 2-week toxicity studies in mice

Macitentan Dose (mg/kg/day)	Time	Macitentan AUC_{0-24} (μg·h/mL)		ACT-132577 AUC_{0-24} (μg·h/mL)	
		Male	Female	Male	Female
2-week toxicity study in the CD-1 mouse [T-05.154]					
150	Day 1	638	1000	856	1130
450	Day 1	959	1590	1290	2390
1500	Day 1	1670	2110	3110	3310
150	Week 2	529	918	948	1230
450	Week 2	735	1020	1380	2180
1500	Week 2	879	1110	2030	2930
2-week toxicity study in the B6C3F1 mouse [T-08.024]					
5	Day 1	49	60	93	118
35	Day 1	136	174	246	304
230	Day 1	861	697	1120	957
1500 (batch 12)	Day 1	1510	1860	2030	2530
1500 (batch 4)	Day 1	1490	1680	2070	2030
5	Week 2	24	33	70	79
35	Week 2	115	113	232	257
230	Week 2	560	843	1020	1370
1500 (batch 12)	Week 2	1140	1690	2570	2960
1500 (batch 4)	Week 2	1010	1390	2190	2660

Values are based on means from 2–3 mice/sex/dose per sampling time point.

Summary of macitentan, ACT-132577 and ACT-373898 exposures in 13-week toxicity studies in mice

Macitentan Dose (mg/kg/day)	Time	Macitentan AUC_{0-24} (µg·h/mL)		ACT-132577 AUC_{0-24} (µg·h/mL)		ACT-373898 AUC_{0-24} (µg·h/mL)	
		Male	Female	Male	Female	Male	Female
13-week toxicity study in the CD-1 mouse [T-05.155]							
75	Day 1	728	667	725	980	–	–
300	Day 1	821	1160	1280	1370	–	–
900	Day 1	1050	1440	1750	2480	–	–
75	Week 13	428	670	706	928	–	–
300	Week 13	528	823	1110	1410	–	–
900	Week 13	571	953	1680	2610	–	–
13-week follow-up toxicity study in the CD-1 mouse [T-07.208]							
5[(1)]	Day 1	14	13	38	37	0.005	0.005
20[(1)]	Day 1	62	94	128	172	0.020	0.020
75[(1)]	Day 1	122	194	262	356	0.066	0.092
5	Week 3	24	46	77.3	95.9	0.016	0.016
20	Week 3	68	112	188	242	0.032	0.052
75[(2)]	Week 3	159	296	397	477	0.146	0.271
5	Week 13	20	36	72	90	–	–
20	Week 13	76	127	219	255	–	–
75	Week 13	224	358	535	641	–	–
13-week toxicity study in the B6C3F1 mouse [T-08.388]							
10	Day 1	84	115	140	195	0.011	0.022
50	Day 1	357	333	446	475	0.071	0.060
400	Day 1	1120	1020	1230	1160	0.238	0.266
1500	Day 1	1290	1510	1470	1910	0.393	0.762
10	Week 13	66[(3)]	100	185	238	0.017	0.038
50	Week 13	307	428	632	768	0.130	0.249
400	Week 13	653	1140	1600	1720	0.456	1.25
1500	Week 13	1090	1340	2710	2970	1.34	3.42

Values are based on means from 2–3 mice/sex/dose per sampling time point.

(1) Actual doses administered on Day 1 as revealed by formulation analysis were lower than intended.

(2) For ACT-373898: Following the highest dose of 75 mg/kg/day, the plasma concentrations are missing at 1 and 7 h after dosing; however, AUC and C_{max} could still be estimated.

(3) Sampling interval 0–10 h.

– , not analyzed.

Summary of macitentan, ACT-132577, and ACT-373898 exposures in the 4-, 13-, and 26-week toxicity studies in rats

Macitentan Dose (mg/kg/day)	Time	Macitentan AUC_{0-24} (µg·h/mL)		ACT-132577 AUC_{0-24} (µg·h/mL)		ACT-373898 AUC_{0-24} (µg·h/mL)	
		Male	Female	Male	Female	Male	Female
4-week toxicity study in the Sprague Dawley rat, Study No. [T-04.043]							
50	Day 1	213	726	398	279	0.009	0.157
150	Day 1	382	1160	699	479	0.039	0.337
450	Day 1	595	1590	1640	776	0.067	0.530
1500	Day 1	979	2500	1140	1190	0.133	0.886
50	Week 4	114	490	288	342	0.033	0.500
150	Week 4	200	776	499	556	0.117	1.06
450	Week 4	327	1280	695	497	0.403	1.90
1500	Week 4	365	1590	925	1680	0.654	2.64
13-week toxicity study in the Wistar rat, Study No. [T-04.076]							
10	Day 1	17.2	92.6	55.2	62.8	–	–
50	Day 1	73.3	220	286	160	–	–
250	Day 1	185	636	803	451	–	–
1500 [(1)]	Day 1	560	1600	2390	1360	0.048	0.198
10	Week 13	11.3	66.9	44.9	52.4	–	–
50	Week 13	34.1	170	142	169	–	–
250	Week 13	74.2	446	324	490	–	–
26-week toxicity study in the Wistar rat, Study No. [T-05.045]							
10	Day 1	21.9	81.0	91.0	53.2	BLQ	0.039
50	Day 1	67.5	365	294	210	BLQ	0.152
250	Day 1	202	940	853	514	0.040	0.222
10	Week 26	9.11	67.4	41.0	62.1	BLQ	0.063
50	Week 26	30.2	229	122	214	0.014	0.266
250	Week 26	39.5	371	210	467	0.022	0.436

Values are based on means from 3 rats/sex/dose per sampling time point.
[(1)] 1500-mg/kg/day group was prematurely terminated in Week 2 due to poor condition.
– , not analyzed; BLQ; below limit of quantification

Summary of macitentan, ACT-132577, and ACT-373898 exposures in 4-, 13-, and 39-week toxicity studies in dogs

Macitentan Dose (mg/kg/day)	Time	Macitentan AUC_{0-24} (µg·h/mL)		ACT-132577 AUC_{0-24} (µg·h/mL)		ACT-373898 AUC_{0-24} (µg·h/mL)	
		Male	Female	Male	Female	Male	Female
4-week toxicity study in the dog, Study No. [T-04.049]							
5	Day 1	17.5	11.6	104	104	–	–
50	Day 1	134	94.8	759	989	–	–
500 (1)	Day 1	692	504	3,000	3,370	–	–
5	Week 4	14.7	10.5	146	164	–	–
50	Week 4	39.1	46.5	1360	1220	–	–
250 (1)	Week 4	44.9	84.4	2530	3,130	–	–
13-week toxicity study in the dog, Study No. [T-04.077]							
2	Day 1	8.46	7.61	39.0	52.3	BLQ	0.005
5	Day 1	19.8	25.9	124	103	0.007	0.011
30	Day 1	99.6	63.3	467	550	0.040	0.050
100	Day 1	267	215	1490	1200	0.093	0.240
2	Week 13	9.16	7.33	56.7	66.4	–	–
5	Week 13	15.4	24.4	178	160	–	–
30	Week 13	55.1	34.3	966	843	–	–
100	Week 13	60.2	101	1990	1580	–	–
39-week toxicity study in the dog, Study No. [T-05.027]							
5	Day 1	11.8	9.5	105	91.7	0.012	0.003
30	Day 1	53.6	48.8	467	479	0.048	0.075
100/75 (2)	Day 1	106	140	849	1360	0.075	0.172
5	Week 39	10	6.14	114	77.8	0.088	0.057
30	Week 39	22.9	21.9	465	341	0.034	0.047
100/75 (2)	Week 39	31.3	37.9	942	1200	0.072	0.121

Values are based on means from 3–4 dogs/sex/dose.

(1) From Day 14 onwards, the highest dose level was reduced from 500 to 250 mg/kg/day.

(2) 100 mg/kg/day dose reduced to 75 mg/kg/day during Week 20.

BLQ, below limit of quantification ; –, not analyzed .

7 Genetic Toxicology

The following studies were all reviewed under IND 77258. All were conducted in accordance with GLP and employed appropriate strains and controls.

There was no evidence for genotoxicity with ACT-064992 in a battery of *in vitro* tests that included the bacterial reverse mutation assay in *Salmonella typhimurium* (Ames test, Study #T-07.106), the mouse lymphoma assay (Study #T-04.045), and the chromosome aberration test in human lymphocytes (Study #T-07.107). For some tests, different drug substance batches were used; all *in vitro* tests were conducted with and without metabolic activation.

An additional Ames test (Study #T-04.072), performed with the metabolite ACT-080803, also gave no evidence of a mutagenic effect.

The mutagenic potential of the metabolite ACT-373898 was assessed as well [T-12.056]. ACT-373898 did not produce an increase in the number of revertants in any of the strains tested in the presence and absence of metabolic activation.

There was no evidence of chromosome damage or effects on the spindle apparatus in an *in vivo* micronucleus test in rat bone marrow (Study #T-04.044).

8 Carcinogenicity

Carcinogenicity was assessed in mice and rats. Both studies were in accordance with GLP and had prior concurrence, with the Executive Carcinogenicity Assessment Committee, regarding the strain, number of animals employed, and doses evaluated. Both studies were considered negative by the Committee.

Study title: ACT-064992: 104-Week Oncogenicity (Gavage) Study in the B6C3F1 Mouse

Study no.:	T-09.160
Study report location:	NDA 204410 SDN 000
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/24/09
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	178194 / Batch 13
CAC concurrence:	yes, 5/21/09

Key Study Findings

The test item did not increase the incidence of neoplastic lesions when tested up to a MTD. High morbidity/mortality in group 5 females (allocation A: 35/60, 400 mg/kg/day) led to early termination of this dose group in week 79. The cause of death could not be established in most of the animals. The remaining treatment groups did not show significant differences in mortality compared to controls.

AUC exposure ratios, at Week 26 for the 100 mg/kg dose, for ACT-064992 (parent), ACT-132577 (active metabolite) and ACT-373898 (inactive metabolite) were 139x, 77x and 1x (females) and 75x, 58x and 0.4x (males), respectively..

