

CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-779

Pharmacology Review(s)



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21779
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: June 30, 2004
PRODUCT: Ventavis
INTENDED CLINICAL POPULATION: Treatment of arterial pulmonary hypertension
SPONSOR: Cotherix
DOCUMENTS REVIEWED: eCTD
REVIEW DIVISION: Division of Cardio-Renal Drug Products (HFD-110)
PHARM/TOX REVIEWER: James M. Willard, Ph.D.
PHARM/TOX SUPERVISOR: Albert Defelice, Ph.D.
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PROJECT MANAGER: Melissa Robb

Date of review submission to Division File System (DFS):

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EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability: In the opinion of this reviewer, the drug is approvable
- B. Recommendation for nonclinical studies: There was an uneven quality to the nonclinical studies, since this drug has many IND submissions to the FDA dating back to 1983. Many of the studies were performed in Germany by a previous sponsor with different indications in mind. Not all the studies were GLP or quality assured. Despite these problems, the data was of sufficient quality to determine that it is reasonably safe to proceed with the proposed protocol.
- C. Recommendations on labeling: Three considerations need to be included in the labeling for this compound. 1) potential for interactions with other anti-coagulant medications, 2) in the Han-Wistar rat strains studied, compound induced skeletal and digit abnormalities, but not in Sprague-Dawley rat strains and 3) If used with other drugs that are active on the cardiovascular system, there may be potential for cardiovascular collapse. There is some potential for birth defects with this drug and caution should be used in prescribing in pregnancy. (see labeling below for exact wording)

Carcinogenesis, Mutagenesis, Impairment of Fertility

Iloprost was not mutagenic in bacterial and mammalian cells in the presence or absence of extrinsic metabolic activation. Iloprost did not cause chromosomal aberrations *in vitro* in human lymphocytes and was not clastogenic *in vivo* in mice. **C**

Pregnancy

Pregnancy Category C. In developmental toxicity studies in pregnant Han-Wistar rats, continuous intravenous administration of iloprost at a dosage of 0.01 mg/kg daily (serum levels not available) led to shortened digits of the thoracic extremity in fetuses and pups. In comparable studies in pregnant Sprague-Dawley rats with which received iloprost clathrate (13% iloprost by weight), orally at dosages of up to 50 mg/kg/day (Cmax of 90 ng/ml), and in pregnant rabbits, at intravenous dosages of up to 0.5 mg/kg/day (Cmax of **J**

86 ng/ml), and in pregnant monkeys, at dosages of up to 0.04 mg/kg/day (serum levels of 1 ng/ml), no such digital anomalies or other gross-structural abnormalities were observed in the fetuses/pups. However, in gravid Sprague-Dawley rats iloprost clathrate (13% iloprost) significantly increased the number of non-viable fetuses at a maternally toxic oral dosage of 250 mg/kg/day, and in Han-Wistar rats was found to be embryolethal in 15 of 44 litters at an intravenous dosage of 1 mg/kg/day [

3]. There are no adequate and well controlled studies in pregnant women. Ventavis® should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers

It is not known whether Ventavis® is excreted in human milk. In studies with Han-Wistar rats, higher mortality was observed in pups of lactating dams receiving iloprost intravenously at 1 mg/kg daily. In Sprague-Dawley rats, higher mortality was also observed in nursing pups at a maternally toxic oral dose of 250 mg/kg/day of iloprost clathrate (13% iloprost by weight). It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, and because of the potential for serious adverse reactions in nursing infants from Ventavis®, a decision [

3]

II. Summary of nonclinical findings

- A. Brief overview of nonclinical findings: Iloprost in high concentrations was able to cause death by vascular collapse after the induction of severe hypotension. Reproductive toxicity was low, however, although rabbits and monkeys showed no large issues, in the Han-Wistar rats there were problems with drug exposure linked to crooked digits, and a hint of such problems in the cynomolgus monkeys. This is an issue that may require further study by the sponsor. Genotoxicity was low, with one assay, the V79 chinese hamster lung cells giving a weak positive result. This is thought to be due to the existence of prostacyclin receptors in this cell line.
- B. Pharmacologic activity: Iloprost is an analog of prostacyclin, PGI₂, and acts to relax smooth muscle. From this property it is a potent vasodilator. It is also involved in reproduction and is important in the implantation process post-fertilization. Iloprost is rapidly absorbed, but also rapidly eliminated from the system, hence the proposed 6 to 9 x per day dosing.
- C. Nonclinical safety issues relevant to clinical use: severe hypotension is worth watching for, particularly in the presence of anti-hypertensive medications, as well as bleeding, since iloprost effects platelet function and interferes with clotting. Another issue to be aware of is the crooked digits found in the Han-

Wistar strain of rats. This issue should be examined further to try to determine the susceptibility.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21779

Review number: 1

Sequence number/date/type of submission: 000/June 30, 2004/N

Information to sponsor: Yes (X) No ()

Sponsor and/or agent: CoTherix

Manufacturer for drug substance: Schering AG

Reviewer name: James M. Willard, Ph.D., Pharmacologist/Toxicologist

Division name: Division of Cardio-Renal Drug Products

HFD #: HFD-110

Review completion date:

Drug: Ventavis (iloprost) inhalation solution

Trade name: Ventavis (iloprost) inhalation solution

Generic name: Iloprost

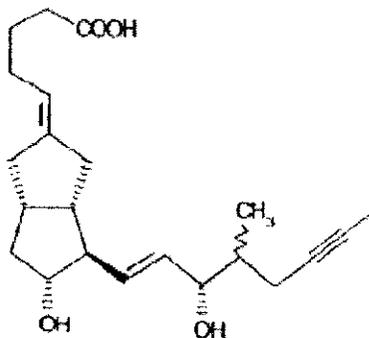
Code name:

Chemical name: 5-[(E)-(1S, 5S, 6R, 7R)-7-Hydroxy-6-[(E)-(3S, 4RS)-3-hydroxy-4-methyl-1-octen-6-ynyl]-bicyclo[3.3.0]oct-3-ylidene)-pentanoic acid

CAS registry number:

Molecular formula/molecular weight: C₂₂H₃₂O₄/360.49

Structure:



Relevant INDs/NDAs/DMFs: I65820, DMF there are — IND's for iloprost dating back to 1983.

Drug class: prostacyclin

Intended clinical population: Arterial Pulmonary Hypertension

Clinical formulation:

Ventavis is an aqueous, sterile, ready-to-use nebuliser solution for inhalation. The drug product contains per ml 10 µg iloprost as trometamol salt dissolved in a solution containing 0.81 mg ethanol 96 %, 9.00 mg sodium chloride, 0.51 mg hydrochloric acid 1N and 1 mg water for injection.

Route of administration: inhalation

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies previously reviewed (found in appendices):

Report # AG 23: Effects of intravenous infusion of iloprost and the E(4S) and E(4R) diastereomers of iloprost, ZK 95302 and ZK 91404, on blood pressure and heart rate in the anaesthetized rat.

Report # AF18: Iloprost, ZK 91403, ZK 91404, ZK 95302, ZK 115891. Evaluation of the report entitled "Vasodilatory action, hypotensive action and inhibition of platelet aggregation of iloprost and its isomers: by [redacted] Dated November 1989.

