CENTER FOR DRUG EVALUATION AND RESEARCH

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PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Center for Drug Evaluation and Research
Office of Drug Evaluation I
Division of Cardiovascular and Renal Products

Memorandum

Date: March 20, 2013

From: Thomas Papoian, PhD, DABT
Supervisory Pharmacologist

To: NDA 203496 (Treprostinil diethanolamine)

Subject: Listing of diethanolamine (diolamine) as known by the State of California to cause cancer (according to Proposition 65) - June 22, 2012

1. Background

Treprostinil diethanolamine (NDA 203496; United Therapeutics Corp.), an analogue of prostacyclin (PGI_2) with potent vasodilatory as well as platelet antiaggregatory effects, was submitted to this Division (DCRP) on Dec. 24, 2011, as a sustained release oral tablet for the treatment of pulmonary arterial hypertension (PAH). Treprostinil had been approved previously for the treatment of PAH by the subcutaneous, intravenous, and inhalational routes of administration. The current oral formulation is different from the previous sodium salt formulations in that it uses the diethanolamine salt (b) (4)

On March 7, 2013, this reviewer was notified by Mr. Dan Brum (DCRP Regulatory Project Manager) that on June 22, 2012, diethanolamine (CAS No. 111-42-2) was listed by the Office of Environmental Health Hazard Assessment (OEHHA) of the State of California as a chemical known to cause cancer. During the course of the NDA review cycle, the sponsor coincidentally changed the name of the drug to treprostinil diolamine, one of several commonly used chemical names for diethanolamine.

Other chemical names for diethanolamine include:

- Diolamine
- Bis(hydroxyethyl)amine
- N,N-Bis(2-hydroxyethyl)amine
- 2,2'-Dihydroxydiethylamine
- β,β'-Dihydroxydiethylamine
- N-Ethylethanamine
- 2-[(2-Hydroxyethyl)amino]ethanol

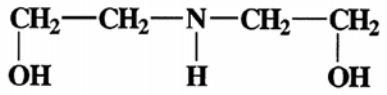
- 2,2'-Iminobisethanol
- Iminodiethanol

Proposition 65, the Safe Drinking Water and Toxic Enforcement Act of 1986, was enacted as a California State ballot initiative in November 1986. The Proposition was intended to protect California citizens and the state's drinking water sources from chemicals known to cause cancer, birth defects or other reproductive harm, and to inform citizens about exposures to such chemicals. Proposition 65 requires the Governor to publish, at least annually, a list of chemicals known to the State to cause cancer or reproductive toxicity.

The safety, particularly carcinogenic risk, of treprostinil diethanolamine (now diolamine) when given to PAH patients at recommended doses is reviewed below.

2. Toxicity Profile of Diethanolamine

Diethanolamine



CAS No. 111-42-2
Chemical formula: $C_4H_{11}NO_2$
MW: 105.14

Diethanolamine (DEA) is an organic compound synthesized from a reaction of ethylene oxide and ammonia. In contrast to naturally-occurring ethanolamine (monoethanolamine; MEA), a common head group for cell membrane phospholipids that is synthesized endogenously, diethanolamine is not known to occur naturally. However, it is widely used in the preparation of diethanolamides and diethanolamide salts of long-chain fatty acids, such as coconut oil diethanolamine condensates (cocomide DEA), that are formulated into soaps, detergents, cosmetics, shampoos, hair conditioners, and in many other industrial uses (reviewed in IARC, 2012). For example, diethanolamine as a contaminant can constitute up to 18% of cocomide DEA.

CFSAN/FDA permits use of diethanolamine as a component of adhesives in food packaging and as an indirect food additive when food comes into contact with paper products containing diethanolamine (21 CFR Parts 175.105, 176.170, 176.180).

A search of currently approved drug labels did not find any drugs stating that they contained diethanolamine or diolamine. However, according to the FDA's database of inactive ingredients used in approved brand-name and generic drug products (i.e., Inactive Ingredient Guide), 1.5% aqueous diethanolamine solutions are used as solvents for drugs given intravenously, in topical creams at 0.3%, and in ophthalmic solutions.

Animal toxicity studies with diethanolamine have been conducted going back many decades (reviewed in Mathews et al., 1997; IARC 2000, 2012a). In 1992, the National Toxicology

Program (NTP) conducted 13-week subchronic toxicity studies with diethanolamine in $B6C3F_1$ mice and F344/N rats following dermal and oral (via drinking water) administration (Melnick et al., 1994a and 1994b). Doses administered were as follows:

- Mice:
  - Drinking water = 630-10,000 ppm = 100-1700 mg/kg
  - Dermal (5X/wk) = 80-1250 mg/kg
- Rats:
  - Drinking water = 160-5000 ppm = 15-440 mg/kg
  - Dermal (5X/wk) = 32-500 mg/kg

Results (as stated in the PubMed abstracts) showed that diethanolamine induced dose-dependent toxic effects in multiple organs in both species (Table 1).

Table 1

Results of 13-Week Toxicity Studies with Diethanolamine in Mice and Rats
(Melnick et al., 1994a and 1994b)

Species	**Tissue**	**Effects**	**NOAEL Dose Achieved? (Yes/No)**
Mouse	Liver	Hepatocellular cytological alterations and necrosis; multiple hepatocyte changes, including enlarged cells that were frequently multinucleated, increased nuclear pleomorphism, increased eosinophilia and disruption of hepatic cords	No
	Kidney	Nephropathy and tubular epithelial necrosis in males	Yes
	Heart	Cardiac myocyte degeneration	Yes
	Skin	Site of application: ulceration, inflammation, hyperkeratosis, and acanthosis	No
Rat	Blood	Poorly regenerative, microcytic anemia	No
	Kidney	Increased weight, tubular necrosis, decreased renal function, and/or tubular mineralization	No
	Brain and spinal cord	Demyelination	Yes
	Testis	Degeneration of the seminiferous tubules	Yes
	Skin	Site of application: ulceration, inflammation, hyperkeratosis and acanthosis	No

The predominant target organs of toxicity were liver in mice, and kidney in both species, the two tissues with the highest concentrations (up to one-third of the administered oral dose) of diethanolamine. Interestingly, no liver lesions were seen in rats by either route of exposure. In rats, diethanolamine produced greater toxicity when given in the drinking water when compared to topical application. This was attributed largely to the limited dermal absorption of the

chemical through rat skin. However, little difference in toxicity between the two routes (oral or dermal) was noted in mice, which was thought to be due to thinner skin in mice vs. rats.

Subsequent absorption and bioavailability studies in mice and rats were conducted using oral, dermal and intravenous dosing in rats, and dermal dosing only in mice (Mathews, et al., 1997). Dermal application of diethanolamine used a wire mesh to prevent oral absorption through grooming, in contrast to the NTP carcinogenicity studies were grooming of the applied area was allowed. Results showed that diethanolamine is 100% absorbed from the GI tract, absorption through skin increased with increasing doses (i.e., diethanolamine enhances its own absorption), absorption of total dose through thinner mouse skin (26.8-58.1%) is greater than thicker rat skin (2.9-16.2%), and that diethanolamine has significant potential for bioaccumulation following repeated exposure, a reflection of diethanolamine's long half-life (7 days for various tissues and up to 54 days in blood). Published exposure estimates of diethanolamine from human daily use of shampoo products (e.g., cocomide DEA) varied widely from 8-200 mg/kg/day to 0.2-2.0 µg/kg/day (reviewed in IARC, 2012a).

Diethanolamine is excreted essentially unchanged, whereas the naturally-occurring ethanolamine is converted to CO_2 (10-15%), with the remainder incorporated into phospholipids. The toxicity of diethanolamine was thought to be due to high tissue accumulation, and incorporation and accumulation of *O*-phosphorylated and *N*-methylated diethanolamine into aberrant phospholipids resulting in alterations in membrane structure and function (Mathews, et al., 1997; reviewed in IARC, 2012).

Due to this reported toxicity in subchronic animal studies and continuing widespread human exposure, particularly in the industrial workplace and through skin care products, diethanolamine was selected by NTP for carcinogenic evaluation (NTP, 1999). Male and female $B6C3F_1$ mice and F344/N rats were administered diethanolamine in ethanol dermally 5 days/week for 2 years. Mice received diethanolamine at 0, 40, 80, or 160 mg/kg, and rats received 0, 16, 32, or 64 mg/kg (males) or 0, 8, 16, or 32 mg/kg (females).

Other than irritation at the site of application, no significant pathologic findings or increased tumors were seen in rats, presumably due to limited dermal absorption of diethanolamine through rat skin.

In mice, however, incidences of the following tumors, when compared to vehicle controls, were either significantly increased or showed a positive trend:

Male mice:

- Hepatocellular adenoma and hepatocellular adenoma and carcinoma (combined) in all dose groups (significantly increased).
- Hepatoblastoma in mid-dose (80) and high-dose (160 mg/kg) groups (significantly increased).
- Renal tubule adenoma (positive trend).

Female mice:

- Hepatocellular adenoma and hepatocellular adenoma and carcinoma (combined) in all dose groups (significantly increased).

A summary of the neoplastic and non-neoplastic findings from the NTP mouse and rat carcinogenicity studies with diethanolamine is shown in Table 2 below.

Table 2 (NTP. 1999)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Diethanolamine

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses in ethanol by dermal application	0, 16, 32, or 64 mg/kg	0, 8, 16, or 32 mg/kg	0, 40, 80, or 160 mg/kg	0, 40, 80, or 160 mg/kg
Body weights	64 mg/kg groups generally less than vehicle control groups	Dosed groups generally similar to vehicle control group	80 and 160 mg/kg groups less than vehicle control group	Dosed groups generally less than vehicle control group
Survival rates	14/50, 10/50, 21/50, 22/50	25/50, 29/50, 29/50, 24/50	40/50, 43/50, 34/50, 30/50	44/50, 33/50, 33/50, 23/50
Nonneoplastic effects	Skin: acanthosis (0/50, 2/50, 4/50, 10/50); hyperkeratosis (0/50, 3/50, 5/50, 11/50); exudate (0/50, 3/50, 2/50, 7/50)	Skin: hyperkeratosis (3/50, 13/50, 23/50, 23/50); exudate (1/50, 7/50, 7/50, 7/50) Kidney: nephropathy (40/50, 47/50, 48/50, 48/50); severity (1.2, 1.5, 1.9, 2.7)	Liver: cytoplasmic alteration (1/50, 17/50, 17/50, 12/50); syncytial alteration (0/50, 28/50, 38/50, 23/50) Kidney: renal tubule hyperplasia (standard and extended evaluation combined (3/50, 7/50, 7/50, 10/50) Thyroid gland: follicular cell hyperplasia (18/50, 22/49, 30/50, 42/50) Skin: hyperkeratosis (0/50, 13/50, 10/50, 17/50)	Liver: syncytial alteration (0/50, 2/50, 17/50, 18/50) Thyroid gland: follicular cell hyperplasia (18/50, 28/49, 32/50, 39/50) Skin: hyperkeratosis (1/50, 3/50, 8/50, 16/50)
Neoplastic effects	None	None	Liver: hepatocellular adenoma (31/50, 42/50, 49/50, 45/50); hepatocellular carcinoma (12/50, 17/50, 33/50, 34/50); hepatoblastoma (0/50, 2/50, 8/50, 5/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (39/50, 47/50, 50/50, 49/50) Kidney: adenoma (standard evaluation - 1/50, 4/50, 6/50, 6/50; standard and extended evaluation combined - 1/50, 6/50, 8/50, 7/50); adenoma or carcinoma (combined) (standard evaluation -3/50, 5/50, 6/50, 8/50; standard and extended evaluation combined - 3/50, 7/50, 8/50, 9/50)	Liver: hepatocellular adenoma (32/50, 50/50, 48/50, 48/50); hepatocellular carcinoma (5/50, 19/50, 38/50, 42/50); hepatocellular adenoma or carcinoma (33/50, 50/50, 50/50, 50/50)

The NTP Working Group noted that tumors of the kidney and hepatoblastomas are considered rare tumors in rodents. NTP concluded that ethanolamine showed "clear evidence" of carcinogenic activity (NTP, 1999).

A full battery of genetic toxicity studies with diethanolamine were also conducted by NTP and were all found to be negative (i.e., Ames test, mouse lymphoma assay, sister chromatid exchanges, chromosomal aberrations in CHO cells, and mouse micronucleous assay) (NTP, 1999).

Given that diethanolamine is a contaminant in coconut oil diethanolamine condensates (cocomide DEA) that are used in many everyday household products, NTP conducted mouse and rat carcinogenicity studies with coconut oil acid diethanolamine condensate (NTP, 2001). Results were similar to those seen with diethanolamine, that is, no tumors were seen in rats, but increased hepatocellular adenomas, hepatocellular carcinomas, hepatoblastomas, and renal tubule adenomas and renal tubule carcinomas were seen in mice. The increases were attributed to the free diethanolamine present as a contaminant at levels up to 18.2% (IARC, 2012b).

3. Possible Mechanisms for Mouse Tumor Data

Subsequent to the publication of the NTP mouse tumor results in 1999, several investigators, including some from chemical companies, published reports examining possible modes of action for the carcinogenic effects seen in mice with diethanolamine (reviewed in IARC, 2000). These studies in mice showed that diethanolamine induced a choline deficiency in B6C3F_1 mice, and that diethanolamine inhibited uptake of choline into mammalian cells in culture. These data together with previous reports that choline deficiency in the diet of rodents predisposes to development of hepatocellular carcinomas, and of "inadequate evidence" for carcinogenicity in humans, prompted the International Agency for Research on Cancer (IARC), an agency that is part of the World Health Organization (WHO), to conclude that diethanolamine-induced choline deficiency provided a plausible mechanism for the tumorigenesis seen in mice, but not rats, and that diethanolamine is not classifiable as to its carcinogenicity to humans" (IARC, 2000).

Subsequent data was published supporting the premise that diethanolamine induced mouse liver tumors by a non-genotoxic mechanism that involves its ability to cause choline deficiency (Leung et al., 2005). The evidence included the following: (1) diethanolamine decreased hepatic choline metabolites and S-adenosylmethionine (SAM) levels in mice, similar to those observed in choline-deficient mice, whereas no effect was seen in the rat, a species in which diethanolamine was not carcinogenic; (2) all doses of diethanolamine that induced tumors in mice were shown to cause choline deficiency; (3) diethanolamine decreased phosphatidylcholine synthesis by blocking the cellular uptake of choline in vitro, but not in the presence of excess choline; and (4) diethanolamine induced transformation in the Syrian hamster embryo cells, increased S-phase DNA synthesis in mouse hepatocytes, and decreased gap junctional intracellular communication in primary cultured mouse and rat hepatocytes, but all these events were prevented with choline supplementation. Finally, it was suggested that rodents are more

susceptible to choline deficiency than humans due to quantitative differences in the enzyme kinetics controlling choline metabolism.

In 2012, IARC re-evaluated more recent evidence relating to the diethanolamine's proposed carcinogenic mode of action in mice and its possible relevance for humans (IARC, 2012a). In addition to effects of diethanolamine on structure and function of biological membranes, the proposed mechanism for its carcinogenic effects in mice postulated that depletion of intracellular choline levels leads to reduced availability of the methyl donor SAM, resulting in hypomethylation of DNA, altered expression of genes that regulate cell growth, and possible tumor formation through epigenetic mechanisms.

The arguments for this proposed mechanism based on the published literature were summarized as follows (reviewed in IARC, 2012):

- Dermal exposure of mice to maximum tolerated doses of diethanolamine resulted in reductions in levels of choline, choline metabolites and SAM in the liver.
- The induction of morphological transformation in SHE cells by diethanolamine was prevented by the addition of excess choline.
- The inhibition of choline uptake by diethanolamine in SHE and Chinese hamster ovary cells was prevented by the addition of excess choline to the culture medium.
- DNA methylation status was similarly altered (mainly hypomethylations) in isolated mouse hepatocytes grown in the presence of diethanolamine or in choline-deficient medium.
- Increases in DNA synthesis in primary cultures of mouse or rat hepatocytes incubated with diethanolamine were prevented by the addition of excess choline.
- N-nitrosodiethanolamine, a potent carcinogen created by the reaction between diethanolamine and sodium nitrite, was not detected in mice that were administered diethanolamine by dermal application with or without sodium nitrite in their drinking-water, indicating the mouse tumors were not due to in vivo formation of this carcinogen.

However, "uncertainties" were raised about the choline deficiency mechanism based on the published literature (reviewed in IARC, 2012):

- No effect on hepatic levels of SAM was observed in mice administered a dose of diethanolamine (40 mg/kg) that produced a significant increase in hepatocellular neoplasms.
- Studies of induced choline deficiency have not been evaluated in mouse kidney, the second site of tumor induction by diethanolamine in mice.
- Although rats are highly sensitive to choline deficiency, the 2 year carcinogenicity study of diethanolamine found no evidence of a liver tumor response in this species.
- The hallmark of dietary choline deficiency is a fatty liver. However, a fatty liver was not diagnosed in rats or mice exposed to diethanolamine.
- The detection of mutations in the β-catenin gene in liver tumors from diethanolamine-exposed mice indicates that in vivo mutagenesis may be involved. No studies have been reported on the mutational profile in liver tumors induced in mice fed a choline-deficient diet.

Given that there appeared to be "weak evidence" for a genotoxic mechanism for the induction of liver tumors by diethanolamine in mice, and "moderate experimental evidence" for a choline deficiency mechanism, IARC concluded that relevance of this mechanism for humans "cannot be excluded", especially for subgroups that are highly susceptible to dietary choline deficiency. The final evaluation of IARC in 2012 was that: (1) there is "inadequate evidence" for carcinogenicity of diethanolamine in humans, (2) there is "sufficient evidence" in animals, and (3) diethanolamine is "possibly carcinogenic" to humans (Group 2B).

Based on this 2012 IARC report, and according to Prop 65, California's Office of Environmental Health Hazard Assessment (OEHHA) on June 22, 2012, listed diethanolamine (CAS No. 111-42-2) as a chemical known to cause cancer in either humans or animals.

4. Assessment of Potential Carcinogenic Risk from Use of Treprostinil Diethanolamine by PAH Patients

The lowest dose that produced liver tumors (hepatocellular adenoma and carcinoma) in the NTP mouse carcinogenicity study was 8 mg/kg/day when given dermally 5X/wk for 2 years. Depending on dose (i.e., there was increased absorption with increasing dose), the percentage of diethanolamine absorbed through the skin of mice was determined to be in the range of 26.8-58.1%. Given that absorption of diethanolamine in the GI tract has been reported to be 100% (Mathews, et al., 1997), and taking the lowest value of 26.8% absorbed, the orally equivalent dose of a 8 mg/kg/day dermally-applied dose would be 2.1 mg/kg/day (= 6.3 mg/m^2), the lowest orally-equivalent dose in which mouse liver tumors were seen.

The recommended dose of treprostinil diethanolamine for PAH patients is 3.4 mg BID or 6.8 mg/day. This equals 0.11 mg/kg, when based on a 60 kg individual. The proportion of diethanolamine (MW 105.14) to treprostinil diethanolamine (MW 495.65) is 0.21. Therefore, a daily dose of 0.11 mg/kg/day of treprostinil diethanolamine constitutes a daily dose of 0.02 mg/kg/day diethanolamine in humans (= 0.74 mg/m^2). Therefore, the lowest orally-equivalent dose of diethanolamine that produced liver tumors in mice (6.3 mg/m^2) is **9X** the recommended daily dose of diethanolamine in PAH patients (0.74 mg/m^2). It should be noted that a NOAEL dose for liver tumors in mice was not identified, therefore, the safety margin is likely to be less than 9X the human dose.

As part of the carcinogenic assessment for treprostinil diethanolamine (NDA 203496), a 26-week transgenic mouse study was conducted in Tg.rasH2 mice at oral doses of 5, 10 and 20 mg/kg/day in males and 3, 7.5 and 15 mg/kg/day in females. When compared to vehicle controls, no significantly increased incidence of tumors was seen, whereas the positive control urethane showed the expected increase in tumor incidence. As mentioned, the proportion of diethanolamine (MW 105.14) to treprostinil diethanolamine (MW 495.65) is 0.21. The highest NOAEL dose of 20 mg/kg/day treprostinil diethanolamine from the transgenic mouse study represents 4.2 mg/kg/day diethanolamine (= 12.6 mg/m^2). When compared to the recommended daily dose of diethanolamine in PAH patients (0.74 mg/m^2), the NOAEL from the 26-week transgenic mouse study represents a safety margin of **17X**. Further, there were no other non-neoplastic findings in the livers of these mice. However, given that diethanolamine has significant potential for bioaccumulation following repeated exposure, a reflection of

diethanolamine's long half-life, and its non-genotoxic mode of action, tumorigenic effects may not become manifest within a 6 month study. A two year carcinogenicity study with treprostinil diethanolamine in rats has been initiated, but not yet completed. Results from this rat study should be informative regarding carcinogenic potential of the diethanolamine salt as a component of treprostinil given orally, but without the issue of limited dermal absorption seen in the NTP rat study. However, the high dose selected in the study is limited by the pharmacological (i.e., vasodilatory) and/or toxicological (i.e., GI toxicity) effects of treprostinil as a prostacyclin (PGI_2) analogue.

5. Conclusions

Treprostinil as the sodium salt (Remodulin and Tyvaso) has been approved for continuous subcutaneous, continuous intravenous, and inhalational routes of administration. However, rodent carcinogenic studies were not previously conducted due to the difficulty of conducting lifetime continuous s.c. or i.v. administration in rodents. Also, the expected lifespan of untreated PAH patients was stated by the sponsor to be only 3 years at the time of approval. The exemption for not having to conduct rodent carcinogenicity studies in this case was according to an ICH carcinogenicity guidance (ICH-S1A; March 1996) that no long-term carcinogenicity studies may be required in instances where the life-expectancy in the indicated population is short (i.e., less than 2 to 3 years).

However, for the current oral formulation using the diethanolamine counterion, it was agreed by the FDA (Nov. 2005) and EMA (Feb. 2006) that carcinogenicity studies need to be ongoing at the time of NDA submission. Six-month transgenic mouse studies were submitted with the NDA and reviewed. Two-year rat carcinogenicity studies are ongoing.

The tumors seen in mice after two years of dermal administration of diethanolamine in the NTP study was postulated to be due to non-genotoxic mechanisms as a result of depleted intracellular choline levels leading to reduced availability of the methyl donor SAM, hypomethylation of DNA, and altered expression of genes that regulate cell growth (IARC, 2000a). Patients with choline deficiency appear to be at greater risk, but whether some patients with PAH are deficient in dietary choline (e.g., vegetarians) has not been examined. Also, doses of diethanolamine associated with liver tumors in mice were at least 9X the human daily dose, although a NOAEL dose was not identified. However, the risk of cancer for PAH patients with relatively short life expectancies, if correct, may be limited.

6. Additional Comment

The cancer risk from exposure to diethanolamine in PAH patients given treprostinil diethanolamine may be relatively limited given: (1) the non-genotoxic mechanism of depleted intracellular choline levels for its tumorigenic effect in mice, (2) its relevancy for PAH patients who may not be choline deficient, and (3) the short life expectancy of PAH patients. However, there is another concern for use of diethanolamine in other pharmaceutical products, such as the 1.5% aqueous diethanolamine solutions that are currently used as solvents for drugs given intravenously.

As mentioned, diethanolamine was found in rodents to be toxic to many tissues, including liver, kidney, heart, skin, blood, brain, spinal cord, testis, and skin. The toxicity of diethanolamine was thought to be due to high tissue accumulation resulting from its long half-life, and incorporation and accumulation of diethanolamine into aberrant phospholipids leading to alterations in membrane structure and function (Mathews, et al., 1997; reviewed in IARC, 2012). Such effects are likely to occur at any dose, and may result in serious toxicities, particularly when given intravenously or repeatedly over longer periods of time. It is not clear how safety was determined for its use as a drug solvent for intravenous use in products that may include formulations for generic drugs or as part of drug compounding. Although occupational exposure limits via skin and inhalational exposures have been established (IARC, 2012a), detailed information regarding human effects following intended therapeutic administration of diethanolamine does not appear to be readily available, but is worthy of further investigation as a separate matter.

7. References

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/s/

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03/21/2013

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 203496
Supporting document/s:	# 0 (original submission); #s 3, 4 & 5 (non-clinical - carcinogenicity and safety pharm. info.) # 14 (GI irritation study)
Applicant's letter date:	12/24/2011
CDER stamp date:	12/27/2011
Product:	(b) (4) TM (treprostinil diolamine) sustained release tablets for oral administration
Indication:	Treatment of pulmonary arterial hypertension
Applicant:	United Therapeutics Corp., Research Triangle Park, NC
Review Division:	Division of Cardiovascular and Renal Products
Reviewer:	Xavier Joseph, D.V.M.
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Project Manager:	Dan Brum, Pharm.D.

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EXECUTIVE SUMMARY

1.1 Introduction

Treprostinil diethanolamine (UT-15C) is being developed as a sustained release oral tablet for the treatment of pulmonary arterial hypertension (PAH). Treprostinil sodium (Remodulin® Injection), a chemically stable analogue of prostacyclin (PGI_2) with potent vasodilatory as well as platelet antiaggregatory effects, has been approved for chronic administration either by continuous subcutaneous or intravenous infusion for the treatment of PAH. Tyvaso® (treprostinil) Inhalation Solution has also been approved for the treatment of PAH by the inhalation route. The active pharmaceutical ingredient present in treprostinil diethanolamine and treprostinil sodium is shown to be identical (ionized treprostinil) irrespective of the salt used.

1.2 Brief Discussion of Nonclinical Findings

In a comparative study in anesthetized rats, the effects of treprostinil (treprostinil sodium) and UT-15C (treprostinil diethanolamine) on systemic arterial blood pressure and heart rate were studied to determine whether changing the salt form of treprostinil would change the bioactivity of treprostinil. The results indicated that the diethanolamine salt produced a similar cardiovascular profile to treprostinil following iv administration. This study also showed that UT-15C was active when administered via the intraduodenal (ID) route; the action was slower than the iv route while the duration of action was longer compared to iv route.

The hemodynamic effects of six human metabolites were evaluated in the same rat model. All metabolites evaluated had a significantly reduced activity (~ 1000 fold) compared to UT-15C.

A cardiovascular safety pharmacology study in beagle dogs, did not reveal any changes in ECG morphology or cardiac rhythm. UT-15C did not inhibit hERG-mediated current at doses up to 300 µM, the highest dose used in the study.

Cardiovascular safety studies conducted with diethanolamine alone did not produce any changes in arterial pressure, heart rate, EKG parameters including QTc, at doses up to 4 mg/kg/day,

The bioavailability (BA) studies in rats showed that UT-15C has an absolute BA of about 10% when given orally in solution. Intraportal vein BA was found to be approximately 40%, suggesting a substantial first pass effect.

Tissue distribution studies using dual-radiolabeled UT-15C showed that the distribution of drug-derived radioactivity for both labels was widespread, and both labels crossed blood/brain and blood/testes barriers.

In vitro protein binding studies using dual-labeled UT-15C showed that [14C]-treprostinil component of UT-15C was highly bound to human plasma proteins with mean binding of approximately 96%, while the [3H]-diethanolamine component was minimally bound to human plasma proteins (mean binding of 6.1%).

In vitro metabolite characterization studies showed that treprostinil was rapidly metabolized by rat, dog and human liver microsomes to five metabolites present in all species, suggesting similar metabolic potential of treprostinil across these species. Metabolism studies further showed that *in vitro* CYP2C8 was the major isozyme involved in the metabolism of UT-15C (about 95% disappearance of parent) while CYP2C9 played a minor role (about 22% disappearance of the parent).

In a 13-week oral toxicity study of UT-15C SR tablets in dogs (0, 10, 30 or 50 mg/dog/day), a high dose female was euthanized moribund on Day 16 due to GI toxicity. (Intussusception was seen at necropsy.) Reversible, dose dependent clinical signs related to GI toxicity observed included vomiting, diarrhea and fecal abnormalities. Treatment-related microscopic lesions were limited to the digestive tract. The GI lesions consisted of moderate, chronic inflammation within the colon accompanied by mild ulceration, congestion and lymphatic dilatation at the site of intussusception. No other toxicologically significant microscopic lesions were noted. The NOAEL was considered to be 10 mg/dog/day or 1 mg/kg/day. GI toxicity symptoms (including intussusception or rectal prolapse seen at necropsy) were also noted in a 14-day oral toxicity in dogs. These GI findings were similar to that seen with Remodulin given sc or iv, and have been previously reported in the Beagle dog as a possible class effect of prostacyclins or prostacyclin analogues in this species. UT-15C-treatment-related gastric mucosal necrosis with accompanying inflammation was noted in rodents.

In an *in vivo* genotoxicity assay, the test drug did not induce a significant increase in the incidence of micronucleated PCEs in either male or female rats.

Oral administration of UT-15C to Tg.rasH2 transgenic mice daily for 26 weeks did not significantly increase the incidence of tumors.

In the rabbit embryo-fetal development study, the total fetal skeletal malformations were significantly increased at 1.5 and 3.0 mg/kg/day, and the NOAEL for teratogenicity was 0.5 mg/kg/day (5-times the human exposure at the mean dose of 3.4 mg BID).

In an *in vitro* study in freshly excised rat colon tissue, the extent of irritation caused by UT-15C SR tablets (2.5 mg) on mucosal membrane was assessed by determining the LDH release and by histological evaluation of tissues. Exposure to the 2.5 mg UT-15C for 180 minutes, under the conditions of the study, did not result in increased LDH release or histologic evidence of tissue irritation when compared to the negative control, while the positive control, KCl, produced significantly higher amounts of LDH and severe histologic lesions. In this study, longer treatment times were not used, and also, it was not known how much of the drug was actually released.

1.3 Recommendations

1.3.1 Approvability

There are no approvability issues for treprostinil diethanolamine based on nonclinical toxicity testing program.

