CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-387

PHARMACOLOGY REVIEW(S)

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-387
DATE RECEIVED BY CENTER:	6/30/2008
PRODUCT:	Tyvaso™ (treprostinil sodium) Inhalation Solution (0.6 mg/ml)
INTENDED CLINICAL POPULATION:	Pulmonary arterial hypertension (PAH) patients with NYHA Class III — symptoms
SPONSOR:	United Therapeutics Corporation, Research Triangle Park, NC 27709
REVIEW DIVISION:	Division of Cardiovascular and Renal Products
PHARM/TOX REVIEWER:	Xavier Joseph
PHARM/TOX SUPERVISOR:	Charles A. Resnick
DIVISION DIRECTOR:	Norman Stockbridge
PROJECT MANAGER:	Dan Brum

b(4)

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Xavier Joseph, D.V.M.
March 23, 2009

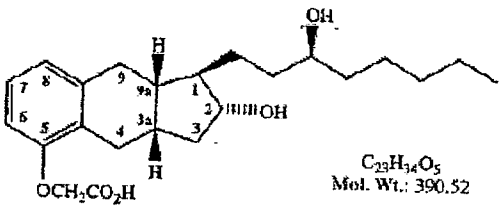
ORIGINAL NDA DATED: June 26, 2008
CENTER RECEIPT DATE: June 30, 2008
REVIEWER RECEIPT DATE: July 7, 2008

SPONSOR: United Therapeutics Corporation
One Park Drive
Research Triangle Park, NC 27709

DRUG PRODUCT: Trade name - Tyvaso™ Inhalation Solution

DRUG SUBSTANCE: Generic name – treprostinil sodium
Code names – UT-15, LRX-15 and 15AU81

Chemical Structure



FORMULATION: Tyvaso™ Inhalation Solution contains 0.6 mg treprostinil/ml, sodium chloride, sodium citrate (dihydrate), 1N hydrochloric acid, sodium hydroxide and water for injection. (1N sodium hydroxide is used for adjusting the pH of the product.) The sodium salt of treprostinil, the active ingredient, is formed during the drug product manufacturing procedure. Treprostinil inhalation solution (2.9 ml) is packaged into ampoules. [Remodulin® (treprostinil sodium) Injection, the marketed product, has the same formulation as Tyvaso except for the

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MODE OF ADMINISTRATION: Tyvaso is administered by oral inhalation using an Optineb-ir ultrasonic, pulsed delivery nebulizer; each pulse of Tyvaso contains 6 µg of treprostinil.

PHARMACOLOGICAL CLASS: Prostacyclin (PGI_2) analogue (vasodilator)

PROPOSED INDICATIONS: For the treatment of pulmonary arterial hypertension (PAH) in patients with NYHA Class III — symptoms. b(4)

PROPOSED DOSAGE REGIMEN: Treatment should be started with 3 breaths (18 µg of treprostinil) per treatment session given 4 times daily. If 3 breaths are not tolerated, the dose may be reduced to 1 or 2 breaths and later increased to 3 breaths as tolerated. The dosage should be increased to 6 breaths and subsequently to the target maintenance dose of 9 breaths (54 µg of treprostinil) per inhalation session given 4 times daily, as tolerated. The maximum dose studied in clinical trials was 12 breaths (72 µg of treprostinil) per inhalation session.

IND UNDER WHICH CLINICAL TRIALS WERE CONDUCTED: IND 70,362 (for inhalation therapy of PAH patients)

RELATED NDAs: United Therapeutics' NDA 21-272 – Remodulin for the sc treatment of PAH patients, and United Therapeutics' NDA 21-272 SE3 – Remodulin for the iv treatment of PAH patients.

EXECUTIVE SUMMARY

Tyvaso™ (treprostinil sodium for inhalation), a chemically stable tricyclic benzindene analogue of prostacyclin (PGI_2), with potent systemic and pulmonary vasodilatory as well as platelet antiaggregatory effects, is being developed for inhalation treatment of pulmonary arterial hypertension (PAH) patients. An injectable formulation of treprostinil sodium has been approved for marketing (Remodulin®) for the treatment of PAH patients either by continuous subcutaneous (sc) or intravenous (iv) routes. Infusion site pain and reactions, and catheter-related infection or sepsis were reported to be the most common adverse events among patients treated sc or iv with treprostinil. With inhalation therapy, by directly applying the drug at the primary site of manifestation of the condition, the adverse effects associated with sc or iv infusion can be avoided.

I. Recommendations

A. Recommendation on Approvability

Tyvaso™ is approvable from a nonclinical perspective.

B. Recommendations for Additional Nonclinical Studies

None

C. Recommendations for Labeling

1. Sponsor's proposed text under section **8. USE IN SPECIFIC POPULATION, 8.1 Pregnancy** presently read as follows:

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We recommend that the above text be revised to read as follows:

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[redacted]

2. Under section **13. NONCLINICAL TOXICOLOGY, 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility,** sponsor's proposed text presently reads as follows:

[redacted]

b(4)

[redacted]

We recommend that the above text be revised to read as follows and to add a new section 13.3 on Developmental Toxicology.