Report # AH35: Effects of iloprost and the E(4S) and E(4R) diastereoisomers of iloprost, ZK 95302 and ZK 91404 on ADP and collagen induced aggregation of human and rat platelets.

Report No. AG42: Pharmacokinetics of iloprost and its diastereomers in rats after i.v. infusion of 0.3 microg/kg/min and of 3 microg/kg/min ZK 91404 over 30 minutes.

Report # 8994: Investigation of the biotransformation of iloprost and its diastereoisomers in the dog during a six-hour intravenous infusion of each compound.

Report # AL26: Comparative toxicity of ZK 36374 (iloprost clathrate, diastereoisomer mixture), ZK 91404 (4R-form) and ZK 95302 (4S-form) in male and female rats after a single i.v. application.

Report AI61: Comparative acute toxicity of ZK 96944 (iloprost clathrate, diastereoisomer-mixture), ZK 153485 (4S-form) and ZK 155534 (4R-form) in male and female rats after a single i.g. application.

Report # 97004: Combined study of fertility and embryonic development and pre- and postnatal development, including maternal function, of iloprost clathrate in female rats.

Report # 97005: Oral development toxicity study of iloprost clathrate in rabbits.

Studies reviewed within this submission:

Study # A05405: Pharmacokinetics of iloprost in the rat during once daily inhalation with a nebulizing solution over a period of 6 months.

Report # AE74: Evaluation of the report entitled: study report on pharmacokinetics of iloprost in rats of []

Report # 4387: Pharmacokinetics and biotransformation of ZK 36374 in the rat. II. Excretion after oral and intravenous administration of 10 microg/kg

Report # 5493: Pharmacokinetics and pharmacodynamics of the stable prostacyclin analogue, ZK 36 374, in the cat.

Study # 4309

Investigation of ZK 34 798 and ZK 36 374 for neurotropic side-effects after a single intravenous and intraperitoneal administration in the mouse by comparison with prostaglandin-I₂-sodium salt.

Study # 5933

The action of ZK 36 374 (iloprost) upon the cardio-and haemodynamics of anaesthetized cats in acute passive anaphylaxis

Study # 7156

Activation of the rennin-angiotensin system and cardiovascular effects of iloprost, ZK 96.480 and ZK 31.472 on conscious, normo-tensive rats

Study # 9704

Comparative study on iloprost, PGE₁ and PGF_{2a}: influence on pulmonary arterial pressure and edema formation during continuous infusion of isolated, perfused rat lungs

Study # 4474

Influence of ZK36374 – a stable prostacyclin-derivative upon renal function in the rat: comparison with PGI₂ (Na-salt). Effect upon fluid and electrolyte-excretion.

Study # 5962

Pharmacological actions of ciloprost (now Iloprost), a stable prostacyclin-analogue on the gastrointestinal tract

Study # 4654

Investigation into the actions of ZK 36 374 and ZK 34 798 by comparison with ZK 31 472 (PGI₂) and ZK 53 258 (PGE₂), upon the cardiovascular system of the anesthetized cat after the pulmonary pressure had been experimentally increased through ADP (adenosine-5-diphosphoric acid-trisodium salt).

Study # 8268

In vitro pharmacology: high throughput profile study of iloprost, E(4)S Iloprost, and E(4)R Iloprost.

8.4.2.1 Single Dose Toxicity Study Reports	6	48
<u>Report No. 5326</u> : Rabbit, male and female, intravenous	6	49
<u>Report No. AL54</u> : Evaluation of the report: "Acute (IV) toxicity study for iloprost in monkeys"	6	56
<u>Report No. AL83</u> : Evaluation of the report: "Acute (IV) drip infusion toxicity study for iloprost in monkeys"	6	85
<u>Report No. AL26</u> : Comparative toxicity of ZK 36374 (iloprost, diastereoisomer mixture), ZK 91404 (4R-form) and ZK 95302 (4S-form) in male and female rats after a single i.v. application	6	120
<u>Report No. AI61</u> : Comparative acute toxicity of ZK 96944 (iloprost clathrate, diastereoisomer-mixture), ZK 153485 (4S-form) and ZK 155534 (4R-form) in male and female rats after a single i.g. application	6	186

Four week inhalation toxicity study with iloprost nebulising suspension in the rat.

— Project 709413: Iloprost: six month inhalation study with a nebulising solution in the rat

Systemic tolerance study in monkeys (*Macaca fascicularis*) after daily per os (intra-gastric) administration over 14-15 days.

Systemic tolerance study of ZK 36.374 in monkey (*Macaca fascicularis*) after continuous intravenous infusion using the — osmotic-pump over a period of four weeks.

Systemic tolerance study in beagle dogs after continuous sub-cutaneous infusion with the — osmotic pump over 26 weeks.

Systemic tolerance study (including local tolerance) in beagle dogs after daily oral administration of retard formulation of iloprost (iloprost clathrate, SH K 529 I/M) over 53 weeks (2 applications per day at a 6-hour interval)

Evaluation of ZK 36.374 in the Ames Salmonella/microsome mutagenicity test.

Evaluation of ZK 36.374 in the Ames Salmonella/microsome mutagenicity test with preincubation

Mutagenicity evaluation of ZK 36.374 in the reverse mutation assay with *E. coli* strain WP2uvrA.

Test report of study LMP 261: ZK 36.374 – detection of gene mutations in somatic mammalian cells in culture: HGPRT-test with V79 cells.

Evaluation of the clastogenic potential in the human lymphocyte test.

Studies on the mutagenic potential of ZK 36.374 in the mouse micronucleus test.

ZK 36.374 – Study of the fertility and general reproductive performance in the rat after continuous intravenous infusion with the — Osmotic-Pump in female animals for 14 days before start of mating to day 7 of gestation.

ZK 36.374 – study of the fertility and general reproductive performance in the rat after treatment of males during the pre-mating and mating period by continuous intravenous infusion.

Combined study of fertility and embryonic development and pre- and postnatal development, including maternal function, of iloprost clathrate in female rats.

Commentary on the report of [redacted], dated June 5, 1989 (— Project # 14/45): ZK 36.374 – Embryotoxicity including teratogenicity study in the monkey (Cynomolgus) after continuous intravenous infusion from day 20 to 50 of gestation.

Oral development toxicity study of iloprost clathrate in rabbits.

ZK 36.374 – peri- and postnatal study in the rat after continuous intravenous infusion with the — Osmotic pump from day 15 of gestation to day 22 post partum.

Iloprost clathrate: tumorigenicity study in mice after daily dietary administration of the ground extended release formulation SH K 529 over approximately 96 weeks.

Iloprost clathrate. Tumorigenicity study in rats after daily dietary administration of the ground extended release formulation SH K 529 over approximately 2 years.

Evaluation of the report entitled: Antigenicity study of iloprost in guinea pigs.