The nonclinical developmental program for UT-15C did not reveal any toxicity that was not identified previously for treprostinil sodium. The GI toxicity in dog has been previously reported with Remodulin, and was considered to be a class effect of prostacyclins or prostacyclin analogues in this species. In the embryo-fetal development study in rats, the NOAEL for teratogenicity was 20 mg/kg/day (55 times the human exposure at the mean dose of 3.4 mg BID). In the rabbit embryo-fetal study, the total fetal skeletal malformations were significantly increased at 1.5 and 3.0 mg/kg/day, and the NOAEL for teratogenicity was 0.5 mg/kg/day (5 times the human exposure at the mean dose of 3.4 mg BID). The above observed effects may have been secondary to maternal toxicity (decreased body weight and food consumption, and increased incidences of abortion and deaths at mid and high doses). We do not consider the reprotoxicity findings to constitute an approvability issue. Nonclinical studies have shown no genotoxic or carcinogenic potential for the drug. Moreover, treprostinil sodium has been in the market for a considerable time.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Sponsor's labeling, along with the recommended changes to the labeling (in **bold letters**) are given below.

Section 8.1 Pregnancy

Pregnancy Category C - (b) (4) .

Animal reproductive studies with treprostinil diolamine have shown an adverse effect on the fetus (b) (4) there are no adequate and well-controlled studies in humans.

In rats, treatment with treprostinil diolamine had no effect on reproductive performance or sperm motility at doses up to 10 mg/kg/day. **The exposures at this dose level are about 10 (male) to 18 (female) fold (b) (4) human exposure at the mean dose of 3.4 mg BID.**
In pregnant rats, reversible, dose-dependent decreases in body weight gain and food consumption were observed during the first four days of dosing in animals administered 10, 20 and 30 mg/kg/day treprostinil diolamine. In a dose range-finding study, there was a 17% decrease in

the pregnancy rate in the animals administered 20 and 30 mg/kg/day. One dam in each of the 20 and 30 mg/kg/day had litters with no viable fetuses. In the definitive study **(0, 5, 10 and 20 mg/kg/day)**, there were four treatment-related deaths, and a 32% decrease in the pregnancy rate for rats administered 20 mg/kg/day. There was an 8% decrease in the pregnancy rate in the animals administered 10 mg/kg/day. Across both studies, an increase in post-implantation loss was observed in animals administered 10 to 30 mg/kg/day, and a significant decrease in the mean number of live births was seen at dose levels ≥10 mg/kg/day. The no observed adverse effect level was 5 mg/kg/day (maternal, fetal viability and growth), and 20 mg/kg/day (teratogenicity), the highest dose tested in the definitive study. **The exposures at 5 and 20 mg/kg/day doses represent 13 and 55 times, respectively, the human exposure.**

For F_1 progeny, a decreased copulation index was observed at the 5 and 10 mg/kg/day treprostinil diolamine dose levels in rats. The no observed effect levels for physical development, reflex development, exploratory behavior, learning and memory, and sexual maturation was 10 mg/kg/day. The no observed effect level for F_1 progeny general development (based on body weight) was 10 mg/kg/day for females and ≤ 2.5 mg/kg/day for males; the no observed effect level for F_1 reproductive performance was 2.5 mg/kg/day or **6 times the human exposure.**

In pregnant rabbits, the primary maternal adverse effects were gastrointestinal disturbance; dose-dependent decreases in mean body weight, body weight gain, and food consumption were observed. During the post-dose phase, the effect was reversed. In a dose range-finding study, there was a 17% decrease in the pregnancy rate for animals administered 4 mg/kg/day. A dose-dependent increase in post-implantation loss was observed. Two dams administered 4 mg/kg/day had litters with no viable fetuses; the mean fetal weight was slightly decreased in animals administered 4 mg/kg/day. In the definitive study, mean fetal weights were significantly decreased in animals administered 0.5 to 3 mg/kg/day of treprostinil diolamine. At doses of 1.5 and 3 mg/kg/day, external fetal and soft tissue malformations were observed in a few fetuses, and the total fetal skeletal malformations were significantly increased. The no observed adverse effect level was less than 0.5 mg/kg/day (maternal), 1.5 mg/kg/day (fetal viability and growth), and 0.5 mg/kg/day (teratogenecity). **The 0.5 mg/kg/day dose represents about 5 times the human exposure.**

Section 8.2 Labor and Delivery

No treprostinil treatment-related effects on labor and delivery were seen in animal studies. The effect of Tradename on labor and delivery in humans is unknown.

Section 13 NONCLINICAL TOXICOLOGY

Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Oral administration of treprostinil diethanolamine to Tg.rasH2 mice at 0, 5, 10 and 20 mg/kg/day in males and 0, 3, 7.5 and 15 mg/kg/day in females daily for 26 weeks did not significantly increase the incidence of tumors. The exposures obtained at the highest dose levels used in males and females are about 8- and 17-fold, respectively, (b) (4) the human exposure at the mean dose of 3.4 mg BID.

In vitro genotoxicity studies with high doses of treprostinil did not demonstrate any mutagenic or clastogenic effects. Treprostinil diolamine was tested *in vivo* in a rat micronucleus assay and did not induce an increased incidence of micronucleated polychromatic erythrocytes.

No adverse effect doses for fertility, fetal viability / growth, fetal development (teratogenicity), and postnatal development were determined in rats. In pregnant rabbits, external fetal and soft tissue malformations and fetal skeletal malformation occurred with the no observed adverse effect level for these adverse effects of 0.5 mg/kg/day **(5 times the human exposure)**. *[see Use in Specific Populations (8.1)]*

2 Drug Information

2.1 Drug : (b) (4) Sustained Release Tablets

CAS Registry Number (Optional): 830354-48-8

Generic Name: Treprostinil diolamine

Code Name: UT-15C

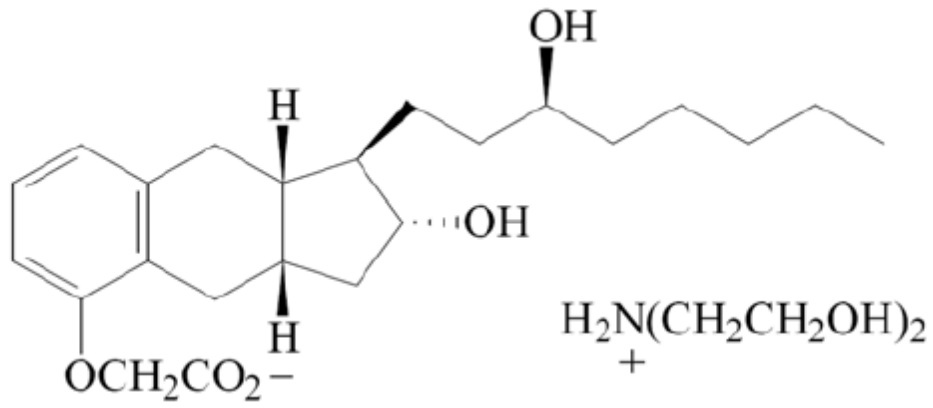
Chemical Names:

1. Acetic acid, 2-[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]-, compd. with 2,2'-iminobis[ethanol] (1:1)

2. 2-hydroxy-*N*-(2-hydroxyethyl)ethanaminium 2-({(1*R*,2*R*,3a*S*,9a*S*)-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-2,3,3a,4,9,9a-hexahydro-1*H*-cyclopenta[*b*]naphthalen-5-yl}oxy)acetate

Molecular Formula/Molecular Weight: $C_{23}H_{34}O_5.C_4H11NO_2$/495.66

Structure or Biochemical Description:



Pharmacologic Class: Prostacyclin analogue

2.2 Relevant INDs, NDAs, BLAs and DMFs: NDA 21272 (Remodulin Injection);
NDA 22387 (Tyvaso)

2.3 Drug Formulation: Treprostinil diethanolamine tablets are formulated as (b) (4) tablet strengths, which contain 0.125, 0.25, (b) (4) 1.0 or 2.5 mg of treprostinil. The formulations also contain xylitol, maltodextrin, sodium lauryl sulfate, magnesium stearate, cellulose acetate, triethyl citrate, polyvinyl alcohol, titanium dioxide, polyethylene glycol and talc. In addition tablets may contain colorants FD&C Blue#2, iron oxide yellow and iron oxide red.

Pharmacology

[Sponsor's summaries of pharmacology and pharmacokinetics are provided below. Sponsor's Tables and Figures are used for this review.]

UT-15C (treprostinil diethanolamine) is being developed as a sustained release (SR) oral tablet for the treatment of pulmonary arterial hypertension (PAH), an orphan disease with a global prevalence of approximately 50,000–100,000 patients. UT-15C is a novel salt form of Remodulin® (treprostinil) Injection and Tyvaso® (treprostinil) inhalation solution, which are approved in the United States for the treatment of patients with PAH.

The pharmacology of treprostinil, the active ingredient in Remodulin and Tyvaso, has been extensively evaluated by the continuous subcutaneous, intravenous, and inhalation routes of administration. Given that the only change to the drug substance synthesis route is the (b) (4) and treprostinil is not altered, the bioactive form of treprostinil diethanolamine and treprostinil sodium is predicted to be identical. Once in solution, both treprostinil sodium and treprostinil diethanolamine are disassociated from their respective salt counterions and exist as the ionized form of treprostinil. As a result, the bioactive form present in the bloodstream is identical irrespective of the selection of the counterion. Therefore, in addition to nonclinical studies conducted with UT-15C, an extensive amount of information on treprostinil sodium is available. United Therapeutics has conducted primary and secondary pharmacodynamic studies and safety pharmacology studies with treprostinil sodium in various in vitro, ex vivo, and in vivo test systems to investigate the potential adverse effects of treprostinil sodium.

Following the overview of treprostinil sodium pharmacology, a summary of UT-15C pharmacology is provided.

Summary of Treprostinil Sodium Pharmacology

In support of the intended clinical route for Remodulin and Tyvaso in the treatment of PAH, the preclinical pharmacology of treprostinil sodium was extensively evaluated in a series of primary pharmacology, secondary pharmacology, and safety pharmacology studies. A brief summary is provided below.

To support the development of Remodulin, primary pharmacodynamic studies with treprostinil were conducted in various in vitro, ex vivo, and in vivo test systems to investigate the potential effects of treprostinil sodium on vascular function, pulmonary artery smooth muscle cell (PASMC) proliferation, and platelet aggregation, with particular emphasis on pulmonary hemodynamics. The primary pharmacodynamic effects of treprostinil sodium paralleled those of naturally occurring eicosanoids such as prostacyclin (PGI2), which is generated in vascular and pulmonary smooth muscle where it moderates vascular tone and inhibits platelet aggregation. These effects have been summarized in Smyth, et al. (2006). The mechanism of action for the effects of treprostinil sodium on the cardiovascular and other systems has been demonstrated to be similar to PGI2.

Nonclinical pharmacology studies revealed that treprostinil exhibits potent pharmacological activity when dosed intravenously and reduces blood pressure in multiple species (rats, cats, rabbits, dogs, pigs) and that this is accompanied by reflex tachycardia. These same effects were observed with intravenous treprostinil in a cardiovascular safety pharmacology study in conscious, unrestrained dogs monitored by telemetry, and also in repeat-dose inhalation toxicity studies in dogs. Metabolites of treprostinil were tested for pharmacologic activity and found to be much less hemodynamically active than treprostinil itself; specifically, metabolite M388 was 100- to 1000-fold less active than treprostinil, metabolites M392 and M566 were 1000-fold less active than treprostinil, and metabolite M334 was at least 10000-fold less active than treprostinil (i.e., did not affect blood pressure or heart rate at the highest dose tested) (WHRI, 2008a; WHRI, 2008b). Metabolites M348 and M374 likewise had little cardiovascular activity, having some 0.01-0.05% the activity of parent drug. It is concluded that the metabolites contributed very little to pharmacologic activity in this model.

There were no adverse effects of parenterally administered treprostinil in autonomic, respiratory, gastrointestinal, uterine motility, inflammatory, or platelet aggregation secondary pharmacologic evaluations.

To support the development of Tyvaso, an ICH-recommended three-test battery of new safety pharmacology studies was performed. In a cardiovascular safety pharmacology study, treprostinil did not affect cardiac repolarization or prolong QT interval, even at concentrations that exceeded peak systemic exposure and estimated peak cardiac exposure in patients at the recommended therapeutic dose. In addition, the only effect on cardiovascular function during repeat-dose inhalation toxicity studies in dogs was an increased heart rate that was considered to be secondary to drug-induced vasodilation. Also, treprostinil did not inhibit hERG-mediated current (Study 060213.MSY) and did not prolong action potential duration in rabbit Purkinje fibers (Study060214.MSY) at concentrations that greatly exceed peak systemic exposure in patients administered treprostinil at the recommended dose. Reversible bradypnea and decreased respiratory minute volume were noted in an evaluation of the effects of inhaled treprostinil on the respiratory system in rats administered treprostinil via inhalation (Study 690478). Similar clinical signs were also noted in repeat dose toxicology studies with inhaled treprostinil in rats (CTBR 78656) but not in dogs (Study 78655 and Study 78657). The clinical significance of the effects on respiratory function in rats is unclear; however they occurred at exposure intensities greater than those that might occur in patients using Tyvaso at the maximum recommended dose.

In summary, there were no effects of treprostinil (parenteral or inhaled) in pharmacologic studies that precluded development or approval of either Remodulin or Tyvaso.

Summary of UT-15C (Treprostinil Diethanolamine) Pharmacology

UT-15C is a tricyclic benzindene analogue of prostacyclin formulated as a sustained –release osmotic tablet for oral administration to patients.

Treprostinil sodium is administered via both the parenteral and inhaled routes of administration, while the diethanolamine salt of treprostinil, UT-15C, is administered orally. In support of intended oral use, the primary pharmacology and safety pharmacology of UT 15C were

evaluated. All pivotal safety pharmacology studies were conducted in compliance with Good Laboratory Practice (GLP) regulations as set forth in the Title 21 of the U.S. Code of Federal Regulations, Part 58.

In the pre-clinical comparative study in the anesthetized rat (WHRI, 2003), treprostinil and UT-15C had identical hemodynamic activity and potency in causing dose-dependent falls in systemic arterial blood pressure following bolus intravenous administration. In addition, this study showed that the diethanolamine salt of treprostinil is active by the intraduodenal (ID) route.

Primary Pharmacology

Rat Blood Pressure Model Study - Intravenous and Intraduodenal Administration of UT-15C Compared with Intravenous Administration of Treprostinil (William Harvey Research Institute, July 2003)

The objective of the study (WHRI, 2003) was to investigate the effects of treprostinil (treprostinil sodium) and UT-15C (treprostinil diethanolamine) on systemic arterial blood pressure and heart rate in the rat following intravenous (iv) administration. A second objective of the study was to determine whether UT-15C was active when administered via the intraduodenal (ID) route. This study was undertaken to confirm that changing the salt form of treprostinil from sodium to diethanolamine would not change the bioactivity of treprostinil. The iv route was selected for the comparison to remove confounding variations in bioavailability of the two salts. An additional arm with ID administration was included to assess lower gastrointestinal (GI) absorption of UT-15C.

Anesthetized male Wistar rats were cannulated and connected to a pressure transducer to monitor mean arterial blood pressure (MAP) and heart rate (HR), which were continuously recorded on a data acquisition system throughout the study. The right jugular vein was cannulated for the administration of UT-15C and treprostinil (volume of administration was 1 mL/kg). After a 15-minute stabilization period, treprostinil (1, 10, or 100 μg/kg or as molar salt equivalent) was administered as an iv bolus. Hemodynamic parameters were monitored for three hours.

For the ID dosing arm, a rubber-dosing catheter was placed within the first centimeter of the duodenal lumen by passing the catheter down the esophagus into the stomach and through the pyloric sphincter. UT-15C (126.9, 253.8, or 507.6 μg/kg) was administered as a slow bolus injection (final volume of 1 mL) over a period of 1 to 2 minutes, ensuring that study drug passed into and remained within the duodenum. These doses were chosen on the basis of an anticipated bioavailability of 25% by this route. Hemodynamic parameters were monitored for three hours. Intravenous Administration of UT-15C and Treprostinil: Intravenous bolus administration of the maximal doses of treprostinil (100 μg/kg) or UT-15C (126.9 μg/kg, adjusted for its molecular weight) studied caused a fall in MAP of approximately 75 mmHg within 1 minute, the magnitude of the responses thus being comparable at equimolar doses. The blood pressure responses were dose-related for both compounds. The duration of the response for the free acid (t1/2 approximately 15 minutes) and its diethanolamine salt (t1/2 approximately 30 minutes) at

these doses were likewise in a similar range. Thus, the diethanolamine salt produces a similar cardiovascular profile to treprostinil following iv administration.

Intraduodenal Administration of UT-15C: Intraduodenal solution administration of the highest dose of UT-15C (507.6 μg/kg) caused a maximal fall in MAP of approximately 35 mmHg, being less than the rapid peak response observed with the maximal dose administered by the iv route. The onset of action was fairly rapid (within 5 minutes of administration) by the ID route but slower than by the iv route, while the duration of action (t1/2 approximately 70 minutes) at the highest dose of UT-15C was longer than that observed with iv administration in the maximal dose studies (t1/2 approximately 30 minutes). Moreover, this period was substantially longer than the duration of action (t1/2 approximately 5 minutes) for an equivalent fall in MAP following iv administration of UT-15C at the dose of 12.7 μg/kg.

This longer duration of action by the ID route presumably reflects the period required for UT-15C absorption from the duodenal lumen; therefore, direct comparisons of the peak fall in MAP by the ID and iv routes cannot be used to evaluate bioavailability.

In summary, the diethanolamine salt of treprostinil retains pharmacological activity and has a similar cardiovascular profile to treprostinil in this model, and is active by the ID route.

Evaluation of the Effects of UT-15C and its Metabolites on Systemic Haemodynamics Following Intravenous Administration in the Anaesthetized Rat

The hemodynamic effects of six metabolites (M334, M388, M392, M566, M348 and M374), identified from human pharmacokinetic studies with treprostinil and UT-15C, were also evaluated in the same rat model. The objective of the study was to determine the potency and duration of action of these metabolites in causing a reduction in systemic MAP and increases in HR following bolus iv administration in comparison to the activity of UT-15C. Overall, no highly active metabolite of treprostinil or UT-15C has been identified, as all the metabolites evaluated had a significantly reduced activity (~1000-fold) compared with UT-15C. Thus, it would appear that the observed pharmacological profile of treprostinil or UT-15C reflects the activity of the parent molecule, treprostinil, and that the contribution to that profile of any known metabolite that would be formed in vivo from UT-15C or treprostinil would be minimal.

A recent exploratory pharmacology study (Study 15205) utilized administration of a thromboxane agonist to elevate pulmonary artery pressure (PAP) in the rat as a model for PAH. Treprostinil was administered to the rats by oral gavage (UT-15C), nose-only inhalation (Tyvaso) or in a combined oral and inhalation regimen, followed by PAP assessment. In this study, when treprostinil therapy was administered either via inhalation or oral gavage, the treprostinil therapy generally reduced PAP during the PAH condition. Also, additional PAP reduction was observed (either greater reduction or more sustained duration) when oral

treprostinil was administered in addition to a low dose of inhaled treprostinil as part of a combination dosing regimen.

Safety Pharmacology

Selected effects of UT-15C on the cardiovascular system, neurobehavioral system, respiratory system, and the gastrointestinal system were evaluated. In addition, effects of the diethanolamine salt alone have been evaluated in the hERG assay.

In a cardiovascular safety pharmacology study in dogs (Study 1259DU16.002), oral administration of 1 mg/kg/dose UT-15C twice daily to male beagle dogs was associated with a mild, transient decrease in arterial pressure and increase in heart rate following each dose. Administration of UT- 15C at 3 mg/kg/dose twice daily to male beagle dogs was associated with a decrease in arterial pressure following the first and second doses followed by an increase 6–7 hours following the second dose. An increase in heart rate was noted for approximately 4 hours following each dose. Oral administration of UT-15C at 5 mg/kg/dose twice daily to male beagle dogs was associated with an increased heart rate for approximately 4 hours following the first dose and for approximately 15 hours following the second dose. Arterial pressure was decreased following the first dose but increased following the second dose. Transient, minimal, yet statistically significant increases in QTc (5–7%) were noted following the second dose of 3 mg/kg/dose UT-15C and following the first and second doses of 5 mg/kg/dose UT-15C. Qualitative analysis of the ECGs at selected time points did not show any changes in cardiac rhythm or ECG morphology. Also, UT-15C did not inhibit hERG-mediated current at doses up to 300 µM, the highest dose used in the study (Study 110106.DMK).

Data from a diethanolamine cardiovascular safety pharmacology study (Study 1259DU 16.003) demonstrated that diethanolamine administered in the absence of treprostinil was not associated with any definitive changes in arterial pressure, heart rate or electro-cardiogram parameters, including QTc, at doses up to 2 mg/kg/dose or 4 mg/kg/day, which is greater than the amount of diethanolamine present in the highest UT-15C dose administered in the UT-15C cardiovascular safety pharmacology study (Study 1259DU16.002). In addition, diethanolamine did not inhibit hERG-mediated current at doses up to 300 µM, the highest dose used in the study (Study 110204.DMK).

No apparent neuropharmacological or toxicological signs or effects upon body temperature were observed through 24 hours in any rats receiving the vehicle at 5 ml/kg or UT-15C at 3.8 mg/kg by oral administration. Decreased motor activity and a statistically significant ($p \leq 0.05$) decrease in body temperature were observed at 12.7 mg/kg. Decreased motor activity, abdominal tone, grip strength and a statistically significant ($p \leq 0.05$) decrease in body temperature were observed at 63.5 mg/kg (Study 0200RU16.001).

A transient increase, followed by a more gradual and persistent decrease in the respiratory rate was observed in anesthetized guinea pigs administered UT-15C intravenously at a dose of 1.27 mg/kg (Study 1082GU16.001). A 10-fold higher dose (12.7 mg/kg) caused death in two animals and pronounced depression of the respiratory rate, tidal volume, and minute volume at 30

minutes in surviving animals. The low dose of 0.127 mg/kg did not produce any biologically relevant changes in pulmonary function.

In conscious rats given UT-15C orally (Study 1275RU16.001), UT-15C at 1.16 mg/kg/day did not induce any biologically relevant effects on respiratory rate, tidal volume or minute volume in conscious male rats, although dose related decreases in respiratory rate and minute volume were observed following the oral administration of UT-15C at 2.5, 5, 15 and 25 mg/kg/day. Tidal volume was not affected. Based on these observations, the NOEL was 1.16 mg/kg/day.

UT-15C administered orally to mice at 8.9, 31.7, or 95.2 mg/kg produced decreases of 9%, 47% and 42%, respectively, in gastrointestinal motility when compared with the control group. The differences from control at the 31.7 and 95.2 mg/kg dose levels were statistically significant and considered biologically relevant. The no adverse effect level (NOAEL) was 8.9 mg/kg (Study 0239MU16.001).

Pharmacokinetics

The pharmacokinetic (PK) profile of the diethanolamine salt of treprostinil, UT-15C, has been evaluated with multiple single-dose and repeat-dose toxicokinetic studies in mice, rats and dogs. Toxicokinetic studies were also performed in pregnant rats and rabbits to determine the toxicokinetics of UT-15C on pregnant and lactating females and/or the development of offspring. In addition, several in vitro distribution and metabolism studies were performed; no formal nonclinical studies for excretion were conducted, although UT-15C excretion has been assessed in humans. A drug-drug interaction study to evaluate the effects of Revatio® (sildenafil citrate) or Tracleer® (bosentan) on UT-15C metabolism was also performed.

Absorption*:* The extent and rate of UT-15C absorption was evaluated in an early screening study (Study 02-UNIT.P04 ~ 02-UNIT.P03R1) that was initiated to determine the extent and nature of intestinal transport of UT-15C utilizing the human carcinoma cell line (Caco-2) as a model of human intestinal permeability. Results from this study were unclear, so a more formal GLP study was initiated to fully understand UT-15C permeability in this system. In this GLP study (Study 7049-123), 10 μM (4.96 μg/mL) UT-15C absorption through Caco-2 monolayers was determined to be moderate with neither preferential exsorption nor absorption detected; the transport is not restricted by MDR1-mediated efflux, suggesting UT-15C transport via a passive route. Control compounds confirmed the viability of the cell monolayers and MDR1 efflux potential.

Bioavailability: Bioavailability (BA) was determined in an early study (Study 02-UNIT.P01R2 ~ Report 1) in a small number of rats. The results indicated that UT-15C has an absolute BA of approximately 10% when given orally in solution. Intraportal vein bioavailability was approximately 40%, suggesting a substantial first pass effect. When administered by the intraduodenal (ID) route, BA was 24%, suggesting that degradation of treprostinil in the stomach or gastric emptying may influence the extent of systemic absorption. By comparing the intraportal vein and intraduodenal bioavailability, it appears that approximately 60% of UT-15C

is absorbed in the gastrointestinal (GI) tract. The average ID bioavailability is almost three times greater than the oral bioavailability suggesting that degradation of treprostinil in the stomach or gastric emptying may influence the extent of systemic absorption. The systemic clearance of treprostinil was greater than the hepatic blood flow signifying that extra-hepatic clearance mechanisms were involved in the elimination of the compound.

A separate study (Study 03-UNIT.P07–Report 1) was initiated in dogs to evaluate the BA and PK profile of two oral UT-15C sustained release (SR) dosage forms (8-hour release tablet and capsule) in comparison to an immediate release (IR) solution of UT-15C given orally, via an ID port, or by intravenous (iv) infusion over 1.5 hours. Oral BA of treprostinil after one tablet (10 mg/dog) and one capsule (10 mg/dog) were similar to that of the 2.5 mg/dog oral solution dose. The maximum plasma concentration (Cmax) was delayed slightly with the capsule, and was delayed substantially with the tablet. Dose-adjusted Cmax had the rank order of: oral solution > capsule > tablet. The tablet and capsule dosage forms may be useful for reducing Cmax and prolonging plasma concentration versus time profiles without reducing the extent of oral BA. Plasma clearance of treprostinil in dogs is similar to hepatic plasma flow, and the stomach has no role as a barrier to BA of treprostinil in dogs.

In dogs, several initial studies were performed using different 8-hr UT-15C SR tablet and capsule prototypes in doses ranging from 30 to 120 mg/dog/day in 14-day studies (Studies 7049-110 and 7049-113). A bridging study (Study 7049-117) was performed to compare the 8-hr and 12-hr UT-15C SR tablet formulations in dogs. Data indicate that systemic exposure in dogs was comparable between the 8- and 12-hr prototypes indicating that the previously collected 14-day toxicology data are applicable to the new 12-hr sustained release prototypes. Moreover, the total daily dose administered with the 12-hr SR tablet prototypes is expected to be lower, 2 mg (1 mg every 12 hours) vs. 2.37 mg (0.79 mg every 8 hours) and with it, total daily exposure. Therefore, the toxicology data for the 8-hr tablet can be considered directly applicable to the 12-hr SR tablet prototypes; thus, bridging the UT-15C 8-hr and 12-hr SR tablet prototypes.

Distribution: A study (No. 7049-125) utilizing dual-radiolabeled [14C, 3H]-UT-15C was conducted to assess the extent of absorption and tissue distribution in thirty male Long Evans rats following administration of a single oral solution dose of 1.51 mg (145 μCi) [14C]-treprostinil/kg and 0.519 mg (714 μCi) [3H]-diethanolamine/kg. The distribution of drug-derived radioactivity for both labels was widespread. Tissues with the highest [14C]-radioactivity (treprostinil) concentrations, excluding gastrointestinal contents, were small intestine, liver, large intestine with cecum, and stomach. The highest [3H]-radioactivity (diethanolamine) concentrations in tissues, excluding gastrointestinal contents, were spleen, adrenal glands, thyroid, kidneys, and liver, although [3H]-diethanolamine–derived radioactivity was quantifiable in all tissues analyzed at all time points. Concentrations of both [14C]-treprostinil and [3H]-diethanolamine-derived radioactivity were observed in brain and testes through 504 hours, which suggests that the radioactivity crossed the blood/brain and blood/testis barrier. Both [14C]-treprostinil and [3H]-diethanolamine exhibited limited, if any, melanin binding.

Protein Binding: The in vitro protein binding and protein binding interaction of the [14C]-treprostinil and [3H]-diethanolamine components of UT-15C in human plasma was conducted (Study 7049-127). Data indicated that the [14C]-treprostinil component of UT-15C was highly

bound to human plasma proteins with mean binding approximately 96%. The [3H]-diethanolamine component of UT-15C was minimally bound to human plasma proteins, with the greatest mean percent bound value of 6.1% observed at 0.01 μg/mL of treprostinil. There was no evidence for concentration dependent protein binding of either component of UT-15C over the target treprostinil concentration range of 0.01 to 10 μg/mL. UT-15C (25 ng/mL) had no effect on the extensive binding of [3H]-warfarin to human plasma proteins, confirming that UT-15C does not affect warfarin plasma protein binding. The moderate binding of [3H]-digoxin was not reduced in the presence of UT-15C (between 1 and 25 ng/mL), confirming that UT-15C did not affect digoxin plasma protein binding.

Metabolism: *In vitro studies* - Metabolite characterization was performed in Study 04640, which showed that treprostinil was rapidly metabolized by rat, dog, and human liver microsomes to five metabolites present in all species. All five metabolites were detected in the liver microsomal incubates of all three species, suggesting similar metabolic potential of treprostinil across these species, although individual metabolites were detected at different levels in each microsomal system. The major in vitro metabolic routes of treprostinil included hydroxylation (M05, M07, M08), oxidation of a hydroxyl group to form a ketomoiety (M09), and combination of hydroxylation and oxidation (M06).

The in vitro metabolism of UT-15C was further evaluated in Study 49251 and Study 49252. In Study 49251, a panel of cDNA-expressed, cytochrome P450 (CYP) enzymes (CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and CYP4A11) were incubated with UT-15C to determine the specific enzyme(s) involved in UT-15C metabolism. Results indicated that in vitro CYP2C8 was the major isozyme involved in the metabolism of UT-15C (approximately 95% disappearance of parent) while CYP2C9 played a minor role (approximately 22% disappearance of parent). All other isozymes tested (CYP1A2, CYP2A6, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP4E11) did not play a significant role (i.e. ≤ 11%) in the metabolism of UT-15C.

In Study 49252, the inhibitory potential of UT-15C towards human hepatic microsomal CYP isoforms (CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) was determined in human liver microsomes. In vitro results indicated that treprostinil (at concentrations of 0.1 to 10,000 ng/mL) had no inhibitory effect on the majority of the isozymes (except for CYP2C8, of which the activity was inhibited by approximately 48% in the presence of treprostinil at 10,000 ng/mL). Weak inhibitory effects toward most of the isozymes were found at the 100,000 ng/mL concentrations, which is approximately 4,000- to 100,000-fold higher than expected plasma concentrations achieved following oral dosing. Therefore, UT-15C would appear to have little potential to cause interactions with drugs metabolized by these enzymes.