Long-term studies have not been performed to evaluate the carcinogenic potential of treprostinil. In vitro and in vivo genetic toxicology studies did not demonstrate any mutagenic or clastogenic effects of treprostinil. Treprostinil sodium did not affect fertility or mating performance of male or female rats given continuous subcutaneous (sc) infusions at rates of up to 450 ng treprostinil/kg/min [about 59 times the recommended starting human sc infusion rate (1.25 ng/kg/min) and 8 times the average rate (9.3 ng/kg/min) achieved in clinical trials, on a ng/m^2 basis.] In this study, males were dosed from 8 weeks prior to mating and through the 2-week mating period. Females were dosed from 2 weeks prior to mating until gestational day 6.

13.3 Developmental Toxicity

In pregnant rats, continuous sc infusions of treprostinil sodium during organogenesis and late gestational development, at rates as high as 900 ng treprostinil/kg/min (about 117 times the recommended starting human sc infusion rate and about 16 times the average rate achieved in clinical trials, on a ng/m^2 basis), resulted in no evidence of harm to the fetus. In pregnant rabbits, effects of continuous sc infusions of treprostinil during organogenesis were limited to an increased incidence of fetal skeletal variations (bilateral full rib or right rudimentary rib on lumbar 1) associated with maternal toxicity (reduction in body weight and food consumption) at an infusion rate of 150 ng treprostinil/kg/min (about 41 times the starting human dose rate for sc infusion and 5 times the average rate used in clinical trials, on a ng/m^2 basis).

II. Summary of Nonclinical Findings

Nonclinical studies conducted with treprostinil were reviewed under NDA 21-272 for Remodulin® Injection. The sponsor is referencing the above NDA for the support of the present NDA for Tyvaso. Additional nonclinical studies conducted with treprostinil, including safety pharmacology studies (hERG assay, cardiac action potential assay in rabbit Purkinje fibers and *in vivo* cardiovascular and respiratory studies) and repeat-dose inhalation toxicity studies in rats and dogs are included in this Tyvaso NDA and are reviewed below.

In the *in vitro* hERG assay, treprostinil was tested (at different concentrations) to determine its potential for the inhibition of hERG-mediated I_{Kr} current (the most common cause of increased cardiac action potential duration that could lead to QT interval prolongation and fatal ventricular arrhythmias in animals and humans) in cultured human embryonic kidney cells. Treprostinil did not inhibit hERG-mediated current at concentrations up to 100 **µM** (39,052 ng/ml), while terfenadine, the positive control, at 60 **nM**, inhibited hERG current by 85%.

The *in vitro* effects of treprostinil on cardiac action potential duration were determined in isolated rabbit Purkinje fibers. Treprostinil did not prolong action potential duration at concentrations up to 300 µM (117,156 ng/ml), while the positive control dl-sotalol, at 50 µM, significantly prolonged the action potential duration. (The Cmax value in patients receiving Tyvaso at the maximum recommended dose is 1.8 ng/ml.)

Female beagle dogs were given single iv bolus injections of treprostinil sodium solution at dose levels of 0, 2, 20 or 200 µg/kg and cardiovascular parameters were evaluated. Treprostinil caused an initial drop in arterial blood pressure and a compensatory increase of heart rates at all dose levels during the first hour after dosing. The initial drop in arterial blood pressure was followed by a period (up to 6 hours) of elevated systolic, diastolic and mean blood pressure at the 200 µg/kg dose level. Increased incidences of atrioventricular block were observed in 1 of 4 dogs at 2 µg/kg and in 1 of 4 dogs at 200 µg/kg; however, PR interval was not affected. Treprostinil did not affect QT interval at any dose level.

In a study to evaluate potential effects on respiratory function, nebulized treprostinil sodium was administered to groups of male rats, via nose-only inhalation, at an aerosol concentration of about 50 µg/L for 2, 3 or 4 hours. Treprostinil, at achieved inhaled dose levels of 300, 416 and 569 µg/kg, produced significant decreases in respiratory rates and derived minute volumes compared to predose and control values. All values returned to predose and control levels 24 hours following treatment.

In a 13-week inhalation toxicity study, groups of rats were exposed, by nose only administration, to aerosol concentrations of 7.1 µg/L for 20 minutes/day, 44.0 µg/L for 30 minutes/day, and 40.3 µg/L for 225 minutes/day, resulting in estimated achieved dose levels of 7, 67 and 464 µg/kg/day, respectively. Control animals were exposed to conditioned room air for the same duration as the high dose animals. At the termination

of the study, there were reductions in body weight gain at all dose levels [17 - 65% (dose-dependent) in males and 22 - 23% in females]. Other treatment-related findings included decreased food consumption (mid and high dose groups), decreased platelet, white cell and lymphocyte counts (high dose males), increased reticulocyte counts (high dose males and females), increased adrenal and lung weights and decreased testis and thymus weights (mid and high dose groups).