Studies not reviewed within this submission:

|

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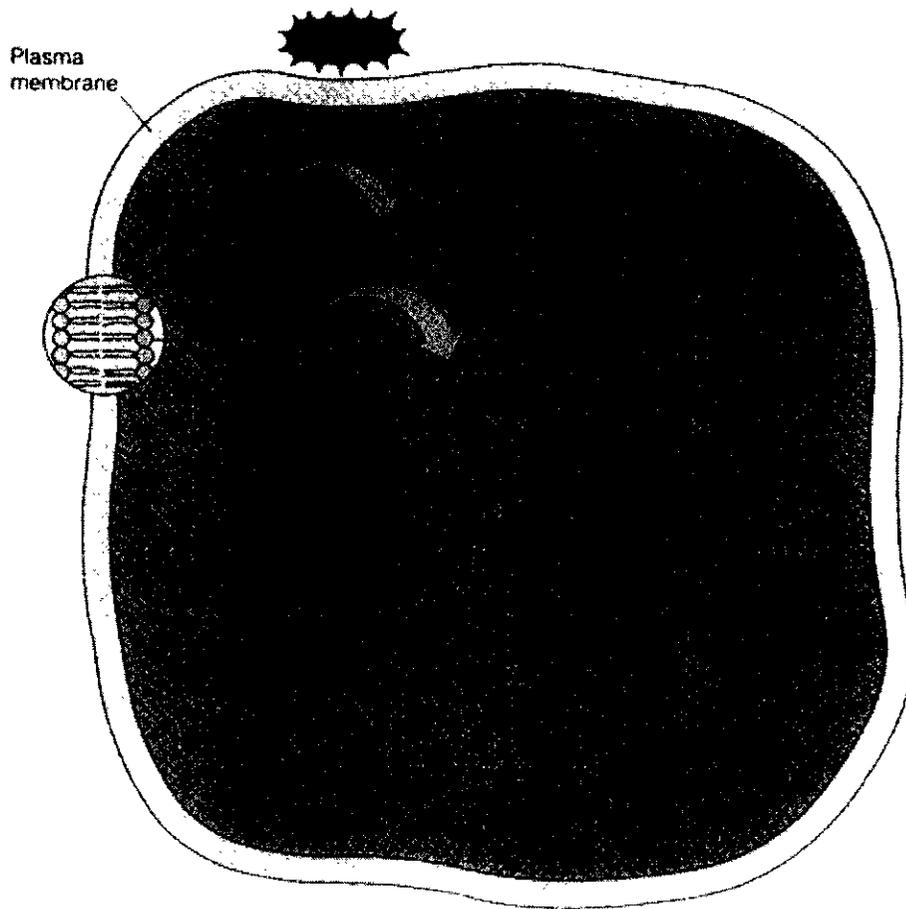
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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary



2.6.2.2 Primary pharmacodynamics

Mechanism of action: Iloprost is an analog of the prostaglandin PGI₂, also known as prostacyclin. PGI₂: receptor binding leads to adenylyl cyclase activation, increased cAMP leads to activation of protein kinases leads to decreased intracellular Ca⁺⁺, and decreased platelet aggregation

Drug activity related to proposed indication: PGI₂ is a potent vasodilator and that is how it relieves pulmonary hypertension.

2.6.2.3 Secondary pharmacodynamics

PGI₂: receptor binding leads to adenylyl cyclase activation, increased cAMP leads to activation of protein kinases leads to decreased intracellular Ca⁺⁺, and decreased platelet aggregation. Iloprost can lead to an increased risk of hemorrhage

Eicosanoid	Source*	Effects
PGI ₂	Endothelial cells	Antithrombotic and anti-inflammatory Induces vasodilation
	Vascular smooth muscle cells	Inhibits platelet aggregation Inhibits platelet mitogen release Suppresses smooth muscle cell proliferation Inhibits leukocyte adhesion

2.6.2.4 Safety pharmacology

Note: many of the safety pharmacology studies were done early in the development of iloprost, and date from the late 1970's. With changes in the proposed therapeutic usage and assay methodologies, many of the studies are of marginal usefulness.

Neurological effects:

Study # 4309

Investigation of ZK 34 798 and ZK 36 374 for neurotropic side-effects after a single intravenous and intraperitoneal administration in the mouse by comparison with prostaglandin-I₂-sodium salt.

Results: Intraperitoneal administration seemed to lead to more effects than intravenous. The primary effect noted was depression (motor and effect). It was speculated that this

was related to the hypotensive effects of the compounds tested. Study is from 1980 and is not well documented.

Cardiovascular effects:

Study # 5933

The action of ZK 36 374 (iloprost) upon the cardio- and haemodynamics of anaesthetized cats in acute passive anaphylaxis

Results: This study was designed to determine the effects of iloprost on cats passively sensitized with antioalbumin. Generally this leads to a circulatory collapse led by a marked reduction in cardiac output, a fall in arterial blood pressure, increase in left ventricular diastolic pressure and increased peripheral vascular resistance. Iloprost at 0.2 and 1.0 microg/kg/min IV infusion led to a dose dependent reduction in the anaphylactic cardio-vascular response in the cat.

Study # 7156

Activation of the rennin-angiotensin system and cardiovascular effects of iloprost, ZK 96.480 and ZK 31.472 on conscious, normo-tensive rats

Results: Iloprost treatment leads to the lowering of blood pressure in a dose-dependent manner with IV infusions.

Pulmonary effects:

Study # 9704

Comparative study on iloprost, PGE1 and PGF2a: influence on pulmonary arterial pressure and edema formation during continuous infusion of isolated, perfused rat lungs

Results: Iloprost had no effects on lung function parameters.

Renal effects:

Study # 4474

Influence of ZK36374 – a stable prostacyclin-derivative upon renal function in the rat: comparison with PGI2 (Na-salt). Effect upon fluid and electrolyte-excretion.

Iloprost (ZK36374) was found to reduce urinary volume and output of sodium and potassium in a manner similar to PGI2.

Gastrointestinal effects:

Study # 5962

Pharmacological actions of ciloprost (now Iloprost), a stable prostacyclin-analogue on the gastrointestinal tract

Iloprost was found to reduce the production of gastric acid and to inhibit gastrointestinal transit.

Abuse liability: not done

Other:

Study # 4654

Investigation into the actions of ZK 36 374 and ZK 34 798 by comparison with ZK 31 472 (PGI₂) and ZK 53 258 (PGE₂), upon the cardiovascular system of the anesthetized cat after the pulmonary pressure had been experimentally increased through ADP (adenosine-5-diphosphoric acid-trisodium salt).

Results: Iloprost (ZK 36 374) was able to improve the ADP induced micro-thromboembolic and other vasoconstrictor activities.

Study # 8268

Study Title: *In Vitro* Pharmacology: High-Throughput Profile

Study of iloprost, E(4)S-iloprost and E(4)R-iloprost

Receptor binding results: In general, iloprost and neither of the diastereomers, E(4)S-iloprost and E(4)R-iloprost, had much interaction with the receptors in the battery of receptors screened. H₁ histamine receptors were inhibited to 77.8 and 73.4 % binding by 10 μM iloprost, although the mixture did not inhibit binding. Purinergic P₂Y receptors were inhibited to 77.8 % by the S form, but not by the R form or the mixture. Serotonergic 5-HT_{5A} receptors were inhibited to 73% binding by E(4)R-iloprost, and 78.6% by the mixture, but only 91.1 % by E(4)S-iloprost. No other serotonergic, histaminergic, or purinergic receptors were affected by iloprost, E(4)S-iloprost and E(4)R-iloprost.