The extent of induction of specific P450 marker isoenzymes (for CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A4) following exposure of human hepatocytes to UT-15C was assessed in Study 7049-122. Exposure of human hepatocytes to 1 or 5 μg/mL UT-15C did not result in notable induction of enzyme activities associated with CYP1A2, CYP2B6, CYP2C9, CYP2C19, or CYP3A4 isoforms in vitro.

Studies in male healthy volunteers: The metabolic profile of UT-15C was also evaluated in humans following administration of dual labeled UT-15C to eight male healthy volunteers (TDE-PH-107). Specifically, six metabolites were identified based on LC/MS/MS analysis of urine, feces and plasma samples. These metabolites included M392, M334, M348, M374, M566 and M388 which accounted for 17.3, 24.6, 23.7, 9.35, 2.54 and 0.50% of the total administered radioactivity, respectively. These metabolites were unique to those identified in the 04640 study with the exception of M388 which was identical to M09. The biological activity of these metabolites was assessed in a rat blood pressure model and found to have significantly reduced (~1000-fold lower) activity than the parent compound, UT-15C (WHRI, 2008).

Excretion: Nonclinical excretion studies were not formally conducted with UT-15C, although excretion was assessed in humans. Following administration of dual labeled UT-15C to eight male healthy volunteers (TDE-PH-107), the predominant route of excretion of [14C]treprostinil- and [3H]diethanolamine-derived radioactivity was via urine, with total combined recovery in urine and feces of 96.8% for [14C]treprostinil derived radioactivity up to 288 hours post-dose and 64.4% for [3H] diethanolamine -derived radioactivity up to 576 hours post-dose with recovery in individual subjects ranging from 36.7 to 75.4%, indicating that recovery of [3H]diethanolamine-derived radioactivity was incomplete.

Overall, these studies demonstrated that the predominant route of excretion for the treprostinil and diethanolamine components of UT-15C was via urine (78.2% and 62.1% for treprostinil and diethanolamine, respectively) with fecal recovery of treprostinil and diethanolamine accounting for 18.6% and 2.25%, respectively.

Drug Interaction Studies: In Study 7049-124, the ability of Revatio (sildenafil citrate), Tracleer (bosentan), and UT-15C (treprostinil diethanolamine) to induce enzyme activities associated with CYP2C8, CYP2C9, CYP2C19, and CYP3A4 were determined in primary cultures of human hepatocytes and compared with the effects of rifampicin (prototypical inducer). In addition, the in vitro effects of repeat dosing of UT-15C on the rate of metabolism of Revatio and Tracleer were investigated, as were the effects of Revatio and Tracleer exposure on UT-15C metabolism. In summary, the rate of Tracleer or Revatio metabolism was unaffected by exposure of hepatocytes to UT-15C. The rate of UT-15C metabolism was unaffected by exposure of hepatocytes to Revatio, Tracleer, or rifampicin control for 72 hours. Therefore, it was proposed based on the results of this experiment that there are no significant metabolic drug interactions between UT-15C and either sildenafil or bosentan.

General Toxicology

Single-Dose Toxicity

Rats: Groups of Sprague-Dawley rats (2/sex/group) were given oral administration of UT-15C at doses of 0, 5, 10 or 15 mg/kg three times (about 5 hours apart) on one day for a total dose of 0, 15, 30 and 45 mg/kg/day. Parameters evaluated for drug effects included survival, clinical signs, body weight, food consumption and necropsy findings. All animals survived to scheduled sacrifice on day 14. Clinical signs at 30 mg/ kg/day and above included clear oral discharge, nonformed feces, brown hair coat and cold to touch on the day of dosing and the first day post dose. The test groups showed transient mean body weight loss related to reduced food consumption on the day after dosing. Food intake subsequently improved and weight gain followed. Necropsy showed no evidence of direct organ toxicity.

Dog: In dogs given UT-15C sustained release tablets orally in 3 divided doses (one dog/sex/ group), about 5 hours apart, for a single day at total dose levels of 0, 30, 60, 90 and 150 mg/dog/day, all dogs survived to 14-day observation period. Treatment-related, dose dependent findings included abnormal feces and decrease in food consumption. Abnormal feces (nonformed feces, yellow or green discolored liquid and mucoid feces) was noted at dose levels of 60 mg/dog/day and above in both sexes. Additionally, vomiting was observed in the female at 90 mg/dog/day, and in both sexes at the 150 mg/dog/day dose level. Decrease in food consumption was observed in both sexes at all dose levels. Necropsy showed no significant findings.

Repeat-Dose Toxicity

Thirteen-Week Oral Toxicity Study of UT-15C in Rats With a 4-Week Recovery Period

Testing Facility: (b) (4)

Study Number: TPU00016

Study Dates: February 18, 2010 – February 04, 2011

GLP Compliance: yes

QA Report: yes

Drug Lot # and % Purity: 02D09014; purity - 100%

Species/Strain: Male and female Sprague Dawley Crl:CD(SD) rats were obtained from (b) (4)

Number, Age and Weight at Start of Study: 15/sex/group; additional 5 rats/sex in the control and high dose groups for the recovery phase; about 8 weeks of age; males 284 – 331 g and females 192- 226 g. Additional animals (12/sex/group) were included for toxicokinetic evaluations. The study design is given below.

Experimental Design for the Toxicity and Toxicokinetic Phases

Group No.	No. of Animals				Dose Material	Dose Levels		Installment Dose Volume (mL/kg/dose)	Dose Concentration (mg/mL)	Adjusted Dose Concentration[b] (mg/mL)
	Toxicity (Recovery)		Toxicokinetic			Daily Dose Level (mg/kg/day)	Installment Dose Level (mg/kg/dose)[a]			
	Males	Females	Males	Females						
1	15 (5)	15 (5)	12	12	Sterile Water for Injection	0	0	5	0	0
2	15	15	12	12	UT-15C	10	5	5	1	1.269
3	15	15	12	12	UT-15C	20	10	5	2	2.538
4	15 (5)	15 (5)	12	12	UT-15C	30/25[c]	15/12.5[c]	5	3/2.5[c]	3.807/3.173[c]

[a]Doses were administered approximately 6 hours apart each day.
[b]Dose levels and concentrations are expressed as the free acid of UT-15C and were adjusted by 1.269 (the conversion factor of the free acid to the salt).
[c]The dose level for Group 4 females was lowered to 25 mg/kg/day (12.5 mg/kg/dose) beginning on 08 March 2010 (Study Day 6), due to mortality/adverse findings in these animals at 30 mg/kg/day (15 mg/kg/dose).

Animal Housing: The animals were housed individually in suspended stainless steel cages. PMI Nutrition International Certified Rodent Chow® #5002 and tap water were provided *ad libitum*.

Methods:

Doses: UT-15C was administered by oral gavage twice daily (about 6 hours apart) at total dose levels of 0 (sterile water for injection), 10, 20 and 30/25* mg/kg/day for 13 weeks. (* = The dose level for the high dose females was lowered from 30 to 25 mg/kg/day on day 6 due to mortality. Dose levels are expressed as the free acid of UT-15C.)

Selection of doses: Doses were selected based on the recommendations of the Division of Cardiovascular and Renal Products, FDA. The Executive CAC reviewed the results of an initial 13-week oral dose-range finding toxicity study in rats, and concluded that the study was inadequate to determine the dose levels for the carcinogenicity study because all doses tested were tolerated and no MTD was determined. The Committee recommended that the sponsor repeat the 13-week study at doses high enough to establish an MTD or that result in steady state AUCs at least 25-fold higher than the steady state AUC in humans. The sponsor repeated the study at higher doses than used previously.

Observation and Measurements

Mortality: twice daily

Clinical signs: Cage-side observations were made three times daily, up to 2 hours after each dose. An additional cage-side observation was performed on each animal before the second daily dose.

Body weight and food consumption: Weekly

Ophthalmology examinations: Pretest and during the last week of dosing

Clinical pathology: Clinical pathology (hematology, coagulation analyses, clinical chemistry and urinalysis) parameters were evaluated on Days 41/42, 91/92 and 119.

Toxicokinetics: Blood samples were collected for TK evaluations predose and at 1, 2, 4, 6, 7, 8, 10, 12 and 24 hours post-dose on Days 1 and 90.

Gross Necropsy: Complete necropsies were performed on all toxicity and recovery phase animals. All organs were examined grossly. The following organs were weighed: adrenal gland, brain, epididymis, heart, kidney, liver, lung, ovary, pituitary gland, prostate, salivary gland, seminal vesicle, spleen, testis, thymus, thyroid with parathyroid and uterus.

The following tissues were collected from all toxicity and recovery phase animals and preserved in 10% neutral buffered formalin.

Tissue Collection and Preservation

Adrenal gland (paired)	Mammary gland
Animal identification	Nerve, optic[b]
Aorta	Nerve, sciatic
Bone, femur	Ovary (paired)
Bone, sternum	Pancreas
Bone marrow, sternum	Parathyroid gland[c]
Bone marrow smear[a]	Pituitary gland
Brain (cerebrum, cerebellum, brain stem, medulla)	Prostate gland
Cervix	Salivary gland (paired)
Epididymis (paired)	Seminal vesicle (paired)
Esophagus	Skeletal muscle (thigh)
Eye (paired)[b]	Skin (inguinal)
Harderian gland (paired)	Spinal cord (cervical, thoracic, lumbar)
Heart	Spleen
Intestine, cecum	Stomach (nonglandular and glandular)
Intestine, colon	Testis (paired)[d]
Intestine, duodenum	Thymus
Intestine, ileum with Peyer's patch[c]	Thyroid gland (paired)
Intestine, jejunum	Tongue
Intestine, rectum	Trachea
Kidney (paired)	Urinary bladder
Liver	Uterus
Lung	Vagina
Lymph node, mandibular	Gross lesions/masses
Lymph node, mesenteric	

[a]Bone marrow smears were collected from the femur at scheduled necropsies only (for possible examination).
[b]Preserved in Davidson's fixative and then transferred to 10% neutral buffered formalin.
[c]Examined only if present in the routine section.
[d]Preserved in modified Davidson's fixative and then transferred to 10% neutral buffered formalin.

Histopathology: All tissues and organs collected from control and high dose group animals, gross lesions from all groups and the adrenal and thyroid glands from low and mid dose groups were examined microscopically. Tissues from all animals that were found dead or killed in *extremis* were also examined microscopically.

Results

Mortality:

The mortality data is presented in the following Table.

Mortality Incidence

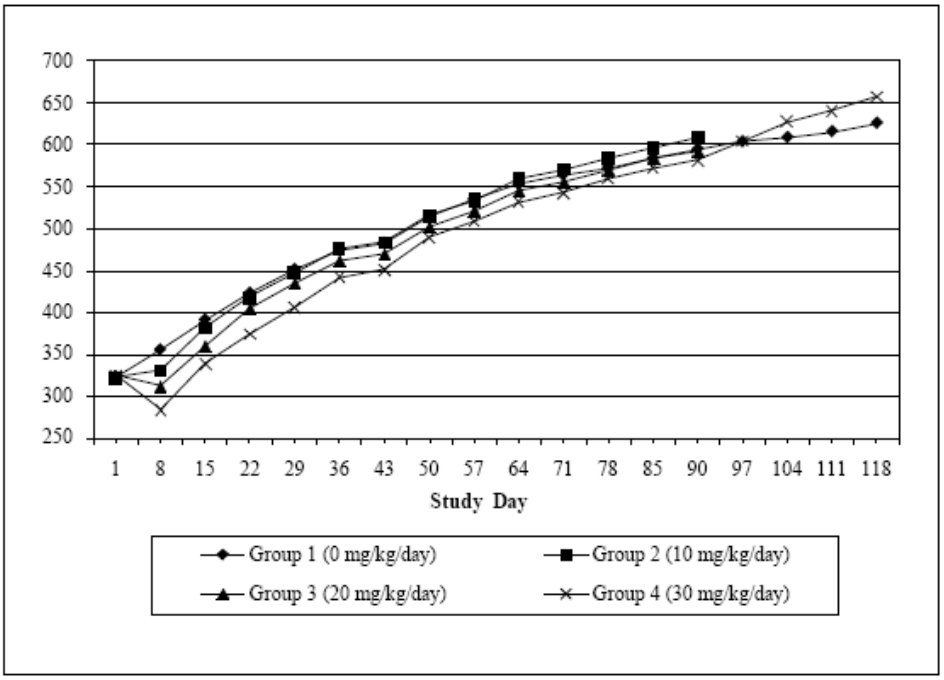
Group No.	1		2		3		4	
Dose mg/kg/day	0		10		20		30/25	
	M	F	M	F	M	F	M	F
Toxicity (Recovery)								
No. of Animals	15 (5)	15 (5)	15	15	15	15	15 (5)	15 (5)
Mortality							2 (10%)	7 (35%)
Toxicokinetic Phase								
No. of Animals	12	12	12	12	12	12	12	12
Mortality					1 (8%)	3 (25%)	1 (8%)	6 (50%)

Treatment-related mortality in the main toxicity phase of the study was noted for 2 high dose males (10%) and 7 high dose females (35%) during the dosing phase. (As noted before, because of the increased mortality, the dose for the high dose females was lowered on Day 6.) The majority of these animals were euthanized moribund; however, one male and one female were found dead. There was no mortality in the lower dose groups. In the TK phase, there were deaths in the mid dose (8% males and 25% females) and high dose (8% males and 50% females) groups. Gross necropsy findings observed in high dose animals that were found dead or euthanized moribund included lung discoloration, enlarged and/or discolored adrenals, gastrointestinal distension, glandular stomach foci, small spleen and thymus and discolored, gelatinous thymus. Histologically, focal or multifocal gastric mucosal necrosis with accompanying inflammation was seen in most of the animals. Lymphoid depletion was noted in all animals, particularly in the spleen, thymus and lymphoid tissues of GI tract. Two high dose females showed cardiac lesions.

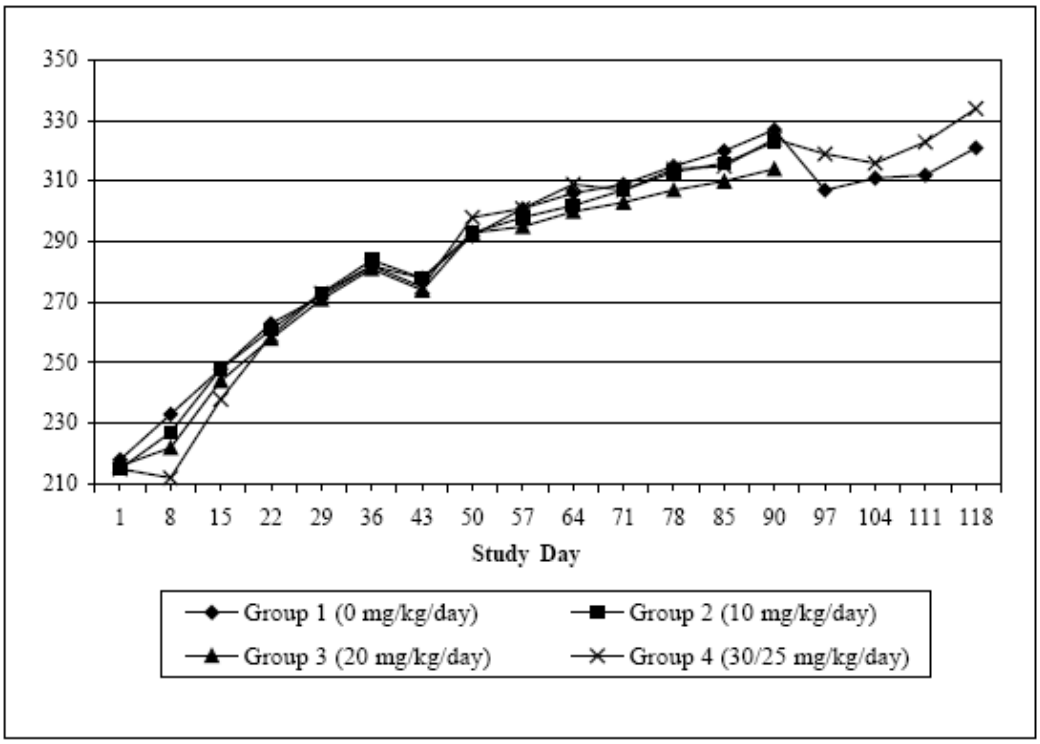
Clinical signs: Dose-related clinical signs noted during the study included decreased activity, wobbly gait, soft stools, red/swollen pinnae, reddened extremities and piloerection. These clinical signs were not observed during the recovery period.

A decrease in mean body weight was noted for all treated male groups and high dose female group during the first week of treatment; the mean body weights increased thereafter (see Figures below). However, mean body weights were generally lower than control in high dose males through Day 43 of the study. Dose-related decreases in food consumption were seen in all treated groups during the first week of treatment; subsequent increases in food consumption were noted for all groups during the remainder of the study.

Body Weights (Sponsor's Figure)



Female Group Mean Body Weights (g)



Hematology: When compared to controls, dose dependent decreases in platelets (28-59%) and increases in leukocytes (24-83%) and lymphocytes (29-91%) were noted in both sexes. These changes were reversible. The decrease in platelet count is a known pharmacological effect of the test drug. No significant clinical chemistry findings were observed in the study.

Urinalysis: Increased urine volumes, compared to controls, were noted at study termination in mid and high dose males (169 and 116%,, respectively) and females (219% increase at the mid and high doses). At the end of the recovery period, urine volume was still increased (83-106%).

Organ weights: Increases in adrenal, thyroid, heart, lung and spleen weights in treated males and females, and increases in thymus, ovary and liver weights in treated females were observed.

Gross necropsy findings: Gross pathological findings observed at the study termination included thickening of the intestinal tract and enlarged adrenal and thyroid glands with higher incidences in mid and high dose males and females. Enlargement of adrenal glands and thickening of the stomach were noted in recovery phase animals.

Microscopic findings: The incidences of significant histopathologic findings observed in the main study animals (scheduled sacrifice) are given in the following Table.

Incidences of Treatment-related Microscopic Lesions

	Main Phase								Recovery Phase			
Group No.	1		2		3		4		1		4	
Dose mg/kg/day	0		10		20		30/25		0		30/25	
	M	F	M	F	M	F	M	F	M	F	M	F
Adrenal												
Animals examined for this tissue	15	15	15	15	15	15	13	10	5	5	5	3
Congestion		1					2					
Minimal		1					2					
Mild												
Hypertrophy	1		15	14	13	15	13	10			1	
Cortical, Minimal			15	14	13	15	12	9			1	
Cortical, Mild							1	1				
Cortical, Multifocal, Minimal	1											
Within Normal limits	14	14	0	1	2	0	0	0	5	5	4	3
Thyroid												
Animals examined for this tissue	15	15	15	15	15	15	13	10	5	5	5	3
Distension			3	6	4	1	8	3				
Follicular, Minimal			3	6	4	1	8	3				
Within Normal Limits	15	15	12	9	11	14	5	7	5	5	5	3
Heart												
Animals examined for this tissue	15	15	0	0	0	0	13	10	5	5	5	3
Cardiomyopathy							1				1	
Minimal							1				1	
Degeneration, Myocardial	5	1					6	1	1		3	
Minimal	5	1					4	1	1		3	
Mild							2					
Fibrosis									1			
Minimal									1			
Hemorrhage							2					
Minimal							1					
Mild							1					
Infiltration, Mononuclear Cell	5	2					3		1			
Minimal	5	2					3		1			
Inflammation												
Acute, Mild												
Chronic/Active, Minimal												
Thrombosis							2					
Mild							2					
Within Normal Limits	6	12					3	9	2	5	1	3

Adrenal glands in all high dose males and females showed minimal to mild hypertrophy of cortical cells. Minimal hypertrophy was also noted in 93% of the animals at the mid dose and 97% at the low dose levels. At the end of the recovery period, one of five high dose males had minimal adrenal cortical hypertrophy. The hypertrophy corresponded to the increased

microvesiculation in the adrenal gland and was considered to be an adaptive change to repeated hypotension.

In the thyroid gland, a reversible increase of minimal follicular distension was observed in a number of animals (both sexes) from all treated groups, although a dose response was not seen.

Myocardial degeneration and mononuclear cell infiltration were observed in control and high dose males, mostly at similar incidence rates. Hearts from low and mid-dose groups were not examined. Cardiomyopathy (1/13), hemorrhage (2/13) and thrombosis (2/13) were seen only in high dose males. Myocardial degeneration is common background lesion in male rats. The thrombotic lesions seen in high dose males consisted of mild mural thrombosis in the region of the left atrioventricular valve.

Toxicokinetics: Toxicokinetic data are presented below.

Plasma Treprostinil

Day	Dose mg/kg BID	Group	AUC_{0-t} (ng•h/mL)	±	SE	CL/F (mL/h/kg)	C_{max1} (ng/mL)	±	SE	C_{max2} (ng/mL)	±	SE	t_{max1} (h)	t_{max2} (h)
1	5	2-Female	356	±	64.7	NC	14.1	±	1.76	34.2	±	7.26	1.00	8.00
	5	2-Male	218	±	23.4	NC	8.08	±	0.835	29.0	±	11.3	2.00	8.00
	10	3-Female	2503	±	755	NC	41.0	±	10.5	288	±	147	1.00	7.00
	10	3-Male	414	±	50.4	NC	15.7	±	3.74	46.9	±	14.1	1.00	10.00
	15	4-Female	1794	±	965	NC	33.0	±	15.0	190	±	156	2.00	24.00
	15	4-Male	494	±	84.5	NC	18.8	±	5.22	55.4	±	12.7	2.00	8.00
Day	Dose mg/kg BID	Group	AUC_{0-t} (ng•h/mL)	±	SE	CL/F (mL/h/kg)	C_{max1} (ng/mL)	±	SE	C_{max2} (ng/mL)	±	SE	t_{max1} (h)	t_{max2} (h)
90	5	2-Female	463	±	77.3	21609	25.7	±	7.57	41.5	±	12.2	2.00	8.00
	5	2-Male	491	±	37.2	20386	34.1	±	5.92	42.0	±	17.1	2.00	8.00
	10	3-Female	1347	±	346	14849	66.2	±	24.5	83.4	±	31.7	4.00	12.00
	10	3-Male	827	±	101	24178	52.2	±	4.95	64.5	±	11.0	4.00	7.00
	12.5	4-Female	1055	±	127	23689	46.8	±	28.3	61.1	±	21.8	4.00	10.00
	15	4-Male	1329	±	115	22579	72.1	±	35.3	85.7	±	20.3	2.00	10.00

Overall, AUC generally increased in a relatively dose-proportional manner on Days 1 and 90. Female exposure was generally higher than in males on Day 1, and may be related to the increased mortality seen in females.

UT-15C: 28-Day Repeated Dose Oral Toxicity and Toxicokinetic Study in Tg.rasH2 Non-Transgenic Mice With a Preliminary 5-Day Range-Finding Toxicity Study

These studies were performed at [redacted] (b) (4) to determine the dose levels for the 26-week oral carcinogenicity study in hemizygous rasH2 mice.

[redacted] (b) (4) Study Number: AC23ZN.2G3R.BTL
Study Dates: January 26, 2009 - June 19, 2009

5-day Toxicity Study Groups of wild-type rasH2 mice (5/sex/group) were given oral gavage administration of either the vehicle alone (sterile water for injection, USP) or UT-15C at doses of 10, 75, 150, 250 or 500 mg/kg/day for 5 consecutive days. The incidences of mortality observed in the study are given below.

Dose (mg/kg/day)	Mortality Incidence (%) Male	Female
Vehicle	0	0
10	0	0
75	20	20
150	0	80
250	100	100
500	100	100

Most of the deaths occurred between days 2 and 3 of treatment. From the mortality data from mice treated at 150 mg/kg/day of UT-15C, it appeared that the lethal effect of the drug was more pronounced in females than in males. Necropsies of dead animals failed to reveal any evidence of gavage error that could account for any of the early deaths observed in the study.

Clinical signs observed in the study included decreased motor activity, hunched appearance and rapid and shallow breathing at or above 75 mg/kg/day, while labored breathing was noted at 150 mg/kg/day and above.

Based on the results of the above 5-day study, the dose levels of 0, 10, 30, 50 and 75 mg/ kg/day were selected for the 28-day repeated dose oral toxicity study.

28-day toxicity study: Groups (10/sex/group) of mice were given oral gavage administration of vehicle alone (sterile water for injection, USP) or UT-15C at doses of 10, 30, 50 or 75 mg/kg/day (doses expressed as the free acid of UT-15C) for 28 consecutive days (Main study). Additional groups of animals (38/sex/group in test-article treated groups and 8/sex in the control group), dosed for 25 consecutive days, were used for the toxicokinetic (TK) evaluations.

Evaluation parameters included mortality, clinical signs, body weight, food consumption, clinical pathology, organ weights, gross and histopathology, and TK evaluations. Blood was collected on days 1 and 25 from TK animals. After completion of bleeding, these animals were discarded without necropsy. All surviving main study animals were bled on the day of scheduled sacrifice (Day 29) for clinical pathology evaluation. A complete necropsy was performed on all animals, protocol-specified tissues were weighed and processed for histopathologic evaluation. Tissues from all mice found dead or moribund or terminally sacrificed, from the vehicle control and the two highest dose groups (50 and 75 mg/kg/day) were evaluated microscopically. Target organs of toxicity (nasal cavity, kidneys, stomach and testes) from mice treated at 10 and 30 mg/kg/day were similarly evaluated.

In the main study, there were no deaths in the control or low dose groups (10 and 30 mg/kg/day). Main and TK studies combined, drug-related mortalities were seen in both sexes at 30 mg/kg/day and above, more pronounced in females.

The mortality data is summarized below.

Deaths	30 mg/kg/day		50 mg/kg/day		75 mg/kg/day	
	M	F	M	F	M	F
Main Study						
Found dead	0/10	0/10	1/10	2/10	2/10	4/10†
Moribund sacrificed	0/10	0/10	0/10	2/10	8/10†	6/10†
Total Unscheduled Deaths	**0/10**	**0/10**	**1/10**	**4/10***	**10/10†**	**10/10†**
TK Study						
Found dead	1/38	0/38	3/38	8/38	5/38	6/38
Moribund sacrificed	0/38	2/38	1/38	3/38	15/38†	14/38†
Total Unscheduled Deaths	**1/38**	**2/38**	**4/38**	**11/38***	**20/38†**	**20/38†**

x/10 or x/38 = number of animals with the observations /total number of animals used/group

*= Statistically significant ($p < 0.05$) when compared to the vehicle control group (Group 1).

†= Incidence not subjected to statistical analysis because group was terminated early.

The cumulative incidences of deaths (main and TK studies combined) are given below.

Dose (mg/kg/day)	Mortality Incidence (%) Male	Female
Vehicle	0	0
10	0	0
30	2	4
50	10	31
75	62	62

Due to the high mortality observed at 75 mg/kg/day during Days 1-3 (males) or Days 1-2 (females), all remaining animals from this group were sacrificed on Day 4 (males) or Day 3 (females). Gross necropsy findings included stomach, duodenal, ileal and jejunal discoloration and pale discoloration of kidneys and spleen in males (75 mg/kg/day) and females (50 and 75 mg/kg/day).

Treatment-related clinical signs included decreased motor activity, hunched appearance, and rapid and shallow breathing. The incidences of these clinical signs were dose-dependent.

Administration of UT-15C for 28 consecutive days caused dose-related increases in body weight gains (10-16%) in treated females, but not in males. During the treatment period, food consumption was decreased in males at 30 and 50 mg/kg/day dose levels, but not in females.

Treatment-related findings in hematology parameters included increased WBC count (45%), compared to control, in males at 50 mg/kg/day dose level, and dose-related increases in RBC, hemoglobin and hematocrit values in treated females.

Dose-related reductions in absolute (7-12%) and relative (6-8%) kidney weights in treated males from all groups and reduction in absolute testes weight (11%) in males treated at 50 mg/kg/day were noted.

There were no notable gross pathologic findings in the study except for the discoloration of the GI tract, kidneys and spleen, and small size of the spleen at 50 and 75 mg/kg/day.

Microscopic lesions were noted in the stomach, kidneys, nasal cavity, testes, spleen, thymus and liver.

Stomach: Erosion, vacuolation and inflammation were noted in the glandular portion of the stomach, while hyperplasia and hyperkeratosis were noted in the non-glandular portion. Edema of the submucosa and infiltration of inflammatory cells were noted in both portions of stomach. These lesions were observed at 50 and 75 mg/kg/day dose levels in both sexes. The incidence and severity of these lesions are given below.

Stomach:

	Male					Female				
Dose Groups	1	2	3	4	5	1	2	3	4	5
Total examined	10	10	10	10	10	10	10	10	10	10
Stomach, glandular										
Erosion										
Minimal	0	0	0	0	0	0	0	0	0	1
Mild	0	0	0	0	1	0	0	0	1	2
Moderate	0	0	0	0	0	0	0	0	0	2
Vacuolation										
Minimal	0	0	0	0	3	0	0	0	1	1
Mild	0	0	0	0	0	0	0	0	0	2
Inflammation, subacute										
Mild	0	0	0	0	0	0	0	0	2	0
Infiltration, inflammatory cells, submucosa										
Minimal	0	0	0	0	1	0	0	0	0	4
Mild	0	0	0	0	1	0	0	0	1	0
Edema, submucosa										
Minimal	0	0	0	0	4	0	0	0	0	6
Mild	0	0	0	0	0	0	0	0	1	0
Stomach, non glandular										
Hyperplasia										
Minimal	0	0	0	1	2	0	0	0	0	0
Hyperkeratosis										
Minimal	0	0	0	1	2	0	0	0	0	0
Pustule	0	0	0	0	1	0	0	0	0	0
Edema, submucosa										
Minimal	0	0	0	0	6	0	0	0	3	4
Infiltration, inflammatory cells										
Minimal	0	0	0	0	5	0	0	0	1	2
Mild	0	0	0	0	1	0	0	0	1	0

Note: Group 1 = Vehicle Control Group 2 = Low dose (10 mg/kg/day) Group 3 = Low Middle dose (30 mg/kg/day)
Group 4 = High Middle dose (50 mg/kg/day) Group 5= High dose (75 mg/kg/day)

Kidneys: Nephrosis was noted in the kidneys primarily at the cortico-medullar interface. The lesion was characterized by swelling of the tubular epithelium, coagulation of the cytoplasm, vacuolation, loss of nuclei and/or presence of hyalinized eosinophilic material within the tubular lumen. These lesions were seen at 75 mg/kg/day in males and at 50 and 75 mg/kg/day in females.