Microscopically, lesions observed in the respiratory tract included squamous metaplasia in the larynx, hemorrhage and macrophage accumulation in the lung and hyperplasia/hypertrophy of goblet cells in the nasal cavity (all dose groups); degeneration/regeneration of the respiratory epithelium in the nasal cavity (mid and high dose groups); and respiratory epithelium ulceration and olfactory epithelium degeneration in the nasal cavity (high dose). Other histopathological findings included myocardial degeneration/fibrosis in the heart and degeneration of seminiferous epithelium in the testis (all treated groups); oligo/aspermia in the epididymis and cortical hypertrophy in the adrenals (mid and high dose groups); and vacuolation of the zona glomerulosa in the adrenals (high dose). Treatment-related microscopic findings were still present in the adrenals, heart, testis, larynx, lung and nasal cavity of high dose recovery group animals. Epididymal lesions showed reversibility. A NOAEL was not achieved in this study.

Dose-related increases in 24 hr AUC values were noted in the study. At each dose level, the AUC values were generally similar after the 1st, 30th and 80th doses except for high dose males in which the levels were twice as high at week 13 (80th dose) than at day 30. The mean AUC values in males at mid and high dose levels were higher than the respective values in females. The terminal phase half-life values for treprostinil ranged from 20 to 50 minutes.

In another 13-week inhalation toxicity study, groups of dogs were exposed, by oronasal inhalation, to treprostinil aerosol concentrations of 0.025 mg/L for 15 minutes and 0.224 mg/L for 6 and 30 minutes daily for 13 weeks, resulting in achieved dose levels of 107, 322 and 1558 µg/kg/day, respectively. The controls were exposed to the vehicle for the same duration as the high dose group. One high dose female died on day 22; the death was attributed to choking on food. Decreased activity, salivation, tremors, vomiting and labored breathing were noted at the high dose. There were no effects on EKG. Although a dose-related sinus tachycardia was seen at mid and high dose levels during the early part of the study, by day 80, tachycardia had decreased and was limited to the high dose group. There were no treatment-related findings in hematology, clinical chemistry, respiratory minute volume, urinalysis, organ weight or gross pathology parameters.

Microscopically, treatment-related lesions were observed only in the respiratory tract, especially in the nasal cavity and larynx of mid and high dose group animals. Focal or multifocal respiratory epithelial degeneration/regeneration in the nasal cavity was observed in these groups, the incidence and/or severity being higher at the highest dose. Other nasal cavity findings, observed only at the highest dose level, included goblet cell hyperplasia/hypertrophy, ulceration in the squamous or respiratory epithelium, and degeneration/necrosis in the squamous epithelium. Degeneration of the ciliated

epithelium in the larynx was noted in mid and high dose males and females. Lesions in the larynx were still present in 1 of 2 male dogs (not in females) after the recovery period. Although lung hemorrhage (minimal in severity) was observed in 1 of 6 low dose dogs, since the incidence rate for this lesion is same or lower than the concurrent or historical control incidence rates, and also because the low dose did not produce any nasal or larynx lesions, the low dose (107 µg/kg/day) could be considered as a NOAEL for the dog study.

The Cmax and AUC values increased between day 1 and week 6, but generally no additional increases were observed between weeks 6 and 13. The terminal phase half-life values ranged from 18 to 26 minutes.

Animal reproduction and developmental toxicity studies have not been conducted with treprostinil administered by the inhalation route. Animal reproduction and developmental toxicity studies were conducted previously with treprostinil administered by continuous sc infusion. In those studies, treprostinil did not affect fertility or mating performance of male or female rats at infusion rates of up to 450 ng/kg/min (about 59 times the recommended starting human sc infusion rate and 8 times the average rate achieved in clinical trials). In pregnant rats, continuous sc infusions of treprostinil sodium during organogenesis and late gestational development, at rates as high as 900 ng treprostinil/ kg/min (about 117 times the recommended starting human sc infusion rate and about 16 times the average rate achieved in clinical trials), resulted in no evidence of harm to the fetus. In pregnant rabbits, effects of continuous sc infusions of treprostinil during organogenesis were limited to an increased incidence of fetal skeletal variations associated with maternal toxicity at an infusion rate of 150 ng treprostinil/kg/min (about 41 times the starting human dose rate for sc infusion and 5 times the average rate used in clinical trials).

The sponsor is planning to conduct a 2-year bioassay in rats by the inhalation route. The range-finding study for the selection of the doses for the carcinogenicity study is being repeated as per the recommendations of the Executive CAC. *In vitro* and *in vivo* genetic toxicology studies did not demonstrate any mutagenic or clastogenic effects of treprostinil.