2.6.2.5 Pharmacodynamic drug interactions

Studies of Iloprost with other anti-coagulant medications indicate that the concomitant usage with iloprost will lead to an increase in the anti-clotting effects of anticoagulant drugs and should be used with caution.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.2.2 Safety pharmacology

Effects of iloprost have been studied on the central and autonomic nervous system and on the cardiac, respiratory, renal, gastrointestinal and female reproductive systems. The results of these studies can be summarized as follows:

- Symptoms of depression (probably due to exaggerated pharmacological effects, e.g. peripheral vasodilatation and hypotension) and, at high doses, of CNS/autonomic stimulation in rats (4309)
- No effects on cardiac action potential, no pro-arrhythmic effects *in vitro* (6058, 9648, 5791), and *in vivo* in rats and mice (6066, 6788)
- No deleterious effects on respiratory function *in vitro* (6915, 4157) and *in vivo* in rabbits (4169)
- Decreased urine flow and sodium excretion at hypotensive doses, which is rapidly reversed on cessation of administration in rats (4474)
- Contractile effects on isolated ileum, little effect or decreased motility and antidiarrhoeic/anti-enteropooling effects (depending on model system) *in vivo* in rats and rabbits (4157, 5962, 4169).
- Uterine contractile (guinea-pig) or biphasic (human) effects *in vitro* (4157, 8991, 9105). No effect on uterine pressure and motility *in vivo* in anaesthetized rabbits (4169), induction of abortion at near-lethal doses in guinea pigs (4447).

None of the studies on the effects of iloprost in various organ systems (safety pharmacology studies) indicate a potential of the compound to induce serious adverse effects in the therapeutic dose range

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Iloprost is a prostacyclin analog that will be administered via a nebulizer and is rapidly absorbed from the lungs. It is also rapidly eliminated, and thus is planned to be given in up to 9 daily doses.

2.6.4.2 Methods of Analysis

[see under individual study reviews]

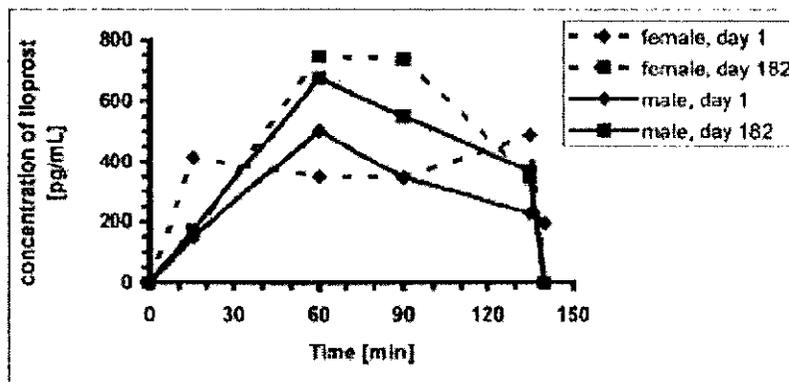
2.6.4.3 Absorption

Only one study was submitted by the sponsor of absorption of iloprost from a nebulized solution, although 2 studies are mentioned in the summary of pharmacokinetic data. Study # A05405: Pharmacokinetics of iloprost in the rat during once daily inhalation with a nebulizing solution over a period of 6 months.

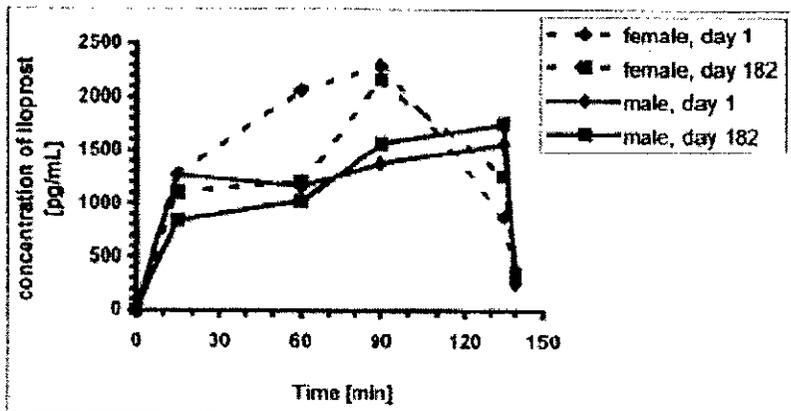
Iloprost was rapidly taken up from the lungs, with a T_{max} time of 60 minutes. Absorption of iloprost stopped almost immediately after removal from exposure to the nebulized drug. C_{max} levels were proportionate to drug exposure times.

Gender	Nebuliser concentration	Minutes Exposure	Dose in Lungs (microg/kg)	Days in Study	Cmax	AUC
F	.0505 microg/L	135	3.6	1	489	51
F	.0505 microg/L	135	3.6	182	747	69
M	.0505 microg/L	135	3.6	1	504	43
M	.0505 microg/L	135	3.6	182	676	60
F	0.345 microg/L	135	24.6	1	2292	224
F	0.345 microg/L	135	24.6	182	2158	191
M	0.345 microg/L	135	24.6	1	1565	173
M	0.345 microg/L	135	24.6	182	1750	167
F	0.345 microg/L	240	43.7	1	1784	307
F	0.345 microg/L	240	43.7	182	2998	604
M	0.345 microg/L	240	43.7	1	2148	280
M	0.345 microg/L	240	43.7	182	1467	295

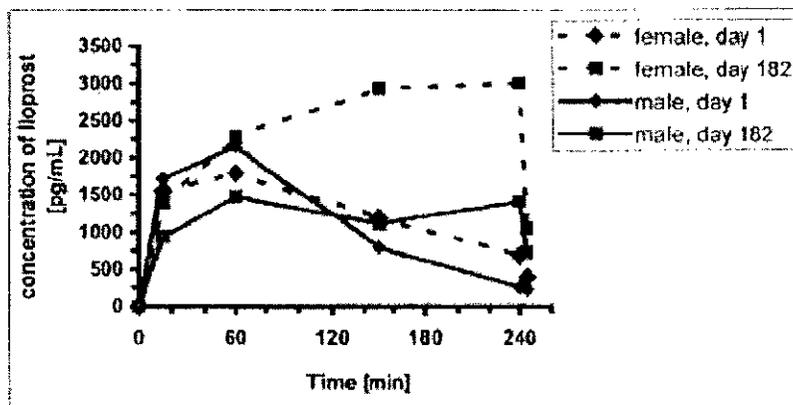
TF 1: Mean serum concentration time profile of iloprost after daily inhalation administration of an aerosol containing 0.0505 µg/L iloprost for about 135 min (group 3/low dose)



TF 2: Mean serum concentration time profile of iloprost after daily inhalation of an aerosol containing 0.345 µg/L iloprost for about 135 min (group 4/intermediate dose)



TF 3: Mean serum concentration time profile of iloprost after daily inhalation of an aerosol containing 0.345 µg/L iloprost for about 240 min (group 5/high dose)



2.6.4.4 Distribution:

Report # AE74: Evaluation of the report entitled: study report on pharmacokinetics of iloprost in rats of \

3H-Iloprost was given to rats via intravenous administration. It was rapidly distributed to liver, kidneys, muscle, and stomach, lungs, heart and placenta. Elimination was rapid from these tissues with a half-life of 45 minutes. Low levels of iloprost cross the placental barrier and also partition into milk.