Kidneys:

	Male					Female				
Dose Groups	1	2	3	4	5	1	2	3	4	5
Total examined	10	10	10	10	10	10	10	10	10	10
Nephrosis										
Minimal	0	0	0	0	4	0	0	0	0	1
Mild	0	0	0	0	4	0	0	0	2	2
Moderate	0	0	0	0	2	0	0	0	1	5

Note: Group 1 = Vehicle Control Group 2 = Low dose (10 mg/kg/day)
Group 3 = Low Middle dose (30 mg/kg/day) Group 4 = High Middle dose (50 mg/kg/day)
Group 5= High dose (75 mg/kg/day)

Nasal cavity: Exudative inflammation of the nasal cavities, characterized by accumulation of sloughed necrotic epithelial cells, fibrin, mucin and dead neutrophils within the cavities, was noted in males at dose levels of 10 mg/kg/day and above and in females at 30 mg/kg/day and above.

Nasal Cavity:

	Male					Female				
Dose Groups	1	2	3	4	5	1	2	3	4	5
Total examined	10	10	10	10	10	10	10	10	10	10
Inflammation, acute, exudative										
Minimal	0	1	0	0	2	0	0	1	2	2
Mild	0	0	0	0	2	0	0	0	0	2
Moderate	0	0	0	1	2	0	0	0	2	2
Marked	0	0	0	0	2	0	0	0	1	2

Note: Group 1 = Vehicle Control Group 2 = Low dose (10 mg/kg/day)
Group 3 = Low Middle dose (30 mg/kg/day) Group 4 = High Middle dose (50 mg/kg/day)
Group 5= High dose (75 mg/kg/day)

Testes: Testicular degeneration, characterized by vacuolation, swelling of spermatids and presence of multinucleated cells, was noted in males at dose levels of 50 mg/kg/day and above.

Testes:

Dose Groups	1	2	3	4	5
Total examined	10	10	10	10	10
Degeneration					
Mild	0	0	0	1	4
Mineralization					
Mild	0	0	0	1	0

Note: Group 1 = Vehicle Control Group 2 = Low dose (10 mg/kg/day)
Group 3 = Low Middle dose (30 mg/kg/day) Group 4 = High Middle dose (50 mg/kg/day)
Group 5= High dose (75 mg/kg/day)

Other Lesions:

Spleen and Thymus: Depletion and necrosis of lymphoid tissue in spleen, and atrophy and necrosis of thymus were noted in moribund and early death animals. According to the pathologist, although these lesions were noted in the test article treated dose groups of both sexes, lesions are considered to be secondary to stress since they are often seen in moribund and early death animals. Hence, spleen and thymus lesions are not considered test article related.

Spleen

	Male			Female		
Dose Groups	1	4	5	1	4	5
Total examined	10	10	10	10	10	10
Hemosiderin pigmentation						
Minimal	0	0	0	0	1	0
Mild	0	0	0	0	1	0
Lymphoid depletion						
Minimal	0	0	2	0	1	1
Mild	0	1	3	0	3	8
Lymphoid necrosis						
Minimal	0	1	0	0	0	1
Mild	0	0	3	0	3	8

Note: Group 1 = Vehicle Control
Group 4 = High Middle dose (50 mg/kg/day) Group 5= High dose (75 mg/kg/day)

Thymus

	Male			Female		
Dose Groups	1	4	5	1	4	5
Total examined	10	10	10	10	10	10
Atrophy						
Mild	0	0	7	0	2	3
Moderate	0	1	1	0	1	1
Necrosis						
Mild	0	0	7	0	2	3
Moderate	0	1	1	0	1	1

Note: Group 1 = Vehicle Control
Group 4 = High Middle dose (50 mg/kg/day) Group 5= High dose (75 mg/kg/day)

Liver: Glycogen depletion and centrilobular infiltration of lipid were noted in the livers of moribund and early death mice of both sexes. According to the pathologist, these lesions were simply indicative of lack of food consumption in moribund or early death animals, and were not directly induced by the test article.

Liver:

	Male			Female		
Dose Groups	1	4	5	1	4	5
Total examined	10	10	10	10	10	10
Lipid infiltration, minimal	0	0	6	0	0	0
Tension lipidosis	0	0	1	0	0	0
Glycogen depletion						
Minimal	0					
Mild	0	0	6	0	2	7
Multifocal necrosis, minimal	0	1	2	0	0	0

Note: Group 1 = Vehicle Control
Group 4 = High Middle dose (50 mg/kg/day) Group 5= High dose (75 mg/kg/day)

Summary of TK parameters

Day	Dose (mg/kg/day)	Group	AUC_{0-24} (ng•h/mL)	AUC_{last} (ng•h/mL)	±	SE	CL/F (mL/h/kg)	C_{max} (ng/mL)	±	SE	$t_{1/2}$ (h)	t_{max} (h)
1	10	2-Female	78.8	45.0	±	9.71	NC	5.62	±	2.54	NC	12
	10	2-Male	72.5	72.5	±	14.5	NC	5.05	±	1.64	NC	12
	30	3-Female	146	146	±	42.3	NC	8.47	±	3.08	NC	24
	30	3-Male	91.8	91.8	±	12.5	NC	5.98	±	1.35	NC	2
	50	4-Female	609	609	±	317	NC	64.7	±	26.1	NC	2
	50	4-Male	155	155	±	33.8	NC	11.8	±	3.55	NC	2
	75	5-Female	1103	1103	±	215	NC	128	±	40.3	NC	4
	75	5-Male	294	294	±	41.4	NC	17.5	±	4.55	NC	12
Day	Dose (mg/kg/day)	Group	AUC_{ss} (ng•h/mL)	AUC_{last} (ng•h/mL)	±	SE	CL/F (mL/h/kg)	Cmax (ng/mL)	±	SE	$t_{1/2}$ (h)	t_{max} (h)
25	10	2-Female	92.4	52.6	±	10.3	108201	6.64	±	3.20	NC	12
	10	2-Male	42.5	34.6	±	5.87	235035	4.57	±	1.81	3.08	2
	30	3-Female	339	339	±	33.2	88532	29.2	±	5.92	3.25	7
	30	3-Male	170	170	±	41.1	176512	19.8	±	9.96	4.09	7
	50	4-Female	535	535	±	192	93449	39.0	±	22.0	NC	12
	50	4-Male	353	353	±	59.6	141516	31.2	±	10.1	5.61	4

NC = Not Calculated

Generally, exposure increased in a more than dose proportional manner, although this was not consistent across genders. Overall, exposure was higher in females compared to males.

Comparison of Systemic Exposures in Mice and Humans

	Dose mg/m²/day	Multiple of Human	*C_{max} (ng/mL)		Multiple of Human		?AUC (h*ng/mL)		Multiple of Human	
Patients(n=8) 6 mg BID	7.400	****	5.225		****		35.596		****	
Mice			***Male***	***Female***	***Male***	***Female***	***Male***	***Female***	***Male***	***Female***
10.0 mg/kg/day	30.000	4.054	4.570	6.640	0.875	1.271	34.600	52.600	0.972	1.478
30.0 mg/kg/day	90.000	12.162	19.800	29.200	3.789	5.589	170.000	339.000	4.776	9.524

Assumes 60 kg human.
* Represents mean C_{max} for males and females at Day 25 in mice and for chronic dosing in humans
? Represents mean AUC_{last} for males and females at Day 25 for mice and AUC_{0-12} for chronic dosing in humans

Based on the mortality data and pathology findings, the maximum tolerated dose (MTD) of UT-15C after daily oral administration for 28 days in Tg.rasH2 non-transgenic male and female mice was considered to be 10 mg/kg/day (the exposure achieved at this dose level was about 1 – 2 fold higher than the human exposure at 6 mg BID). Drug-related deaths were obtained in male and female mice treated at dose levels of 30 mg/kg/day and above (exposure 5-10 fold higher than the human exposure).

Nine-Month Oral Toxicity Study of UT-15C (Sustained Release Tablet Formulation) in Dogs With 1-Month Recovery Period

Testing Facility: (b) (4)

Study Number: TPU00009

Study Dates: January 31, 2006 – December 1, 2006

GLP Compliance: The study was conducted in compliance with the GLP regulations with the following exception: the characterization of the test article, stability analyses and end-of study analysis of the dose formulations were not conducted in compliance with GLP regulations, but the analyses were performed according to current Good Manufacturing Practice procedures.

QA Report: yes

Drug Lot #s and % Purity: PD0290-116, PD0290-098 & PD0290-117. Control placebo tablets - lot #s PD0290-083 and PD0290-097 – purity not provided

Species/Strain: Dog/ Beagle, obtained from (b) (4)

Number, Age and Weight at Start of Study: 4/sex/group; additional 2 dogs/sex in the control and high dose groups for the recovery phase; about 6 months of age; males 5.9 – 7.7 kg and females 5.9 to 7.5 kg.

Animal Housing:

The animals were housed individually in suspended stainless steel cages. Dogs were given PMI Nutrition International Certified Canine Diet #5007® as a daily ration. About 400 g feed was left in the cage for at least 4 hours daily and then removed. Supplemental diet (Hill's Science Diet® or Nutri-Cal®) was offered with the daily ration or in place of daily ration or for an extended time period to enhance the nutritional intake of specific animals with low food consumption of less than 100 g/day.

Methods

Doses: 0, 6, 10 and 30 mg/dog/day (doses expressed as free acid of UT-15C). Beginning on day 127, the dose levels for the low- and mid- dose males were increased to 8 and 15 mg/dog/day, respectively; for the high dose males, the dose was increased to 35 mg/ dog/day starting from Day 115. (It was stated that dose levels for males were adjusted during the study to account for increases in body weight, with a target of maintaining approximately 1, 1.5 and 4.5 mg/kg/day for low, mid and high doses, respectively, for males and females.)

The control and the sustained release test article tablets were administered twice daily (about 6 hours apart). The dose was fixed with the same daily dose being administered to all animals within a group.

The dose levels were selected based on the results of a 13-week oral toxicity study (0, 10, 30 and 50 mg UT-15C/dog/day) in dogs. In that study, one high dose female was euthanized moribund on Day 16. This animal displayed diarrhea, vomitus, decreased activity, dehydration, cool to touch and prolapsed rectum prior to euthanasia. Intussusception of the large intestine was noted at necropsy and was confirmed by the microscopic examination. The other gastrointestinal lesions observed in this dog included chronic inflammation within the colon accompanied by mild ulceration, congestion and lymphatic dilatation at the site of the intussusception. No other significant microscopic findings were noted. Since lethality was noted at the high dose (50 mg/dog/day) in the 13 week study, a dose level of 30/35 mg/dog/day was selected as the high dose for the 9-month dog study.

Statistical methods: The data on body weight, food consumption, hematology, clinical chemistry, urinalysis and organ weights were initially analyzed for homogeneity of variance using Levene's test followed by the Shapiro-Wilk test for normality. If parametric the assumption was fulfilled, a single-factor ANOVA was applied. If the parametric ANOVA was significant, Dunnett's test was used to identify significant differences between the control and treated groups. If the parametric assumption was not satisfied, then the Kruskal-Wallis non-parametric ANOVA procedure was used to determine the intergroup differences. If this test was significant, then the data was analyzed by the Dunn's test.

Observations and Measurements

Mortality: twice daily, in the morning and afternoon.

Clinical signs: Detailed clinical observations were performed pretest and weekly prior to dosing during the treatment and recovery periods. Cage-side observations were performed twice daily before dosing and between 2-4 hours post dose. A final detailed clinical observation was performed for each animal on the day of scheduled euthanasia.

Body weight: pretest, weekly during the treatment and recovery periods, and on the day of scheduled euthanasia.

Food consumption: daily throughout the treatment and recovery periods

Ophthalmology examination: pretest, during weeks 13 and 26, and at main study termination

EKG measurements: pretest, during weeks 13, 26 and the last week of dosing

Clinical pathology: Hematology, clinical chemistry and urinalysis parameters were determined pretest, and on days 87, 177, 274/275 and 305. The following parameters were evaluated:

Hematology – RBC, WBC (total and differential), reticulocyte and platelet counts; hemoglobin, hematocrit, MCH, MCHC, MCV and red blood cell morphology; prothrombin time and activated partial thromboplastin time

Clinical chemistry – alkaline and aspartate aminotransferases, alkaline phosphatase, gamma glutamyltransferase, total bilirubin, urea nitrogen, creatinine, calcium, phosphorus, total protein, albumin, globulin, A/G ratio, glucose, cholesterol, triglycerides, sodium, potassium and chloride

Urinalysis – color, specific gravity, microscopic evaluation of urine sediment, total volume, pH, protein, glucose, bilirubin, ketones, nitrites, leukocytes, blood and uribilinogen

Toxicokinetics: Blood samples were collected for TK evaluations predose and at 1, 2, 4, 6, 7, 8, 10, 12 and 24 hr post dose on Days 1, 180 and 270.

Gross necropsy: Complete necropsies were performed on all animals. All organs were examined macroscopically.

Organ weights: Adrenal gland, brain, epididymis, heart, kidney, liver, lung, ovary, pituitary gland, prostate gland, salivary gland (mandibular), spleen, testis, thymus, thyroid with parathyroid and uterus were weighed.

The following tissues/organs were collected from all animals (main study and recovery phase animals) and preserved in 10% neutral buffered formalin: adrenals, aorta, bone (femur and sternum), bone marrow (sternum), brain, cervix, epididymis, esophagus, eye, gallbladder, heart, intestine (duodenum, ileum, jejunum, cecum, colon and rectum), kidney, lacrimal gland, liver, lung, lymph node (mandibular and mesenteric), mammary gland, nerve (optic and sciatic), ovary, pancreas, parathyroid, pituitary, prostate, salivary gland (mandibular), skeletal muscle (thigh), skin, spinal cord, spleen, stomach, testis, thymus, thyroid, tongue, trachea, urinary bladder, uterus, vagina and gross lesions.

(Notes - 1) Bone marrow smears were prepared from the 7th rib for possible examination, but were not examined. 2) Eyes were first preserved in Davidson's fixative and then transferred to 10% neutral buffered formalin.)

Histopathology: All tissues/gross lesions collected at necropsy from all animals were processed and examined microscopically.

Results

Mortality: All animals survived until scheduled sacrifice.

Clinical signs: Dose-related increased incidences of diarrhea with mucoid stools were seen in both male and female dogs. Mucoid stools were more noticeable after the second daily dose. Empty or partially filled tablet capsulations were seen in the cage/tray of animals from all groups.

Body weights: The mean body weights for high dose males and females decreased from the pre-test levels during the first 5-6 weeks of treatment (9 to 10% reduction) and then the animals started to show gain in weight. The low- and mid-dose groups gained body weight during this period, but at a slightly lower rate than the control group. At the end of the dosing period, the high dose males had a mean body weight about 5% less than that of the control, while the mean body weights for low and mid dose males were about 9% lower than control. At the end of the treatment period, all treated female groups had about 10-12% lower body weight than control. The mean body weights for the high dose males and females were lower than control at the end of the recovery period.

Food consumption: Food consumption in high dose males and females was significantly lower than that in control during the first 10-20 days of the study; thereafter the food consumption improved. However, the food consumption in the high dose group remained slightly lower than control throughout the remainder of the treatment period. Supplemental diet was offered to those specific animals with food consumption < 100 g/ day (mostly high dose animals) during days 9-50. Food consumption in the low and mid dose groups was generally comparable to that in the control group. During the recovery period, the food consumption was comparable to control in high dose females but lower than control in high dose males.

Ophthalmology: There were no treatment-related effects.

EKG: There were no significant changes noted in PR interval, QRS duration, QT interval or QTc throughout the study. On Day 269, the heart rate was lower in low dose group females compared to control females (24% change). Correspondingly, the RR interval was longer in low dose group females than in control females (30% change). Since there was no dose response relationship, these findings are considered not of any biologic significance.

Hematology: Statistically significant reductions in hemoglobin, hematocrit and reticulocyte and erythrocyte counts, and increases in leukocyte and segmented neutrophils, compared to control, were noted in mid and high dose male groups on Day 87 but not on Days 177 or 274. Increased activated PTT value was noted in high dose males on Day 274. However, all the values for the above parameters were within the historical control ranges for the laboratory.

Clinical chemistry: Significant reductions in glucose levels, compared to control, were noted in mid and high dose males and in high dose females on day177, but not at other time intervals. No dose-response relationship was noted for this finding.

Urinalysis: Total volume was increased in high dose males and females on days 87 or 177.

Gross necropsy findings: No significant treatment-related gross lesions were noted.

Organ weights: Significant increase in absolute adrenal weight (48% higher than control value), but not in the relative weight, was noted in high dose males.

Microscopic findings: No test article-related microscopic findings were noted. Siderotic plaques were observed in the spleen of mid and high dose males and females, and also in one control female. Siderotic plaques are a common spontaneous lesion seen in dogs consisting of small areas of hemorrhage and fibrosis in or on the surface of spleen and are not typically considered a toxicity-associated lesion. No dose-response relationship was noted for this lesion.

Toxicokinetics: Treprostinil toxicokinetic parameters are given in the table below. On Day 1, mean treprostinil AUC values for both sexes increased more than proportionately versus total daily dose. However, by Day 270 the increase in mean treprostinil AUC values following the high dose increased less than proportionately versus total daily dose. At similar daily dose levels, female dogs had higher systemic exposure than males.

Summary of Treprostinil Pharmacokinetic Parameters in Beagle Dogs – Mean Value (CV)

		Day 1		Day 180		Day 270	
Parameter	UT-15C Dose[a] M/F (mg/dog/day)	Female	Male	Female	Male	Female	Male
C_{max1} (ng/mL)	6/6[b]	1.13 (19.2%)	1.09 (20.2%)	0.83 (8.9%)	1.28 (48.8%)	0.65 (68.8%)	1.17 (36.3%)
	10/10[b]	3.83 (45.3%)	2.2 (21.9%)	3.42 (62.4%)	3.33 (14.4%)	2.95 (52.9%)	3.58 (31.3%)
	30/30[c]	16.87 (83.4%)	9.75 (52.7%)	9.25 (47.4%)	5.9 (30.6%)	7.64 (55%)	5.25 (29.8%)
T max1 (hr)	6/6[b]	2.25 (55.9%)	1.75 (85.7%)	2.75 (54.5%)	1.5 (38.5%)	4.67 (49.5%)	3.25 (68.2%)
	10/10[b]	1.5 (38.5%)	2.75 (54.5%)	1.75 (28.6%)	1.25 (40%)	2.25 (55.9%)	1.75 (28.6%)
	30/30[c]	3.5 (61.9%)	4.17 (53.5%)	2.33 (79.8%)	2.17 (89.6%)	2.33 (58.6%)	2 (100%)
C_{max2} (ng/mL)	6/6[b]	1.43 (24.9%)	1.01 (22.8%)	0.6 (130.1%)	1.04 (25%)	1.71 (64.3%)	1.49 (57.3%)
	10/10[b]	2.8 (30.4%)	2.48 (56.3%)	2.14 (44.2%)	0.85 (137.5%)	3.66 (74.8%)	1.45 (24.4%)
	30/30[c]	15 (64.4%)	5.81 (69%)	7.2 (60.6%)	4.09 (92.6%)	5.35 (50.2%)	3.95 (51.3%)
T max2 (hr)	6/6[b]	8.5 (28%)	8.75 (25.3%)	11 (12.9%)	10.25 (23.1%)	9.25 (24%)	10.75 (23.3%)
	10/10[b]	9 (27.2%)	10.5 (9.5%)	9.75 (21.1%)	9 (15.7%)	13.25 (56.3%)	9.75 (21.1%)
	30/30[c]	8.67 (17.4%)	8.5 (24.4%)	9.5 (18.5%)	10.5 (18.8%)	9.5 (18.5%)	12 (52.4%)
AUC_{24} (ng/mL·hr)	6/6[b]	13.71 (27.1%)	9.63 (44.1%)	7.14 (71.5%)	10.78 (18.5%)	15.69 (81.5%)	14.46 (39.1%)
	10/10[b]	35.39 (17.4%)	26.86 (25%)	20.36 (41.3%)	13.83 (67.4%)	45.48 (62.7%)	20.99 (39.8%)
	30/30[c]	148.57 (52.2%)	91.07 (38.9%)	80.91 (56.8%)	49.26 (60.9%)	58.39 (24.8%)	44.57 (26.8%)
T 1/2 (hr)	6/6[b]	- -	- -	- -	2.97 -	2.05 -	- -
	10/10[b]	- -	- -	- -	3.05 -	- -	- -
	30/30[c]	4.82 (4.2%)	6.24 (11.6%)	4.25 (18.7%)	3.53 -	- -	- -
CL/F (mL/min)	6/6[b]	7762 (29.8%)	11670 (33.9%)	21634 (69.7%)	12657 (16.4%)	9298 (55%)	10827 (52.7%)
	10/10[b]	4836 (20%)	6566 (29.5%)	9383 (42.3%)	24250 (54.5%)	4791 (53.2%)	13380 (37.4%)
	30/30[c]	4220 (58%)	6216 (48.9%)	7788 (47.6%)	14501 (38.7%)	8984 (22.9%)	14189 (35.9%)

[a]Doses are expressed as the free acid treprostinil.
[b]Beginning on Day 127, the dose levels for the low- and mid-dose males were raised to 8 and 15 mg/dog/day, respectively, to attempt to ensure dose consistency (based on mg/kg body weight per day).
[c]Beginning on Day 115, the dose level for the high-dose males was raised to 35 mg/dog/day.

GI Toxicity in Dogs

As noted earlier, in a 13-week oral toxicity study of UT-15C SR tablets (0, 10, 30 or 50 mg/dog/day), a high dose female was euthanized moribund on Day 16 due to GI toxicity. Reversible, dose dependent clinical signs related to GI toxicity observed included vomiting, diarrhea and fecal abnormalities. Test-related microscopic lesions were limited to the digestive tract and the confirmation of the intussusception of the animal that was euthanized moribund. The GI lesions consisted of moderate, chronic inflammation within the colon accompanied by mild ulceration, congestion and lymphatic dilatation at the site of intussusception. No other toxicologically significant microscopic lesions were noted. The NOAEL was considered to be 10 mg/dog/day or 1 mg/kg/day.

In a 14-day study in dogs, UT-15 SR tablets or SR capsules were administered 3 times a day at total doses of 0, 30, 60 and 120 mg UT-15C/dog/day. Mortality due to GI toxicity was observed at 120 mg/dog/day for both tablets and capsules. GI toxicity was characterized by body weight loss, low food consumption, lethargy, bloody, mucoid, liquid feces, vomiting and dehydration with GI intussusceptions or rectal prolapse seen at necropsy, and hemorrhage, inflammation and congestion noted microscopically in the GI tract. These treatment-related findings were similar to that seen with Remodulin given sc or iv, and have been previously reported in the Beagle dog as a possible class effect of prostacyclins or prostacyclin analogues in this species. The NOAEL was 30 mg/dog/day or 3 mg/kg/day.

Genetic Toxicology

In vitro genotoxicity studies with UT-15C were not conducted since these studies were previously done using high doses of the free acid, treprostinil. In *in vitro* studies, treprostinil was non-mutagenic in a GLP Ames test at concentrations up to 5000 µg/plate with and without S9 metabolic activation, and in the Mouse Lymphoma assay at concentration up to 400 µg/ml without S9 metabolic activation and up to 300 µg/ml in the presence of S9.

UT-15C was tested *in vivo* in the rat micronucleus assay, which evaluated the potential of the test drug to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow of rats.

Mammalian Erythrocyte Micronucleus Test

Study no: AB18RN.125.BTL
Study report location: United Therapeutics, Corp., NC
Conducting laboratory and location: (b) (4)
Date of study initiation: October 19, 2005
GLP compliance: yes
QA statement: yes
Drug, lot#, and purity: UT-15C, D-129-053, 99.3%

Key Study Findings

Oral administration of two equal doses (about 6 hours apart) of UT-15 in Sprague Dawley rats, at total doses of 0, 12.5, 25 and 50 mg/kg, and euthanized 24 and 48 hours after dosing, did not induce a significant increase in the incidence of micronucleated PCEs in either male or female rats.

Methods

Doses in definitive study: total doses of 0, 12.5, 25 and 50 mg/kg (free acid treprostinil)
Frequency of dosing: two equal doses (approximately 6 hours apart) of 6.25, 12.5 and 25 mg/kg/dose. (Animals from all groups were euthanized 24 hours post dose. Additional animals (5/sex/group) assigned to control and high dose groups were sacrificed 48 hours post dose.)
Route of administration: oral gavage
Dose volume: 10 ml/kg/dose
Formulation/Vehicle: dissolved in sterile water for injection (vehicle)
Species/Strain: Rat/Sprague Dawley
Number/Sex/Group: 5 rats/sex/group. Additional animals included in the control and high dose groups were used for the 48 hours post dose sacrifice. (Moreover, additional 5 male and 5 female rats were treated with the test drug at 50 mg/kg, to be used as replacement rats for bone marrow collection in the event of mortality.

Basis of dose selection: In a dose range-finding study in rats, oral gavage administration of 2 equal doses of UT-15, at total doses of 0, 45, 75, 125 and 250 mg/kg, produced mortality at doses of 75 mg/kg and above. Based on the above finding, doses of 0, 12.5, 25 and 50 mg/kg were selected for the definitive study.
Negative control: Sterile water for injection
Positive control: Cyclophosphamide monohydrate/40 mg/kg/ single oral administration

Bone marrow evaluation

At the termination of the study, rats were sacrificed and bone marrow slides were prepared. The slides were stained with acridine orange.

Bone marrow cells [polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) collected 24 and 48 hours after treatment were examined microscopically for the presence of micronuclei.

The incidence of micronucleated PCEs per 2000 PCEs for each rat, and per 10,000 PCEs for each treatment group were determined. Statistical significance was determined using the Kastenbaum-Bowman tables which are based on the binomial distribution.

As an indicator of bone marrow toxicity, the proportion of PCEs to total erythrocytes was determined for each animal and treatment group.

Study Validity

The criteria for a valid test are as follows:

The mean incidence of micronucleated PCEs must not exceed 5/2000 PCEs in the vehicle control. The incidence of micronucleated PCEs in the positive control must be significantly increased relative to the vehicle control group.

A test article is judged negative if no statistically significant increase in micronucleated PCEs above the concurrent vehicle control values and no evidence of dose response are observed at any sampling time.

Results

Mortality was observed in 2 of the 15 females at 50 mg/kg. Lethargy and piloerection was observed in all males and females at 25 and 50 mg/kg. Crusty eyes and diarrhea were seen at the high dose in both sexes.

The incidence of micronucleated PCEs per 10,000 PCEs scored (2000 PCEs/animal) and the proportion of PCEs per total erythrocytes are summarized for each treatment group by sacrifice time in the following Table.

Table 8.0-4: Summary of Bone Marrow Micronucleus Analysis Following Oral Administration of Treprostinil diethanolamine (UT-15C) in Sprague Dawley Rats

Treatment**	Sex	Time (hr)	Number of Rats	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes Number per 1000 PCEs (Mean +/- SD)	Number per PCEs Scored[1]
Water	M	24	5	0.557 ± 0.04	---	0.2 ± 0.27	2 / 10000
2 x 10 mL/kg	F	24	5	0.607 ± 0.05	---	0.5 ± 0.35	5 / 10000
Treprostinil diethanolamine (UT-15C)							
12.5 mg/kg	M	24	5	0.630 ± 0.04	13	0.3 ± 0.27	3 / 10000
(2 x 6.25 mg/kg)	F	24	5	0.548 ± 0.03	-10	0.3 ± 0.27	3 / 10000
25 mg/kg	M	24	5	0.582 ± 0.02	4	0.1 ± 0.22	1 / 10000
(2 x 12.5 mg/kg)	F	24	5	0.593 ± 0.03	-2	0.1 ± 0.22	1 / 10000
50 mg/kg	M	24	5	0.551 ± 0.07	-1	0.3 ± 0.27	3 / 10000
(2 x 25 mg/kg)	F	24	5	0.535 ± 0.03	-12	0.0 ± 0.00	0 / 10000
Cyclophosphamide							
40 mg/kg	M	24	5	0.480 ± 0.08	-14	17.3 ± 4.21	*173 / 10000
	F	24	5	0.400 ± 0.07	-34	15.3 ± 2.75	*153 / 10000
Water	M	48	5	0.530 ± 0.04	---	0.1 ± 0.22	1 / 10000
2 x 10 mL/kg	F	48	5	0.568 ± 0.04	---	0.2 ± 0.27	2 / 10000
Treprostinil diethanolamine (UT-15C)							
50 mg/kg	M	48	5	0.634 ± 0.03	20	0.4 ± 0.22	4 / 10000
(2 x 25 mg/kg)	F	48	5	0.626 ± 0.03	10	0.1 ± 0.22	1 / 10000

[1]*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables)
**Test article doses expressed as "mg treprostinil free acid/kg body weight"

The data show that the number of micronucleated PCEs per 10,000 PCEs in the drug-treated groups was not statistically increased relative to their respective vehicle controls in either male or female rats, regardless of dose levels or bone marrow collection times ($p > 0.05$). Reductions in the ratio of PCEs to total erythrocytes up to 12% were observed in some of the test-drug treated groups compared to control groups, suggesting that the test article did not inhibit erythropoiesis.

The positive control induced a significant increase in micronucleated PCEs in both male and female rats ($p \leq 0.05$)

Mortality observed at the high dose may indicate systemic exposure of animals to the test drug.

In this study, all the criteria for a valid study were met as described in the protocol. Based on the results of the study, UT-15C was considered to be negative in the micronucleus test.

Carcinogenicity

26-Week Transgenic Mouse Study

Key Findings: Oral administration of UT-15C to Tg.rasH2 mice at 5, 10 and 20 mg/kg/ day in males and 3, 7.5 and 15 mg/kg/day in females daily for 26 weeks did not significantly increase the incidence of tumors.