Nonclinical Safety Issues Relevant to Clinical Use

13-week inhalation toxicity studies with treprostinil revealed respiratory tract lesions, attributed to local irritation, in both rats and dogs. (In previous studies with treprostinil, local irritation was noted at the site of infusion when the drug was administered subcutaneously.) In the rat, no-observable-adverse-effect-levels (NOAELs) for respiratory or other treatment-related findings (myocardial, adrenal or testicular lesions) were not demonstrated. Myocardial lesions were considered to be due to reflex tachycardia induced by the exaggerated pharmacological action of treprostinil because of higher cardiac exposure to the drug when given by inhalation. Adrenal lesions were considered to be related to the stress of dosing and/or to the prolonged period of exposure

of rats in the inhalation apparatus (225 minutes/day at the high dose level vs 6 to 8 minutes/day in humans; lesions were mainly limited to the high dose).

The histopathological findings in the adrenal, heart, respiratory or reproductive organs observed in the 13-week inhalation study were not seen in a previous 26-week continuous sc infusion study (50, 150, 450 ng treprostinil/kg/min) in rats. This may be related to the higher plasma drug concentration obtained in the inhalation study; the mean maximum plasma treprostinil concentration obtained at the high dose in the rat inhalation study (307 ng/ml) was about 15-fold higher than the mean steady state concentration achieved at the high dose (20 ng/ml) in the 26-week sc continuous infusion study. (The Cmax value in patients receiving Tyvaso at the maximum recommended dose appears to be about 2 ng/ml.)

The NOAEL in the dog (107 µg/kg/day) for the respiratory findings is about 12 times higher than the maximum dose used in clinical studies [72 µg/inhalation session (4.8µg/kg/day)], on a mg/m^2 basis. The other treatment-related findings (myocardial, adrenal or testicular lesions) observed in the rat were not seen in the dog even at doses as high as 178 times the maximum clinical trial dose, on a body surface area basis. Since the above lesions were not seen in dogs even at relatively high dose multiples, and also because the plasma drug levels in patients at the recommended therapeutic dose level are far less than that seen in rats in the inhalation toxicity study, we do not consider the rat toxicity findings to constitute an approvability issue. Also, the long term clinical experience with treprostinil supports the safety of the drug.

In conclusion, there are no approvability issues for Tyvaso based on the non-clinical toxicity-testing program.

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NONCLINICAL SUPPORT FOR THE PROPOSED NDA FOR TYVASO

Nonclinical studies conducted with treprostinil sodium were reviewed under NDA 21-272 for Remodulin® Injection for sc and iv administration. The sponsor is referencing the above NDA for the support of the present NDA for Tyvaso. Additional nonclinical studies conducted with treprostinil sodium included safety pharmacology studies (an *in vitro* hERG assay, an *ex vivo* action potential assay in rabbit Purkinje fibers, a cardiovascular iv safety pharmacology study in dogs and a respiratory safety pharmacology study in rats) and repeat-dose inhalation toxicity studies in rats and dogs. These studies are summarized below.

SAFETY PHARMACOLOGY STUDIES

The hERG (human ether-a-go-go-related gene) assay and the action potential assay in rabbit Purkinje fibers were conducted b(4)

In the hERG assay, the effects of treprostinil on cloned hERG potassium channels expressed in cultures of human embryonic kidney cells were determined using electrophysiological procedures. The cardiac potassium channel, hERG, is responsible for a rapid delayed rectifier current (I_{Kr}) in ventricles. The inhibition of I_{Kr} current is the most common cause of cardiac action potential prolongation by non-cardiac drugs. Increased action potential duration causes prolongation of the QT interval which has been shown to be associated with a lethal ventricular arrhythmia, *torsade de pointes*. In this study, treprostinil was tested at concentrations of 0, 5, 10, 50 and 100 μM to determine its potential for the inhibition of hERG-mediated current. Treprostinil, at different concentrations, inhibited hERG current at the following rates: 0.5% inhibition for the vehicle control, 0.7% at 5 μM, 0.5% at 10 μM, 1.9% at 50 μM and 1.4% at 100 μM. Terfenadine, the positive control, at 60 nM, inhibited hERG current by 85.0%. In summary, treprostinil did not inhibit hERG-mediated current at concentrations up to 100 μM [39,052 ng/ml; the Cmax value in patients receiving Tyvaso at the maximum recommended dose is 1.8 ng/ml.]

The *in vitro* effects of treprostinil on cardiac action potentials were determined in isolated rabbit Purkinje fibers. Increased action potential duration (APD) is associated with prolongation of QT interval. Rabbit Purkinje fibers have been shown to be more sensitive to drug-induced APD prolongation than Purkinje fibers from other species. In rabbit Purkinje fibers, the action potential wave form and the underlying ionic currents are similar to those of humans. In this study, the test drug treprostinil, at concentrations of 3, 30 and 300 μM, was added to isolated rabbit Purkinje fiber preparations (n = 4) at two stimulus intervals [basic cycle lengths (BCL) of 1 and 0.5 s] and the effects of treprostinil on action potential parameters were compared to the time-matched vehicle controls. Treprostinil at 3 μM did not induce any significant changes in APD_{60} and APD_{90} (action

potential duration measured at 60 and 90% repolarization) when compared to vehicle controls. Treprostinil at 30 μM produced shortening in APD_{60} and APD_{90} at 1 s BCL, but not at 0.5 s BCL. Treprostinil at 300 μM induced significant shortening in APD_{60} and APD_{90} at both 1 and 0.5 s BCL. Treprostinil at all three concentrations did not significantly change the resting membrane potentials of the Purkinje fibers, the action potential amplitude and the maximum rate of depolarization (dV/dt max). The positive control dl-sotalol (50 μM) significantly prolonged the APD_{60} and the APD_{90} at both stimulus intervals without significant changes in resting membrane potential, action potential amplitude and the maximum rate of depolarization. In conclusion, treprostinil did not prolong action potential duration at concentrations up to 300 μM (117,156 ng/ml).