Adequacy of Carcinogenicity Study

Mice were dosed up to a demonstrated maximum dose based on MTD. Conduct of the study was consistent with FDA Guidances. Appropriate tissues were examined.

Appropriateness of Test Models

The strain of mouse utilized, and the general study design, is appropriate for the study.

Methods

Doses:	5, 30, 100, and 400 mg/kg/day
Frequency of dosing:	daily
Dose volume:	10 mL/kg
Route of administration:	oral, gavage
Formulation/Vehicle:	0.5% methylcellulose
Basis of dose selection:	MTD
Species/Strain:	Mouse, B6C3F1 (SPF)
Number/Sex/Group:	60
Age:	3-4 weeks
Animal housing:	Individually in Makrolon type-2 cages with wire mesh tops
Paradigm for dietary restriction:	none, fed *ad libitum*
Dual control employed:	no
Interim sacrifice:	no
Satellite groups:	24/sex/group for TK
Deviation from study protocol:	no serious deviations

Observations and Results

Mortality

Mice were observed twice daily, at the beginning and at the end of each working day during the entire study period. High morbidity/mortality in group 5 females (allocation A: 35/60) led to early termination of this dose group in week 79. The cause of death could not be established in most of the animals. The remaining treatment groups did not show significant differences in mortality compared to controls.

Clinical Signs

Clinical signs were evaluated weekly during the entire study period. The time of onset, location, dimensions, appearance and progression of each palpable tumor was recorded. An individual record was maintained of the clinical condition of each animal. Test item-related clinical signs of respiratory distress (e.g. breathing noises, labored breathing, tachypnea, cyanosis (blue discoloration of the whole body)) were recorded in females at 400 mg/kg/day (from week 33 onwards) and 100 mg/kg/day (from week 47 onwards) and in males at 400 mg/kg/day (from week 40 onwards).

Body Weights

Body weights were determined weekly during acclimatization week 1 and 3, twice during acclimatization week 2, on treatment day 1, weekly up to week 17, every four weeks thereafter and before necropsy.

Mean body weight and mean body weight gain of both sexes at 5 mg/kg/day were not affected by the treatment with the test item. Both sexes showed a test item-related decrease in mean body weight and mean body weight gain during the treatment phase at dose levels ≥30 mg/kg/day. Mean body weight was statistically significantly decreased in males at 400 mg/kg/day starting on treatment day 286 and in males at 100

mg/kg/day starting on treatment day 650. Males at 100 and 400 mg/kg/day showed a statistically significant decrease in mean body weight of -7% and -17%, respectively, as compared to control on treatment day 728 (end of treatment phase).

Mean body weight of males at 30 mg/kg/day was slightly decreased towards the end of treatment phase (-4% as compared to control on treatment day 728). Mean body weight gain of males at 30, 100 and 400 mg/kg/day was decreased - partly attaining statistical significance - during the treatment phase with a statistically significant decrease at the end of treatment phase on treatment day 728 of -15%, -18%, and -47%, respectively.

Mean body weight was statistically significantly decreased in females at 400 mg/kg/day starting on treatment day 230, in females at 100 mg/kg/day starting on treatment day 398, and in females at 30 mg/kg/day starting on treatment day 482. Females at 30 and 100 mg/kg/day showed a statistically significant decrease in mean body weight of -8% and -19% as compared to control on treatment day 728. Females at 400 mg/kg/day showed a statistically significant decrease in mean body weight of -35% as compared to control on treatment day 538 (latest possible comparison in that early terminated group).

The mean body weight gain was statistically significantly decreased in females at 400 mg/kg/day starting on treatment day 230, in females at 100 mg/kg/day starting on treatment day 286, and in females at 30 mg/kg/day starting on treatment day 314 (no statistical significance on treatment day 370). Females at 30 and 100 mg/kg/day showed a statistically significant decrease in mean body weight gain of -20% and -42% as compared to control on treatment day 728. Females at 400 mg/kg/day showed a statistically significant decrease in mean body weight of -70% as compared to control on treatment day 538 (latest possible comparison in that early terminated group).

Food Consumption

Food consumption was measured once during acclimatization, starting on treatment day 1, weekly up to start of week 18 (day 120), every 4 weeks thereafter. Food consumption was decreased - partly attaining statistical significance - in males at 400 mg/kg/day (starting on treatment day 533) and in females at 400 and 100 mg/kg/day (starting on treatment day 281).

Gross Pathology

A high incidence of discoloration of the body was noted in unscheduled deaths of females at 100 and 400 mg/kg/day. At ≥30 mg/kg/day, the incidence of thickened uterus was statistically significantly increased in females after 28 weeks. After 104 weeks (and in week 79 for group 5 females), cystic-thickened uterus wall was increased in females at ≥30 mg/kg/day.

<u>Organ weights</u>

Terminal body weights were statistically significantly decreased in both sexes at 100 mg/kg/day and in males at 400 mg/kg/day after 104 weeks. Females at 400 mg/kg/day - terminated in week 79 - showed a markedly low terminal body weight.

The following test item-related changes - partly attaining statistical significance - on absolute and relative organ weights were recorded in allocation B animals after 28 weeks as well as in allocation A animals after week 104 (data are given as percent difference from control, female group 5 of allocation A not included):

			MALES (Group)				FEMALES (Group)			
Organ	**Weight**	**After Week**	**2**	**3**	**4**	**5**	**2**	**3**	**4**	**5**
Liver	Absolute weight	28	+10%#	+9%#	+16%**	+23%**				+11%**
		104								
	Rel. to body weight	28	+5%#	+9%**	+12%**	+24%**				+15%**
		104					+22%*	+22%*	+20%#	
	Rel. to brain weight	28	+9%#	+9%#	+17%**	+22%**				+13%**
		104								
Spleen	Absolute weight	28				+12%*		+12%*	+13%**	+14%**
		104					+143%*	+115%#		
	Rel. to body weight	28				+12%*		+14%**	+13%*	+19%**
		104					+137%*	+125%#	+33%#	
	Rel. to brain weight	28						+13%*	+14%**	+16%**
		104					+153%*			

			MALES (Group)				FEMALES (Group)			
Organ	**Weight**	**After Week**	**2**	**3**	**4**	**5**	**2**	**3**	**4**	**5**
Uterus	Absolute weight	28						+45%**	+53%**	+38%**
		104					+91%**	+82%*	+139%**	
	Rel. to body weight	28					+20%#	+46%**	+53%**	+43%**
		104					+94%**	+90%*	+177%**	
	Rel. to brain weight	28						+46%**	+54%**	+41%**
		104					+93%**	+81%*	+133%**	

Histopathology

All surviving allocation A and B animals as well as all allocation A and B animals which were found dead or killed moribund were weighed and necropsied. Descriptions of all macroscopic abnormalities were recorded. All animals surviving to the end of the observation period and all moribund animals were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination.

Samples of the following tissues and organs were collected from all decedents of allocation A, as well as all animals (allocation A and B) at scheduled necropsy. Unless otherwise indicated, tissues and organs were fixed in neutral phosphate buffered 4% formaldehyde solution. Additional tissues (such as ear tattoo) were retained in accordance with standard operating procedures but not processed or examined further.

Tissues / Organs	Weight	Collect	Examine
Adrenal glands	X	X	X
Aorta		X	X
Bone (sternum, femur including joint)		X	X
Bone marrow (femur)		X	X
Brain - including section of medulla/pons, cerebral and cerebellar cortex[1]	X	X	X
Cecum		X	X
Colon		X	X
Duodenum		X	X
Epididymides (fixed in Davidson`s solution)	X	X	X
Esophagus		X	X
Eyes with optic nerve (fixed in Davidson's solution)		X	X
Harderian gland (fixed in Davidson's solution)		X	X
Heart including auricles	X	X	X
Ileum, with Peyer's patches		X	X
Jejunum with Peyer's patches		X	X
Kidneys	X	X	X
Larynx		X	X
Lacrimal gland, exorbital		X	X
Liver with gall bladder	X	X	X
Lungs, filled with formalin at necropsy	X	X	X
Lymph nodes – mesenteric and mandibular		X	X
Mammary gland area		X	X
Nasal cavity (4 levels)		X	X[2]
Ovaries with oviducts	X	X	X
Pancreas		X	X
Pharynx		X	X
Pituitary gland		X	X
Preputial / clitorial glands		X	X
Prostate gland	X	X	X
Rectum		X	X
Salivary glands - mandibular, sublingual, parotid		X	X
Sciatic nerve		X	X
Seminal vesicles incl. coagulating glands		X	X
Skeletal muscle		X	X
Skin and subcutaneous tissue (location different from mammary gland)		X	X
Spinal cord - cervical, midthoracic, lumbar		X	X
Spleen	X	X	X
Stomach (non-glandular and glandular)		X	X
Testes (fixed in Davidson's solution)	X	X	X
Thymus	X	X	X
Thyroid (incl. parathyroid gland, if possible)		X	X
Tongue		X	X
Trachea		X	X
Ureter		X	X
Urinary bladder, filled with formalin at necropsy		X	X

cont.

Tissues / Organs	Weight	Collect	Examine
Uterus (horn, corpus, cervix)	X	X	X
Vagina		X	X
Zymbal's glands		X	X
All gross lesions		X	X

Peer Review

A pathology peer review was conducted under non-GLP conditions by a peer reviewing Pathologist.

Neoplastic findings

Sponsor's evaluation
There were no neoplastic lesions that could be attributed to treatment with the test item. There was only one neoplastic lesion that revealed a statistically significant increase in the trend test according to PETO and LIN and RAHMAN (i.e. $p < 0.025$ for rare tumors or $p < 0.005$ for common tumors). It was considered not test item-related because it occurred in a non-protocol organ (body cavities) and in a single animal:

- Females. Body cavities: Hemangioma. Distribution: 0/12, 0/16, 0/8, 1/3 in groups 1, 2, 3, and 4, respectively.