Testing Facility: [redacted] (b) (4)

Study Number: Contract Lab's Number – [redacted] (b) (4)

Study Dates: Experimental Initiation Date – September 28, 2010 (males)
September 30, 2010 (females)
Experimental Completion Date – October 03, 2011

GLP Compliance: The study was conducted in accordance with US FDA GLP regulations and also in compliance with OECD Principles of GLP and the Japanese Ministry of Health, Labor and Welfare GLP regulations with minor exceptions that had no effect on the outcome of the study.

QA Report: Yes

Drug Lot # and Purity: Lot # - 02B10007; Purity – 100.3%

Species/Strain: Main Study – Tg.rasH2 mice
[CByB6F1-Tg(HRAS)2Jic (+/- hemizygous c-Ha-ras)]
TK Study – CByB6F1 Tg(HRAS)2Jic (-/- homozygous c-Ha-ras;
Tg.rasH2 wildtype non-transgenic littermates).
Both groups of mice, about 7 to 8 weeks of age, were obtained from [redacted] (b) (4)

Number, Age and Weight at Start of Study:
Main Study - 25/sex/group; 9 weeks of age; body weights – males 21.5 to 27.7 g and females 16.7 to 21.3 g. (positive control group – 15/sex)
TK Study – control group - 5/sex; treatment groups – 23/sex/group; 9-10 weeks of age; body weights – males 21.2 to 30.4 g and females 17.1 to 23.1 g (The Experimental Design for the study is given below.)

Experimental Design

Group	Dose levels (mg/kg/day)	Dose Concentration (mg/mL)	Number of Animals			
			Main Study (Tg.rasH2)		TK Study (wildtype littermates)**	
			Male	Female	Male	Female
Group 1 (Vehicle)	0	0	25	25	5	5
Group 2 (Positive Control)	1000 (Urethane)*	100	15	15	-	-
Group 3	3.0	0.3	0	25	0	23
Group 4	5.0	0.5	25	0	23	0
Group 5	7.5	0.75	0	25	0	23
Group 6	10.0	1.0	25	0	23	0
Group 7	15.0	1.5	0	25	0	23
Group 8	20.0	2.0	25	0	23	0
Total			115	115	74	74

- Not Applicable
*The Positive Control animals were administered a total of 3 intraperitoneal (i.p.) injections on Study Days (SD) 1, 3, and 5.
**Extra TK animals (2/sex/group) were used to try to ensure adequate animals for TK bleeding

Animal Housing: Animals were housed individually in polycarbonate cages with Sani-Chip hardwood bedding, after a 2-week acclimation period. Animals had *ad libitum* access to Harlan TEKLAD Global Diet # 2018CM (Certified 18% Protein Rodent Diet, Harlan Teklad, Madison, WI) and drinking water (WSSC Potomac plant, Potomac, MD).

Methods

Doses: Males - 0 (vehicle control - sterile water for injection), 5, 10 and 20 mg/kg/day
Females – 0, 3, 7.5 and 15 mg/kg/day

Route of administration: Oral gavage

[Positive control animals were treated with urethane (1000 mg/kg) on Study Days (SDs) 1, 3 and 5 by intraperitoneal injection.)]

Basis of dose selection: Dose levels for the 26-week mouse bioassay were selected based on the results of a preliminary 5-day range-finding study and a 28-day oral toxicity and TK study in wildtype rasH2 mice. In the 28-day study [0 (water), 10, 30, 50 and 75 mg/kg/day], treatment-related mortalities were seen in both sexes at 30 mg/kg/day and above, the incidence being

higher in females than in males. At 50 mg/kg/day and above, treatment–related histopathologic lesions were observed in the stomach (erosion and inflammation of the glandular part, and hyperplasia of the non-glandular portion), kidney (nephrosis) and testes (testicular degeneration). Toxicokinetic evaluation showed the exposure was higher in females than in males. Based on the above results, the Executive CAC recommended doses of 0 (water), 5, 10 and 20 mg/kg/day in males, and 3, 7.5 and 15 mg/kg/day in females, by oral gavage, based on MTD (mortality).

Frequency of drug administration: once daily for 26 weeks at a dose volume of 10 mL/kg

Drug formulations and stability: The drug formulations were made a total of 13 times during the course of the study, and used within their established stability period. All dose formulations analyzed met the acceptance criteria of 90-110% of target concentration and ≤ 5% relative standard deviation.

Deviations from original study protocol: Minor deviations from original protocol occurred during the study but were not considered to have compromised the validity or integrity of the study.

Observations and Measurements

Mortality and clinical signs: All animals were observed twice daily, at least 6 hours apart, for moribundity and mortality. Main study animals were observed for clinical signs of toxicity within 2 hours after dose administration. A detailed hand-on examination was performed weekly.

Body weight and food consumption: Body weights for Main study and TK animals were recorded weekly during the first 13 weeks and biweekly thereafter. Body weights of TK animals were recorded for dose volume calculation only. Food consumption of Main study animals was recorded weekly throughout the study. Food consumption was not recorded for TK animals.

Toxicokinetic evaluation: Blood samples were collected from drug-treated TK animals (up to 3 animals/sex/dose/timepoint) on Days 176-177 before dosing and at 2, 4, 7, 12, 18 and 24 hours post-dose. Vehicle control TK animals (3/sex) were bled once at 2-hr post-dose on Day 176. After blood collection, TK animals were discarded without necropsy.

Gross pathology: All surviving Main study animals, with the exception of the positive controls, were sacrificed after the last treatment on Days 183 or 184 and subjected to a complete necropsy. The adrenals, brain, heart, kidneys, liver, spleen, and testes/ovaries were weighed. The following tissues/organs from main study animals were collected and fixed in 10% neutral buffered formalin for histopathologic evaluation.

Tissues Preserved for Microscopic Evaluation

Adrenal glands	Pancreas
Aorta	Parathyroid glands
Bone (femur and sternum)	Pituitary gland
Bone marrow (femur and sternum)	Prostate gland
Brain	Salivary gland
Epididymides	Sciatic nerve
Esophagus	Seminal vesicles
Eyes	Skeletal muscle (thigh)
Gall bladder	Skin, mammary area
Gross lesions including masses	Small intestine (duodenum, jejunum, and ileum)
Harderian gland	Spinal cord (cervical, thoracic, and lumbar)
Heart	Spleen
Kidneys	Stomach
Large intestine (cecum, colon, rectum)	Testes
Liver	Thymus
Lungs and bronchi	Thyroid glands
Lymph nodes (mesenteric, mediastinal, and mandibular)	Trachea
Mammary gland (Females only)	Urinary bladder
Nasal cavity	Uterus
Ovaries	Vagina

All surviving positive control animals were sacrificed on Days 106 (females) or 108 (males). A full necropsy was performed on these animals, but no organ weights were recorded. All main study animals that were found dead or sacrificed in moribund condition were also necropsied and all tissues, including gross lesions, preserved for histology evaluation. Organs were not weighed from these animals. TK animals that were found dead on the study did not receive a full necropsy, but were examined for evidence of gavage error.

Histopathology: All tissues collected from the control and drug-treated groups, and also selected tissues from the positive control animals (lungs and spleen) were evaluated histologically.

Results

Mortality: There was no treatment-related increased incidence of mortality in either sex of the Main and TK studies following treatment with the test drug when compared to control. In the Main study, the following animals were found dead or sacrificed in moribund condition: 1/25 mid-dose male (Day 160); 1/25 low dose female (Day 121); 2/25 mid dose females (Days 8 and 120) and 1/25 high dose female (Day 177). The incidences of mortality [day of death, mode of death and cause of death (if known)] are given in the following two Tables.

Incidence of Mortality - Males

Day of Death	Mode of Death	Group 1	Group 2	Group 4	Group 6	Group 8
Day 47	Found Dead	-	1	-	-	-
Day 58	Moribund Sacrifice	-	1	-	-	-
Day 67	Moribund Sacrifice	-	1	-	-	-
Day 77	Moribund Sacrifice	-	1	-	-	-
Day 84	Found Dead	-	1	-	-	-
Day 91	Moribund Sacrifice	-	1	-	-	-
Day 99	Moribund Sacrifice	-	1	-	-	-
Day 108	Scheduled Sacrifice	-	8	-	-	-
Day 160	Found Dead	-	-	-	1 (Spleen Hemangios-arcoma)	-
Day 183 or 184	Terminal Sacrifice	25	-	25	24	25
TOTAL Unscheduled Deaths		0	7*	0	1	0

* $p<0.05$ (Fisher's Exact Test) when incidence of early unscheduled death was compared to Group 1.

- = Not applicable

() = Cause of Death

Group 1 - 0 mg/kg/day
Group 2 - 1000 mg/kg/day (urethane)
Group 4 - 5 mg/kg/day
Group 6 - 10 mg/kg/day
Group 8 - 20 mg/kg/day

Incidence of Mortality - Females

Day of Death	Mode of Death	Group 1	Group 2	Group 3	Group 5	Group 7
8	Found Dead	-	-	-	1 (Unknown)	-
69	Found Dead	-	1	-	-	-
85	Found Dead	-	2	-	-	-
97	Found Dead	-	1	-	-	-
106	Scheduled Sacrifice	-	11	-	-	-
120	Moribund Sacrifice	-	-	-	1 (Nasal Cavity Carcinoma)	-
121	Moribund Sacrifice	-	-	1 (Salivary Gland Sarcoma)	-	-
177	Found Dead	-	-	-	-	1 (Unknown)
Day 183 or 184	Terminal Sacrifice	25	-	24	23	24
TOTAL Unscheduled Deaths		0	4*	1	2	1

* $p<0.05$ (Fisher's Exact Test) when incidence of early unscheduled death was compared to Group 1.

- = Not applicable

() = Cause of Death

Group 1 - 0 mg/kg/day
Group 2 - 1000 mg/kg/day (urethane)
Group 3 – 3 mg/kg/day
Group 5 - 7.5 mg/kg/day
Group 7 – 15 mg/kg/day

In the TK study, three animals were found dead or killed in moribund condition: one control male (Day 113), one high dose male (Day 105), and one mid dose female (Day 108).

Statistically significant increases in mortality were noted in both sexes of the positive control group (7/15 males and 4/15 females; $p < 0.05$) compared to the control group.

Dose-related increased incidences of clinical signs, compared to control, were generally observed in both sexes of all treated groups. These clinical signs included decreased motor activity, hunched posture and labored/rapid and shallow breathing. The onset of these signs was earlier in the mid and high dose groups, compared to low dose groups, in both sexes. Most of these clinical observations lasted until the end of the study.

Body weights: There were no statistically significant treatment-related differences in body weight in either sex between control and treated groups. Also, no significant differences in the absolute or percent body weight gain for the duration of the study were noted in treated groups when

compared to control. The mean body weight of the positive control was significantly increased, compared to control, in males (Days 50-85; 7-12%) and females (Days 29-50; 4-5%).

Food consumption: Food consumption was significantly lower in high dose females (16-44%), compared to control, from Day 106 until the end of the study.

Organ weights: Statistically significant differences in organ weights, compared to control, included a decrease in absolute brain weight in low, mid and high dose males (2.6, 2.5 and 3.1%, respectively), and mid and high dose females (4.4 and 3.3%). The relative liver weight was significantly higher than control in mid and high dose males (5.2 and 5.0%). The absolute and relative spleen weights were increased in high dose males (47.9 and 53.6%, respectively).

(It is noted that there is no histopathological findings in the brain or liver that would account for the changes in weight. Moreover, these changes are considered to be small compared to control values and there was no dose relationship. The spleen weight change in the high dose male group is attributed to a high incidence of extra-medullary hematopoiesis in this group compared to control.)

Gross pathology: There were no drug treatment-related increased incidences of gross lesions in the study. The pulmonary and splenic lesions observed in the positive control group were expected lesions related to treatment with urethane.

Microscopic findings: The incidences of neoplastic and non-neoplastic lesions observed in the study are given below.

Neoplastic lesions in lungs: The incidences of pulmonary tumors (adenomas and carcinomas) observed in the study are provided in the following Table. A statistically significant increase ($p < 0.05$) in the incidence of pulmonary tumors was noted in the positive control males and females when compared to vehicle control mice. The incidences of all pulmonary tumors in the vehicle and drug treated groups were comparable and were within the historical control range established at the Contract Lab (b) (4)

Incidences of Pulmonary Tumors (all groups including positive control)

MALE						
	Group 1	Group 2	Group 4	Group 6	Group 8	HCR
Adenoma, single	2	0	4	1	0	0-6
Adenoma, multiple	0	15[1]	0	0	1	0-1
Carcinoma	0	4[1]	0	0	0	0-2
Adenomas and Carcinomas	2	15[1,2]	4	1	1	0-7
FEMALE						
	Group 1	Group 2	Group 3	Group 5	Group 7	HCR
Adenoma, single	1	0	2	2	2	0-6
Adenoma, multiple	1	15[1]	0	0	0	0-1
Carcinoma	0	10[1]	0	0	0	0-1
Adenomas and Carcinomas	2	15[1,2]	2	2	2	0-6

25 mice examined in each of the vehicle and test article treated groups of both sexes; 15 mice examined in each of the positive control groups.
Group 1 male and female – 0 mg/kg/day
Group 2 male and female – Positive Control (urethane, 1000 mg/kg/day)
Group 3 female – 3 mg/kg/day
Group 4 male – 5 mg/kg/day
Group 5 female– 7.5 mg/kg/day
Group 6 male – 10 mg/kg/day
Group 7 female – 15 mg/kg/day
Group 8 male – 20 mg/kg/day
HCR: BioReliance Historical Control Range (See Appendix B)
[1] Statistically significant compared to control Group 1 (see Appendix A)
[2] Multiple adenomas and/or carcinomas were present in the same animal in some of the urethane treated mice

Neoplastic lesions in spleen: The incidences of splenic hemangiosarcomas in all groups are provided. There was a statistically significant increase ($p \leq 0.05$) in the incidence of splenic hemangiosarcomas in the positive control males and females when compared to control. There were no statistically significant differences for the incidences of this tumor between vehicle control and drug treated groups..

Incidences of Splenic Tumors (all groups including positive control)

MALE						
	Group 1	Group 2	Group 4	Group 6	Group 8	HCR
Hemangiosarcoma	0	9[1]	2	3	1	0-4
FEMALE						
	Group 1	Group 2	Group 3	Group 5	Group 7	HCR
Hemangiosarcoma	2	14[1]	0	0	3	0-4

25 mice examined in each of the vehicle and test article treated dose groups of both sexes. 15 mice examined in each of the positive control dose groups.
Group 1 male and female – 0 mg/kg/day
Group 2 male and female – Positive Control (urethane, 1000 mg/kg/day)
Group 3 female – 3 mg/kg/day
Group 4 male – 5 mg/kg/day
Group 5 female– 7.5 mg/kg/day
Group 6 male – 10 mg/kg/day
Group 7 female – 15 mg/kg/day
Group 8 male – 20 mg/kg/day
HCR: BioReliance Historical Control Range (See Appendix B)
[1] Statistically significant compared to control Group 1 (See Appendix A)

Hemangiomas and hemangiosarcomas in multiple organs (including spleen): The incidence of multiple organ hemangiomas and hemangiosarcomas are presented below. There were no treatment related increased incidence of these tumors when compared to control.

Hemangiomas and Hemangiosarcomas (vehicle control and drug-treated groups)

MALE					
	Group 1	Group 4	Group 6	Group 8	HCR
Hemangiosarcomas					
Spleen	0	2	3	1	0-4
Testes	0	1	0	0	0-1
Combined incidence	0	3	3	1	0-4
FEMALE					
	Group 1	Group 3	Group 5	Group 7	HCR
Hemangiosarcomas					
Spleen	2	0	0	3	0-4
Hemangioma, Skin	0	1	0	0	0-1[1]
Combined incidence	2	1	0	3	0-5[1]

25 mice examined in each of the vehicle and test article treated dose groups of both sexes.
Group 1 male and female – 0 mg/kg/day
Group 3 female – 3 mg/kg/day
Group 4 male – 5 mg/kg/day
Group 5 female– 7.5 mg/kg/day
Group 6 male – 10 mg/kg/day
Group 7 female – 15 mg/kg/day
Group 8 male – 20 mg/kg/day
HCR: BioReliance Historical Control Range (See Appendix B)
[1]Skin hemangiosarcomas; skin hemangiomas not previously reported in females

Other tumors in multiple organs: The incidences of other tumors in multiple organs are presented below. The incidences of these tumors fall within the historical control ranges established at (b) (4) No statistically significant differences were observed between control and treated groups for the incidences of these tumors.

Other Tumors Observed in the Study (vehicle control and drug-treated groups)

Male					
	Group 1	Group 4	Group 6	Group 8	HCR
Nasal cavity, adenocarcinoma	0	0	1	1	0-2[1]
Ear, papilloma	0	0	1	0	0-1
Harderian gland, adenoma	0	0	1	2	0-2
Stomach, papilloma	0	0	1	0	0-1
Stomach, squamous cell carcinoma	0	0	1	0	0-1
Thyroid, follicular adenoma	1	0	0	0	NR
Female					
	Group 1	Group 3	Group 5	Group 7	HCR
Nasal cavity, adenocarcinoma	1	0	0	0	0-1
Nasal cavity, carcinoma	0	0	1	0	NR
Ear, papilloma	0	1	0	0	NR
Harderian gland, adenoma	1	0	1	0	0-4
Harderian gland, carcinoma	0	0	0	1	0-2
Lungs with bronchi, Mesothelioma	0	1	0	0	0-1
Salivary gland, sarcoma	0	1	0	0	NR
Salivary gland, mesothelioma	1	0	0	0	0-1

25 mice examined in each of the vehicle and test article treated dose groups of both sexes.
Group 1 male and female – 0 mg/kg/day
Group 3 female – 3 mg/kg/day
Group 4 male – 5 mg/kg/day
Group 5 female– 7.5 mg/kg/day
Group 6 male – 10 mg/kg/day
Group 7 female – 15 mg/kg/day
Group 8 male – 20 mg/kg/day
[1] Only nasal cavity adenomas recorded in HCR for males
HCR: BioReliance Historical Control Range (See Appendix B)
NR=Not previously reported.

Non-neoplastic lesions: Kidneys – Treatment-related non-neoplastic lesions were limited to kidneys, observed mainly in the high dose male group (Table below). These lesions included regenerative and degenerative changes in the renal tubules of the subcapsular cortex. The regenerative changes involved small clusters of tubules that were basophilic with hyperchromatic nuclei. The degenerative change was characterized by dilatation of the tubules (lined by attenuated epithelium) that contained sparse proteinaceous material. Single isolated necrotic tubules were also noted occasionally. One animal each from the control and mid dose male groups, and one high dose female also showed minimal or mild degree of these lesions. It is noted that kidney was considered to be a target organ for males and females in the 28-day

rangefinding study conducted with this test article. There were no other treatment-related increased incidences of non-neoplastic lesions observed in the study.

Non-Neoplastic Kidney Lesions (vehicle control and drug-treated male groups)

Male				
	Group 1	Group 4	Group 6	Group 8
Regeneration				
Minimal	1	0	0	12
Mild	0	0	1	2
Moderate	0	0	0	4
Degeneration				
Minimal	0	0	0	9
Mild	0	0	1	5
Moderate	0	0	0	1
Necrosis				
Minimal	0	0	0	3

25 mice examined in each of the vehicle and test article treated dose groups of both sexes.
Group 1 male – 0 mg/kg/day
Group 4 male – 5 mg/kg/day
Group 6 male – 10 mg/kg/day
Group 8 male – 20 mg/kg/day

Toxicokinetics: The toxicokinetic parameters are summarized below.

Group	CL/F (mL/h/kg)	AUC_{0-t} (ng•h/mL)	V_{ss}/F (mL/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)
3	44816	63.1	456641	6.38	7	3.60
5	46552	161	552160	9.93	12	5.07
7	36700	409	NC	28.2	12	NC
4	133092	36.4	1129659	5.61	7	3.96
6	110503	90.5	1106477	7.71	4	5.32
8	107953	185	1100687	17.5	4	5.47

Group 3 female – 3 mg/kg/day
Group 4 male – 5 mg/kg/day
Group 5 female– 7.5 mg/kg/day
Group 6 male – 10 mg/kg/day
Group 7 female – 15 mg/kg/day
Group 8 male – 20 mg/kg/day
NC = Not calculated

The results indicate that the systemic clearance in female mice was lower than that observed in male mice. The AUC values of treprostinil in female mice were about 2-fold higher than those observed in male mice, possibly accounting for the higher incidence of mortality seen previously

in females when compared to males. The AUC and Cmax values increased in a proportional manner. The $t_{½}$ was similar across dose groups in both sexes.

The mean dose for UT-15C in patients in a controlled clinical trial at 12 weeks was 3.4 mg BID and the AUC at this dose level was 24.35 ng.h/mL. The NOAELs for kidney lesions in this mouse study were 10 mg/kg/day (AUC 90.5 ng.h/mL) for males and 15 mg/kg/day (AUC 409 ng.h/mL) for females, indicating that the exposures obtained at these levels were about 4-17 fold higher than the human exposure at the mean dose of 3.4 mg BID.

Systemic Exposure Comparisons in Mice and Humans

	Dose (mg/kg/day)		AUC (ng.h/ml)		Multiple of Human Exposure	
	M	F	M	F	M	F
Mouse	10	15	90.5	409	4	17
Human	0.11		24.35		----	

Mouse doses = NOAELs for kidney lesions
Clinical dose = the mean dose (3.4 mg BID) used in patients.

The FDA statistical analyses of the mouse tumor data (see review by MA Rahman; dated 5/31/2012) showed no statistically significant dose response relationships for the incidence of any of the observed tumor types in either sex. The pairwise comparisons also did not show statistically significant increased incidence of tumors in treated groups compared to the vehicle control group in either sex. There was no treatment-related increased incidence of mortality in either sex.

The positive control group showed statistically significant increased mortality ($p < 0.05$) and increased incidences ($p < 0.05$) of pulmonary (adenoma, carcinoma and hemangiosarcoma) and splenic (hemangiosarcoma) tumors in both sexes.

Reproductive and Developmental Toxicology

Fertility and Early Embryonic Developmental Study of UT-15C in Rats

Testing Facility: [redacted] (b) (4)

[redacted] (b) (4) Study Number: 7049-130

Study Dates: Initiation Date – May 23, 2007
Completion Date – May 13,2008

GLP Compliance: The study was conducted in accordance with the GLP regulations of the US FDA and the Japanese Ministry of Health, Labor and Welfare GLP Standards.

QA Report: Yes

Animals: Male and female Crl:CD (SD) rats were obtained from [redacted] (b) (4) After a 7-day acclimation and observation period, the animals were assigned to study groups as shown below (25/sex/group for the main study and 12/sex/group for the toxicokinetic evaluation) and the dosing was initiated.

Group	No. of Animals		Daily Dose Level [a,b] (mg/kg/day)	Installment Dose Level [a,b] (mg/kg/dose)	Dose Concentration [a,b] (mg/mL)	Daily Dose Volume (mL/kg/day)	Installment Dose Volume (mL/kg/dose)
	Male	Female					
Main study animals							
1 (Control) [c]	25	25	0	0	0	10	5
2 (Low)	25	25	2.5	1.25	0.25	10	5
3 (Mid)	25	25	5	2.5	0.5	10	5
4 (High)	25	25	10	5	1	10	5
Toxicokinetic animals							
5 (Control)[c]	12	12	0	0	0	10	5
6 (Low)	12	12	2.5	1.25	0.25	10	5
7 (Mid)	12	12	5	2.5	0.5	10	5
8 (High)	12	12	10	5	1	10	5

a Doses were expressed as treprostinil, the free acid of UT-15C [the conversion factor from free acid to the salt (i.e., UT-15C for dose administration) was 1.269].
b Animals were dosed at a volume of 5 mL/kg/dose; animals were dosed two times daily (10 mL/kg/day) approximately 6 hours apart between doses at approximately the same time of the day.
c Control animals were dosed with the vehicle using the same dosing regimen.

At the initiation of treatment, the rats were 9 weeks old and their body weights ranged from 279 to 339 g for the males and 195 to 236 g for the females.

Male and female rats were housed individually (except during acclimation and mating periods) in stainless steel cages, and were given Certified Rodent Diet #8728C (Harlan Teklad). Water was available ad libitum.

Dose Levels and Mode of Administration: UT-15C (treprostinil diethanolamine, Lot No. DB-R06017, purity 99.3%), dissolved in sterile water for injection (USP), was administered by oral gavage twice daily (about 6 hours apart) at dose levels of 0, 2.5, 5 and 10 mg/kg/day (dose volume of 10 mL/kg/day). The doses were expressed as the free acid of UT-15C.

Males were dosed for at least 28 days prior to mating, throughout the mating phase, and through the day prior to termination (i.e., at least 10 weeks). Females were dosed for at least 14 days prior to mating, throughout the mating phase and through Gestation Day (GD) 7.

[Dose levels for the present study were selected based on the findings in previous embryo-fetal developmental studies with UT-15C in rats. In the definitive study, the NOAEL for maternal toxicity was 5 mg/kg/day. The NOEL for fetal viability and growth was 5 mg/kg/day based on increased postimplantation loss observed at 10 mg/kg/day. In this current study, it was anticipated that the 10 mg/kg/day dose level would produce drug-related effects. Hence, 10 mg/kg/day was selected as the high dose for the study.]

Dosing Formulation Stability: Dosing formulations were prepared at least once weekly. Stability has been previously established to be up to 30 days under room temperature conditions.

Observations and measurements: *Clinical signs:* Animals were checked twice daily for mortality, abnormalities and clinical signs. Detailed observations were done once during the predose phase and at body weight intervals during the study for the main study animals.

Daily cageside observations were done one hour postdose (after each dose) during the first 14 days of drug administration for each sex and twice weekly for the remaining weeks of dosing.

Body weights were recorded once during pretreatment phase, on the first day of treatment and twice weekly for the males and females during the premating and mating phases. Females that were confirmed to have mated were weighed on GD 0, 3, 7, 10 and 13.

Food consumption for males was determined weekly during the premating treatment phase. For the females, food consumption was determined weekly during the premating treatment phase and measured beginning at GD 0 at each gestation body weight interval.

Estrous cycle determination: During the 14-day premating phase for the treated females, daily vaginal smears were assessed for stage of estrus. Estrous cycle determination continued until the confirmation of mating occurred.

Mating and confirmation of mating: Rats were mated by placing one female with a male from the same group. The maximum time for mating was 3 weeks. Mating was confirmed by the presence of a retained or dropped copulatory plug or by the observation of vaginal sperm. The day in which sperm or plug was observed was designated as GD 0.

Unscheduled deaths: All females that died prior to the scheduled cesarean section were examined grossly for abnormalities. The uterus and ovaries were examined for implantations and corpora lutea, respectively. The placenta or amniotic sac was examined for any abnormalities. An evaluation of the uterus was made to indicate early or late resorbing fetuses, dead fetuses or apparently normally developing fetuses.

Male Reproductive Assessment: The first surviving 10 males/group were evaluated for epididymal sperm motility and sperm concentration using the computer-assisted sperm analysis (CASA) System IVOS in the epididymal fluid.

Sacrifice at cesarean section: Performed on GD 13. Complete necropsies were conducted and uterine contents examined.

Disposition of Males: After 10 weeks of treatment, a complete necropsy was performed all surviving males.

Organ Weights: Protocol-specified organ weights were recorded at the scheduled sacrifice.

Control Tissues: All cervical, thoracic and abdominal tissues from the first five surviving control animals/sex were retained in 10% neutral-buffered formalin for possible examination. However, these tissues were not examined.

Toxicokinetic Evaluation: Blood samples were taken on Day 1 and the last day of dosing from males, and on Day 1 and GD 7 from females. Three animals/sex/group were bled just prior to the first daily dose and at 1, 2, 3, 4, 6, 7, 8, 9, 10, 12 and 24 hours after the first daily dose.

Statistical Evaluation: One-way analysis of variance (ANOVA) was used to analyze the data. If the ANOVA was significant , Dunnett's t-test was used for group comparisons. Levene's test was done to test for variance homogeneity. In the case of significant heterogeneity of variance, rank transformation was used to stabilize the variance.

Results

In –life Observations - Males

There were no unscheduled deaths. All males survived until the study termination.

During the premating phase, treatment-related clinical signs consisted primarily of red skin of the extremities (ears, paws, nose and tail) following the daily dosings (1 hr post-dose). As dosing continued, the incidence of red skin on the nose and tail began to resolve, but redness of the paws and ears persisted primarily at the mid and high dose levels throughout the entire dosing phase. Soft feces was noted in the mid and high dose groups during the first week of dosing.

At the initiation of dosing (Days 0 to 4), significant reductions in body weight were noted at the mid dose (4%) and high dose (11%) levels compared to control. Although decreased body weights were noted at the mid dose (during the fist week of treatment) and high dose (the first 3 weeks of treatment) levels during the beginning of the study, no significant weight differences were noted between control and treated groups during the rest of the study.

Treatment with UT-15C produced significantly decreased food consumption at mid and high dose levels during the first week of dosing (Days 0 to 7). During the subsequent weeks of the premating phase, mean food consumption was generally similar across all male groups.

In-life Observations – Females- Premating phase

There were no unscheduled deaths during premating phase.

Similar to the males, treatment-related clinical signs in females consisted of red skin of the extremities (ears and paws) following dosing in all drug treated groups. Red skin of the nose and paws was also observed, at a lower incidence, at mid and high dose levels. The above findings persisted throughout the 14-day premating phase.

In females, the body weight changes were generally similar across groups. Treatment with UT-15C produced significant, dose-related decreases in mean food consumption in all treated groups during the first week of dosing. Mean food consumption was significantly decreased in all treated groups over the entire premating phase (Days 0 to 14).

Gestation Phase

There was one unscheduled death during the gestation phase. A mid dose female was found dead on GD 3. On the preceding day, the clinical signs exhibited included hypoactivity, irregular respiration, and clear oral discharge. There were no remarkable necropsy findings. Since the death occurred on GD 3 (prior to implantation but after confirmed mating), it was too early to determine the pregnancy status.

In the remaining females, remarkable clinical signs during gestation included a dose-related increase in the incidence of red ears in all treated groups following the daily dosings.