A cardiovascular safety pharmacology study in dogs was conducted _____ b(4)
_____ Treprostinil sodium solution was administered as a single iv bolus injection to 4 female beagle dogs (radiotelemetry-implanted) at dose levels of 0, 2, 20 or 200 μg/kg, according to a Latin-square design, such that each dog received each treatment once, with at least a 3-day washout period between doses. Heart rate, arterial blood pressure and EKG parameters were evaluated every 10 minutes for about 24 hours post-dose. As expected, treprostinil caused an initial drop in arterial blood pressure (systolic, diastolic and mean) and a compensatory increase in heart rate at all dose levels during the first hour after dosing. Elevated heart rates (up to 39%) were seen at the 200 μg/kg dose level 3 hr post-dose. The initial drop in arterial blood pressure was followed by a period (up to 6 hours) of elevated systolic (up to 15%), diastolic (27%) and mean (23%) blood pressures at 200 μg/kg dose level. The increase in blood pressure is considered to be due to a compensatory sympathetic reflex mechanism after the initial drop in pressure. Increased incidences of atrioventricular block were observed in one dog at 2 μg/kg and another dog at 200 μg/kg. However, PR interval was not affected. Prolongation of QRS complex was observed at 200 μg/kg. Treprostinil did not affect QT interval at any dose level.

[In a separate study, the hemodynamic activity of treprostinil and four of its metabolites (M334, M388, M392 and M566) on mean arterial pressure (MAP) and heart rate (HR) were investigated in anesthetized rats given iv bolus doses of treprostinil at 0.13, 1.3, 12.7 and 127 μg/kg or each metabolite at 10, 100, 1000 or 10000 μg/kg. At the lowest dose levels, neither treprostinil (0.13 μg/kg) nor its metabolites (10 μg/kg) affected MAP or HR. At the highest dose levels, treprostinil (127 μg/kg) and its 3 metabolites (M388, M392 and M566; 10000 μg/kg) caused a fall in MAP within 1 minute and a subsequent rise in HR, indicating that the metabolites were much less active than treprostinil itself. The metabolite M344 did not affect MAP or HR at any dose level.]

A respiratory safety pharmacology study was conducted in rats _____ b(4)
_____ . To evaluate the potential effects on respiratory function, nebulized treprostinil sodium was administered to groups of male rats via nose-only inhalation at an aerosol concentration of about 50 μg/L for 2, 3 or 4 hours. An additional group of male rats was given citrate buffer vehicle for 4 hours. Respiratory rate and tidal volume were measured, and the minute volume was calculated. Treprostinil, at achieved inhaled dose levels of 300, 416 and 569 μg/kg, produced significant decreases

in respiratory rates and derived minute volumes compared to predose and control values. These decreases occurred within 15 minutes of aerosol administration and rates and volumes remained decreased for the duration of exposure. However, all values returned to predose and control levels 24 hours following treatment.

INHALATION TOXICITY STUDIES

13-week inhalation toxicity studies were conducted in rats and dogs (with a 4-week recovery period) . These studies were reviewed earlier under IND 70,362, and are summarized below. b(4)

Thirteen-Week Study in Rats With 4-Week Recovery Period

Groups of Sprague-Dawley rats [15/sex/group for the main study; 9/sex/treatment for the toxicokinetic (TK) evaluations and 5/sex in the control and high dose groups for reversibility studies] were exposed, by nose only administration, to treprostinil aerosol concentrations of 7 µg/L for 20 minutes/day, 44 µg/L for 30 minutes/day and 40 µg/L for 225 minutes/day for 13 weeks, resulting in estimated achieved dose levels of 7, 67 and 464 µg/kg/day. Control animals were exposed to conditioned room air for the same duration as the high dose animals. All animals were observed twice daily for mortality and clinical signs. Body weight and food consumption were recorded weekly. Respiratory minute volume was recorded on 5 rats/sex/group for 15 minutes prior to dosing and then for the first 15 minutes post-dose during weeks 1, 6 and 13. Ophthalmoscopic examination was performed on all rats before the initiation of treatment and during weeks 13 and 17 (recovery group). Hematology, serum chemistry and urinalysis parameters were evaluated during weeks 4 and 13 and at the end of the recovery period. Plasma samples were collected from TK rats just before dosing and at 5, 15, 30, 75 and 105 minutes post-dose on days 1, 30 and 80 for TK evaluations.