2.6.4.5 Metabolism :

Report # 4387: Pharmacokinetics and biotransformation of ZK 36374 in the rat. II. Excretion after oral and intravenous administration of 10 microg/kg

Report # 5493: Pharmacokinetics and pharmacodynamics of the stable prostacyclin analogue, ZK 36 374, in the cat.

Metabolism was examined in the rat, where the primary modification is beta-oxidation of the upper side chain. Iloprost is also hydroxylated or conjugated.

2.6.4.6 Excretion:

When iloprost is given intravenously, approximately 75% is excreted renally, the rest in the feces. 98% was excreted within 24 hrs of administration. With oral administration, approximately 50% is excreted renally, 35% in the feces in the first 24 hrs for elimination of about 85% of the dosed iloprost. Orally, about 15% of the dose was not recovered.

2.6.4.7 Pharmacokinetic drug interactions

Iloprost produces hypotension and also reduces platelet aggregation. Interactions with anti-hypertensive medications and other drugs that reduce blood pressure could be significant, as well as potential interactions with anti-coagulant medications.

2.6.4.8 Other Pharmacokinetic Studies

2.6.4.9 Discussion and Conclusions:

Iloprost is apparently readily absorbed from the lung in the rat. It is also eliminated quickly from the system. It is not known how it will be eliminated after inhalation. After iv administration, 98% eliminated 3:1 in urine:feces within 24 hrs, and after oral administration 85% is eliminated 1.5:1 in urine:feces within 24 hrs.

2.6.4.10 Tables and figures to include comparative TK summary

No data

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Gender	Nebuliser concentration	Minutes Exposure	Dose in Lungs (microg/kg)	Days in Study	Cmax	AUC
F	.0505 microg/L	135	3.6	1	489	51
F	.0505 microg/L	135	3.6	182	747	69
M	.0505 microg/L	135	3.6	1	504	43
M	.0505 microg/L	135	3.6	182	676	60
F	0.345 microg/L	135	24.6	1	2292	224
F	0.345 microg/L	135	24.6	182	2158	191
M	0.345 microg/L	135	24.6	1	1565	173
M	0.345 microg/L	135	24.6	182	1750	167
F	0.345 microg/L	240	43.7	1	1784	307
F	0.345 microg/L	240	43.7	182	2998	604
M	0.345 microg/L	240	43.7	1	2148	280
M	0.345 microg/L	240	43.7	182	1467	295

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: Overall, iloprost is potentially of low toxicity. Most deaths occurred due to extreme hypotension, or in the oral formulations high doses induced strong gastrointestinal effects (diarrhea, torsion of the GI tract in a few cases). There was, however, only one 4 week and one six-month study of toxicity in the rat using a nebulized formulation.

Genetic toxicology: Only one assay gave a positive response, the chromosomal aberration assay with Chinese Hamster Lung Cells. These cells have receptors for prostacyclin, at the cell surface as well as internal receptors that upon binding prostacyclin are translocated to the nucleus and bind to the DNA. Therefore, a positive response by this cell line might be expected, but difficult to interpret from a genotoxicity perspective.

Carcinogenicity: Iloprost was dosed to rats and mice in their food as iloprost clathrate (i.e. iloprost in a beta-cyclodextrin cage). The dosage was low with serum levels of iloprost being 1.5 ng/ml in rats and 3.9 ng/ml in mice. No signs of carcinogenicity were seen in the assays.

Iloprost was also dosed to rats and mice via oral gavage. Control, 5, 50, and 125 mg/kg/day doses were given to mice and rats. In mice, there was no increase in mortality, nor any effects noted due to Iloprost. In rats, there was increased mortality in the 125 mg/kg group, and the males were reduced to 100 mg/kg

Reproductive toxicology: Rabbits and Sprague-Dawley rats did not show any significant effects of iloprost exposure to either mothers or fetuses. However, Han-Wistar rats did show anomalies such as crooked digits and incomplete sternum formation. This shows that although prostacyclin and analogs of prostacyclin are involved in the reproductive process (primarily implantation), there can be some variation in sensitivity and this may have to be considered in the labeling.

Special toxicology:

2.6.6.2 Single-dose toxicity

8.4.2.1 Single Dose Toxicity Study Reports.....	6	48
<u>Report No. 5326</u> : Rabbit, male and female, intravenous	6	49
<u>Report No. AL54</u> : Evaluation of the report: "Acute (IV) toxicity study for iloprost in monkeys".....	6	56
<u>Report No. AL83</u> : Evaluation of the report: "Acute (IV) drip infusion toxicity study for iloprost in monkeys".....	6	85
<u>Report No. AL26</u> : Comparative toxicity of ZK 36374 (iloprost, diastereoisomer mixture), ZK 91404 (4R-form) and ZK 95302 (4S-form) in male and female rats after a single i.v. application.....	6	120
<u>Report No. AI61</u> : Comparative acute toxicity of ZK 96944 (iloprost clathrate, diastereoisomer-mixture), ZK 153485 (4S-form) and ZK 155534 (4R-form) in male and female rats after a single i.g. application.....	6	186

Acute toxicity studies were carried out with mice, rats, rabbits, and cynomolgus monkeys. Many of these studies are flawed, with the sponsor commenting at one point on the contract lab: "There is no rational reasoning for the application schedule used". Most of these studies have some weakness, and the data below should be taken to estimate where toxic effects may be anticipated to occur.

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Table 1: Design and results (LD₅₀) of systemic toxicity tests with a single intravenous (i.v.) and intragastric (i.g.) administration

Species	Number of animals per dose and sex	Route of administration	LD ₅₀ -values and 95% confidence limits (mg/kg)	Schering report
Mouse	10M	i.v.	201 (179-244)	5359
	10F	i.v.	204 (168-247)	5389
Rat	5M/5F	i.v.	119 (86-164)	5362
	5M/5F	i.v.	M: 128 (92-216) F: 144 (116-201)	AL62
Rabbit	3M/3F	i.v.	9.8 (5.5-16.0)	5326
Monkey	1M/1F	i.v.	> 5	AL54
Mouse	3M	i.g.	> 100	4300
Rat	3M	i.g.	> 100	4301

M = male, F = female

Table 2. Species LD50 values and Human Equivalents for comparison purposes.

Species	LD50	Human Equivalent
Mouse i.v.	201 mg/kg	16.3 mg/kg
Rat i.v.	119 mg/kg	19.3 mg/kg
Rabbit i.v.	9.8 mg/kg	3.2 mg/kg
Monkey i.v.	>5mg/kg	>1.6 mg/kg
Mouse i.g.	>100 mg/kg	>8.1 mg/kg
Rat i.g.	>100 mg/kg	>16.2 mg/kg

In Study AL83, cynomolgus monkeys had an LD50 estimated at 3.75 mg/kg, however, only 2 monkeys were tested at that dose level. This would be a human equivalent dose of approximately 1 mg/kg, which would be reasonable to consider as a potential human LD50 via intravenous administration until there is evidence to the contrary for purposes of calculating a safety margin for dosing.