FDA evaluation
Independent statistical evaluation performed at FDA (reviewer: Mohammad Atiar Rahman, Ph.D.) did not find evidence for any significant increases in tumor incidence.

Non Neoplastic findings

In main test animals assigned for the 104-week terminal sacrifice, there were minor alterations in nasal cavities. Increased secretion was observed in group 4 females and group 5 males in nasal cavity level 1 and in group 4 males and females and group 5 males in nasal cavity level 2. Hyaline inclusions were observed at the transition from respiratory to olfactory mucosa of nasal cavity level 1, and in the olfactory mucosa of levels 2 and 3 in males and females of groups 3 to 5. Main locations were the dorsal meatus in all levels as well as the septum and turbinates in levels 2 and 3. At the cellular level, the inclusions were considered to be located in sustentacular cells.

Focal or multifocal proliferation of the nasal mucosa ("mucosal hyperplasia") mainly affecting respiratory epithelium and submucosal glands was observed in all dose groups and/or in all nasal cavity levels. Increased incidences of inflammatory infiltration in the submucosa / submucosal glands were observed in all dose groups, mainly in nasal cavity level 2. Occasionally, there were other findings like inflammatory secretion (pus), focal or multifocal epithelial disorganization / degeneration or focal squamous metaplasia. These lesions, however, were distributed randomly.

Findings in the nasal cavity are summarized below.

Incidence and Mean Severity of Main Findings in Nasal Cavities. Main Test Animals, including Deaths. From group 5 females, only decedents are included.

Finding / Groups	1		2		3		4		5	
Total Affected / Mean Severity	(60) M	(60) F	(60) M	(60) F	(60) M	(60) F	(60) M	(60) F	(60) M	(35) F*
Nasal Cavity Level 1										
Secretion	23/1.4	7/1.0	4/1.0	1/1.0	5/1.6	12/1.3	24/1.5	28/1.3	46/1.5	9/1.1
Hyaline inclusions	27/1.1	15/1.1	14/1.4	11/1.5	26/1.5	34/2.1	48/1.9	54/1.9	40/1.4	32/2.0
Mucosal hyperplasia	3/1.0	5/1.0	16/1.1	9/1.1	21/1.3	26/1.5	36/1.3	16/1.0	9/1.2	4/1.0
Nasal Cavity Level 2										
Secretion	3/1.7	0	1/1.0	3/1.0	2/1.0	5/1.0	14/1.2	17/1.4	30/1.5	1/2.0
Hyaline inclusions	10/1.2	24/1.3	9/1.2	15/1.1	28/1.6	34/2.2	52/2.4	57/2.4	54/1.6	33/2.1
Mucosal hyperplasia	9/1.1	9/1.0	25/1.2	23/1.2	28/1.3	36/1.3	40/1.4	28/1.1	20/1.2	7/1.0
Inflammation, mucosal glands	8/1.0	6/1.0	19/1.0	12/1.2	13/1.2	21/1.1	30/1.2	25/1.0	14/1.0	5/1.0
Nasal Cavity Level 3										
Hyaline inclusions	4/1.0	15/1.1	8/1.3	18/1.3	12/1.8	35/2.4	50/2.1	58/2.2	55/1.5	34/2.4
Mucosal hyperplasia	0	1/1.0	1/1.0	6/1.3	4/1.3	11/1.6	10/1.1	3/1.0	0	2/1.0

Similar lesions were seen also in the nasal cavities of toxicokinetic animals (dosed only 26 weeks) and group 5 females that were sacrificed in week 79, whereby there was mainly the presence of hyaline inclusions.

In the liver of main test and toxicokinetic animals, the incidence of hepatocellular hypertrophy was increased in males of groups 2 to 5.

Toxicokinetics

Blood samples (approximately 0.3 mL) were collected from the retro orbital plexus from all allocation B (toxicokinetic) animals under light isoflurane anesthesia, on Day 1 and during Week 26. The determination of ACT-064992 and its metabolites ACT-132577 and ACT-373898 was done by LC-MS/MS. Results are presented below.

Toxicokinetic parameters and dose dependence of exposures of ACT-064992 and its metabolites ACT-132577 and ACT-373898 after oral administration of ACT-064992 to mice

Compound	Dose	Week	Sex	Tmax	Cmax	AUClast	Tlast	Cmax/D	AUClast/D
	(mg/kg)			(h)	(µg/mL)	(h*µg/mL)	(h)	(µg/mL)/ (mg/kg)	(h*µg/mL)/ (mg/kg)
ACT-064992	5	1	f	2	5.10	33.1	10	1.0	6.6
ACT-064992	5	1	m	1	5.05	25.6	10	1.0	5.1
ACT-064992	5	26	f	1	8.25	60.7	24	1.7	12
ACT-064992	5	26	m	1	7.97	31.1	10	1.6	6.2
ACT-064992	30	1	f	1	21.8	218	24	0.7	7.3
ACT-064992	30	1	m	2	19.6	203	24	0.7	6.8
ACT-064992	30	26	f	1	52.0	392	24	1.7	13
ACT-064992	30	26	m	1	37.9	261	24	1.3	8.7
ACT-064992	100	1	f	2	37.2	460	24	0.4	4.6
ACT-064992	100	1	m	7	45.1	465	24	0.5	4.7
ACT-064992	100	26	f	1	71.4	751	24	0.7	7.5
ACT-064992	100	26	m	1	57.7	405	24	0.6	4.0
ACT-064992	400	1	f	7	96.0	1160	24	0.2	2.9
ACT-064992	400	1	m	4	88.3	880	24	0.2	2.2
ACT-064992	400	26	f	2	105	1110	24	0.3	2.8
ACT-064992	400	26	m	1	83.3	751	24	0.2	1.9
ACT-132577	5	1	f	4	6.78	97.3	24	1.4	19
ACT-132577	5	1	m	4	5.72	79.0	24	1.1	16
ACT-132577	5	26	f	4	8.88	129	24	1.8	26
ACT-132577	5	26	m	2	9.31	106	24	1.9	21
ACT-132577	30	1	f	10	21.2	346	24	0.7	12
ACT-132577	30	1	m	7	20.0	357	24	0.7	12
ACT-132577	30	26	f	7	40.6	609	24	1.4	20
ACT-132577	30	26	m	4	39.3	574	24	1.3	19

(cont.)

Compound	Dose	Week	Sex	Tmax	Cmax	AUClast	Tlast	Cmax/D	AUClast/D
	(mg/kg)			(h)	(µg/mL)	(h*µg/mL)	(h)	(µg/mL)/ (mg/kg)	(h*µg/mL)/ (mg/kg)
ACT-132577	100	1	f	10	39.1	619	24	0.4	6.2
ACT-132577	100	1	m	7	38.6	652	24	0.4	6.5
ACT-132577	100	26	f	10	73.7	1190	24	0.7	12
ACT-132577	100	26	m	7	62.5	907	24	0.6	9.1
ACT-132577	400	1	f	10	107	1520	24	0.3	3.8
ACT-132577	400	1	m	10	72.8	1220	24	0.2	3.0
ACT-132577	400	26	f	10	124	1920	24	0.3	4.8
ACT-132577	400	26	m	10	110	1750	24	0.3	4.4
ACT-373898	5	1	f	2	0.00168	0.00885	7	0.0003	0.002
ACT-373898	5	1	m	1	0.00113	0.00164	2	0.0002	0.0003
ACT-373898	5	26	f	2	0.00337	0.0231	10	0.0007	0.005
ACT-373898	5	26	m	1	0.00205	0.00535	4	0.0004	0.001
ACT-373898	30	1	f	2	0.00586	0.0432	10	0.0002	0.001
ACT-373898	30	1	m	1	0.00398	0.0286	10	0.0001	0.001
ACT-373898	30	26	f	2	0.0249	0.229	24	0.0008	0.008
ACT-373898	30	26	m	1	0.0129	0.186	24	0.0004	0.006
ACT-373898	100	1	f	7	0.00959	0.127	24	0.0001	0.001
ACT-373898	100	1	m	7	0.00823	0.0608	10	0.00008	0.0006
ACT-373898	100	26	f	4	0.0489	0.491	24	0.0005	0.005
ACT-373898	100	26	m	2	0.0225	0.145	10	0.0002	0.002
ACT-373898	400	1	f	7	0.0335	0.420	24	0.00008	0.001
ACT-373898	400	1	m	4	0.0161	0.195	24	0.00004	0.0005
ACT-373898	400	26	f	2	0.126	1.20	24	0.0003	0.003
ACT-373898	400	26	m	2	0.0552	0.538	24	0.0001	0.001

Dosing Solution Analysis

With the exception of group 5 from 24-JUN-2009 (111.5%), groups 2, 3, 4 and 5 from 07-OCT- 2009 (72.4%, 80.3%, 77.1% and 81.8%), group 2 from 10-MAR-2010 (89.4%) and group 5 from 09-FEB-2011 (89.8%), for all other samples (samples taken after the end of preparation, mean of top, middle and bottom) the results as that of the nominal concentration were in the range from 90.0% to 105.8% and therefore within the specified range of 90.0% to 110%.

Study title: ACT-064992: 104-Week Oncogenicity (Gavage) Study in the Wistar Rat

Study no.:	T-09.159
Study report location:	NDA 204410 SDN 000
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5/27/09
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	178194 / Batch 13 (a, dosed until 05-Apr-2011) 178194 / Batch 15 (b, dosed from 06-Apr-2011) 100.3% (will be assumed to be 100% for dose calculation) (a and b)
CAC concurrence:	yes, 5/19/09

Key Study Findings

The test item did not increase the incidence of neoplastic lesions when tested up to a MTD. High morbidity/mortality in Group 4 and Group 5 females led to dose reductions to 25 mg/kg and 50 mg/kg in these Groups, respectively. The cause of death could not be established in most of the animals. The low dose group did not show significant differences in mortality compared to controls.

AUC exposure ratios, at Week 26 for the 50 mg/kg dose, for ACT-064992 (parent), ACT-132577 (active metabolite) and ACT-373898 (inactive metabolite) were 42x, 14.6x and 0.64x (females) and 8.3x, 10.6x and 0,08x (males), respectively.