Complete necropsy was performed on all animals found dead during the study and those sacrificed at the termination of the study, select organs weighed, and the following tissues were collected and preserved: adrenals, aorta, bone and marrow (sternum), brain, bronchi, cecum, colon, duodenum, epididymides, esophagus, eyes, Harderian glands, heart, ileum, jejunum, kidneys, larynx, lacrimal glands, liver, lungs, lymph nodes, mammary gland, nasal cavities, optic nerves, ovaries, pancreas, pharynx, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, vagina and all gross lesions. All tissues from control and high dose group rats, and also respiratory tract tissues, adrenals, epididymides, heart, sternum, testis, thymus and all gross lesions from low and mid dose group rats, were examined microscopically.

Five rats (1 low dose male, 2 HD males and 2HD females) died during the study. Two deaths (low dose male and 1 HD female) were attributed to bleeding procedures; the cause of the other deaths was not determined. Labored breathing and decreased activity and muscle tone were observed in high dose rats. At the termination of the study, body

weight gains at 7, 67 and 464 µg/kg/day were 17, 36 and 65% lower than control for males and 22, 23 and 23% lower than control for females, respectively. Treatment-related decreases in food consumption were observed in mid and high dose males and females. Respiratory minute volume was reduced at mid and high dose levels during weeks 6 and 13. Decreased platelet, white cell and lymphocyte counts in high dose males, and increased reticulocyte counts in high dose males and females were observed. Organ weight findings included significantly higher than control adrenal (mid and high dose male and female groups) and lung weights (high dose – both sexes) and lower than control testis (high dose) and thymus weights (mid and high dose groups – both sexes). Apart from the above organ weight findings, there were no gross pathology findings at necropsy.

Microscopically, the lesions observed in the respiratory tract included squamous metaplasia in the larynx, hemorrhage and macrophage accumulation in the lung and hyperplasia/hypertrophy of goblet cells in the nasal cavity (all dose groups); degeneration/regeneration of the respiratory epithelium in the nasal cavity (mid and high dose groups); and respiratory epithelium ulceration and olfactory epithelium degeneration in the nasal cavity (high dose; Table 1). Other histopathological findings included myocardial degeneration/fibrosis in the heart and degeneration of seminiferous epithelium in the testis (all treated groups); oligo/aspermia in the epididymis and cortical hypertrophy in the adrenals (mid and high dose groups); and vacuolation of the zona glomerulosa in the adrenals (high dose; Table 2).

Treatment-related body weight, food consumption, platelet count and epididymal findings appeared to be reversible. However, treatment-related microscopic findings in the adrenals, heart, testis, larynx, lung and nasal cavity of high dose animals were also present in recovery animals.

A NOAEL was not achieved in this study.

Table 1.

Incidence and Severity of Treprostinil-related Histopathological Changes in Respiratory Tract Organs

Tissue/findings	sex	Male				Female			
Group ID		1	2	3	4	1	2	3	4
Dose level (µg/kg/day)		0	10	70	526	0	10	70	526
Larynx	No examined	15	15	15	16	15	15	15	15
Squamous metaplasia	Total No. affected	—	3	11	13	—	1	5	13
	Minimal	—	3	3	4	—	1	3	6
	Slight	—	—	8	9	—	—	2	7
Lung	No examined	15	15	15	16	15	15	15	15
Hemorrhage	Total No. affected	—	3	7	7	—	2	1	5
	Minimal	—	3	6	3	—	2	—	3
	Slight	—	—	1	4	—	—	1	2
Macrophage accumulation	Total No. affected	—	5	7	12	—	1	1	9
	Minimal	—	5	7	10	—	1	1	9
	Slight	—	—	—	2	—	—	—	—
Nasal cavity	No examined	15	15	15	16	15	15	15	15
Degeneration/regeneration: respiratory epithelium									
	Total No. affected	—	—	2	16	—	—	—	15
	Minimal	—	—	2	5	—	—	—	3
	Slight	—	—	—	9	—	—	—	11
	Moderate	—	—	—	2	—	—	—	1
Infiltration: neutrophilic cells	Total No. affected	—	—	—	6	—	—	—	1
	Minimal	—	—	—	6	—	—	—	—
	Slight	—	—	—	—	—	—	—	1
Ulceration: respiratory epithelium									
	Total No. affected	—	—	—	2	—	—	—	2
	Minimal	—	—	—	—	—	—	—	1
	Slight	—	—	—	2	—	—	—	1
Degeneration/regeneration: olfactory epithelium									
	Total No. affected	—	—	—	3	—	—	—	3
	Minimal	—	—	—	—	—	—	—	3
	Slight	—	—	—	3	—	—	—	—
Hyperplasia/hypertrophy: goblet cells									
	Total No. affected	—	6	1	10	—	5	4	7
	Minimal	—	6	1	5	—	5	4	7
	Slight	—	—	—	5	—	—	—	—

Table 2.