Over the entire gestation period (GD 0 to 13), mean female body weights were similar across groups. During the first week of gestation (GD 0 to 7; dosing phase), mean food consumption was decreased in all treated groups compared to control, but during the second week of gestation

(GD 7 to 13; nondosing phase) mean food consumption in the treated groups were generally similar to or greater than control.

There were no effects of treatment on male or female reproductive organ weights. Mean gravid uterine weights were similar across groups.

Reproductive Performance

There were no biologically relevant effects on the estrous cycle. Three control, one low dose, five mid dose and six high dose females were observed to be in prolonged diestrus (4 or more consecutive days) at some time during the premating phase. Of these females, only one control female did not confirm to have mated and was subsequently confirmed not to be pregnant. All other females that were found to be in prolonged diestrus were confirmed to have mated and had pregnancies with viable fetuses. (One mid dose female had no viable fetuses.)

Treatment with UT-15C had no effect on reproductive performance. The majority of females were confirmed mated within the first 4 days of mating. The pregnancy rates were 91% for control, 96% for low dose and 100% each for mid and high doses (Table below). There were no abortions or early deliveries. One mid dose female had no viable fetuses. Mean number of corpora lutea, implantation sites, and percent preimplantation loss were generally similar across groups, indicating no treatment –related effect on fertility. Mean postimplantation loss and mean percent of live fetuses were similar across groups, indicating that the test drug had no effect on embryo/fetal viability. There were no dead fetuses in the study.

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Table 12
Summary of Cesarean Section Data

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 2.5 MG/KG/DAY	GROUP 3 5 MG/KG/DAY	GROUP 4 10 MG/KG/DAY
Females Paired	N	25	25	25	25
Mated	N	23	25	25	24
Pregnant	N	21	24	24[a]	24
	%	91	96	100	100
Aborted	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Died	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Delivered Early	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Pregnant at C-section	N	21	24	24	24
Dams with Viable Fetuses	N	21	24	23	24
	%	100	100	96	100
Dams with no Viable Fetuses	N	0	0	1	0
	%	0.0	0.0	4.2	0.0
Corpora Lutea	MEAN	16.2	15.3	16.5	16.0
	S.D.	2.0	2.0	2.8	2.5
	N	21	24	24	24
	TOTAL	340	367	395	385
Implantation Sites	MEAN	15.2	14.4	15.3	14.5
	S.D.	1.7	2.0	2.1	3.0
	N	21	24	24	24
	TOTAL	320	346	368	347
Preimplantation Loss	MEAN%	5.5	5.5	6.2	9.7
	S.D.	6.6	7.5	9.3	13.6

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

[a] Confirmed-mated Female B22953 was found dead on GD 3 (prior to probable date of implantation); thus, this female was excluded from group mean percent pregnant as it was too early to determine pregnancy status.

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TABLE 12
SUMMARY OF CESAREAN SECTION DATA

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 2.5 MG/KG/DAY	GROUP 3 5 MG/KG/DAY	GROUP 4 10 MG/KG/DAY
Pregnant at C-section	N	21	24	24	24
Resorptions: Total	MEAN	0.9	0.8	1.4	0.8
	S.D.	1.0	0.7	3.0	0.8
	N	21	24	24	24
	TOTAL	18	18	33	18
	MEAN%	5.6	5.5	9.3	5.0
	S.D.	6.2	5.4	20.0	5.7
Early	MEAN	0.9	0.8	1.4	0.7
	S.D.	1.0	0.7	3.0	0.8
	N	21	24	24	24
	TOTAL	18	18	33	16
	MEAN%	5.6	5.5	9.3	4.4
	S.D.	6.2	5.4	20.0	5.1
Late	MEAN	0.0	0.0	0.0	0.1
	S.D.	0.0	0.0	0.0	0.3
	N	21	24	24	24
	TOTAL	0	0	0	2
	MEAN%	0.0	0.0	0.0	0.6
	S.D.	0.0	0.0	0.0	2.0
Dead Fetuses	TOTAL	0	0	0	0
Postimplantation Loss	MEAN%	5.6	5.5	9.3	5.0
	S.D.	6.2	5.4	20.0	5.7

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

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TABLE 12
SUMMARY OF CESAREAN SECTION DATA

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 2.5 MG/KG/DAY	GROUP 3 5 MG/KG/DAY	GROUP 4 10 MG/KG/DAY
Pregnant at C-section	N	21	24	24	24
Live Fetuses	MEAN	14.4	13.7	14.0	13.7
	S.D.	1.8	2.3	3.8	2.9
	N	21	24	24	24
	TOTAL	302	328	335	329
	MEAN%	94.4	94.5	90.7	95.0
	S.D.	6.2	5.4	20.0	5.7

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

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No treatment-related differences were observed in sperm motility evaluation. Group mean values were comparable between the study groups and ranged from 88 to 95%. For the sperm count evaluation, no apparent treatment-related differences were observed in the number of sperm per gram of cauda epididymis

Pharmacokinetic evaluation showed that there were dose-related increases in Cmax and AUC values within each sex on both sampling days. However, female rats generally had higher systemic exposure to treprostinil than male rats throughout the study, which was more marked on the last day of dosing.

Plasma Treprostinil Pharmacokinetic Parameters in Female and Male Rats

Parameter	Dose (mg/kg/day)	First Day*		Last Day**	
		Female	Male	Female	Male
C_{max1} (ng/mL)	2.5	4.31	2.72	6.57	3.37
	5.0	6.44	4.1	11.9	12.85
	10.0	8.77	6.3	26.5	19.2
T_{max1} (hr)	2.5	2.0	6.0	3.0	3.0
	5.0	1.0	4.0	3.0	4.0
	10.0	1.0	1.0	6.0	1.0
C_{max2} (ng/mL)	2.5	5.91	3.21	7.2	5.24
	5.0	10.21	6.52	12.7	12.84
	10.0	10.22	11.44	36.73	20.13
T_{max2} (hr)	2.5	7.0	8.0	7.0	7.0
	5.0	8.0	8.0	8.0	9.0
	10.0	7.0	8.0	7.0	10.0
AUC_{24}□ (hr*ng/mL)	2.5	68.35	39.37	66.84	57.85
	5.0	140.39	71.26	129.74	153.35
	10.0	140.54	116	438.97	251.01
CL/F (mL/min/kg)	2.5	420	736	624	720
	5.0	-	642	642	544
	10.0	-	-	380	664

* Dosing Day 1 for female rats and male rats
** Last day of dosing for female rats was Gestation Day 7 and for male rats was Day 70
□ AUC_{24} for first day of dosing and AUC_{ss} for last day of dosing

Embryo-Fetal Development Study of UT-15C in Rats

Testing Facility: (b) (4)

Study Number: (b) (4) (contract Lab's number)

Study Dates: Initiation Date – March 01, 2005
Completion Date – August 31, 2005

GLP Compliance: The study was conducted in accordance with the FDA GLP Regulations , Title 21 of the U.S.CFR part 58 and Japanese Ministry of Health, Labor and Welfare GLP Standards Ordinance No. 21.

QA Report: yes

Animals: Time-mated female Crl:CD®(SD)IGS BR rats were received from (b) (4) On gestation day (GD) 0, the animals were 10 to 12 weeks old, and their body weights (females) ranged from 200 to 250 g. Mated females were randomly assigned to 4 main study groups (25 rats/group). Additional time-mated rats were assigned for toxicokinetic (TK) analyses (12/group) as follows.

Group Designation and Dose Levels

Group	No. of Animals Female	Daily Dose Level [a,b] (mg/kg/day)	Installment Dose level [a,b] (mg/kg/dose)	Dose Concentration [a,b] (mg/mL)	Daily Dose Volume (mL/kg/day)	Installment Dose Volume (mL/kg/dose)	Dosing Schedule Days of Gestation
Main Study animals							
1 (Control) [c]	25	0	0	0	10	5	6-17
2 (Low)	25	5	2.5	0.5	10	5	6-17
3 (Mid)	25	10	5	1	10	5	6-17
4 (High)	25	20	10	2	10	5	6-17
Toxicokinetic animals							
5 (Low)	12	5	2.5	0.5	10	5	6-17
6 (Mid)	12	10	5	1	10	5	6-17
7 (High)	12	20	10	2	10	5	6-17

a Doses were expressed as the free acid of UT-15C [the conversion factor from free acid to the salt (i.e. UT-15C for dose administration) is 1.269].
b Animals were dosed at a volume of 5 mL/kg/dose; animals were dosed two times daily (10 mL/kg/day) with approximately 6 hours between doses, at approximately the same time of day for at least 12 days (see Dose Administration).
c Control animals were dosed with the vehicle using the same dosing regimen.

Female rats were housed individually in suspended, stainless steel cages and were given food and water *at libitum.*

Dose Levels and Mode of Administration: Treprostinil diethanolamine (UT-15C; Lot No. D-129-053; purity 99.3%), dissolved in the vehicle article (sterile water for injection), was administered by oral gavage twice daily (6 hours apart), on gestation days 6 through 17, at dose levels of 0 (vehicle), 5, 10 and 20 mg/kg/day (expressed as free acid). The drug was administered at a dose volume of 10 mL/kg/day.

[Note: Dose levels for the present study were selected based on the results of a previous oral gavage dose range-finding developmental toxicity study with UT-15C in rats. In that study, groups of Crl:CD®(SD)IGS BR female rats were given oral doses of UT-15 at 0, 5, 10, 20 and 30 mg/kg/day, twice daily on GD 6 through 17 at a dose volume of 10 mL/kg/day. There was a dose-dependent decrease in body weight gain and food consumption at doses of 10 mg/kg/day and above. Mean gravid uterine weight was decreased at 20 and 30 mg/kg/day. One dam each in the 20 and 30 mg/kg/day groups had litters with no viable fetuses. There was an increase in postimplantation loss at 20 and 30 mg/kg/day. The mean number of live fetuses was lower in the 30 mg/kg/day group. There were no fetal external variations or malformations. A dose of 20 mg/kg/day was selected as the high dose for the definitive study.]

Dosing Formulations and Stability: Dosing formulations were prepared weekly. Stability was previously established at concentrations ranging from 0.1 and 40 mg/mL for up to 30 days under room temperature conditions. Verification of uniformity for concentrations of 0.1 and 3.0 mg/mL has been previously established. All dose concentrations were within 3% of target level.

Observations and Measurements: Animals were checked twice daily for mortality, abnormalities and signs of toxicity. Detailed observations were done at each body weight interval for main study animals. Cageside observations were done twice daily 1 hour post dose for main study animals.

Animals were weighed on GD 0, 4, 6, 8, 10, 12, 14, 16, 18 and 20. (main study animals only on GD 20)

Beginning on GD 4, food consumption was measured at body weight intervals (main study animals only).

Blood was collected for TK evaluations according to the following schedule. On GD 17, the first set of 3 TK rats per group was bled prior to dosing and at 4 and 9 hours (post-first dose); the second set of 3 TK rats per group was bled at 1, 6 and 10 hours; the third set of 3 TK rats per group was bled at 2, 7 and 12 hours; and the fourth set of 3 TK rats was bled at 3, 8 and 24 hours.

Cesarean section was performed on main study animals on GD 20. Uterine weights were recorded and the uterine contents were examined. Abnormal maternal tissues were saved in 10% neutral-buffered formalin.

Each fetus (live or dead) from the main study rats was sexed, weighed and examined for external abnormalities. Live fetuses were sacrificed via ip injection of a barbiturate followed by exsanguination.

Abnormalities were judged to be malformations or variations. Malformations are developmental deviations which (1) are gross structural changes, (2) are incompatible with life, or (3) may affect the quality of life. Variations are structural deviations which are thought to have no effect on body conformity or well being of the animal.

All surviving TK dams were sacrificed after the Day 18 blood collection. After determining the pregnancy status of each animal, carcasses were discarded without necropsy.

A necropsy was done on all main study animals that died or were sacrificed at an unscheduled interval.

The following statistical methods were used. One-way analysis of variance (ANOVA) was used to analyze the data. Levene's test was used to test for variance homogeneity. In the case of heterogeneity of variance at $p < 0.05$, rank transformation was used. If the ANOVA was significant ($p < 0.05$), Dunnett's t-test was used for control versus treated group comparisons. The fetal and the litter incidences of findings were statistically analyzed using the Cochran-Armitage test for linear trend followed by the Fisher–Irwin exact test.

Results

Maternal Toxicity

There were four unscheduled deaths from the high dose group during the study, which occurred during the second half of the dosing phase (GD 12 to 14). Remarkable clinical signs observed in these animals included hunched posture, hypoactivity, discolored red skin, labored respiration, cold to touch, alopecia and red/black vaginal discharge. No necropsy findings were noted in these animals.

For the remaining animals, maternal clinical observations included dose-related increased incidence of post-dose discolored red skin, alopecia and red crust on the nose in drug-treated rats. In addition, rough hair coat, hunched appearance, hypoactivity and red/black vaginal discharge were noted at the high dose.

The body weights were significantly lower ($p<0.01$) than control at mid (6-8%) and high dose (8-9%) levels during GD 8 and 18. This effect was found to be reversible since on GD 20, the mean body weights were generally similar across all groups. There was a significant ($p<0.01$) dose-dependent decrease in food consumption in drug-treated groups during GD 6-18. However, this effect was reversible since the food consumption in treated groups was significantly higher than control during the post dosing phase (GD 18-20).

Remarkable necropsy findings were limited to dilated kidneys in one low dose rat. The clinical findings observed in this rat included discolored red skin during the course of the study. The findings of dilated kidneys were not seen in the higher dose groups.

Although not statistically significant, the mean gravid uterine weights were slightly decreased at mid and high dose levels (6-8%). The mean corrected maternal body weights (terminal body weights minus gravid uterine weights) were similar across all groups.

The pregnancy rates were 96, 100, 92 and 68% for the 0, 5, 10 and 20 mg/kg/day groups, respectively. There were no abortions or early deliveries. All pregnant dams had litters with viable fetuses.

Developmental Toxicity

The numbers of corpora lutea and implantation sites and the preimplantation loss were generally similar across all groups. There was a significant increase in postimplantation loss in the mid dose group, which was attributed to significant increase in early resorptions. The mean number of live fetuses was significantly lower in the mid dose group. Mean fetal weights from the treated groups were not significantly different from control.

There were no external anomalies (fetal and litter incidences) observed in the study (Table below).

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Table 9
Summary of Fetal External Observations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 10 MG/KG/DAY	GROUP 4 20 MG/KG/DAY
Litters Evaluated	N	24	25	23	14
Fetuses Evaluated	N	312	331	281	176
Live	N	312	331	281	176
Dead	N	0	0	0	0
TOTAL FETAL EXTERNAL OBSERVATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P≤0.05 ** - P≤0.01.
N - NUMBER

The total fetal and litter incidences soft tissue variations were generally similar across all groups. Notable soft tissue variations included one fetus each with small/missing cardiac lobe of the lung and dark kidneys in the control group, and a few pups with dilated ureters in the control, low and mid dose levels. Dilated lateral and third ventricles were observed in control and treated groups with generally similar fetal incidence, however, the litter incidence showed a significant positive trend across treated groups. These variations are considered incidental findings and are not attributed to drug treatment.

There were no fetal soft tissue malformations.

Covance 7049-120

Table 10
Summary of Fetal Soft Tissue Variations

BODY EXAMS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 10 MG/KG/DAY	GROUP 4 20 MG/KG/DAY
Litters Evaluated	N	24	25	23	14
Fetuses Evaluated	N	156	165	142	89
Live	N	156	165	142	89
Dead	N	0	0	0	0
CARDIAC LOBE OF LUNG SMALL/MISSING					
Fetal Incidence	N	1	0	0	0
	%	0.6	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	4.2	0.0	0.0	0.0
KIDNEY(S) - DARK					
Fetal Incidence	N	1	0	0	0
	%	0.6	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	4.2	0.0	0.0	0.0
DILATED URETER(S)					
Fetal Incidence	N	2	6	1	0
	%	1.3	3.6	0.7	0.0
Litter Incidence	N	2	4	1	0
	%	8.3	16	4.3	0.0
TOTAL FETAL SOFT TISSUE VARIATIONS					
Fetal Incidence -	N	4	6	1	0
	%	2.6	3.6	0.7	0.0
Litter Incidence -	N	4	4	1	0
	%	17	16	4.3	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P≤0.05 ** - P≤0.01.
N - NUMBER
- - SIGNIFICANT NEGATIVE TREND

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TABLE 10
SUMMARY OF FETAL SOFT TISSUE VARIATIONS

HEAD EXAMS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 10 MG/KG/DAY	GROUP 4 20 MG/KG/DAY
Litters Evaluated	N	24	25	23	14
Fetuses Evaluated	N	156	165	142	99
Live	N	156	165	142	99
Dead	N	0	0	0	0
DILATED LATERAL VENTRICLE(S)					
Fetal Incidence +	N	1	2	4	3
	%	0.6	1.2	2.8	3.4
Litter Incidence +	N	1	2	3	3
	%	4.2	8.0	13	21
DILATED THIRD VENTRICLE					
Fetal Incidence	N	1	2	0	1
	%	0.6	1.2	0.0	1.1
Litter Incidence	N	1	1	0	1
	%	4.2	4.0	0.0	7.1
TOTAL HEAD EXAM VARIATIONS					
Fetal Incidence	N	2	3	4	4
	%	1.3	1.8	2.8	4.5
Litter Incidence +	N	2	2	3	4
	%	8.3	8.0	13	29

+ = SIGNIFICANT POSITIVE TREND
N = NUMBER

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Table 11
Summary of Fetal Soft Tissue Malformations

BODY EXAMS

DOSE LEVEL		GROUP 1 0 MG/KG	GROUP 2 5 MG/KG	GROUP 3 10 MG/KG	GROUP 4 20 MG/KG
Litters Evaluated	N	24	25	23	14
Fetuses Evaluated	N	156	165	142	99
Live	N	156	165	142	99
Dead	N	0	0	0	0
TOTAL FETAL SOFT TISSUE MALFORMATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
N = NUMBER

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TABLE 11
SUMMARY OF FETAL SOFT TISSUE MALFORMATIONS

HEAD EXAM

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 10 MG/KG/DAY	GROUP 4 20 MG/KG/DAY
Litters Evaluated	N	24	25	23	14
Fetuses Evaluated	N	156	165	142	99
Live	N	156	165	142	99
Dead	N	0	0	0	0
TOTAL HEAD EXAM MALFORMATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

N = NUMBER

The total fetal and litter incidences of skeletal variations were generally similar across all groups; however, the following variations were significantly ($p<0.01$) increased as shown in the following Tables.

a) fetal incidence of less than four caudal vertebrae ossified at 20 mg/kg/day
b) fetal incidence of incomplete ossification of sternebrae at 20 mg/kg/day and
c) fetal incidence of the 13th rudimentary rib(s) at 5 mg/kg/day.

These variations, except for the fetal incidence of the 13th rudimentary ribs at the low dose, were within the historical control of the contract laboratory. Since the increases in variations generally correlated with the decreased mean maternal body weight and gravid uterine weights, they are considered to represent a delay in fetal development.

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Table 12
Summary of Fetal Skeletal Variations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 10 MG/KG/DAY	GROUP 4 20 MG/KG/DAY
Litters Evaluated	N	24	25	23	14
Fetuses Evaluated	N	156	166	139	97
Live	N	156	166	139	97
Dead	N	0	0	0	0
INCOMPLETE OSSIFICATION OF SKULL					
Fetal Incidence	N	27	21	16	9
	%	17	13	12	10
Litter Incidence	N	12	12	7	6
	%	50	48	30	43
UNOSSIFIED HYOID BODY					
Fetal Incidence	N	13	6	5	8
	%	8.3	3.6	3.6	9.2
Litter Incidence	N	8	3	4	3
	%	33	12	17	21
LESS THAN FOUR CAUDAL VERTEBRAE OSSIFIED					
Fetal Incidence +	N	38	30	43	37
	%	24	18	31	43**
Litter Incidence	N	13	11	14	10
	%	54	44	61	71
INCOMPLETE OSSIFICATION OF VERTEBRAL ARCH(ES)					
Fetal Incidence -	N	40	33	34	11
	%	26	20	24	13*
Litter Incidence	N	15	13	14	6
	%	63	52	61	43
BIPARTITE VERTEBRAL CENTRUM(A)					
Fetal Incidence	N	1	2	4	2
	%	0.6	1.2	2.9	2.3
Litter Incidence	N	1	2	4	1
	%	4.2	8.0	17	7.1

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
+ = SIGNIFICANT POSITIVE TREND
- = SIGNIFICANT NEGATIVE TREND
N = NUMBER

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TABLE 12
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 10 MG/KG/DAY	GROUP 4 20 MG/KG/DAY
Litters Evaluated	N	24	25	23	14
Fetuses Evaluated	N	156	166	139	87
Live	N	156	166	139	87
Dead	N	0	0	0	0
25 PRESACRAL VERTEBRAE					
Fetal Incidence	N	1	0	0	1
	%	0.6	0.0	0.0	1.1
Litter Incidence	N	1	0	0	1
	%	4.2	0.0	0.0	7.1
27 PRESACRAL VERTEBRAE					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.7	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	4.3	0.0
5TH STERNEBRA UNOSSIFIED					
Fetal Incidence	N	31	29	29	23
	%	20	17	21	26
Litter Incidence	N	14	13	14	11
	%	58	52	61	79
5TH/6TH STERNEBRA(E) INCOMPLETE OSSIFICATION					
Fetal Incidence	N	79	72	76	48
	%	51	43	55	55
Litter Incidence	N	21	20	19	13
	%	88	80	79	93
OTHER STERNEBRA(E) INCOMPLETE OSSIFICATION					
Fetal Incidence +	N	4	8	7	11
	%	2.6	4.8	5.0	13**
Litter Incidence	N	4	5	6	3
	%	17	20	26	21

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P≤0.05 ** - P≤0.01.
+ - SIGNIFICANT POSITIVE TREND
N - NUMBER

TABLE 12
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 10 MG/KG/DAY	GROUP 4 20 MG/KG/DAY
Litters Evaluated	N	24	25	23	14
Fetuses Evaluated	N	156	166	139	97
Live	N	156	166	139	97
Dead	N	0	0	0	0
OTHER STERNEBRA(E) UNOSSIFIED					
Fetal Incidence	N	3	1	1	2
	%	1.9	0.6	0.7	2.3
Litter Incidence	N	2	1	1	2
	%	8.3	4.0	4.3	14
6TH STERNEBRA UNOSSIFIED					
Fetal Incidence	N	3	2	2	3
	%	1.9	1.2	1.4	3.4
Litter Incidence	N	3	1	1	1
	%	13	4.0	4.3	7.1
STERNEBRA(E) ASYMMETRICALLY OSSIFIED					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.6	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	4.0	0.0	0.0
7TH CERVICAL RIB(S)					
Fetal Incidence	N	0	0	2	1
	%	0.0	0.0	1.4	1.1
Litter Incidence	N	0	0	2	1
	%	0.0	0.0	8.7	7.1
WAVY/BENT RIB(S)					
Fetal Incidence	N	11	9	6	4
	%	7.1	5.4	4.3	4.6
Litter Incidence	N	7	7	4	2
	%	29	28	17	14

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P≤0.05 ** - P≤0.01.
N - NUMBER

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TABLE 12
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 10 MG/KG/DAY	GROUP 4 20 MG/KG/DAY
Litters Evaluated	N	24	25	23	14
Fetuses Evaluated	N	156	166	139	97
Live	N	156	166	139	97
Dead	N	0	0	0	0
14TH RUDIMENTARY RIB(S)					
Petal Incidence	N	7	3	7	4
	%	4.5	1.8	5.0	4.6
Litter Incidence	N	4	2	6	4
	%	17	8.0	26	29
13TH RUDIMENTARY RIB(S)					
Petal Incidence	N	0	5	0	0
	%	0.0	3.0*	0.0	0.0
Litter Incidence	N	0	3	0	0
	%	0.0	12	0.0	0.0
INCOMPLETE OSSIFICATION OF RIB(S)					
Petal Incidence -	N	4	0	0	0
	%	2.6	0.0	0.0	0.0
Litter Incidence	N	2	0	0	0
	%	8.3	0.0	0.0	0.0
LESS THAN FOUR METATARSALS OSSIFIED					
Petal Incidence	N	0	1	0	0
	%	0.0	0.6	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	4.0	0.0	0.0
INCOMPLETE OSSIFICATION OF ISCHIUM(A)					
Petal Incidence	N	3	1	4	0
	%	1.9	0.6	2.9	0.0
Litter Incidence	N	2	1	3	0
	%	8.3	4.0	13	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P≤0.05 ** - P≤0.01.
- - SIGNIFICANT NEGATIVE TREND
N - NUMBER

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TABLE 12
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 10 MG/KG/DAY	GROUP 4 20 MG/KG/DAY
Litters Evaluated	N	24	25	23	14
Fetuses Evaluated	N	156	166	139	97
Live	N	156	166	139	97
Dead	N	0	0	0	0
UNOSSIFIED PUBIS(ES)					
Petal Incidence	N	1	0	1	0
	%	0.6	0.0	0.7	0.0
Litter Incidence	N	1	0	1	0
	%	4.2	0.0	4.3	0.0
TOTAL FETAL SKELETAL VARIATIONS					
Petal Incidence	N	118	111	106	69
	%	76	67	76	79
Litter Incidence	N	24	25	23	14
	%	100	100	100	100

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P≤0.05 ** - P≤0.01.
N - NUMBER

There were no skeletal malformations in the study.

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Table 13
Summary of Fetal Skeletal Malformations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 10 MG/KG/DAY	GROUP 4 20 MG/KG/DAY
Litters Evaluated	N	24	25	23	14
Fetuses Evaluated	N	156	166	139	87
Live	N	156	166	139	87
Dead	N	0	0	0	0
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

N = NUMBER

The pharmacokinetic parameters in female rats on gestation day 17 are provided below.

Table 2: Summary of Treprostinil Pharmacokinetic Parameters in Female Rats on Gestation Day 17 by Dose Group

Dose Group (mg/kg/day)	Gender	C_{max1} (ng/mL)	T_{max1} (hr)	C_{max2} (ng/mL)	T_{max2} (hr)	AUC_{ss} (hr*ng/mL)	CL/F (mL/min/kg)
5	Female	15.6	2	30.1	8	315.3	264.4
10	Female	28.9	6	53.7	8	582.7	286.1
20	Female	86.7	4	171.4	9	1316.4	253.3

Following the first daily dose of UT-15C, the Cmax1 values were obtained between 2 to 6 hr. Following second daily dose, the Cmax2 values were achieved between 2 to 3 hr. The Cmax2 values were found to be about twice the corresponding Cmax1 values, indicating that the plasma treprostinil concentrations at 6 hr after the first daily dose either had not declined or had declined minimally from peak concentrations. The steady state AUC values increased proportionately versus total daily dose. The results indicated pharmacokinetic linearity over the dosage range of 5 to 20 mg/kg/day.

The NOAEL for maternal toxicity was found to be 5 mg/kg/day. The NOEL for fetal viability and growth was 5 mg/kg/day. The NOAEL for teratogenicity was 20 mg/kg/day.

Embryo-Fetal Development Study of UT-15C in Rabbits

Testing Facility: [redacted] (b) (4)

Study Number: [redacted] (b) (4) (contract Lab's number)

Study Dates: Initiation Date – March 22, 2005
Completion Date – August 31, 2005

GLP Compliance: The study was conducted in accordance with the FDA GLP Regulations, Title 21 of the U.S.CFR part 58 and Japanese Ministry of Health, Labor and Welfare GLP Standards Ordinance No. 21.

QA Report: yes

Animals: Time-mated female Hra:(NZW)SPF rabbits were obtained from [redacted] (b) (4) The females were mated at the supplier and the day of confirmation of mating was designated as GD 0. The females were received prior to GD 3. Animals were assigned to 4 main study groups (20/group) and 3 TK groups (3/group) as follows.

Group	No. of Animals Female	Daily Dose Level[a,b] (mg/kg/day)	Installment Dose level[a,b] (mg/kg/dose)	Dose Concentration[a,b] (mg/mL)	Daily Dose Volume (mL/kg/day)	Installment Dose Volume (mL/kg/dose)	Dosing Schedule Days of Gestation
Main study animals							
1 (Control)[c]	20	0	0	0	5	2.5	7-20
2 (Low)	20	0.5	0.25	0.1	5	2.5	7-20
3 (Mid)	20	1.5	0.75	0.3	5	2.5	7-20
4 (High)	20	3	1.5	0.6	5	2.5	7-20
Toxicokinetic animals							
5 (Low)	3	0.5	0.25	0.1	5	2.5	7-20
6 (Mid)	3	1.5	0.75	0.3	5	2.5	7-20
7 (High)	3	3	1.5	0.6	5	2.5	7-20

a Doses were expressed as the free acid of UT-15C (the conversion factor from free acid to the salt [i.e., UT-15C for dose administration] is 1.269).
b Animals were dosed at a volume of 2.5 mL/kg/dose; animals were dosed two times daily (5 mL/kg/day), with approximately 6 hours between doses, at approximately the same time of the day for at least 14 days (see Dose Administration).
c Control animals were dosed with the vehicle using the same dosing regimen.

At the initiation of treatment, the animals were about 5 to 6 moths old and their body weights ranged from 3068 to 3852 g. Female rabbits were individually housed in suspended, stainless cages, and provided food and water *ad libitum*.

Dose Levels and Mode of Administration: Treprostinil diethanolamine (Lot No. D-129-053; purity 99.3%), dissolved in the vehicle (sterile water for injection), was administered by oral gavage twice daily (6 hours apart), on gestation days 7 through 20, at dose levels of 0 (vehicle), 0.5, 1.5 and 3.0 mg/kg/day (expressed as free acid) and at a dose volume of 5 mL/kg/day.

[Doses were selected based on the findings of a previous oral gavage dose-range finding developmental toxicity study in rabbits. In that study, groups (6/group) of time-mated, female Hra: (NZW) SPF rabbits were given oral administration of UT-15C at 0, 5, 10, 15 and 20 mg/ kg/day, twice daily, on gestation days 7 through 20 . All rabbits in the 10, 15 and 20 mg/kg/day groups were found dead or moribund and removed from the study on GD 8 or 9. In the 5 mg/kg/day group, 3 rabbits were found dead (GDs 9 and 20). Due to severe body weight loss, the remaining rabbits from this group were killed on GD 21. There were no remarkable findings at necropsy. Because of high mortality, additional groups of rabbits were added to the study, and were given UT-15C at 0, 0.5, 1, 2 and 4 mg/kg/day. One animal each from the 0.5, 2 and 4 mg/kg/day groups was found dead or sacrificed in moribund condition during the latter phase of the study (GD 15-23). Treatment-related clinical observations included thin appearance, red skin, few or no feces and labored/rapid respiration in all treated groups.