Adequacy of Carcinogenicity Study

Rats were dosed up to a demonstrated maximum dose based on MTD. Conduct of the study was consistent with FDA Guidances. Appropriate tissues were examined.

Appropriateness of Test Models

The strain of mouse utilized, and the general study design, is appropriate for the study.

Methods

Doses:	Group 1: 0 mg/kg/day Group 2: 0 mg/kg/day Group 3: 10 mg/kg/day Group 4: 50/25 mg/kg/day Group 5: 250/50 mg/kg/day (also see chart below)
Frequency of dosing:	Rat, HanRcc: WIST(SPF)
Dose volume:	10 mL/kg
Route of administration:	oral, gavage
Formulation/Vehicle:	0.5% (w/v) methylcellulose
Basis of dose selection:	MTD and 25-fold AUC at 250 mg/kg/day
Species/Strain:	Rat, HanRcc: WIST(SPF)
Number/Sex/Group:	51
Age:	4 weeks
Animal housing:	In groups of three in Makrolon type-4 cages with wire mesh tops
Paradigm for dietary restriction:	no
Dual control employed:	yes, Groups 1 & 2
Interim sacrifice:	no
Satellite groups:	9/sex/Group for TK
Deviation from study protocol:	

Allocation and Dose Levels mg/kg bw/day		Group 1 Control*, b 0	Group 2 Control* 0	Group 3 10	Group 4 50/25a, b	Group 5 250/50a, b
Males	A	1 - 51	61 - 111	112 - 162	172 - 222	232 - 282
	B	52 - 60	---	163 - 171	223 - 231	283 - 291
Females	A	292 - 342	352 - 402	403 - 453	463 - 513	523 - 573
	B	343 - 351	---	454 - 462	514 - 522	574 - 582
	C	583 - 591	---	---	592 - 600	601 - 609

* Control animals were treated with the vehicle, an aqueous solution composed of 0.5% (w/v) methylcellulose, only.

a) Following an interim recovery from 28-Apr-2010 to 11-May-2010 the females of group 4 and 5 / allocation A, only were dosed at a level of 25 or 50 mg/kg/day dose, respectively, starting on 12-May-2010.

b) An additional allocation C for toxicokinetic evaluation of females exposed to 0, 25 and 50 mg/kg/day was introduced. For technical reasons, the allocation C animals exposed 0, 25 and 50 mg/kg/day were allocated to group 6, 7 and 8, respectively (but were reported as group 1, 4 and 5, respectively, as stated in the fifth amendment to study plan).

A Main study animals
B Animals for toxicokinetic evaluations
C Additional females for toxicokinetic evaluations

Observations and Results

Mortality

Rats were observed twice daily, at the beginning and at the end of each working day during the entire study period.

There was no dose-related effect on survival of male animals and in females at the low and mid dose level. The number of female decedents and moribund killed females was increased in group 5. In female groups 1 to 5 there were 12, 11, 16, 18 and 30 decedents / moribund killed animals, respectively, out of 51 animals per group. The number of unscheduled deaths, in which the cause of death or moribund condition could be determined, was 11, 11, 15, 15, 12 for the consecutive dose groups. Therefore, for the increased mortality in group 5 females, no overt pathological changes could be identified. There was no single or preponderant cause of morbidity / mortality that could be attributed to the effect of test item.

Clinical Signs

Signs were evaluated once daily by cage-side clinical observation during acclimatization, twice daily during the first two treatment weeks (immediately after dosing and approx. 6 hours after dosing); once daily thereafter (approx. 6 hours after dosing).

Clinical evaluations were conducted weekly during the entire study period. The time of onset, location, dimensions, appearance and progression of each palpable tumor was recorded. An individual record was maintained of the clinical condition of each animal

Test item-related clinical signs of respiratory distress (as e.g. breathing noises (both sexes at all dose levels), labored breathing (both sexes at all dose levels), tachypnea (both sexes of group 4 and 5), cyanosis (both sexes of group 4 and 5)) were noted in a dose-dependent manner in both sexes at all dose levels (in general with a higher incidence in females) starting in high and mid dose males during treatment week 43 and in high dose females during treatment week 34 (tachypnea was recorded in a single female since week 29). In addition, a significant increase in reduced general condition (e.g. weakened condition, visible weight loss, ruffled fur, hunched posture, decreased activity) was observed in females at 250/50 mg/kg/day (as compared to controls and the remaining dose groups) leading to an increased mortality rate.

Body Weights

Weights were recorded during acclimatization, on treatment day 1, weekly up to start of week 18 (day 120), every four weeks thereafter and before necropsy.

The mean body weight and mean body weight gain of both sexes at 10 and in males at 50 mg/kg/day was not affected by the treatment.

Males at 250 mg/kg/day showed a statistically significant decrease in mean body weight starting on treatment day 428 until the end of treatment period (last recording on day 728: -15%, $p < 0.01$ as compared to controls).

Females at 250/50 mg/kg/day showed a statistically significant decrease in mean body weight starting on treatment day 106 until the end of treatment period (last recording on day 728: -39%** as compared to controls). A statically significant decrease in mean body weight was noted in females at 50/25 mg/kg/day starting on treatment day 232 until the end of treatment period (last recording on day 728: -25%** as compared to control). The marginal decrease in mean body weight partly attaining statistical significance (2 of 5 occasions since study day 624) recorded in males at 50 mg/kg/day starting on treatment day 624 until the end of treatment period was considered to be incidental in the absence of a clear effect on weight gain.

The mean body weight **gain** in males at 10 and 50 mg/kg/day was not affected by the treatment. A statistically significantly decreased mean body weight gain was noted in males at 250 mg/kg/day from treatment day 456 until the end of treatment period (last recording on day 728: -18%** as compared to controls).

A statistically significant decrease in mean body weight **gain** was noted in females at 250/50 mg/kg/day starting on treatment day 78 and in females at 50/25 mg/kg/day starting on treatment day 232 until the end of treatment period (last recording on day 728: -53%** and -35%**, respectively, as compared to control). Females at 10 mg/kg/day showed a minimal, not statistically significant decrease in body weight gain towards the end of treatment period as compared to control.

Food Consumption

Consumption was measured once during acclimatization, weekly from Treatment Day 1 until the start of week 18 (day 120), every 4 weeks thereafter.

There was no effect on the food consumption of males at any dose level.

Females at the mid and high dose showed a test item-related decrease in food consumption. A statistically significant decrease in food consumption was recorded in females at 250/50 mg/kg/day from treatment day 43 until the end of treatment period with a mean of means over the treatment period of -14% as compared to the control (exception: no statistical significance on days 64 - 71 interval). Females at 50/25 mg/kg/day showed a statistical significant decrease in food consumption starting on treatment day 106 until the end of treatment period with a mean of means over the treatment period of -9% as compared to the controls (groups 1 and 2 combined) (exception: no statistical significance on days 309 - 316 interval). The marginally decreased food consumption partly attaining statistical significance (on 3 of 6 occasions since day 589) noted in females at 10 mg/kg/day from treatment day 589 until the end of treatment period was considered to be incidental. The mean of means over the treatment period for this group was lower by 3% as compared to the controls (groups 1 and 2 combined)).

Gross Pathology

All surviving allocation A and B as well as all allocation A and B animals which were found dead, moribund or were sacrificed *in extremis* were weighed and necropsied. Descriptions of all macroscopic abnormalities were recorded. All animals surviving to the end of the observation period and all moribund animals were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination.

The organs from allocation A and B animals listed in the Histopathology table were weighed before fixation and recorded on the scheduled dates of necropsy. Relative organ weights were calculated on the basis of the body weight and brain weight.

The following findings in absolute and relative organ weight recorded in allocation B animals in week 49 as well as in allocation A animals after week 104 are considered to be test item-related (data given as percent difference from control):

		MALES				FEMALES		
Organ	**Weight**	**In/after Week**	**3**	**4**	**5**	**3**	**4**	**5**
Body	terminal weight	49						-17*
		104			-15**	-7*	-25**	-41**
Lungs	absolute weight	49				+7#	+13#	+20*
		104					+8#	+19**
	rel. to body weight	49					+14#	+43**
		104		+10#	+28**	+11#	+47**	+103**
	rel. to brain weight	49					+13#	+22*
		104						+20**

		MALES				FEMALES		
Organ	**Weight**	**In/after Week**	**3**	**4**	**5**	**3**	**4**	**5**
Liver	absolute weight	49		+25**	+18#			
		104	+13**	+6#	+18**		-14**	-22**
	rel. to body weight	49		+18**	+22**	+9#	+15*	+40**
		104	+15**	+13**	+40**	+7#	+15**	+32**
	rel. to brain weight	49		+26**	+21*			
		104	+13**	+7#	+18**		-14**	-22**
Uterus	absolute weight	49						
		104					+36*	+26#
	rel. to body weight	49						
		104					+90**	+116**
	rel. to brain weight	49						
		104					+35*	+26#

Symbols: # no statistically significant difference; * statistically significant at 5%; ** statistically significant at 1%

Macroscopic findings

At necropsy of allocation A, the male animals had an increased incidence of enlarged liver in group 5. The female animals had an increased incidence of hepatodiaphragmatic hernia in group 4. In group 4 and 5 females there were dose-related decreased incidences of brain compression, pituitary gland nodule(s) and skin nodule(s). These changes reflected the dose-dependent reduction in the incidence of pituitary adenomas and of mammary gland neoplasms, respectively

Histopathology

Samples of the following tissues and organs were collected from all decedents of allocation A, as well as all animals (allocation A and B) at scheduled necropsy. Unless otherwise indicated, tissues and organs were fixed in neutral phosphate buffered 4% formaldehyde solution. Additional tissues (such as ear tattoo) were retained in accordance with standard operating procedures but not processed or examined further.