Incidence and Severity of Treprostinil-related Histopathological Changes in Non-Respiratory Tract Organs

Tissue/findings	sex	Male				Female			
Group ID		1	2	3	4	1	2	3	4
Dose level (µg/kg/day)		0	10	70	526	0	10	70	526
Adrenal	No examined	15	15	15	16	15	15	15	15
Hypertrophy: cortical	Total No. affected	—	—	1	12	—	4	3	12
	Minimal	—	—	1	11	—	4	3	11
	Slight	—	—	—	1	—	—	—	1
Vacuolation: zona granulosa	Total No. affected	—	—	—	4	—	—	—	4
	Minimal	—	—	—	3	—	—	—	3
	Slight	—	—	—	—	—	—	—	1
	Moderate	—	—	—	1	—	—	—	—
Heart	No examined	15	15	15	16	15	15	15	15
Degeneration/fibrosis	Total No. affected	—	2	2	8	—	5	4	3
	Minimal	—	2	2	5	—	5	4	2
	Slight	—	—	—	3	—	—	—	1
Testis	No examined	15	15	15	16				
Degeneration: seminiferous epithelium									
	Total No. affected	—	2	2	9				
	Minimal	—	2	—	1				
	Slight	—	—	1	7				
	Moderate	—	—	1	—				
	Marked	—	—	—	1				
Epididymis	No examined	15	15	15	16				
Oligo/aspermia	Total No. affected	—	—	2	7				
	Minimal	—	—	—	3				
	Slight	—	—	—	3				
	Moderate	—	—	—	—				
	Marked	—	—	2	1				

The Cmax and AUC values obtained at different intervals (after 1st, 30th and 80th doses) in the 13 week study are presented below.

	Males			Females		
Achieved Aerosol Conc. (µg/L) =	**7.1**	**44.0**	**40.3**	**7.1**	**44.0**	**40.3**
Inhalation Duration (min/day) =	**20**	**30**	**225**	**20**	**30**	**225**
Estimated Dose (µg/kg/day) =	**7**	**67**	**464**	**7**	**67**	**464**
C_{max} (ng/mL)						
1st dose	13	131	185	11	107	266
30th dose	23	158	279	19	104	169
80th dose	16	122	564	27	79	212
Mean*	20	140	422	23	92	191
AUC_{inf} (h*ng/mL)						
1st dose	6.0	65.3	532	5.5	47.7	665
30th dose	9.0	76.5	632	7.5	48.3	441
80th dose	6.2	68.5	1233	10.0	42.2	521
Mean*	7.6	72.5	933	8.8	45.3	481

*Means calculated using steady-state data for the 30th and 80th doses.

AUC values indicated significant exposure to treprostinil and appeared to increase in direct proportion to dose level. At each dose level, the AUC values were generally similar at different time points (except in high dose males at the 80th dose). The mean AUC values in males at mid and high dose levels were higher than the respective values in females.

The histopathological findings in the adrenal, heart, respiratory and reproductive organs observed in this 13-week inhalation study were not seen in a previous 26-week continuous sc infusion study with treprostinil sodium (50, 150, 450 ng/kg/min) in rats. The mean maximum plasma treprostinil concentration obtained at the high dose (307 ng/ml) in the inhalation study in the rat was about 15-fold higher than the mean steady state concentration achieved at the high dose (20 ng/ml) in the 26-week continuous sc infusion study.

(The sponsor submitted their 13-week inhalation study as a range-finding study in support of dose selection for their 2-year carcinogen bioassay in rats. The Executive CAC found the evaluation of the range-finding study was confounded by its design, i.e., the twin variables of aerosol concentration and the duration of treatment. The Committee recommended that the sponsor repeat the study with a single variable, preferably aerosol concentration.)

Thirteen-Week Inhalation Toxicity Study in Dogs With 4-Week Recovery Period

Groups of beagle dogs (3/sex/group for the main study and 2 additional animals/sex in the control and high dose groups for the recovery phase of the study) were exposed, by oronasal inhalation, to treprostinl aerosol concentrations of 0.025 mg/L for 15 minutes, 0.224 mg/L for 6 or 30 minutes daily, for 13 weeks, resulting in achieved dose levels of 107, 322 and 1558 µg/kg/day, respectively. The control group was exposed to the vehicle formulation for the same duration as the high dose group. Clinical signs, body weight and food consumption were recorded. Blood pressure, EKG, respiratory minute volume, hematology, serum chemistry and urinalysis parameters were assessed. Toxicokinetic evaluations were performed on day 1 and during weeks 6 and 13.

Complete necropsy was performed on all animals, selected organs weighed and more than 40 tissues/animal and all gross lesions were preserved. Histopathological examinations were performed on all tissues from all dogs, including those found dead.