2.6.6.3 Repeat-dose toxicity

Study title: Four week inhalation toxicity study with iloprost nebulising suspension in the rat

Key study findings: While examining the effects of rats receiving a nebulized dose of 0, 0.9, 3.6, 10.8, and 24.6 microg/kg, no clinical sequelae of iloprost was noted except a reduction in serum bilirubin, lipids, cholesterol, and other phospholipids. These changes were not thought to be adverse in nature. Apparently the serum levels of iloprost from this study were taken to be part of a different study which was not reported. Without drug exposure data this study is of little utility.

Study no.: Report # A01317

Volume #, and page #: Vol 5.7, pp. 1-456

Conducting laboratory and location: ☐

☐

Date of study initiation: April 9, 1999

GLP compliance: yes

QA report: yes (X) no ()

Drug, lot #, and % purity:

4.1.1.1 Administration by inhalation

Table 2: Systemic and local tolerance test with repeated inhalative administration of aerosolized iloprost solution

Species	Number of animals per group/sex	Iloprost concentration in nebulizer (µg/mL)	Aerosol concentration of iloprost (Mean/SD) (µg/m ³ air)	MMAD*/GSD (µm)	Achieved dose levels of iloprost** (µg/kg/day)	Achieved dose levels of iloprost (µg/kg/min)	Duration of treatment	Schering report
Rat	5M, 5F	0 (Control)	0	--	0	0	28 days	A01317
		10	11.6/4.1	1.78 / 2.17	0.84	0.006	(135 min daily)	
		10	49.8/11.9	1.78 / 2.33	3.55	0.026		
		10	125.6/38.8	1.37 / 2.49	9.15	0.058		
		20	14.3/12.3	1.80 / 2.34	1.05	0.008		
		20	313.4/72.1	1.29 / 2.28	22.6	0.167		

M = male; F = female * = Mass Median Aerodynamic Diameter/Geometric Standard Deviation

** The dose levels of iloprost achieved during the 135-minute exposure period, based on 100% deposition within the respiratory tract were calculated using the formula

$$D_L = \frac{E_c \times MV \times T}{BW}$$

D_L = Achieved dose level (µg active compound/kg body weight)
 E_c = Actual chamber concentration (µg active compound/L air)
 MV = Minute volume (mL/min) according to Guyton's formula $EW(g)^{0.75} \times 2.1$
 T = Time, duration of daily exposure (minutes)
 BW = Mean body weight on sampling days (g)

For the assessment of local and systemic toxicity of iloprost after inhalative administration, Wistar rats were exposed by inhalation to three target concentrations of iloprost 10 µg/mL formulation (0.9, 3.6 and 10.8 µg/kg) and two concentrations of iloprost 20 µg/mL (0.9 and 24.6 µg/kg) seven days a week for 28 days (A01317). Blood sampling was performed in satellite animals for concomitant determination of iloprost serum levels and pharmacokinetic parameters (C_{max} , t_{max} , AUC) (B784). Inhalation exposure conditions were qualified by chemical analysis of the iloprost aerosol concentration and measurement of the particle size distribution, oxygen concentration, relative humidity, temperature and exposure airflow rate. The

animals were observed for mortality and overt signs of a reaction to treatment. Body weights and food consumption were measured weekly. Clinical laboratory investigations were performed on blood and urine samples from all animals before necropsy. A complete post mortem macroscopic examination was performed, a full list of organ/tissue samples preserved. The respiratory tract from all animals and the full tissue list from all animals in the two high dose groups and the controls were subjected to histopathological examination.

Observation times and results

Mortality: none

Clinical signs: none

Body weights: although body weights did change, they changed also for the controls and may be due to the nebulization treatment modality

Food consumption: although food consumption did change, it changed for the controls as well as the drug treated and may be due to the nebulization treatment modality

Ophthalmoscopy: none

EKG: not done

Hematology: no treatment related effects noted

Clinical chemistry: In males, bilirubin levels were decreased at all dosages and magnesium levels were decreased over controls. In females, phospholipids and cholesterol were decreased compared to controls.

Urinalysis: no treatment related effects noted

Gross pathology: no treatment related effects noted

Organ weights (specify organs weighed if not in histopath table): no treatment related effects noted

Histopathology: Adequate Battery: yes (), no ()—explain
Peer review: yes (), no ()

Toxicokinetics: not done

Other:

Study title: — Project 709413: Iloprost: six month inhalation study with a nebulising solution in the rat

Key study findings: While examining the effects of rats receiving a nebulized dose of 0, 3.55, 27.8, and 43.7 microg/kg, only minor effects were noted. Primary was the increase in absolute and relative lung weights in the mid and high dose groups. Also, hyaline inclusions were found in the nasal epithelium for groups 2 and 5, related to the longer exposure times to the nebulizer of these two groups. Without drug exposure data this study is of little utility.

Study no.: Report # A04447

Volume #, and page #: Vol 5.8, pp. 325-515

Conducting laboratory and location: []

Date of study initiation: June 13, 2000

GLP compliance: yes

QA report: yes (X) no ()

Drug, lot #, and % purity: iloprost, batch # N02140

Doses: 0, 3.6, 24.6, or 43.7 mg/kg/day

Species/strain: Han-Wistar Rats

Number/sex/group or time point (main study): 20 M, 20 F per group

Route, formulation, volume, and infusion rate: inhalation, nebuliser, iloprost in 0.9% saline

Satellite groups used for toxico-kinetics or recovery: 6 M, 6 F per dose group (no controls)

Age: males 8 weeks, females 11 weeks

Weight (nonrodents only):

Unique study design or methodology (if any):

Observation times and results

Mortality: no deaths occurred in the study

Clinical signs: sponsor reported no treatment related effects

Body weights: sponsor reported no treatment related effects

Food consumption: was lower in the iloprost treated groups, only occasionally reaching statistical significance. No effect noted on growth

Ophthalmoscopy: none

EKG: not done

Hematology: several erythrocyte related parameters were decreased dose dependently by iloprost treatment. Erythrocyte count and hematocrit were significantly affected in drug exposed groups (hematocrit only in the intermediate and high dose groups).

Clinical chemistry: many of the changes were minor and non-significant, and were perhaps different at one time point, but not the next one. Changes thought to be due to treatment were: a decrease in glutamate dehydrogenase in females of groups 4 and 5 after 26 weeks of treatment; creatine kinase increased in females of groups 3 and 5 after 13 weeks of treatment; and sodium increased in group 4 females after 26 weeks of treatment.

Urinalysis: Urinary volume was increased in group 4 and 5 males, accompanied by a decrease in specific gravity and osmolality in these groups.

Gross pathology: uterine adipose nodules were found in 4 females from group 5, and one nodule was a metastasis from a renal tubule carcinoma.