Because of potential test item-related effects the following organs were examined in the groups 3 and 4 of allocation B: the heart, kidneys, liver, pancreas, pituitary gland, testes and epididymides, thyroid gland and uterus/vagina. In allocation A the potential target organs examined in groups 3 and 4 were: male and female adrenal glands, female heart, male and female liver, male and female thyroid gland, testes and epididymides in males, and ovaries, uterus and vagina in females.

Tissues / Organs	Weight	Collect	Examine
Adrenal glands	X	X	X
Aorta		X	X
Bone (sternum, femur including joint)		X	X
Bone marrow (sternum, femur)		X	X
Brain - including section of medulla/pons, cerebral and cerebellar cortex[1]	X	X	X
Cecum		X	X
Colon		X	X
Duodenum		X	X
Epididymides (fixed in Davidson's solution)	X	X	X
Esophagus		X	X
Eyes with optic nerve (fixed in Davidson's solution)		X	X
Harderian gland (fixed in Davidson's solution)		X	X
Heart including auricles	X	X	X
Ileum, with Peyer's patches		X	X
Jejunum with Peyer's patches		X	X

cont.

Tissues / Organs	Weight	Collect	Examine
Kidneys	X	X	X
Larynx		X	X
Lacrimal gland, exorbital		X	X
Liver	X	X[§]	X
Lungs, filled with formalin at necropsy	X	X	X
Lymph nodes – mesenteric and mandibular		X	X
Mammary gland area		X	X
Nasal cavity (4 levels)		X	X[**]
Ovaries with oviducts	X	X	X
Pancreas		X	X
Pharynx		X	X
Pituitary gland		X	X
Preputial / clitorial glands		X	X
Prostate gland	X	X	X
Rectum		X	X
Salivary glands - mandibular, sublingual, parotid		X	X
Sciatic nerve		X	X
Seminal vesicles incl. coagulating glands		X	X
Skeletal muscle		X	X
Skin and subcutaneous tissue (location different from mammary gland)		X	X
Spinal cord - cervical, midthoracic, lumbar		X	X
Spleen	X	X	X
Stomach (non-glandular and glandular)		X	X
Testes (fixed in Davidson's solution)	X	X	X
Thymus	X	X	X
Thyroid (incl. parathyroid gland, if possible)		X	X
Tongue		X	X
Trachea		X	X
Ureter		X	X
Urinary bladder, filled with formalin at necropsy		X	X
Uterus	X	X	X

§ Whole liver was collected and fixed
** Level 3 only

Tissues / Organs	Weight	Collect	Examine
Vagina		X	X
Zymbal's glands		X	X
All gross lesions		X	X

Peer Review

A pathology peer review was conducted on the animals of Allocation A by (b) (4) . All tissues from the following animals were reviewed: nos. 1 to 5, 292 to 296, 232 to 236, and 523 to 527. In addition, all neoplastic lesions as well as the target organs (adrenal glands, liver, testes, thyroid gland and uterus / vagina), were reviewed in all animals. A peer review statement was provided to the Study Director and is archived in the study file.

Neoplastic findings

According to the sponsor, statistical trend analysis (Peto et al. 1980) was used to test for positive dose-related trend of the incidence of neoplastic findings. Two control groups were used in this study, the evaluation compared either all groups (groups 1 to 5), or the first control groups versus the treated groups (groups1, 3 to 5), or the second control group versus the treated groups (groups 2 to 5). The results are commented if p-values were below of 0.05. According to Lin and Rahman (1998) stricter p-values of 0.005 for common tumors and 0.025 for rare tumors are to be used as thresholds of statistical significance for positive trends.

FDA evaluation
Independent statistical evaluation performed at FDA (reviewer: Mohammad Atiar Rahman, Ph.D.) did not find evidence for any significant increases in tumor incidence.

The neoplastic findings observed in this study were either solitary cases in single animals, or/and only statistically significant ($p<0.05$) in some comparisons but not in the others. These findings were:

Adrenal Glands - Hemangiosarcoma occurred in one male at 250 mg/kg/day.

Body Cavities - Hemangioma occurred in one male at 250 mg/kg/day and hemangiosarcoma in another male at 250 mg/kg/day.

Jejunum - Leiomyoma occurred in one control female and two females at 250/50 mg/kg/day.

Lung - Metastatic carcinoma occurred in one female at 50/25 mg/kg/day and one male at 250 mg/kg/day.

Lymph Nodes - Lymphangioma occurred in the lymph nodes of one male at 50 mg/kg/day. Hemangioma occurred in the mesenteric lymph nodes of one male at 50 mg/kg/day, one control female, and one female at 250/50 mg/kg/day.

Sublingual Salivary Gland - Adenoma occurred in one female at 250/50 mg/kg/day.

Skin/Subcutis - Sarcoma NOS (“not otherwise specified”) occurred in two control males, one male at 10 mg/kg/day, one male at 250 mg/kg/day and a one female at 250/50 mg/kg/day.

Uterus - Hemangioma occurred in one female at 250/50 mg/kg/day. Stromal polyp occurred in all female groups, but with incidence slightly increased in females at 250/50 mg/kg/day

Non Neoplastic findings

Statistic trend analysis (Armitage 1955) indicated significant positive trend for selected nonneoplastic findings. The findings considered to be toxicologically relevant are presented below:

Adrenal Glands

Incidence of diffuse hypertrophy of the zona glomerulosa in the adrenal cortex was increased in males at 250 mg/kg/day and females at 250/50 mg/kg/day.

Group Number	1		2		3		4		5	
Dose levels mg/kg/day	0		0		10		50/25		250/50	
Sex	M	F	M	F	M	F	M	F	M	F
Incidence	7/51	4/51	4/51	3/51	4/51	3/49	8/51	2/49	18/51	19/51
Mean grade	1.1	1.3	1.0	1.0	1.5	1.3	1.0	1.5	1.0	1.1
Armitage trend test: Groups 1-5, M and F $p < 0.0005$										

Liver

Incidence, and to a minimal extent mean grade, of centrilobular hepatocellular hypertrophy was increased in males at 50 and 250 mg/kg/day and females at 50/25 and at 250/50 mg/kg/day.

Group Number	1		2		3		4		5	
Dose levels mg/kg/day	0		0		10		50/25		250/50	
Sex	M	F	M	F	M	F	M	F	M	F
Incidence	5/51	1/51	2/51	0/51	5/51	0/50	11/51	4/51	34/51	16/51
Mean grade	1.0	1.0	1.0		1.0		1.1	1.0	1.1	1.1
Armitage trend test: Groups 1-5, M and F $p < 0.0005$										

Incidence of centrilobular hepatocellular vacuolation was increased in males at 250 mg/kg/day and females at 250/50 mg/kg/day.

Group Number	1		2		3		4		5	
Dose levels mg/kg/day	0		0		10		50/25		250/50	
Sex	M	F	M	F	M	F	M	F	M	F
Incidence	0/51	0/51	1/51	0/51	3/51	1/50	3/51	3/51	20/51	5/51
Mean grade			3.0		1.3	3.0	2.0	1.7	1.9	1.2
Armitage trend test: Groups 1-5, M $p < 0.0005$, F $p = 0.0006$										

Incidence of hepatodiaphragmatic herniation was increased in females at 50/25 and 250/50 mg/kg/day.

Group Number	1		2		3		4		5	
Dose levels mg/kg/day	0		0		10		50/25		250/50	
Sex	M	F	M	F	M	F	M	F	M	F
Incidence	1/51	2/51	0/51	0/51	0/51	0/50	0/51	9/51	1/51	7/51
Mean grade	3.0	3.5						3.0	4.0	3.3
Armitage trend test: Groups 1-5, F p < 0.0005										

Ovaries

Angiomatous change, characterized by the presence of prominent blood vessels, was more frequent in females at 250/50 mg/kg/day than in the other groups.

Group Number	1		2		3		4		5	
Dose levels mg/kg/day	0		0		10		50/25		250/50	
Sex	M	F	M	F	M	F	M	F	M	F
Incidence		1/51		1/51		0/51		0/51		7/51
Mean grade		1.0		1.0						3.0
Armitage trend test: Groups 1-5, F p < 0.0005										

Testes

Incidence of tubular atrophy was increased in males at all dose groups. At 250 mg/kg/day, hypospermatogenesis was observed in a few males, and incidence of impaction was slightly increased. Tubular dilation, observed in the testes at interim examination, could not be evaluated at terminal examination due to the presence of tubular atrophy. In the epididymides, the occurrence of aspermia or cellular debris at 250 mg/kg/day was only minimally higher than in the other experimental groups.

Group Number	1		2		3		4		5	
Dose levels mg/kg/day	0		0		10		50/25		250/50	
Sex	M	F	M	F	M	F	M	F	M	F
Tubular Atrophy										
Incidence	4/51		3/51		18/51		35/51		42/51	
Mean grade	2.5		2.0		1.9		1.7		2.0	
Armitage trend test: Groups 1-5, M p < 0.0005										
Hypospermatogenesis										
Incidence	0/51		0/51		0/51		0/51		4/51	
Mean grade									4.3	
Armitage trend test: Groups 1-5, M p < 0.0005										
Impaction										
Incidence	0/51		3/51		1/51		4/51		6/51	
Mean grade			1.0		2.0		1.8		1.0	
Armitage trend test: Groups 1-5, M p =0.0080										

Thyroid Gland

Incidence of follicular cell hypertrophy was increased in both sexes at all dose groups. In males at 50 and 250 mg/kg/day, the mean grade of this finding was increased.

Group Number	1		2		3		4		5	
Dose levels mg/kg/day	0		0		10		50/25		250/50	
Sex	M	F	M	F	M	F	M	F	M	F
Incidence	21/50	7/51	30/49	5/51	39/47	14/50	39/48	21/49	48/49	13/46
Mean grade	1.2	1.1	1.2	1.2	1.7	1.2	2.3	1.2	2.2	1.2
Armitage trend test: Groups 1-5, M p < 0.0005, F p = 0.0036										

Incidence of focal follicular cell hyperplasia was increased in males at 250 mg/kg/day.