The pregnancy rate was 100% for the 0, 0.5, 1 and 2 mg/kg/day groups and 83% for the 4 mg/kg/day group. There was a dose-dependent increase in postimplantation loss in treated groups. Two dams in the 4 mg/kg/day group had litters with no viable fetuses, which resulted in an increased mean postimplantation loss and a decreased mean number of live fetuses. The mean fetal weight was slightly decreased at 4 mg/kg/day.

There were no fetal external variations or malformations in the litters of treated dams.

Based on the results of this study, the 3 mg/kg/day dose was selected as the high dose for the definitive developmental toxicity study in rabbits.]

Dosing Formulations and Stability: Dosing formulations were prepared weekly. Stability was previously established for up to 30 days under room temperature conditions.

Observations and Measurements: *Clinical Signs*: All animals were checked twice daily for mortality, abnormalities and signs of toxicity. Detailed examinations were done for main study animals at body weight intervals. On dosing days, cageside observations were done for main study animals about 1 hr postdose after each dose.

Body Weights: All animals were weighed on GD 4, 7, 9, 11, 13, 15, 18, 21, 24, 27, and 29 (main study animals only on GD 24, 27 and 29).

Food Consumption: Beginning on GD 4, food consumption was measured at body weight intervals.

Toxicokinetic Evaluation: On GD 20, TK animals were bled predose and at 1, 2, 3. 4, 6, 7, 8, 9, 10, 12 and 24 hours postdose.

Necropsy and Fetal Examination: A complete necropsy was done on animals that died or were sacrificed at an unscheduled interval. Cesarean section was performed on all surviving animals on GD 29. Gravid uterus was weighed and the uterine contents were examined. Each fetus (live or dead) was weighed and examined for external abnormalities. Live fetuses were sacrificed and the contents of the cranium were examined. The internal organs of the thoracic and abdominal cavities of all fetuses were examined, and the sex of each fetus was determined. Viscera was then discarded. Carcasses were processed for skeletal examination using the Alizarin Red S staining method.

Findings were judged to be variations or malformations. All fetuses were retained in Bouin's fixative or glycerin with thymol.

Abnormal viscera were preserved in 10% neutral-buffered formalin.

Statistical Evaluation: One-way analysis of variance (ANOVA) was used to analyze the data. Levene's test was done to test for variance homogeneity. In case of significant heterogeneity of variance, rank transformation was used. If the ANOVA was significant, Dunnett's test was used for control versus treated groups comparisons.

Results

Maternal Toxicity

There were 7 unscheduled deaths during the study. One high dose rabbit was found dead on GD 9, and 4 were sacrificed after aborting during GD 25 to 27. Two mid dose rabbits were found dead on GD 12 and 14, respectively. Prior to death, notable clinical observations included few or no feces, soft feces, thin appearance, red/black vaginal discharge, and postdose observations of cyanosis, hypoactivity, white discharge (eyes and nose), rapid/audible/labored respiration and red skin (ears). There were no remarkable necropsy findings.

For the remaining rabbits, the postdose clinical observations of rapid respiration and red skin (ears) were more pronounced at the high dose.

A significant reduction in body weight, relative to control, was noted in all drug treated groups during dosing phase (GD 9-21). Mean body weight reduction correlated with a significant dose-dependent decrease in food consumption. During the postdose phase (GD 21-29) the effects on food consumption were reversed, which resulted in increased body weight gain. By the end of the postdose phase (GD 29), mean body weights were generally similar across all groups except for the high dose group, in which the value remained significantly lower (8.4%) than control.

There were no remarkable maternal necropsy findings.

All animals except for one each in the 0.5, 1.5 and 3.0 mg/kg/day groups were pregnant.

Four pregnant does aborted in the high dose group, and another 4 pregnant does in this group had litters with no viable fetuses.

Developmental Toxicology

Cesarean section data are provided in the following Tables. The numbers of corpora lutea, implantation sites and the preimplantation loss were generally similar across all groups. There was a significant increase in postimplantation loss at the high dose, which was attributed to the significantly higher number of early resorptions many of which were from dams with no viable fetuses. The mean number of live fetuses was significantly lower at the high dose. Covariate-adjusted mean fetal weights were significantly decreased in all treated groups.

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Table 7
Summary of Cesarean Section Data

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Females Mated	N	20	20	20	20
Pregnant	N	20	19	19	19
	%	100	95	95	95
Aborted	N	0	0	0	4
	%	0.0	0.0	0.0	20
Died	N	0	0	2	1
	%	0.0	0.0	10	5.0
Delivered Early	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Pregnant at C-section	N	20	19	17	14
Dams with Viable Fetuses	N	20	19	17	10
	%	100	100	100	71
Dams with no Viable Fetuses	N	0	0	0	4
	%	0.0	0.0	0.0	29
Corpora Lutea	MEAN	9.0	9.4	9.8	8.9
	S.D.	2.6	2.0	1.9	2.7
	N	20	19	17	14
	TOTAL	179	179	166	125
Implantation Sites	MEAN	8.1	8.6	8.8	7.8
	S.D.	2.4	1.7	1.9	2.3
	N	20	19	17	14
	TOTAL	161	163	150	109
Preimplantation Loss	MEAN%	10.4	8.4	9.3	11.9
	S.D.	11.8	11.3	13.0	15.5

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

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TABLE 7
SUMMARY OF CESAREAN SECTION DATA

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Pregnant at C-section	N	20	19	17	14
Resorptions: Total	MEAN	0.5	0.3	0.6	3.1
	S.D.	0.6	0.7	1.3	3.3
	N	20	19	17	14
	TOTAL	10	5	11	44
	MEAN%	6.8	2.7	6.6	43.9*
	S.D.	8.7	6.6	12.5	42.1
Early	MEAN	0.4	0.2	0.5	3.0
	S.D.	0.6	0.6	1.3	3.4
	N	20	19	17	14
	TOTAL	7	4	8	42
	MEAN%	5.3	2.0	4.6	42.4*
	S.D.	8.8	6.0	11.4	42.9
Late	MEAN	0.2	0.1	0.2	0.1
	S.D.	0.4	0.2	0.4	0.4
	N	20	19	17	14
	TOTAL	3	1	3	2
	MEAN%	1.5	0.8	2.0	1.4
	S.D.	3.8	3.3	4.6	3.7
Dead Fetuses	TOTAL	0	0	0	0
Postimplantation Loss	MEAN%	6.8	2.7	6.6	43.9*
	S.D.	8.7	6.6	12.5	42.1

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

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TABLE 7
SUMMARY OF CESAREAN SECTION DATA

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Pregnant at C-section	N	20	19	17	14
Live Fetuses	MEAN	7.6	8.3	8.2	4.6
	S.D.	2.5	1.6	1.9	3.9
	N	20	19	17	14
	TOTAL	151	158	139	65
	MEAN%	93.2	97.3	93.4	56.1*
	S.D.	8.7	6.6	12.5	42.1
Females	MEAN	3.9	3.9	4.4	3.1
	S.D.	2.0	1.5	1.8	1.7
	N	20	19	17	10
	TOTAL	78	74	75	31
	MEAN%	52.3	46.4	54.0	47.9
	S.D.	17.8	13.4	17.2	26.5
Males	MEAN	3.7	4.4	3.8	3.4
	S.D.	1.5	1.3	1.6	1.9
	N	20	19	17	10
	TOTAL	73	84	64	34
	MEAN%	47.7	53.6	46.0	52.1
	S.D.	17.8	13.4	17.2	26.5
Sex Ratio M:F		48:52	53:47	46:54	52:48

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

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TABLE 7
SUMMARY OF CESAREAN SECTION DATA

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Pregnant at C-section	N	20	19	17	14
Dams with Viable Fetuses	N	20	19	17	10
Resorptions: Total	MEAN	0.5	0.3	0.6	1.5
	S.D.	0.6	0.7	1.3	1.6
	N	20	19	17	10
	TOTAL	10	5	11	15
	MEAN%	6.8	2.7	6.6	21.4
	S.D.	8.7	6.6	12.5	24.6
Early	MEAN	0.4	0.2	0.5	1.3
	S.D.	0.6	0.6	1.3	1.6
	N	20	19	17	10
	TOTAL	7	4	8	13
	MEAN%	5.3	2.0	4.6	19.4
	S.D.	8.8	6.0	11.4	24.5
Late	MEAN	0.2	0.1	0.2	0.2
	S.D.	0.4	0.2	0.4	0.4
	N	20	19	17	10
	TOTAL	3	1	3	2
	MEAN%	1.5	0.8	2.0	2.0
	S.D.	3.8	3.3	4.6	4.3
Dead Fetuses	TOTAL	0	0	0	0
Postimplantation Loss	MEAN%	6.8	2.7	6.6	21.4
	S.D.	8.7	6.6	12.5	24.6

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
MEANS CALCULATED EXCLUDING DAMS WITH NO VIABLE FETUSES.

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TABLE 7
SUMMARY OF CESAREAN SECTION DATA

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Pregnant at C-section	N	20	19	17	14
Dams with Viable Fetuses	N	20	19	17	10
Live Fetuses	MEAN	7.6	8.3	8.2	6.5
	S.D.	2.5	1.6	1.9	3.0
	N	20	19	17	10
	TOTAL	151	158	139	65
	MEAN%	93.2	97.3	93.4	78.6
	S.D.	8.7	6.6	12.5	24.6
Females	MEAN	3.9	3.9	4.4	3.1
	S.D.	2.0	1.5	1.8	1.7
	N	20	19	17	10
	TOTAL	78	74	75	31
	MEAN%	52.3	46.4	54.0	47.9
	S.D.	17.8	13.4	17.2	26.5
Males	MEAN	3.7	4.4	3.8	3.4
	S.D.	1.5	1.3	1.6	1.9
	N	20	19	17	10
	TOTAL	73	84	64	34
	MEAN%	47.7	53.6	46.0	52.1
	S.D.	17.8	13.4	17.2	26.5
Sex Ratio M:F		48:52	53:47	46:54	52:48

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
MEANS CALCULATED EXCLUDING DAMS WITH NO VIABLE FETUSES.

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Table 8
Summary of Mean Fetal Weights (g)

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
of all Viable Fetuses	MEAN	42.43	37.13	35.20	36.96
	S.D.	6.13	4.71	3.29	4.02
	N	20	19	17	10
Covariate Adjusted	MEAN	42.20	37.70**	35.62**	35.63**
of Male Fetuses	MEAN	41.19	37.52	34.81	37.15
	S.D.	6.86	5.09	3.63	4.96
	N	19	19	17	9
Covariate Adjusted	MEAN	41.08	37.87	35.03**	36.22*
of Female Fetuses	MEAN	42.81	36.57	35.76	36.14
	S.D.	5.86	4.55	4.49	4.73
	N	20	19	17	9
Covariate Adjusted	MEAN	42.46	37.08**	36.12**	35.17**

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

There were no external fetal variations.

The fetal and litter incidences of soft tissue variations are given below.

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Table 11
Summary of Fetal Soft Tissue Variations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Litters Evaluated	N	20	19	17	10
Fetuses Evaluated	N	151	158	139	65
Live	N	151	158	139	65
Dead	N	0	0	0	0
VARIATIONS OF THE MAJOR VESSELS					
Fetal Incidence	N	3	2	2	1
	%	2.0	1.3	1.4	1.5
Litter Incidence	N	3	2	2	1
	%	15	11	12	10
INTERMEDIATE LOBE OF LUNG SMALL/MISSING					
Fetal Incidence	N	1	1	3	0
	%	0.7	0.6	2.2	0.0
Litter Incidence	N	1	1	3	0
	%	5.0	5.3	18	0.0
GALL BLADDER SMALL					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.6	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	5.3	0.0	0.0
MALPOSITIONED KIDNEY(S)					
Fetal Incidence	N	1	0	0	0
	%	0.7	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	5.0	0.0	0.0	0.0
DILATED URETER(S)					
Fetal Incidence	N	1	0	0	1
	%	0.7	0.0	0.0	1.5
Litter Incidence	N	1	0	0	1
	%	5.0	0.0	0.0	10

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
N = NUMBER

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TABLE 11
SUMMARY OF FETAL SOFT TISSUE VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Litters Evaluated	N	20	19	17	10
Fetuses Evaluated	N	151	158	139	65
Live	N	151	158	139	65
Dead	N	0	0	0	0
TOTAL FETAL SOFT TISSUE VARIATIONS					
Fetal Incidence	N	6	4	5	2
	%	4.0	2.5	3.6	3.1
Litter Incidence	N	6	3	4	2
	%	30	16	24	20

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
N = NUMBER

The total fetal and litter incidences of soft tissue variations were similar across control and treated groups. The incidences of variations observed (major vessels, small/missing intermediate lobe of lung, small gallbladder, malpositioned kidneys and dilated ureters) were not dose related and they were also seen in controls.

The total fetal incidences of skeletal variations were significantly increased at mid and high dose levels.

The following variations were significantly increased:

- Fetal incidence of 26 presacral vertebrae at 0.5, 1.5 and 3.0 mg/kg/day
- Fetal incidence of less than 16 caudal vertebrae ossified at 3.0 mg/kg/day
- Fetal incidence of 6th sternebra unossified at 0.5 and 3.0 mg/kg/day
- Fetal incidence of 5th sternebra unossified at 0.5, 1.5 and 3.0 mg/kg/day
- Litter incidence of 5th sternebra unossified at 0.5 mg/kg/day
- Fetal incidence of 5th/6th sternebrae bipartite at 3.0 mg/kg/day
- Fetal incidence of 13th full rib at 1.5 and 3.0 mg/kg/day
- Fetal incidence of 13th rudimentary rib at 1.5 and 3 mg/kg/day
- Fetal and litter incidence of 13th unilateral full rib at 3.0 mg/kg/day

According to the sponsor, since the above increases in variations correlated with the significant decrease in fetal weight, they are considered to represent a delay in fetal development and are not considered a direct effect of the test article.

Incidences of skeletal variations are given below.

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Table 13
Summary of Fetal Skeletal Variations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Litters Evaluated	N	20	19	17	10
Fetuses Evaluated	N	151	158	139	65
Live	N	151	158	139	65
Dead	N	0	0	0	0
UNOSSIFIED HYOID BODY					
Fetal Incidence	N	3	6	4	4
	%	2.0	3.8	2.9	6.2
Litter Incidence	N	2	5	4	2
	%	10	26	24	20
UNOSSIFIED HYOID WING(S)					
Fetal Incidence +	N	0	1	0	2
	%	0.0	0.6	0.0	3.1
Litter Incidence	N	0	1	0	1
	%	0.0	5.3	0.0	10
ANGULATED HYOID WING(S)					
Fetal Incidence	N	1	3	5	2
	%	0.7	1.9	3.6	3.1
Litter Incidence	N	1	3	4	2
	%	5.0	16	24	20
ACCESSORY BONE(S) IN SKULL					
Fetal Incidence	N	0	2	4	1
	%	0.0	1.3	2.9	1.5
Litter Incidence	N	0	2	3	1
	%	0.0	11	18	10
INCOMPLETE OSSIFICATION OF SKULL					
Fetal Incidence	N	0	1	1	0
	%	0.0	0.6	0.7	0.0
Litter Incidence	N	0	1	1	0
	%	0.0	5.3	5.9	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
+ = SIGNIFICANT POSITIVE TREND.
N = NUMBER

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TABLE 13
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Litters Evaluated	N	20	19	17	10
Fetuses Evaluated	N	151	158	139	65
Live	N	151	158	139	65
Dead	N	0	0	0	0
26 PRESACRAL VERTEBRAE					
Fetal Incidence +	N	30	50	62	36
	%	20	32*	45**	55**
Litter Incidence	N	13	14	15	8
	%	65	74	88	80
LESS THAN 16 CAUDAL VERTEBRAE OSSIFIED					
Fetal Incidence	N	7	16	9	9
	%	4.6	10	6.5	14*
Litter Incidence	N	6	9	7	6
	%	30	47	41	60
INCOMPLETE OSSIFICATION OF VERTEBRAL CENTRUM(A)					
Fetal Incidence	N	0	2	1	0
	%	0.0	1.3	0.7	0.0
Litter Incidence	N	0	2	1	0
	%	0.0	11	5.9	0.0
BIPARTITE VERTEBRAL CENTRUM(A)					
Fetal Incidence +	N	0	0	0	2
	%	0.0	0.0	0.0	3.1
Litter Incidence +	N	0	0	0	2
	%	0.0	0.0	0.0	20
HEMICENTRUM(A)					
Fetal Incidence +	N	0	0	0	2
	%	0.0	0.0	0.0	3.1
Litter Incidence +	N	0	0	0	2
	%	0.0	0.0	0.0	20

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
+ = SIGNIFICANT POSITIVE TREND.
N = NUMBER

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TABLE 13
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Litters Evaluated	N	20	19	17	10
Fetuses Evaluated	N	151	158	139	65
Live	N	151	158	139	65
Dead	N	0	0	0	0
UNOSSIFIED VERTEBRAL CENTRUM(A)					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.7	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	5.9	0.0
5TH/6TH STERNEBRA(E) INCOMPLETE OSSIFICATION					
Fetal Incidence	N	38	44	29	20
	%	25	28	21	31
Litter Incidence	N	16	16	13	6
	%	80	84	76	60
6TH STERNEBRA UNOSSIFIED					
Fetal Incidence +	N	8	36	13	26
	%	5.3	23**	9.4	40**
Litter Incidence	N	5	10	8	6
	%	25	53	47	60
5TH STERNEBRA UNOSSIFIED					
Fetal Incidence +	N	6	17	17	9
	%	4.0	11*	12**	14*
Litter Incidence	N	3	10	5	5
	%	15	53*	29	50
STERNEBRA(E) ASYMMETRICALLY OSSIFIED					
Fetal Incidence	N	2	4	0	1
	%	1.3	2.5	0.0	1.5
Litter Incidence	N	2	4	0	1
	%	10	21	0.0	10

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
+ = SIGNIFICANT POSITIVE TREND.
N = NUMBER

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TABLE 13
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Litters Evaluated	N	20	19	17	10
Fetuses Evaluated	N	151	158	139	65
Live	N	151	158	139	65
Dead	N	0	0	0	0
MINOR FUSION OF STERNEBRAE					
Fetal Incidence	N	1	3	4	0
	%	0.7	1.9	2.9	0.0
Litter Incidence	N	1	3	3	0
	%	5.0	16	18	0.0
OTHER STERNEBRA(E) INCOMPLETE OSSIFICATION					
Fetal Incidence	N	1	3	2	1
	%	0.7	1.9	1.4	1.5
Litter Incidence	N	1	1	2	1
	%	5.0	5.3	12	10
STERNEBRAE EXTRA OSSIFICATION SITE(S)					
Fetal Incidence	N	2	1	2	2
	%	1.3	0.6	1.4	3.1
Litter Incidence	N	2	1	1	1
	%	10	5.3	5.9	10
5TH/6TH STERNEBRA(E) BIPARTITE					
Fetal Incidence +	N	0	0	0	3
	%	0.0	0.0	0.0	4.6*
Litter Incidence +	N	0	0	0	2
	%	0.0	0.0	0.0	20
STERNEBRA(E) BIFURCATED					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.7	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	5.9	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
+ = SIGNIFICANT POSITIVE TREND.
N = NUMBER

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TABLE 13
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Litters Evaluated	N	20	19	17	10
Fetuses Evaluated	N	151	158	139	65
Live	N	151	158	139	65
Dead	N	0	0	0	0
SPLIT STERNEBRA(E)					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.7	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	5.9	0.0
13TH FULL RIB(S)					
Fetal Incidence +	N	74	88	101	54
	%	49	56	73**	83**
Litter Incidence	N	18	17	17	9
	%	90	89	100	90
13TH RUDIMENTARY RIB(S)					
Fetal Incidence -	N	28	19	11	2
	%	19	12	7.9**	3.1**
Litter Incidence -	N	13	11	7	2
	%	65	58	41	20*
13TH UNILATERAL FULL RIB					
Fetal Incidence +	N	2	2	4	5
	%	1.3	1.3	2.9	7.7*
Litter Incidence +	N	2	2	3	5
	%	10	11	18	50*
14TH RUDIMENTARY RIB(S)					
Fetal Incidence	N	1	0	0	0
	%	0.7	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	5.0	0.0	0.0	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
+ = SIGNIFICANT POSITIVE TREND.
- = SIGNIFICANT NEGATIVE TREND.
N = NUMBER

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TABLE 13
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Litters Evaluated	N	20	19	17	10
Fetuses Evaluated	N	151	158	139	65
Live	N	151	158	139	65
Dead	N	0	0	0	0
7TH CERVICAL RIB(S)					
Fetal Incidence	N	0	2	2	0
	%	0.0	1.3	1.4	0.0
Litter Incidence	N	0	1	1	0
	%	0.0	5.3	5.9	0.0
DETACHED RIB(S)					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.7	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	5.9	0.0
UNOSSIFIED TALUS(I)					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.6	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	5.3	0.0	0.0
UNOSSIFIED TALUS(I)					
Fetal Incidence	N	1	1	2	0
	%	0.7	0.6	1.4	0.0
Litter Incidence	N	1	1	2	0
	%	5.0	5.3	12	0.0
TOTAL FETAL SKELETAL VARIATIONS					
Fetal Incidence +	N	127	139	133	64
	%	84	88	96**	98**
Litter Incidence	N	20	19	17	10
	%	100	100	100	100

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
+ = SIGNIFICANT POSITIVE TREND.
N = NUMBER

External fetal malformations included a single incidence of filamentous tail and a rudimentary tail in two different high dose fetuses from the same litter. Rudimentary tails were also found in two mid dose fetuses from different litters.

Soft tissue malformations were limited to a diaphragmatic hernia in one control fetus, and an internal hydrocephaly in one mid dose fetus.

The total fetal skeletal malformations were significantly increased ($p \leq 0.05$) at mid and high dose levels. There was a positive trend for increased fetal and litter incidence of misaligned, fused, and/or absent caudal vertebrae in the higher dose groups. Other skeletal malformations were limited to vertebral anomaly with/without associated rib anomaly, misaligned lumbar vertebrae, major fusion of sternebrae and forked/fused ribs among the drug treated groups.

In summary, the sponsor concluded that the above external, soft tissue and skeletal malformations were attributed to treatment with UT-15C. These malformations are presented in the following Tables.

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Table 10
Summary of Fetal External Malformations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Litters Evaluated	N	20	19	17	10
Fetuses Evaluated	N	151	158	139	65
Live	N	151	158	139	65
Dead	N	0	0	0	0
FILAMENTOUS TAIL					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	1.5
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	10
RUDIMENTARY TAIL					
Fetal Incidence	N	0	0	2	1
	%	0.0	0.0	1.4	1.5
Litter Incidence	N	0	0	2	1
	%	0.0	0.0	12	10
TOTAL FETAL EXTERNAL MALFORMATIONS					
Fetal Incidence +	N	0	0	2	2
	%	0.0	0.0	1.4	3.1
Litter Incidence	N	0	0	2	1
	%	0.0	0.0	12	10

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
+ = SIGNIFICANT POSITIVE TREND.
N = NUMBER

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Table 12
Summary of Fetal Soft Tissue Malformations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Litters Evaluated	N	20	19	17	10
Fetuses Evaluated	N	151	158	139	65
Live	N	151	158	139	65
Dead	N	0	0	0	0
INTERNAL HYDROCEPHALY					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.7	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	5.9	0.0
DIAPHRAGMATIC HERNIA					
Fetal Incidence	N	1	0	0	0
	%	0.7	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	5.0	0.0	0.0	0.0
TOTAL FETAL SOFT TISSUE MALFORMATIONS					
Fetal Incidence	N	1	0	1	0
	%	0.7	0.0	0.7	0.0
Litter Incidence	N	1	0	1	0
	%	5.0	0.0	5.9	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
N = NUMBER

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Table 14
Summary of Fetal Skeletal Malformations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Litters Evaluated	N	20	19	17	10
Fetuses Evaluated	N	151	158	139	65
Live	N	151	158	139	65
Dead	N	0	0	0	0
MISALIGNED, FUSED AND/OR ABSENT CAUDAL VERTEBRA(E)					
Fetal Incidence +	N	1	0	4	3
	%	0.7	0.0	2.9	4.6
Litter Incidence +	N	1	0	3	2
	%	5.0	0.0	18	20
VERTEBRAL ANOMALY WITH/WITHOUT ASSOCIATED RIB ANOMALY					
Fetal Incidence	N	0	3	0	1
	%	0.0	1.9	0.0	1.5
Litter Incidence	N	0	2	0	1
	%	0.0	11	0.0	10
MISALIGNED LUMBAR VERTEBRA(E)					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.7	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	5.9	0.0
MAJOR FUSION OF STERNEBRAE					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.7	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	5.9	0.0
FORKED/FUSED RIB(S)					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.6	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	5.3	0.0	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
+ = SIGNIFICANT POSITIVE TREND.
N = NUMBER

Covance 7049-121

TABLE 14
SUMMARY OF FETAL SKELETAL MALFORMATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 0.5 MG/KG/DAY	GROUP 3 1.5 MG/KG/DAY	GROUP 4 3 MG/KG/DAY
Litters Evaluated	N	20	19	17	10
Fetuses Evaluated	N	151	158	139	65
Live	N	151	158	139	65
Dead	N	0	0	0	0
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence +	N	1	4	6	4
	%	0.7	2.5	4.3*	6.2*
Litter Incidence	N	1	2	5	2
	%	5.0	11	29	20

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
+ = SIGNIFICANT POSITIVE TREND.
N = NUMBER

Pharmacokinetics:

The pharmacokinetic parameters on gestation day 20 in female rabbits are given below.

Table 2: Summary of Treprostinil Pharmacokinetic Parameters in Female Rabbits on Gestation Day 20 by Dose Group

	0.5 mg/kg/day		1.5 mg/kg/day		3.0 mg/kg/day	
Parameter	Mean	SD	Mean	SD	Mean	SD
C_{max1} (ng/mL)	9.3	2.7	16.6	5.4	32.8	3.7
T_{max1} (hr)	1.7	1.2	1.0	0.0	1.7	1.2
C_{max2} (ng/mL)	10.4	2.6	20.3	5.1	40.6	4.5
T_{max2} (hr)	7.0	0.0	8.3	1.5	7.0	0.0
AUC_{ss} (hr*ng/mL)	128.9	1.7	278.7	52.2	582.1	89.1
CL/F(mL/min/kg)	64.7	0.8	92.1	19.2	87.4	14.7
V_z/F(L/kg)	53.8	27.3	47.8	11.9	59.2	12
$T_{1/2}$ (hr)	9.6	4.9	6	0.2	7.9	1.7

Mean treprostinil Cmax1 and Cmax2 increased in dose-related manner. Mean Tmax1 values (range of 1.0 to 1.7 hr after the first daily dose) appeared to be less varied compared to Tmax2 values (slightly wider range of 1.0 to 2.3 hr after the second daily dose). The steady state AUC values on gestation day 20 increased less than proportionally versus total daily dose. The less than dose proportional increase in both Cmax and AUC values was suggestive of absorption limited pharmacokinetics over the dosage range of 0.5 to 3.0 mg/kg/day.

The no observable adverse effect level (NOAEL) for UT-15C when administered twice daily via oral gavage to pregnant rabbits during the period of organogenesis is < 0.5 mg/kg/day for maternal toxicity. The NOAEL for fetal viability and growth is 1.5 mg/kg/day based on increased post implantation loss observed at 3.0 mg/kg/day. The NOAEL for teratogenicity is 0.5 mg/kg/day.

Pre-and Post-Natal Development Study in Rats

Testing Facility: (b) (4)

Study Number: (b) (4) (contract Lab's number)

Study Dates: Initiation Date – May 23, 2007
Completion Date – November 12, 2008

GLP Compliance: The study was conducted in accordance with the FDA GLP Regulations , Title 21 of the U.S.CFR part 58 and Japanese Ministry of Health, Labor and Welfare GLP Standards Ordinance No. 21.

QA Report: yes

Animals: Time-mated female Crl:CD®(SD) rats were received from (b) (4) The females were mated at the supplier and the day of confirmation of mating was designated as gestation day (GD) 0. The females were received on or prior to GD 4. At the initiation of treatment, the animals were 10 to 12 weeks old, and their body weights ranged from 207 to 253 g. Animals were randomly assigned to 4 main study groups (25 rats/group). Additional time-mated rats were assigned for toxicokinetic (TK) analyses (12/group). Animals were assigned to the study as follows.

Group Designation and Dose Levels

Group	No. of Animals Female	Daily Dose Level [a,b] (mg/kg/day)	Installment Dose level [a,b] (mg/kg/dose)	Dose Concentration [a,b] (mg/mL)	Daily Dose Volume (mL/kg/day)	Installment Dose Volume (mL/kg/dose)	Dosing Schedule
			Main Study Animals				
1 (Control) [c]	25	0	0	0	10	5	GD6-LD20
2 (Low)	25	2.5	1.25	0.25	10	5	GD6-LD20
3 (Mid)	25	5	2.5	0.5	10	5	GD6-LD20
4 (High)	25	10	5	1	10	5	GD6-LD20
			TK animals				
5 (Control)[c]	12	0	0	0	10	5	GD6-LD20
6 (Low)	12	2.5	1.25	0.25	10	5	GD6-LD20
7 (Mid)	12	5	2.5	0.5	10	5	GD6-LD20
8 (High)	12	10	5	1	10	5	GD6-LD20

a Doses were expressed as treprostinil, the free acid of UT-15C [conversion factor from free acid to the salt (i.e., UT-15C for dose administration) is 1.269].
b Animals were dosed at a volume of 5 mL/kg/dose; animals were dosed two times daily (10 mL/kg/day), approximately 6 hours apart between doses, at approximately the same time of the day.
c Control animals were dosed with the vehicle using the same dosing regimen.

Female rats were individually housed in stainless steel cages, except during mating and lactation. Certified Rodent Diet #8728C (Harlan Teklad) and water were provided ad libitum.