Organ weights (specify organs weighed if not in histopath table): lung weights (absolute and relative) were significantly increased in group 4 and 5 animals (intermediate and high dose). A reduction in spleen to body weight ratio was found in group 4 males.

Histopathology: Adequate Battery: yes (), no ()—explain

Peer review: yes (), no ()

Hyaline inclusions were found in the nasal epithelium of rats in all groups, but was especially noticeable in the group 2 and 5 animals. The exposure time to the nebulizer in these animals was 240 minutes per day, versus 135 minutes per day for groups 1, 3, and 4. Since group 2 and 5 were similar, the effect is probably due to the exposure to the saline mist for an extended period of time.

Toxicokinetics: not reported in this study

Other:

Study title: systemic tolerance study in monkeys (*Macaca fascicularis*) after daily per os (intra-gastric) administration over 14-15 days.

Key study findings: groups of 2 male and 2 female monkeys were given 0.02, 0.2, or 1.0 mg/kg/day of iloprost. One animal died in each treated group. 1.0 mg/kg/day caused the death of 1 animal, QT prolongation (1/3), loss of consciousness (2/4), diarrhea (2/4) and drops in monocyte counts and elevation of bone marrow lymphocytes.

Study no.: 4858/II

Volume #, and page #: 5.8, pp.1-105

Conducting laboratory and location: unknown

Date of study initiation: January 12, 1981

GLP compliance: yes

QA report: yes () no ()

Drug, lot #, and % purity: Unknown

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1. Test/control article and stability data

For i.g. application, solutions of the test compound were used in the present study and formulations were prepared daily prior to application under non-aseptic conditions by dilution of a sterilized stock solution containing 0.2 mg ZK 36.374/ml vehicle with a sterilized NaCl-solution. The vehicle consists of 8.975 mg NaCl, 0.4848 mg trometamol, 0.01 ml ethanol 96% per ml bidistilled water (pH 8.3 adjusted with 1N HCl).

The solution with the substance concentration of 0.1 mg per ml proved to be chemically sufficiently stable over 3 months at temperatures up to 60°C (C J Report No. 4592, dated Feb. 10, 1981).

It may therefore be assumed that a formulation of 0.2 mg ZK 36.374 per ml is chemically sufficiently stable for 3 months.

2. Selection of animal species, reasoning for dosage and route of application

Monkeys were selected for this study because they are standard laboratory animals and the species of choice for non rodent toxicity study to evaluate the potential of new drugs. In addition, background control data are available on the parameters recorded in this study.

The oral (intra-gastric) route of administration was chosen because this compound is intended to be administered to humans also by the same route.

The doses were selected on the basis of the planned phase I study in which the intended human dose is set at 10 mg ZK 36.374/subject (0.2 mg/kg body weight). Thus daily doses of 0.02, 0.2 and 2 mg/kg were selected which corresponds to 0.1, 1 and 10 times of the human intended dose.

3. Experimental procedure

3.1. Animal management and treatment

Number of animals and strain: 2 M, 2 F monkeys per group
(macaca fascicularis)

Animal quality: []

Supplier: []

Acclimatization time: > 4 weeks

Caging conditions: conventional

Animal per cage: 1

Number of groups: 4

Body weight: 2.0 - 6.7 kg

Feed type: []

Feeding time: limited to 1 hour per day

Type of drinking water: tap water

Drinking water offered: ad libitum

Room temperature: 22 - 23°C

Relative humidity: 54 - 75%

Light period: 12 hours from 6 a.m. to 6 p.m.

Identification: permanent tattoo number

Randomization: by lot

The animals were treated once daily in the morning before feeding time orally by gavage (the stomach tube was rinsed with 10 ml tap water) over a period of 14-15 days according to the following application schedule.

Table 1: Application schedule

Dose of ZR 36.374	Application volume (ml/kg)	Concentration (mg/ml)	No. of animals sex
vehicle	10	-	2M / 2F
0.02 mg/kg	10	0.002	2M / 2F
0.2 mg/kg	10	0.02	2M / 2F
1.0 mg/kg	10	0.1	2M / 2F

Since one animal (No. 158M) died on the very first day of treatment with 2.0 mg/kg (group 4), presumably as a consequence of a very strong decrease in blood pressure, and since the other animals in this group also showed a marked drop in blood pressure, the dose of 2.0 mg/kg envisaged for the duration of the experiment was amended to 1.0 mg/kg from the second day of treatment onward.

Animal No. 158M was replaced by animal No. 448M; likewise animal No. 210M (which died on the first day of treatment 20 minutes after administration!) was replaced by animal No. 200M. The death of animal No. 210M may be assumed not to be substance-induced, since this animal was already partly in a state of shock one and a half hours prior to administration.

3.2. Observations, clinical and laboratory studies

3.2.1. Mortality

Recording of premature death.

3.2.2. General observations

All signs of weak health or reactions to treatment were recorded twice daily - before, during, up to 2 3/4 hours after administration (longer when blood pressure was being monitored), and in the afternoon; on public holidays and on the weekends after other work had been attended to.

3.2.3. Body weight gain

Body weight was recorded weekly over 15 days and body weight gain was calculated over this period.

3.2.4. Food consumption

The amount of food consumed by the individual animal was recorded daily over a period of 13 days.

3.2.5. Ophthalmoscopy

The eyes of all animals were examined at day 15.

The ophthalmoscopic examination was performed in [] using a direct ophthalmoscope [] and a fundus-camera, [] for fundus examination and a — sciascope [] for the examination of the lense.

3.2.6. Electrocardiography and heart rate

During week -1 and at day 9 electrocardiograms were recorded for each animal. They consisted of standard limb leads II and III. Heart rate was determined by counting the R-waves per minute.

3.2.7. Blood pressure

Mean and systolic blood pressure were determined using a pressure cuff at days 1, 2 (only groups 1 and 4), 5 and 10 each before treatment and 30 minutes, 1, 2, 3, 3.5 and 4h after treatment.

3.2.8. Hematology

Hemoglobin, hematocrit, erythrocyte, leucocyte and reticulocyte counts, MCV (mean corpuscular volume of the individual erythrocyte), MCH (mean content of hemoglobin in the individual erythrocyte), ESR (erythrocyte sedimentation rate), differential blood count, eosinophil and thrombocyte counts: during week -1 (with exception of reticulocyte, thrombocyte and eosinophil counts and differential blood count) and on day 11.

3.2.9. Bone marrow

Determination of the number of nucleated bone marrow cells per mg bone marrow and a myelogram were performed at days 15 and 16.

3.2.10. Urinalysis

pH, specific gravity, protein, glucose, acetone, blood, urobilinogen and sediment were determined in spontaneous urine at day 12.

Urinalysis was performed using the [] test [

] Specific gravity was measured using a hydrometer and sediment analysis was carried out with a microscope.

3.2.11. Biochemistry

The following parameters were determined in serum:

glutamic oxaloacetic transaminase (GOT)
glutamic pyruvic transaminase (GPT)
γ-glutamyl transferase (γ-GT)
during week -1, day 2 (24 h), day 4 (72 h) and day 11
urea nitrogen and
glucose during week -1 and at day 11
cholesterol
total protein
protein electrophoresis
sodium
potassium
calcium
chloride
at day 11

3.2.12. Coagulation

Thromboplastin time, partial thromboplastin time, thrombin time and fibrinogen were determined at day 11.