Group Number	1		2		3		4		5	
Dose levels mg/kg/day	0		0		10		50/25		250/50	
Sex	M	F	M	F	M	F	M	F	M	F
Incidence	1/50	0/51	3/49	1/51	1/47	1/50	4/48	0/49	15/49	1/46
Mean grade	3.0		2.3	1.0	3.0	1.0	2.5		2.1	1.0
Armitage trend test: Groups 1-5, M $p < 0.0005$.										

Uterus

Incidence of endometrial cysts was increased in females at all dose groups. Endometrial cysts were characterized by single or multiple dilated endometrial glands.

Group Number	1		2		3		4		5	
Dose levels mg/kg/day	0		0		10		50/25		250/50	
Sex	M	F	M	F	M	F	M	F	M	F
Incidence		0/51		4/51		9/51		20/51		9/51
Mean grade				1.8		2.0		1.8		2.0
Armitage trend test: Groups 1-5, F $p = 0.0018$										

Toxicokinetics

Toxicokinetic evaluations were performed based on the plasma concentration vs time profiles of ACT-064992, ACT-132577 and ACT-373898. Toxicokinetic analysis appropriate to the data was performed, and included determination of maximum plasma concentration (Cmax), time of maximum plasma concentration (Tmax), and area under the plasma concentration time-curve (AUClast). Additionally, AUClast and Cmax was compared by calculating ratios in order to evaluate dose proportionality (dose-level related differences), sex differences, and differences between single and repeated dosing (time dependency). Metabolite to parent compound ratio and last time point with measurable concentrations (Tlast) were reported.

Toxicokinetic parameters of ACT-064992 after oral administration of ACT-064992 to rats

Allocation B

Compound	Dose (mg/kg)	Week	Sex	Tmax (h)	Cmax (µg/mL)	AUClast (h*µg/mL)	Tlast (h)	Cmax/D (µg/mL)/(mg/kg)	AUClast/D (h*µg/mL)/(mg/kg)
ACT-064992	10	1	f	4	6.02	77.3	24	0.6	7.7
ACT-064992	10	1	m	2	2.17	20.3	24	0.2	2.0
ACT-064992	10	26	f	2	9.67	104	24	1.0	10
ACT-064992	10	26	m	2	2.78	18.3	24	0.3	1.8
ACT-064992	50	1	f	4	23.8	360	24	0.5	7.2
ACT-064992	50	1	m	4	9.33	71.8	24	0.2	1.4
ACT-064992	50	26	f	4	25.5	227	24	0.5	4.5
ACT-064992	50	26	m	2	6.42	45.1	24	0.1	0.9
ACT-064992	250	1	f	10	53.9	789	24	0.2	3.2
ACT-064992	250	1	m	4	22.8	214	24	0.09	0.9
ACT-064992	250	26	f	4	41.8	506	24	0.2	2.0
ACT-064992	250	26	m	2	13.1	97.4	24	0.05	0.4

Allocation C

Compound	Dose (mg/kg)	Day	Sex	Tmax (h)	Cmax (µg/mL)	AUClast (h*µg/mL)	Tlast (h)	Cmax/D (µg/mL)/(mg/kg)	AUClast/D (h*µg/mL)/(mg/kg)
ACT-064992	25	1	f	4	14.5	213	24	0.6	8.5
ACT-064992	25	28	f	4	16.4	168	24	0.7	6.7
ACT-064992	50	1	f	7	28.3	382	24	0.6	7.6
ACT-064992	50	28	f	2	17.4	207	24	0.4	4.1

Toxicokinetic parameters of metabolite ACT-132577 after oral administration of ACT-064992 to rats

Allocation B

Compound	Dose (mg/kg)	Week	Sex	Tmax (h)	Cmax (µg/mL)	AUClast (h*µg/mL)	Tlast (h)	Cmax/D (µg/mL)/(mg/kg)	AUClast/D (h*µg/mL)/(mg/kg)
ACT-132577	10	1	f	10	2.89	50.8	24	0.3	5.1
ACT-132577	10	1	m	7	5.60	71.2	24	0.6	7.1
ACT-132577	10	26	f	7	6.52	98.2	24	0.7	9.8
ACT-132577	10	26	m	4	6.94	72.7	24	0.7	7.3
ACT-132577	50	1	f	10	10.8	170	24	0.2	3.4
ACT-132577	50	1	m	7	18.6	231	24	0.4	4.6
ACT-132577	50	26	f	4	18.0	227	24	0.4	4.5
ACT-132577	50	26	m	7	14.1	164	24	0.3	3.3
ACT-132577	250	1	f	10	25.6	393	24	0.1	1.6
ACT-132577	250	1	m	10	39.1	494	24	0.2	2.0
ACT-132577	250	26	f	7	33.2	497	24	0.1	2.0
ACT-132577	250	26	m	4	25.7	291	24	0.1	1.2

Allocation C

Compound	Dose (mg/kg)	Day	Sex	Tmax (h)	Cmax (µg/mL)	AUClast (h*µg/mL)	Tlast (h)	Cmax/D (µg/mL)/(mg/kg)	AUClast/D (h*µg/mL)/(mg/kg)
ACT-132577	25	1	f	10	7.50	130	24	0.3	5.2
ACT-132577	25	28	f	4	7.80	121	24	0.3	4.8
ACT-132577	50	1	f	10	13.3	224	24	0.3	4.5
ACT-132577	50	28	f	7	8.60	128	24	0.2	2.6

Toxicokinetic parameters of metabolite ACT-373898 after oral administration of ACT-064992 to rats

Allocation B

Compound	Dose (mg/kg)	Week	Sex	Tmax (h)	Cmax (µg/mL)	AUClast (h*µg/mL)	Tlast (h)	Cmax/D (µg/mL)/(mg/kg)	AUClast/D (h*µg/mL)/(mg/kg)
ACT-373898	10	1	f	2	0.00176	0.0313	24	0.0002	0.003
ACT-373898	10	1	m	NA	NA	NA	NA	NA	NA
ACT-373898	10	26	f	2	0.00491	0.0798	24	0.0005	0.008
ACT-373898	10	26	m	7	0.00135	0.00719	7	0.0001	0.0007
ACT-373898	50	1	f	10	0.00670	0.119	24	0.0001	0.002
ACT-373898	50	1	m	7	0.00156	0.0119	10	0.00003	0.0002
ACT-373898	50	26	f	4	0.0264	0.296	24	0.0005	0.006
ACT-373898	50	26	m	4	0.00231	0.0378	24	0.00005	0.0008
ACT-373898	250	1	f	10	0.0190	0.341	24	0.00008	0.001
ACT-373898	250	1	m	10	0.00532	0.0378	10	0.00002	0.0001
ACT-373898	250	26	f	4	0.0845	0.872	24	0.0003	0.004
ACT-373898	250	26	m	4	0.00816	0.0516	10	0.00003	0.0002

NA = not available

Allocation C

Compound	Dose (mg/kg)	Day	Sex	Tmax (h)	Cmax (µg/mL)	AUClast (h*µg/mL)	Tlast (h)	Cmax/D (µg/mL)/(mg/kg)	AUClast/D (h*µg/mL)/(mg/kg)
ACT-373898	25	1	f	24	0.00311	0.0659	24	0.0001	0.003
ACT-373898	25	28	f	4	0.00784	0.0946	24	0.0003	0.004
ACT-373898	50	1	f	10	0.00782	0.138	24	0.0002	0.003
ACT-373898	50	28	f	10	0.0107	0.161	24	0.0002	0.003

Dosing Solution Analysis

There was no ACT-064992 found in the control sample.

Generally, the formulations were prepared correctly with the achieved concentrations of the majority of samples being within the acceptance criteria of 90% to 110% of nominal concentration. Individual samples of different concentrations at different sampling time-points were outside the acceptance criteria (mean values of respective top/middle/bottom samples per concentration and time point between 84.8% and 89.6%). This is not considered to compromise the validity of the study. The analyzed samples were considered as homogeneous, since their coefficients of variation were in the range from 0.3% to 7.5% and therefore within the specified acceptance criteria of ≤10%.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Male fertility (Study #T-05.087) evaluation was reviewed under IND 77258. Male rats were treated with 10, 50 or 250 mg/kg/day for 10 weeks, then mated to untreated females. Although fertility in the absolute sense was unaffected, increased incidence of early intrauterine death (250 mg/kg) and post-implantation loss (50 and 250 mg/kg) in the mated dams were statistically significant, suggesting an effect is transferred from the treated males to the untreated females. This could indicate that either the sperm is affected directly, or that the female is dosed via semen during copulation and this, in turn, affects implantation. It is not known if macitentan is secreted into semen, and the dose would be expected to be small, however, the exposure to the uterus could be significant if macitentan is secreted. Testicular tubular atrophy was noted in the 50 and 250 mg/kg groups.

There was no effect of treatment on sex ratio, mean litter weight, mean placental weight or mean fetal weight. There was no effect of treatment on the incidence or inter-group distribution of fetal abnormalities. A NOAEL of 10 mg/kg was observed.

Female fertility (Study #T-05.153) evaluation was also reviewed under IND 77258, Administration of 10, 50 and 250 mg/kg/day of ACT-064992 for two weeks to the female Wistar rat elicited slight reductions in food intake in the parental females. There were no adverse effects on fertility or early-embryonic development at any dose level investigated. The no-observed-effect-level for fertility and early embryonic development was 250 mg/kg/day.

9.2 Embryonic Fetal Development

Fetal developmental effects were evaluated in rats and rabbits. Both studies were reviewed under IND 77258.

In rats, treatment with ACT-064992 at dosages of 150 and 450 mg/kg/day during the organogenesis phase of gestation resulted in a specific disruption of embryo-fetal development, which affected all fetuses in these groups. All fetuses had a number of craniofacial abnormalities (collectively described as mandibular arch fusion abnormalities) and there was also a high incidence of cardiovascular abnormalities at both dosages. These findings are consistent with other members of this class of compounds. Based on the results of this study, it was concluded that the no-observed-adverse-effect-level (NOAEL) for maternal toxicity was 450 mg/kg/day, while a NOAEL for embryo-fetal development was not established and lies below 150 mg/kg/day.