Dose Levels and Mode of Administration: Treprostinil diethanolamine (UT-15C; Lot No. DB-R06017 - purity 99.3%), dissolved in the vehicle article (sterile water for injection), was administered by oral gavage twice daily (6 hours apart), beginning on gestation day 6 and continuing through lactation day (LD) 20, at dose levels of 0 (vehicle), 2.5, 5, and 10 mg/kg/day (expressed as free acid). The drug was administered at a dose volume of 10 mL/kg/day.

[Doses were selected based on the findings in a previous developmental toxicity study in rats. In that study, the NOAEL for maternal toxicity was 5 mg/kg/day. The NOEL for fetal viability and growth was 5 mg/kg/day based on increased postimplantation loss observed at 10 mg/kg/day. The NOAEL for teratogenicity was 20 mg/kg/day. In this present study, it was expected that the high dose would show drug-specific effects.]

Dosing Formulations and Stability: Dosing formulations were prepared weekly. Stability was previously established for up to 30 days under room temperature conditions.

Observations and Measurements:

F0 Maternal Animals

Clinical signs: Animals were checked twice daily for mortality, abnormalities and signs of toxicity. Detailed observations were done at each body weight recording interval during the study. For the main study animals, daily cageside observations were done one hour postdose after each dose for the first 2 weeks of dosing and then twice weekly for the remaining period of dosing.

Body Weights: Body weights were recorded on GD 0, 4, 6, 8, 10, 12, 14, 16, 18, and 20 and LD 0, 4, 7, 10, 14, 17 and 21.

Food Consumption: Food consumption of F0 females was recorded during gestation at body weight intervals beginning GD 4. Food consumption was not measured during lactation.

Natural Delivery: F0 females were allowed to deliver naturally. F0 dams that delivered naturally were sacrificed soon after their litters were weaned (Day 21 postpartum) or after a total litter death. If a F0 female failed to produce a viable litter by GD 26, the female was sacrificed. Complete necropsies were conducted on these animals. Abnormal tissues were preserved in 10% neutral-buffered formalin.

F1 Progeny

After the completion of delivery, each live and dead pup was sexed. All dead pups were examined for cervical, thoracic and abdominal viscera abnormalities and preserved in alcohol.

On LD 0, live pups were weighed and examined for external abnormalities. On LD 4, the size of each litter was adjusted to 8 pups (4 per sex, where possible), to reduce possible confounding effects resulting from different litter sizes. On LD 4, 7, 14 and 21, the number of live pups/sex/litter, body weights of all pups, and clinical signs were recorded.

During lactation period, the general growth and physical development were monitored as follows (day testing started): pinna unfolding on Day 1, surface righting reflex on Day 4, hair growth and incisor eruption on Day 7, eye opening on Day 11, and auditory startle on Day 21.

Pups were weaned on LD 21, and 20 pups/sex/group (1 pup/sex/litter) were selected for the F1 maturation phase (7 weeks). Testing for vaginal opening was done beginning on postpartum Day (ppd) 30 and cleavage of the balanopreputial gland testing was conducted beginning on ppd 35.

During the maturation phase, animals were examined at each body weight interval. Body weights of F1 males and females were recorded during maturation and breeding, and in females on GD 0, 7, 14 and 20 and LD 0.

After completion of the 7-week maturation phase, F1 animals were mated, and the day in which copulatory plug or sperm was observed was designated as GD 0.

F1 females were allowed to deliver naturally. The date of delivery, litter size, sex, weight and observations of individual offspring were recorded.

[*Disposition of F1 Offspring*: Pups that were found dead were examined for cervical, thoracic and abdominal viscera abnormalities and preserved in alcohol. Pups culled on LD 4 were euthanized and examined. All F1 pups not selected for the maturation phase were discarded without necropsy.

Disposition of F1 Parental Animals: Complete necropsy was conducted with special attention given to the organs of the reproductive tract. For females found dead or sacrificed in a moribund condition, the uteri and ovaries were examined for implantation sites and corpora lutea, respectively.

Disposition of F2 Offspring: On LD 1, F2 pups were examined and then euthanized and preserved.]

Results:

F0 Maternal Animals

Two control rats and one rat each from the low and mid dose levels were found not pregnant. There were two unscheduled deaths on GD 22; one mid dose female was found dead (confirmed pregnant at necropsy) and a high dose female died while delivering. Prior to death, no remarkable clinical signs were reported.

Treatment-related clinical signs were limited to red skin of the paws and ears primarily at the high dose from GD13 through 17. The mean body weight was significantly lower ($P < 0.01$; 5 to 7%) than concurrent control for the high dose group on gestation days 8 to 20. Food consumption was significantly decreased for the high dose group during gestation.

During lactation, although mean body weight was significantly decreased for the high dose group on LD 0, effects on body weight change were transient. From LD 4 to 21, significantly increased body weight change was observed for the high dose, and the body weight changes were similar across groups.

On LD 5, a high dose female had a total litter loss. Prior to total litter death, the neonates in this litter were reported as weak, thin and no visible milk in their stomach.

The duration of gestation was significantly increased at the high dose level. Though not statistically significant, there was a slight increase in the number of litters with stillborn pups and slight decreases in the viability index and mean number of pups per litter at the high dose.

Covariate-adjusted mean neonatal body weight reduction was noted for males and females at the high dose; these decreases were significant on LD 14 for both sexes.

There were no treatment-related F0 necropsy findings.

F1 Generation

Developmental landmarks (eye opening, hair growth, incisor eruption, and surface righting and auditory reflexes), exploratory behavior (locomotor activity), learning and memory (water maze), and covariate-adjusted mean age for vaginal opening and preputial separation were unaffected by treatment.

At the beginning of the maturation phase, mean body weights were slightly lower in the high dose F1 males. As the maturation phase progressed, significantly decreased mean body weights were observed in all drug-treated male groups. Mean body weights in F1 females were unaffected by treatment.

The reproductive data indicated that mating behavior (copulation) was decreased in the mid and high dose F1 offspring, but the fertility was not affected.

F1 delivery and F2 pup survival and body weight data were unremarkable as were the F1 male and female, and F2 pup necropsy findings.

Toxicokinetic data (given below) indicated that on both sampling days (GD 6 and LD 20), Cmax and AUC values increased in a dose related manner, but generally less than dose proportionately).

Plasma Treprostinil Pharmacokinetic Parameters in Female Rats

Parameter	Dose[a] (mg/kg/day)	Gestation Day 6	Lactation Day 20
C_{max1} (ng/mL)	2.5	4.59	13.31
	5.0	8.53	18.13
	10.0	9.66	41.9
T_{max1} (hr)	2.5	2.0	2.0
	5.0	6.0	3.0
	10.0	1.0	4.0
C_{max2} (ng/mL)	2.5	5.51	18.45
	5.0	11.14	19.3
	10.0	14.75	30.2
T_{max2} (hr)	2.5	8.0	7.0
	5.0	7.0	8.0
	10.0	9.0	9.0
AUC_{24} (hr*ng/mL)	2.5	70.1	156.6
	5.0	149.9	164.75
	10.0	168.51	385.47
CL/F (mL/min/kg)	2.5	594	266
	5.0	556	506
	10.0	989	432

[a] expressed as treprostinil, the free acid of UT-15C ; administered in two daily doses, six hours apart.

The NOAEL for F0 maternal effects is 5 mg/kg/day (corresponding to a LD 20 AUC and Cmax2 of 164.75 hr*ng/mL and 19.3 ng/mL, respectively). The NOEL for physical development (developmental landmarks), learning and memory, reflex development and sexual maturation is 10 mg/kg/day. The NOEL for reproductive performance is 2.5 mg/kg/day.

Special Toxicology Study

An *in vitro* special toxicology study was conducted (b) (4) to assess the extent of irritation caused by UT-15C sustained release (SR) tablet, 2.5 mg, in the freshly excised rat colon tissue, mounted in a vertical Ussing chamber apparatus.

Colon samples (2 cm^2) from the freshly killed rats were dissected out, and serosal muscle layer was stripped off manually before the tissue being placed in the chamber apparatus. For the tissues exposed to UT-15C SR tablets, each tablet was placed inside the chamber, with the laser drilled aperture facing the mucosal side. Positive control consisted of KCl solutions equivalent to tablet strengths of 750, 375 and 100 mg of KCl dissolved in 5 mL. Blank assay buffer was used as a negative control. Positive control for membrane damage (100% LDH release) was 10% solution of Triton-X 100 in assay buffer. Tissue integrity was assessed by measuring the permeability of atenolol (low permeability marker) and antipyrine (high permeability marker). The permeability was tested in the mucosal to serosal direction. The receiver buffer consisted of blank assay buffer at pH 7.4. The buffer volume on both the donor and receiver sides was 5 ml. Solution mixing in the chambers was achieved by infusing a mixture 5% CO_2 and 95% O_2. The chambers were maintained at a temperature of 37^oC.

The extent of irritation caused by the test drug on the mucosal membrane was assessed by determining the LDH release and by histologic evaluation of tissues. Irritation induced by UT-15C was compared to the positive control or negative control.

The experiment was run for 180 minutes. At the end of the assay, samples for the LDH measurement were collected from donor and receiver sides of the chamber. The tissues were fixed for histopathology evaluation.

The amount of LDH released by tissues exposed to 750 and 375 mg KCl were significantly higher than those measured in samples exposed to 2.5 mg UT-15C SR tablets and negative control. No differences were detected between groups exposed to 100 mg KCl, the test drug or negative control. Because of the autolysis of tissue samples exposed to 750 or 375 mg KCl, histopathologic evaluation of these tissues were not conducted. Histopathology findings in the test drug exposed group were comparable to the negative control group, and severe histopathology lesions were seen in tissues exposed to 100 mg KCl. For the 100 mg KCl treated specimens, it appeared that microscopic examination was a more sensitive indicator of damage than LDH release. Under the conditions of this study, exposure to the 2.5 mg UT-15C SR tablet did not result in increased LDH release or microscopic lesions when compared to positive control. It was not clear why longer treatment times were not used, or how much of the drug was actually released.

Integrated Summary and Safety Evaluation

Treprostinil diethanolamine (UT-15C) is being developed as a sustained release oral tablet for the treatment of pulmonary arterial hypertension (PAH). Treprostinil sodium (Remodulin® Injection), a chemically stable analogue of prostacyclin (PGI_2) with potent vasodilatory as well as platelet antiaggregatory effects, has been approved for chronic administration either by continuous subcutaneous or intravenous infusion for the treatment of PAH. Tyvaso® (treprostinil) Inhalation Solution has also been approved for the treatment of PAH by the inhalation route. An oral formulation of treprostinil will allow patients to benefit from the ease of drug administration and to avoid the adverse effects associated with sc or iv infusion.

The active pharmaceutical ingredient present in treprostinil diethanolamine and treprostinil sodium is shown to be identical. Once in solution, both treprostinil sodium and treprostinil diethanolamine are disassociated from their respective counterions and exist as the ionized form of treprostinil. As a result, the bioactive form present in the bloodstream is identical irrespective of the salt used. Nonclinical studies conducted with treprostinil sodium were previously reviewed under Remodulin and Tyvaso NDAs 21272 and 22387, respectively.

In a comparative study in anesthetized rats, the effects of treprostinil (treprostinil sodium) and UT-15C (treprostinil diethanolamine) on systemic arterial blood pressure and heart rate were investigated. The study was undertaken to determine whether changing the salt form of treprostinil from sodium to diethanolamine would change the bioactivity of treprostinil. Intravenous bolus administration of the maximal doses of treprostinil (100 µg/kg) or UT-15C (126.9 µg/kg, adjusted for its molecular weight) studied caused a fall in MAP of about 75 mmHg within one minute; the magnitude of the response thus being comparable at equimolar doses. The blood pressure responses were dose-related for both compounds. The results indicated that the diethanolamine salt produced a similar cardiovascular profile to treprostinil following iv administration. This study also investigated whether UT-15C was active when administered via the intraduodenal (ID) route. Administration of UT-15C at 507.6 µg, by this route, caused a fall in MAP of about 35 mmHg within 5 minutes of administration, slower than the iv route while the duration of action was longer compared to iv route.

The hemodynamic effects of six metabolites (M334, M388, M392, M566, M348 and M374) identified from human pharmacokinetic studies with treprostinil and UT-15C were evaluated in the same rat model. The objective of the study was to determine the potency and duration of action of these metabolites in causing reductions in systemic MAP and increases in heart rates following bolus iv administration when compared to the activity of UT-15C. Generally, no highly active metabolite of treprostinil or UT-15C was identified, as all the metabolites evaluated had a significantly reduced activity (~ 1000 fold) compared to UT-15C. These results indicated that the pharmacological activity of treprostinil or UT-15C reflects the activity of the parent molecule, treprostinil.

In a cardiovascular safety pharmacology study in male beagle dogs, oral administration of 1 mg/kg/dose UT-15C twice daily was associated with a transient decrease in arterial pressure and increase in heart rate following each dose. Administration of UT-15C at 3 mg/kg/dose twice daily produced an increase in heart rate and a decrease in arterial pressure after each dose. Increased blood pressure (6-7 hours) was noted after the second dose. At 5 mg/kg/dose, twice daily, in dogs produced increased heart rate for about 4 hours following the first dose and for about 15 hours after the second dose. Arterial pressure was decreased after the first dose but increased following the second dose. Transient, minimal, statistically significant increases in QTc (5-7%) were noted following the second dose of 3 mg/kg/dose UT-15C and after first and second doses of 5 mg/kg/dose. Analysis of the ECGs at selected time points did not reveal any changes in cardiac rhythm or ECG morphology. Furthermore, UT-15C did not inhibit hERG-mediated current at doses up to 300 µM, the highest dose used in the study.

Cardiovascular safety studies conducted with diethanolamine alone did not produce any changes in arterial pressure, heart rate, EKG parameters including QTc, at doses up to 4 mg/kg/day, which is greater than the amount of diethanolamine present in the highest UT-15C dose administered in the UT-15C cardiovascular safety pharmacology study. Also, diethanolamine did not inhibit hERG-mediated current at doses up to 300 µM.

No neuropharmacological or toxicological signs or effects upon body temperature were observed in rats receiving UT-15C 3.8 mg/kg by oral administration. Decreased motor activity and statistically significant ($p \leq 0.05$) decrease in body temperature were observed at doses of 12.7 mg/kg and above.

In conscious male rats given oral administration of UT-15C at 1.16mg/kg/day, no effects were seen on any biologically relevant effects on respiration rate, tidal volume or minute volume. Dose related decreases in respiratory rate and minute volume were observed following the oral administration of UT-15C at 2.5, 5, 15 and 25 mg/kg/day. Tidal volume was not affected. The NOEL was found to be 1.16 mg/kg/day.

Oral administration of UT-15C in mice at 8.9, 31.7 or 95.2 mg/kg produced decreases (9, 47 and 42%, respectively) in GI motility when compared to control. The differences from control at the two higher dose levels were statistically significant and considered biologically relevant. The NOAEL was 8.9 mg/kg.

The bioavailability (BA) studies in rats showed that UT-15C has an absolute BA of about 10% when given orally in solution at a dose of 1 mg/kg. Intraportal vein BA was found to be approximately 40%, suggesting a substantial first pass effect. When administered by intraduodenal route, BA was 24%, suggesting that degradation of treprostinil in the stomach or gastric emptying may influence the extent of systemic absorption.

A study using dual-radiolabeled UT-15C was conducted to assess the extent of absorption and tissue distribution in rats following administration of a single oral solution dose. The distribution of drug-derived radioactivity for both labels was widespread. Tissues with highest [14C] radioactivity concentrations of treprostinil were small intestine, liver, large intestine with cecum, and stomach. Radioactivity reached maximum concentrations at 1 to 8 hours post dose in most

tissues. The terminal $t_{1/2}$ values in tissues ranged from 27 hours in thyroid to 879 hours in eyes. The highest [3H] radioactivity concentrations of diethanolamine in tissues were spleen, adrenal glands, thyroid, kidneys and liver. Radioactivity reached maximum concentrations at 8 to 48 hours post dose in most tissues. The terminal $t_{1/2}$ values in tissues ranged from 99 hours in prostate to 179 hours in eyes. Both labels crossed blood/brain and blood/testes barriers.

In vitro protein binding studies using dual-labeled UT-15C showed that [14C]-treprostinil component of UT-15C was highly bound to human plasma proteins with mean binding of approximately 96%, while the [3H]-diethanolamine component was minimally bound to human plasma proteins (mean binding of 6.1%).

In vitro metabolite characterization studies showed that treprostinil was rapidly metabolized by rat, dog and human liver microsomes to five metabolites present in all species. All five metabolites were detected in the liver microsomal incubates of all three species, suggesting similar metabolic potential of treprostinil across these species. The major *in vitro* metabolic routes of treprostinil included hydroxylation (M05, M07 and M08), oxidation of a hydroxyl group to form a keto-moiety (M09), and combination of hydroxylation and oxidation (M06). The metabolic profile of UT-15C was also evaluated in humans following administration of dual labeled UT-15C to male healthy volunteers. Six metabolites (M392, M334, MM348, M374, M566 and M388) were identified. These metabolites were unique to those identified in the liver microsomal study with the exception of M388 which was identical to M09.

Metabolism studies further showed that *in vitro* CYP2C8 was the major isozyme involved in the metabolism of UT-15C (about 95% disappearance of parent) while CYP2C9 played a minor role (about 22% disappearance of parent). All other isozymes tested did not play a significant role in the metabolism of UT-15C.

Nonclinical excretion studies were not conducted with UT-15C. Studies in humans showed that the major route of excretion for the treprostinil and diethanolamine components of UT-15C was via urine (78.2% for treprostinil and 62.1% for diethanolamine) with fecal recovery of treprostinil and diethanolamine accounting for 18.6% and 2.25%, respectively.

In a 13-week oral toxicity in rats, UT-15C was administered by oral gavage twice daily at total dose levels of 0 (water), 10, 20 and 30 mg/kg/day. Due to mortality, the dose level for the high dose females was lowered from 30 to 25 mg/kg/day on Day 6. In the main toxicity phase of the study, there were deaths at the high dose in both sexes during dosing phase. In the TK phase, there were deaths at mid and high dose levels. Histological lesions observed in animals that were found dead or euthanized moribund included focal or multifocal gastric mucosal necrosis with accompanying inflammation, and lymphoid depletion, particularly in the spleen, thymus and lymphoid tissues of the GI tract.

Dose-related clinical signs included decreased activity, red/swollen pinnae, reddened extremities and piloerection. Dose-dependent decreases in platelets and increases in leukocytes were noted in both sexes. Increased urine volumes, compared to controls, were noted in mid and high dose males and females. Grossly, thickening of the intestinal tract and enlarged adrenal and thyroid glands were noted in mid and high dose animals. Histologically, adrenal glands in all high dose

males and females showed minimal to mild hypertrophy of cortical cells. The hypertrophy corresponded to the increased microvesiculation in the adrenal gland and was considered to be an adaptive change to repeated hypotension. In the thyroid gland, a reversible increase of minimal follicular distension was observed in a number of animals from all treated groups, although a dose response was not seen. Myocardial degeneration and mononuclear cell infiltration were observed in control and high dose males, mostly at similar incidence rates (hearts from low and mid-dose groups were not examined). Cardiomyopathy (1/13), hemorrhage (2/13) and thrombosis (2/13) were seen only in high dose males. Myocardial degeneration is common background lesion in male rats. The thrombotic lesions seen in high dose males consisted of mild mural thrombosis in the region of the left atrioventricular valve.

Overall, AUC generally increased in a dose-proportional manner on Days 1 and 90. Female exposure was generally higher than that in males on Day 1, and may be related to the increased mortality seen in females.

A 28-day oral toxicity and toxicokinetic study was conducted in wildtype rasH2 mice, with a preliminary 5-day range-finding study, to select the doses for the 26-week mouse carcinogenicity bioassay. For the range-finding study, groups of mice were given oral gavage administration of UT-15C at dose levels of 0 (water), 10, 75, 150, 250 or 500 mg/kg/day for 5 days. Lethality was observed at doses of 75 mg/kg/day and above. Based on these results, dose levels of 0, 10, 30, 50 and 75 mg/kg/day were used for the 28-day oral toxicity study. Treatment-related mortalities were seen in both sexes at 30 mg/kg/day and above, the incidence being higher in females than in males (at 50 mg/kg/day, 10% death in males and 31% in females). Treatment-related histopathological lesions were observed in the stomach (erosion and inflammation of the glandular part, and hyperplasia and hyperkeratosis of the non-glandular portion), kidney (nephrosis), nasal cavity (exudative inflammation) and testes (testicular degeneration) at 50 mg/kg/day. Toxicokinetic evaluation showed that the exposure was higher in females than in males.

In a 14-day study in dogs, UT-15 SR tablets or SR capsules were administered 3 times a day at total doses of 0, 30, 60 and 120 mg UT-15C/dog/day. Mortality due to GI toxicity was observed at 120 mg/dog/day for both tablets and capsules. GI toxicity was characterized by body weight loss, low food consumption, lethargy, bloody, mucoid, liquid feces, vomiting and dehydration with GI intussusceptions or rectal prolapse seen at necropsy. Hemorrhage, inflammation and congestion were noted microscopically in the GI tract. These treatment-related findings were similar to that seen with Remodulin given sc or iv, and have been previously reported in the Beagle dog as a possible class effect of prostacyclins or prostacyclin analogues in this species. The NOAEL was 30 mg/dog/day or 3 mg/kg/day.

In a 13-week oral toxicity study of UT-15C SR tablets (0, 10, 30 or 50 mg/dog/day) in dogs, a high dose female was euthanized moribund on Day 16 due to GI toxicity. (Intussusceptions was seen at necropsy.) Reversible, dose dependent clinical signs related to GI toxicity observed included vomiting, diarrhea and fecal abnormalities. Treatment-related microscopic lesions were limited to the digestive tract. The GI lesions consisted of moderate, chronic inflammation within the colon accompanied by mild ulceration, congestion and lymphatic dilatation at the site of

intussusception. No other toxicologically significant microscopic lesions were noted. The NOAEL was considered to be 10 mg/dog/day or 1 mg/kg/day.

In a 9-month oral toxicity study, dogs were given UT-15C SR tablets twice daily at doses of 6, 10 and 30 mg/dog/day. Starting on study day 115, the high dose males received 35 mg/dog/day, and beginning on day 127, the low and mid dose males received 8 and 15 mg/dog/day, respectively. There was no mortality in the study. Reversible, test article-related clinical observations included mucoid stools, soft stools, diarrhea in both sexes, and increased salivation and reddened lips in the high dose females. There were no treatment-related findings in ECG, ophthalmic examination, clinical pathology and gross and microscopic evaluations. A significant increase in absolute adrenal weight, not in relative weight, was noted in high dose males. At similar daily dose levels, female dogs had higher systemic exposure than males.

In an *in vivo* genotoxicity assay, oral administration of two equal doses (about 6 hours apart) of UT-15C in Sprague Dawley rats, at total doses of 0, 12.5, 25 and 50 mg/kg, and euthanized 24 and 48 hours after dosing, did not induce a significant increase in the incidence of micronucleated PCEs in either male or female rats.

Oral administration of UT-15C to Tg.rasH2 mice at 5, 10 and 20 mg/kg/day in males and 3, 7.5 and 15 mg/kg/day in females daily for 26 weeks did not significantly increase the incidence of tumors. The exposures obtained at the highest dose levels used in males and females are about 8 and 17-fold, respectively, higher than the human exposure (24.35 ng.h/ml) at the mean dose of 3.4 mg BID.

The effects of UT-15C on fertility and early embryonic development were assessed in rats. Oral administration of UT-15C, twice daily (about 6 hours apart), at doses of 0, 2.5, 5 and 10 mg/kg/day to male and female rats during pre-mating, mating and until termination (males) or through gestation day 7 (females) had no effects on reproductive performance, fertility, or embryo/fetal viability. The mean percent sperm motility and caudal epididymal sperm count were not affected by the drug treatment. The NOAEL for reproductive effects was considered to be 10 mg/kg/day. The exposures obtained at this dose level were about 10 (male) to 18 (female) fold higher than the human exposure (24.35 ng.h/mL) at the mean dose of 3.4 mg BID.

Effects of UT-15C on embryo-fetal development were assessed in pregnant rats. UT-15C was administered by oral gavage, twice daily, on gestation days (GD) 6 through 17, at dose levels of 0, 5, 10 and 20 mg/kg/day. There were four treatment-related deaths in the 20 mg/kg/day group during GD 12 to 14. There were no necropsy findings. For the remaining animals, decreased food consumption and body weight gain were noted at 10 and 20 mg/kg/day. There was a significant increase in post-implantation loss at doses of 10 mg/kg/day and above, which was attributed to increases in early resorptions. A significant decrease in the mean number of live births was noted at dose levels $\geq$ 10 mg/kg/day. There were no fetal external, soft tissue or skeletal anomalies observed in the study. The NOAEL for maternal toxicity was 5 mg/kg/day (13 times the human exposure). The NOEL for fetal viability and growth was 5 mg/kg/day. The NOAEL for teratogenicity was 20 mg/kg/day (55 times the human exposure).

Pregnant rabbits were given oral gavage administration of UT-15C, twice daily, at doses of 0.5, 1.5 or 3 mg/kg/day on GD 7 through 20. There were seven deaths during the study [5 deaths from the high dose group and 2 from the mid dose group]. There were no remarkable necropsy findings. A significant reduction in body weight, relative to control, was noted in all drug treated groups during dosing phase, which correlated with a significant dose-dependent decrease in food consumption. These effects were reversed during the post dose phase. Four pregnant high dose does aborted and another 4 pregnant does in this group had litters with no viable fetuses. There was a significant increase in the post-implantation loss at the high dose, which was attributed to an increase in early resorptions. The mean number of live fetuses was significantly lower at the high dose. Mean fetal weights were significantly decreased in all treated groups. There were no fetal external or soft tissue variations. Treatment-related skeletal variations observed were correlated with significant decreases in fetal weights and were considered to represent a delay in fetal development. External fetal and soft tissue malformations were observed in a few of the 1.5 and 3.0 mg/kg/day fetuses. The total fetal skeletal malformations were significantly increased in the 1.5 and 3.0 mg/kg/day fetuses. Based on these findings, the NOAEL was < 0.5 mg/kg/day for maternal toxicity. The NOAEL for fetal viability and growth was 1.5 mg/kg/day. The NOAEL for fetal development (teratogenicity) was 0.5 mg/kg/day (5 times the human exposure).

In a study to assess the effects of UT-15C on pre- and post-natal development, pregnant rats were given oral gavage administration of UT-15C, twice daily, at doses of 0, 2.5, 5 and 10 mg/kg/day, beginning on GD6 and continuing through lactation day (LD) 20. There were no deaths during the gestation phase. During delivery and lactation, a high dose female died while delivering, and another high dose female had a total litter loss on LD5. The duration of gestation was significantly increased at the high dose level. There was a slight increase in the number of litters with stillborn pups and slight decreases in the viability index and the mean number of pups per litter at the high dose. Mean neonatal body weights for both males and females were decreased in the high dose litters. Physical and reflex development, exploratory behavior, learning and memory, vaginal opening and preputial separation were unaffected by treatment. The mating behavior (copulation) was decreased in the 5 and 10 mg/kg/day F1 offspring, but fertility was not affected. F1 delivery and F2 pup survival and body weight data were unremarkable. The NOAEL for F0 maternal effects was 5 mg/kg/day (7 times the human exposure), and the NOEL for reproductive performance was 2.5 mg/kg/day (6 times the human exposure).

In an *in vitro* study in freshly excised rat colon tissue, the extent of irritation caused by UT-15C SR tablets (2.5 mg) on mucosal membrane was assessed by determining the LDH release and by histological evaluation of tissues. Irritation induced by the test drug was compared to the positive control material, KCl (100, 375 and 750 mg), and negative control, blank assay buffer. Tissues mounted in the Ussing chamber apparatus were exposed to test or control substances for 180 minutes. At the end of the assay, samples for LDH measurement were collected from the donor and receiver sides of the chamber. Tissue integrity was assessed by measuring the permeability of atenolol (low permeability marker) and antipyrine (high permeability marker). Results indicated that, under the conditions of the study, exposure to the 2.5 mg UT-15C SR tablet did not result in increased LDH release or histologic evidence of tissue irritation (epithelial degeneration) when compared to the negative control, while the positive control KCl produced significantly higher amounts of LDH at ≥ 375 mg and severe histologic lesions at ≥ 100 mg.

However, it was not clear why longer treatment times were not used, or how much of the drug was actually released (i.e., there was no measurement of the amount of drug remaining in the tablet).

In summary, there are no approvability issues for treprostinil diethanolamine based on nonclinical toxicity testing program.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

XAVIER JOSEPH
10/03/2012

THOMAS PAPOIAN
10/03/2012
Concur.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 203496 **Applicant:** United Therapeutics Corp. **Stamp Date:** 12/27/2011

Drug Name: treprostinil diethanolamine sustained release tablets (b) (4) **NDA Type:** 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		CTD format
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		Electronic submission
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		Carcinogenicity studies in rats and transgenic mice are ongoing. At an End of Phase 1 meeting, it was agreed that the carcinogenicity studies need to be ongoing at the time of approval of a NDA. All other studies, except the juvenile animal studies, are completed.
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		In the 13-week and 9-month dog studies, the current clinical 12-hr sustained release (SR) tablet formulation was used. In dog studies of up to 14-days, UT-15C was given as an oral 8-hr SR tablet or SR capsule formulation to support the initial clinical study using 8-hr SR tablets. A PK bridging study was used to establish the equivalence of the 8 and 12 hr SR tablets.
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		Same route of administration
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		

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
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?		x	No special studies were requested.
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	Human dose multiples comparisons are not made in the labeling
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		Very low levels (b) (4) of impurities have been observed. (b) (4)
11	Has the applicant addressed any abuse potential issues in the submission?		x	
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

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. N/A

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

Final study report of the cardiovascular safety pharmacology study in dogs should be submitted.

Xavier Joseph 1-20-12

Reviewing Pharmacologist Date

Thomas Papoian 1-20-12

Team Leader/Supervisor Date

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

APPEARS THIS WAY ON ORIGINAL

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

XAVIER JOSEPH
01/20/2012

THOMAS PAPOIAN
01/20/2012
I concur.