One high dose female was found dead during treatment on day 22. The death was attributed to the animal choking on its own vomit in the animal mask based on the presence of food material in the trachea. Decreased activity, dehydration, salivation, tremors, dilated pupils, vomiting, labored breathing and abnormal breathing sounds were observed at the high dose. Reversible decreased body weight gain and food consumption were noted for high dose animals. There were no effects on EKG morphology or systolic blood pressure. A dose-related sinus tachycardia was observed in mid and high dose animals until day 30 of treatment. By day 80, tachycardia had decreased and was limited to the high dose group. There were no treatment-related findings in hematology, clinical chemistry, respiratory minute volume, urinalysis, organ weight or gross pathology parameters.

Microscopically, treatment-related lesions were observed in the respiratory tract, especially in the nasal cavity and larynx of mid and high dose group animals (Table 3). Focal or multifocal respiratory epithelial degeneration/regeneration in the nasal cavity was observed in these groups, the incidence and/or severity being higher at the high dose. Other nasal cavity findings, observed only at the high dose level, included goblet cell hyperplasia/hypertrophy, ulceration in the squamous or respiratory epithelium, and degeneration/necrosis in the squamous epithelium. Degeneration of the ciliated epithelium in the larynx was noted in mid and high dose males and females sacrificed at the end of the treatment period as well as in 1 of 2 male dogs (not in females) sacrificed 4 weeks later.

A microscopic finding of lung hemorrhage of minimal severity was not considered by the study pathologist to be treatment related since the lesion was also seen in the control group. However, the combined incidences (both sexes) of lung hemorrhage, [C 1/6 (17%), LD 1/6 (17%), MD 2/6 (33%) and HD 5/6 (83%)], showed a dose relationship at the mid and high dose levels. The historical control data from the previous 6 inhalation studies in dogs, conducted at the same study facility during the period from 2004 to 2007, showed a control incidence rate of 24% for lung hemorrhage. Since the low dose

incidence rate for the lung hemorrhage is the same or lower than the concurrent and historical control incidence rates, and also because the low dose did not cause any nasal cavity or larynx lesions, the low dose (107 µg/kg/day) can be considered as a NOAEL for this study.

Table 3.

		Males				Females			
Dose Group		1	2	3	4	1	2	3	4
Number of dogs examined		3	3	3	3	3	3	3	3
Nasal Cavity									
Respiratory epithelial degeneration/regeneration	**Total**	**0**	**0**	**3**	**3**	**0**	**0**	**2**	**3**
	Minimal			3	0			2	0
	Slight				3				3
Goblet cell hyperplasia/hypertrophy	**Total**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	Minimal				1				
Squamous epithelial ulceration	**Total**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	Minimal				1				
Respiratory epithelial ulceration	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
	Minimal								1
Squamous epithelial degeneration/necrosis	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
	Minimal								1
Larynx									
Degeneration of ciliated epithelium	**Total**	**0**	**0**	**2**	**3**	**0**	**0**	**2**	**1**
	Minimal			2	2			2	1
	Slight				1				

The C_{max} and AUC values were shown to be increased with increasing dose (Table 4). These values increased between day 1 and week 6, but generally no additional increases were observed between weeks 6 and 13. The average terminal phase half-life values ranged from 18 to 26 minutes.

Table 4.

Dose (µg/kg) Duration	Sex	Time	t½ (min)	T_{max} (min)	C_{max} (ng/mL)	AUC_{INF} (min*ng/mL)
Low	Male	Day 1	19.3	15.00	31.8	896
84.7		Week 6	19.2	15.00	58.8	1157
15 min		Week 13	18.0	15.00	44.6	1137
Low	Female	Day 1	17.9	15.00	18.8	612
129.9		Week 6	21.3	15.00	65.0	1327
15 min		Week 13	20.5	16.67	54.5	1301
Mid	Male	Day 1	20.3	9.33	143.6	3529
308.7		Week 6	18.5	6.00	523.7	6686
6 min		Week 13	21.0	7.67	346.7	4993
Mid	Female	Day 1	24.8	9.33	143.3	3565
335.4		Week 6	23.9	7.67	340.0	5744
6 min		Week 13	25.5	6.67	337.7	5004
High	Male	Day 1	20.9	29.00	627.4	31130
1599.0		Week 6	21.0	30.00	1326.8	39643
30 min		Week 13	21.0	30.00	1706.4	32448
High	Female	Day 1	20.9	28.00	622.2	29071
1516.7		Week 6	21.7	30.00	1494.0	40641
30 min		Week 13	22.8	30.00	1169.5	25193

[The achieved dose level values provided in the text are the mean values (sexes combined) for each dose level.]

{Note: In a 7-day range-finding inhalation study in dogs [at achieved dose levels of 0.53, 0.86 and 2.99 (days 1 to 3) or 2.28 mg/kg/day (days 4 to 7)], nasal cavity lesions were observed in all treated groups. Minimal to slight myocardial lesions were seen in the high dose group. (High dose was reduced from 2.99 to 2.28 mg/kg/day on day 4 due to adverse clinical signs.) It is noted that no myocardial lesions were observed in the 13-week inhalation study at doses up to 1.558 mg/kg/day.}

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/s/

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