ACT-064992 exhibited a teratogenic potential in rabbits from the low dose of 2.5 mg/kg/day upwards and is fetotoxic at the high dose of 25.0 mg/kg/day. All fetuses were affected at the 12.5 and 25 mg/kg doses. No marked effects on the dams were seen up to 25.0 mg/kg/day.

9.3 Prenatal and Postnatal Development

Pre- and post-natal developmental effects were evaluated in rats as follows.

Study title: ACT-064992: Study for Effects on Pre- and Postnatal Development including Maternal Function in the Wistar Rat

Study no.:	T-09.617
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/4/10
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	178194 / Batch 13, 100.3% (assumed to be 100% for dose calculation)

Key Study Findings

For F0 dams, a NOAEL of 50 mg/kg/day was established regarding clinical signs and general condition. No NOAEL could be established for reproduction parameters neither for F0 dams nor for the F1- generation. Increased pup mortality and increased pre- and post implantation loss was seen at all dose levels.

Mating of F1 males and females resulted in increased pre-implantation loss in all dose groups.

Methods

Doses:	10, 50, 250 mg/kg
Frequency of dosing:	daily from Day 17 post coitum to Day 20 post-partum
Dose volume:	10 mL/kg
Route of administration:	oral
Formulation/Vehicle:	0.5% (w/v) methylcellulose
Species/Strain:	Rat, RccHanTM: WIST(SPF)
Number/Sex/Group:	24 females/group
Satellite groups:	4/group for TK
Deviation from study protocol:	Erroneously no organs of females of the F1 females necropsied before 21-May-2010 were preserved except the organs with macroscopic abnormalities. This error was corrected on 21- May-2010 and thereafter. Due to microscopic findings in the liver of F1 females, it was decided to also preserve all livers from 12-May-2010 onwards. Additionally, test agent was found to be present in blood of control rats, albeit at very low levels.

Observations

F0 generation

After acclimatization, females were housed with sexually mature males (1:1) in special automatic mating cages (Makrolon type-3) equipped with automatic doors i.e. with synchronized timing to initiate the nightly mating period, until evidence of copulation was observed. This system reduced the variation in the copulation times of the different females. The females were removed and housed individually if: **-** the daily vaginal smear was sperm positive, or **-** a copulation plug was observed. The day of mating was designated day 0 post coitum. Male rats of the same source and strain were used only for mating. These male rats are in the possession of (b) (4) and were not considered part of the test system. The fertility of these males had been proven and was continuously monitored. All F0 dams were allowed to give birth and rear their litters up to day 21 p.p. (weaning).

F1- generation

The offspring were examined as soon as possible after completion of delivery for litter size, number of live, sex and still births, and any gross abnormalities. F1-pups were culled by random selection to yield as far as possible 4 males and 4 females per litter on day 4 post partum. On day 21 p.p., one pup per litter (one male or one female pup in alteration from each litter) was necropsied and tissue was preserved. In F1 pups not used for assessment of reproductive performance, clinical observations, food consumption and body weight was recorded until necropsy on days 45 to 50.

For assessment of reproductive performance, 22 male and 22 female pups per group were selected (at least one of each sex from each of the first 22 weaned litters where possible). Litters of less than eight pups were not used to build a new generation. If there were less than 22 weaned litters, a random selection was made of the available litters to ensure a full complement of 22 males and 22 females per group for the F1 generation. Pairing of the selected 22 animals per sex and group was performed between animals of different litters, siblings were not paired. If a female did not mate during an 8-day pairing period, this female was paired with a male of the same group which had already mated successfully. If mating was not performed during this additional pairing period of a maximum of 8 days, the female was sacrificed 8 days later (after pairing period of females).

For behavioral tests, the pups for assessment of reproductive performance were used.

The following observations were recorded:

Viability / Mortality: Twice daily

Clinical Signs F0: Daily cage-side clinical observations (once daily during the acclimatization and thereafter twice daily cage-side clinical observations were recorded up to day of necropsy). Additionally parental females were observed for behavioral abnormalities in nesting and nursing.

Food Consumption F0: Recorded for the following periods: days 0 - 6, 6 - 11, 11 - 17 and 17 - 21 post coitum, and days 1 - 4, 4 - 7 and 7 - 14 p.p. (since pups begin to consume maternal feed on or about lactation day 14, food consumption was not recorded after this day).

Body Weights F0: Recorded daily

Pup Data: Number of missing (cannibalized) or dead pups: Daily

Abnormal findings: Daily

Body weight: Days 0/1, 4, 7, 14, 21, 28, 35 and 42 p.p.

Body Weight and Food Consumption F1 Animals:

When the last litter was 42 days old, food consumption and body weight were measured weekly during the pre-pairing period (= first 28 days) and after-pairing period (males only). During the gestation period, food consumption and body weights of dams were recorded for the following periods: day 0, 6, 11 and 14 post coitum.

The pups selected for use in F1 parental generation were taking part in the following behavioral tests. These were performed in 22 males and 22 females per group.

Hearing Ability

Hearing ability, performed on day 22 p.p. (+ 1 day). Positive Preyer's reflex (pinna reflex) when exposed to a tone of frequency 10 kHz; volume 80 dB, duration 30 msec.

Locomotor Activity

On day 24 p.p. locomotor activity was measured quantitatively for the same animals. Activity was measured with an Activity Monitor AMS-0151 (FMI, Germany). Activity of the animals (based on beam count) was recorded for 6-minute intervals over a period of 30 minutes. These data and the total activity over 30 minutes were reported.

Water Maze Tests

A water maze tests (Y maze) were performed between days 35 and 42 p.p. (± 1 day, respectively). The test used was a "Y" shaped water maze with one escape ladder. The time needed by the rats to find the escape ladder was recorded for each trial. All animals were given 6 trials on day 1 (learning phase). The ability to find a ladder in a water labyrinth constituted a positive reaction. On the same day a straight "maze" (channel) was used to evaluate swimming speed. The rats were retested 7 days later in a memory trial.

F0 parental females were sacrificed after weaning of the offspring on day 21 p.p. Females which not delivered on the expected date (day 21 post coitum), were sacrificed on day 25 post coitum at the latest. One pup per litter (one male or one female pup in alteration from each litter) was necropsied on day 21 p.p., and tissue was preserved. F-1 pups not used for assessment of reproductive performance were sacrificed when the developmental parameters were completed (day 42 – 50 p.p.). F1-generation females were sacrificed on day 14 post coitum and the reproduction data were recorded. F1-generation males were sacrificed after the F1 females.

Necropsy

Animals were killed by an injection of sodium pentobarbital or by CO2 asphyxiation. All F0 and F1 animals were necropsied and subjected to a detailed macroscopic examination. The uteri of all F0 females and of F1 females without visible implantation sites were placed in a solution of ammonium sulfide to visualize possible hemorrhagic areas of implantation sites. Dead pups, except those excessively cannibalized, were examined macroscopically. Dead or culled pups were discarded.

Organ Weights

At the scheduled sacrifice, the testes and epididymides of all F1 males were weighed.

Tissue Preservation

The following tissues from F0 dams, parental F1 animals and one additional F1 pup per litter (one male or one female pup in alteration from each litter on day 21 p.p.) were preserved in neutral phosphate buffered 4% formaldehyde solution:

Pituitary	Adrenals
Testes with epididymides (in Bouin's fixative)	Prostate
Seminal vesicles with coagulating gland	Ovaries
Uterus (including cervix, vagina and oviducts)	

Toxicokinetics

Blood samples (approximately 0.5 mL) were collected sublingually from all toxicokinetic females under light isoflurane anesthesia on days 4 and 12 p.p. Samples were collected at 1, 4, 6 and 24 hours postdose.

F1 Pups:

Blood was collected on day 4 and 12 p.p.. Blood was pooled by sex and litter. On day 4, when litter size was adjusted to four males and four females, blood from all culled pups was taken. On day 12 p.p. all remaining pups were used for blood sampling. In case ≤ 4 pups per sex were available on day 4 p.p., no blood sampling was done on this day but from all fetuses on day 12 p.p..

Results

Dose formulations:

The analyzed samples were considered as homogeneous, since their coefficients of variation were in the range from 0.7% to 4.0% and therefore within the specified acceptance criteria of <10%. There was no ACT-064992 found in the vehicle control sample. With exception of one individual measurement in group 4, the results regarding actual content of ACT-064992 in the formulations were 91.2% to 96.3% of the nominal concentration in the samples of day 1 and 90.4% to 92.8% in the samples of the lactation week and therefore within the specified range of 90% to 110%.

Clinical signs and mortality: All females of F0 generation survived until scheduled necropsy. No clinical signs or observations were noted.

Food consumption:

No effect on mean food consumption was observed at 10 and 50 mg/kg/day. At 250 mg/kg, mean food consumption was statistically significantly reduced from day 17 post coitum onwards (= start of treatment) and during the entire lactation period. The difference was statistically significant only between days 7 and 14 p.p.

Body weight:

No ACT-064992-related effect on body weights or body weight gain was observed. At the dose levels of 10 and 50 mg/kg, statistically significantly higher mean body weights were observed from days 19 or 18 p.p., respectively, until the end of the period. Body weight gains were higher already towards the end of gestation period and during the lactation period). In the absence of dose dependency, this effect was considered not to be ACT-064992-related.

Reproductive parameters:

Maternal

Gestation duration

No effect on duration of gestation was observed at any dose level.

Post-implantation loss

Post-implantation loss seemed slightly increased at all dose levels, reaching statistical significance only at 250 mg/kg/day.

Dose (mg/kg/day)	0	10	50	250
No. of litters	22	21	23	21
No. of implantations	287	278	302	263
Post-implantation loss (%)	7.0	9.7	10.6	12.5
Total number of post-implantation loss / group	20	27	32	33*
Number of litters affected	9	17**	17*	14
Mean post-implantation loss per litter	0.9	1.3	1.4	1.6

* statistically significant, p<0.05 ** statistically significant, p<0.01

Litter size

In groups 2 - 4, the number of living pups at first litter check was slightly reduced, resulting in reduced birth indices (number of pups borne alive as a percentage of implantations).

Dose (mg/kg/day)	0	10	50	250
Mean number of living pups	12.1	12.0	11.7	11.0
Pups found dead	1	2	0	2
Litters affected	1	2	0	2
Birth index (%)	93.0	90.3	89.4	87.5*

* statistically significant, p<0.05

Postnatal loss (Days 0-4 postpartum)

In all dose groups, postnatal loss between day 0 and 4 was increased.

Dose (mg/kg/day)	0	10	50	250
Postnatal loss	1	8*	10**	13**
Litters affected	1	5	6	6*
Viability index (%)	99.6	96.8*	96.3**	94.3**

* statistically significant, p<0.05 ** statistically significant, p<0.01

Postnatal loss (Days 5-21 postpartum)

No effects on breeding were observed in the low and mid dose group.
At 250 mg/kg/day, breeding loss was statistically significantly increased.

Dose (mg/kg/day)	0	10	50	250
Breeding loss	0	1	1	7**
Litters affected	0	1	1	5*
Weaning indices (%)	100	99.4	99.5	95.7**

* statistically significant, p<0.05 ** statistically significant, p<0.01

F1 generation pups

Mortality and clinical signs

One, 8, 10 and 13 pups, respectively, in order of ascending dose levels, were found dead or were missing on day 4 when compared with the number of living pups at first litter check. Between days 5 and 21, further 0, 1, 1, and 7 pups were found dead or were missing when compared to number of living pups on day 4.

During the lactation period, pups without milk in the stomach were observed in one pup at the former dose level of 10 mg/kg, in 5 pups (from 2 litters) at 50 mg/kg and in 8 pups (from 6 litters) at 250 mg/kg. At a dose level of 250 mg/kg, five of these 8 pups were missing or were found dead at a later point in time, indicating impaired well-being of these animals. This finding was considered to be ACT-064992-related.

Body weight
The body weight of pups at birth was not affected at any dose level. From day 1 pp onwards, no effect on mean pup body weights or body weight gain was observed after exposure of dams to 10 and 50 mg/kg/day.

After 250 mg/kg/day was administered to dams, mean body weight gain of pups was reduced from day 1p.p. onwards, resulting in statistically significant lower body weights on day 21 p.p. compared to controls. From day 21 to 43-50 p.p., mean body weight gain was similar to the control group. The difference between body weights of high dose and control animals stayed constant.

Developmental indices

There were no changes in mean time points of pinna unfolding, incisor eruption, onset of coat development, opening of eyes and descent of testes.

At 250 mg/kg/day administered to dams, a slight but statistically significant delay in balanopreputial separation (27.0 days compared to 26.1 days in the control group) and opening of vagina (34.5 days compared to 32.9 days in the control group) were observed. These effects were considered secondary to the lower pup weights.

Behavioral tests

The Preyer's reflex was present in all animals in all groups indicating that the hearing ability was not affected.

There was no effect on the level of locomotor activity.

There were no differences in learning and memory in all groups.

F1 Generation

Mortality and clinical signs

All animals of the F1 generation survived until scheduled necropsy which was scheduled for females on day 14 post coitum, and for males on days 36 – 37 of the after-pairing period (around day 126 p.p.).

No ACT-064992-related clinical signs or observations were noted.

Food consumption

No effect on food consumption was observed during pre-pairing and gestation period in females.

In male animals, a very slight and statistically not significant reduction of food consumption after 50 and 250 mg/kg/day administered to dams is considered to have contributed to the slower body weight development in these groups until end of after-pairing period.

Body weight

No effect on body weight was observed during pre-pairing and gestation period in females.

Male animals at 50 and 250 mg/kg/day administered to dams started the pre-pairing period with mean body weights slightly lower (5%) than the control animals. At the end of the after-pairing period, body weight in these animals was statistically significantly lower than in group 1 animals (13% and 14%, respectively).

Reproduction Data F1 Generation

Mean number of corpora lutea was similar in all groups.

Pre-implantation loss was increased in groups 2, 3 and 4 and resulted in a reduced number of implantation sites. The values were slightly above the range of the historical control data. Although no clear dose dependence was observed, the effect was considered ACT-064992- related.

Due to the reduced number of implantation sites, the mean number of live embryos at termination was also slightly reduced in groups 2-4.

Post-implantation loss appeared slightly higher in groups 2 – 4 than in the control group.

Summary of Reproduction Data of F1 Generation

Group Dose (mg/kg/day) [a]	1 (0)	2 (10)	3 (50)	4 (250)
No. of females with live fetuses	20	15	10	10
Pre-implantation loss (%)	5.3	9.7*	7.3	13.7**
Number of implantation sites	94.7	90.3*	92.7	86.3**
Post-implantation loss (%)	4.4	5.9	8.7	7.1
Mean number of live embryos	12.1	11.7	11.6	10.5

[a] Indicates the dosages administered to the females of the F0 generation
* $p<0.05$ ** $p<0.01$

Organ weights - F1

A drug-related decrease of absolute and relative organ weight of testes was observed at all dose levels. Additionally, in groups 3 and 4, epididymis weights decreased.

Toxicokinetics

All dams dosed with ACT-064992 were exposed to ACT-064992 and its two metabolites (ACT 373898 and ACT-132577). The maximum concentrations of ACT-064992 and the two metabolites were achieved in plasma at 4-6 hours post-dosing.

All pups which were breast-fed by the dams were also exposed to ACT-064992 and ACT- 132577 but not to ACT-373898.

Toxicokinetic parameters and dose dependence of exposures of ACT-064992 and its metabolites in dams after oral administration of ACT-064992 to dams

Compound	Dose (mg/kg)	Post-Partum Day	Tmax (h)	Cmax (ng/mL)	AUC0-24h (h*ng/mL)	Cmax/D (ng/mL)/ (mg/kg)	AUC0-24h/D (h*ng/mL)/ (mg/kg)
ACT-064992	0	4	4	87.2	1200	NA	NA
	0	12	1	22.3	114[#]	NA	NA
	10	4	4	6360	64500	640	6500
	10	12	4	6970	69100	700	6900
	50	4	4	16600	165000	330	3300
	50	12	4	14800	143000	300	2900
	250	4	6	37300	407000	150	1600
	250	12	6	30500	294000	120	1200
ACT-132577	0	4	6	27.4	548	NA	NA
	0	12	NA	NA	NA	NA	NA
	10	4	4	3270	51700	330	5200
	10	12	6	4380	69800	440	7000
	50	4	6	11800	164000	240	3300
	50	12	4	13400	185000	270	3700
	250	4	4	27800	382000	110	1500
	250	12	6	28800	375000	120	1500
ACT-373898	0	4	NA	NA	NA	NA	NA
	0	12	NA	NA	NA	NA	NA
	10	4	4	3.38	48.8	0.3	4.9
	10	12	4	3.25	60.0	0.3	6.0
	50	4	6	17.3	181	0.4	3.6
	50	12	6	16.4	171	0.3	3.4
	250	4	6	42.0	459	0.2	1.8
	250	12	6	42.7	400	0.2	1.6

****Samples for complete concentration-time profiles were obtained from all available control dams on both post-partum days. Although these dams did not receive any dosing, ACT-064992 was found in several samples on post-partum days 4 and 12. Concentrations up to 87.2 ng/mL were detected and Tmax occurred at 1h or 4h. The concentrations detected in the control animals were very low (less than 2% AUC exposure) as compared to those in animals received the low dose of ACT-064992. ACT-132577 was detected in control dams on post-partum day 4 only and ACT-373898 was not detected on either sampling days. In control pups, no parent compound nor metabolites were detected on either post-partum day 4 or day 12.

10 Special Toxicology Studies

In an *in vitro* system using Balb/c 3T3 fibroblast cell cultures, macitentan exhibited weak phototoxicity at high concentrations (26,600-times above human free concentration of macitentan at 10 mg). An *in vivo* study in hairless rats showed no phototoxic effects up to the high dose level of 60 mg/kg/day corresponding 24-fold the human exposure at 10 mg per day. It can be concluded that there is no relevant risk of phototoxicity for patients treated with macitentan at 10 mg per day.

11 Appendix/Attachments

1. Statistical Review of Carcinogenicity Study Data
2. CDER Computational Toxicology Report

30 Pages Have Been Withheld As A Duplicate Copy Of The Statistical Review of Carcinogenicity Study Data Which Is Located In The Statistical Review Section Of This NDA Approval Package.

To: William T. Link
cc: Albert DeFelice
From: CDER/OPS/OTR/DDSR: The CDER Computational Toxicology Group
Re: NDA 204410
Date: March 11, 2013

Nine chemicals were evaluated by CDER/OPS/OTR/DDSR for genetic toxicity and rodent carcinogenicity using (quantitative) structure-activity relationship [(Q)SAR] models. Overall, the software programs from three providers were used for the (Q)SAR analyses: Derek Nexus 3.0.1 (DX), Leadscope Model Applier 1.5.0-4 (LMA), and MC4PC 2.4.1.4 (MC) or CASE Ultra 1.4.6.0 (CU). The results of the predictions from the specific software programs used for each endpoint were weighted equally to reach the overall conclusions.

(b) (4)

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/s/

WILLIAM T LINK
08/26/2013

ALBERT F DEFELICE
08/26/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 204410 **Applicant: Actelion** **Stamp Date: 10/19/12**

Drug Name: Opsumit **NDA/BLA Type: new NME**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		The performed toxicology studies used liquid formulations with gavage dosing, or in the case of dog studies, (b) (4).
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	n/a		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		Some minor changes may be required pending review of carcinogenicity and reproductive toxicology studies.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		Further information will be requested, as to some impurities which may require further discussion and/or characterization.
11	Has the applicant addressed any abuse potential issues in the submission?	n/a		Low potential for abuse.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	n/a		

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? _yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

WILLIAM T LINK
12/06/2012

ALBERT F DEFELICE
12/21/2012