3.2.13. Necropsy

On completion of treatment all surviving animals were sacrificed under general [] anaesthesia. Each animal was rapidly exsanguinated by incision of the jugular and axillary blood vessels. Immediately after occurrence of death a full post mortem examination was performed.

The animals which died during the experiment were also dissected.

Examinations were carried out on organ and tissue specimens taken from all animals of group 1 (controls) and group 4 (highest dose group), and from the two animals of the other groups which died during the study (No. 3600F, group 2 and No. 85F, group 3). Eye and bone (rib) specimens were only fixed in formalin but were not examined histologically.

3.2.16. Plasma concentration of ZK 36.374

Determination of plasma concentration of ZK 36.374 (1 ml citrated plasma taken 1 hour after administration) was performed in samples collected on days 1, 8 and 12 from all animals.

4. Methods for statistical analysis

The Dunnett-test was used to assess differences between group 1 (control) and the treated groups. Group mean values which differ significantly from the control group are marked by * ($p < 0.05$) or ** ($p < 0.01$).

A 1-factorial covariance analysis was performed for systolic and mean blood pressure, at which only p values smaller than 0.05 are shown (marked by an asterisk (*) in the table).

For the statistical analysis no separation between males and females was performed although differences in body weights were observed between sexes on the basis of individual values. The interpretation of the results was therefore based mainly also on the basis of individual data.

5. Results

In general only pathological findings or distinctly recognizable changes and tendencies toward changes which are attributable to the direct effect of ZK 36.374 will be reported.

All further information about individual data and observations can be obtained from Appendix I and II.

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3.2.14. Organ weights

From all animals sacrificed at termination the following organs were dissected free of fat, removed and weighed, the paired organs being weighed together:

liver, kidneys, heart, lungs, adrenals, thyroid, pituitary, ovaries, uterus, testes, epididymides, prostate together with seminal vesicle.

3.2.15. Microscopical examination

From all control and ZK 36.374 treated animals the following organs or representative samples thereof were fixed and stained:

lung (right main lobe), spleen, thymus, lymph node (Ln. iliacus), cerebrum, cerebellum, medulla oblongata, epididymis (head, tail), prostate, gall-bladder, vein (V. cava caudalis), artery (Aorta thoracalis), muscle (M. gastrocnemius), heart (right and left atrium), thyroid gland, ovary.

fixation: neutral buffered formalin acc. Lillie

staining: hematoxylin-eosin (H.E.)

Oviducts*, uterus (corpus, cervix), vagina, skin, mammary gland, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas (tail), urinary bladder (trigonum), liver, kidney, heart (left papillary muscle, right ventricle wall), adrenals, testes, esophagus.

Fixation: Bouin's fixative

Staining: H.E.

Pituitary:

Fixation: formalin sublimate acc. Brookes

Staining: aldehyde fuchsin acc. Scott (modif.)**

In addition, for the demonstration of fat, frozen sections of liver, kidney, and heart from all the animals in the experiment, stained with oil red O, were examined under the microscope. The frozen sections were prepared from formalin-fixed specimens.

Observation times and results

Mortality: One animal from each group treated with iloprost died

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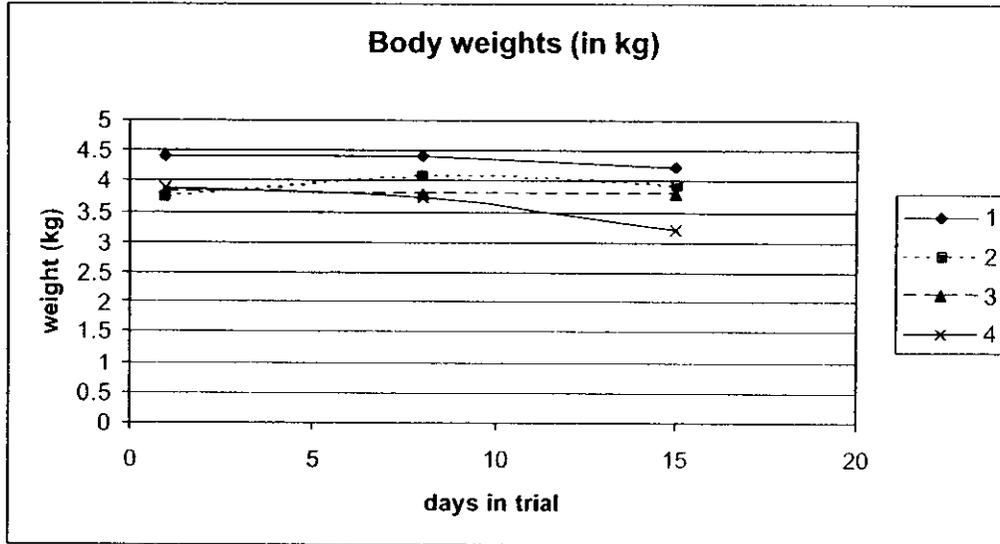
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Clinical signs:

Table 2: Clinical findings (General Observation) after ZK 36.374
 Number of animals with symptoms/Σ of the findings from
 observation times; [] day of first and last occurrence
 of finding; () number and sex of animals affected

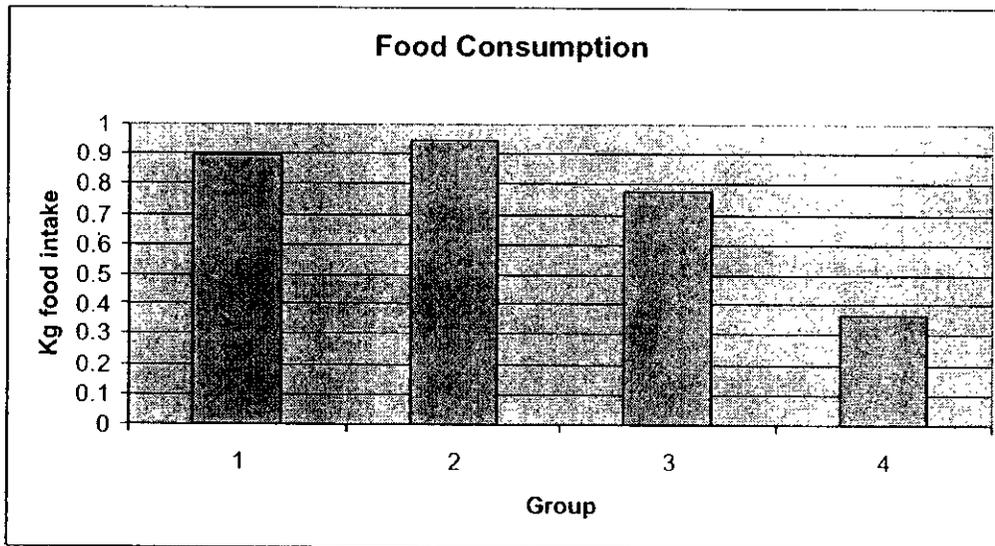
Findings	Group 2	Group 3	Group 4
<u>Before administration:</u>			
Vomiting	1/1[15] (1F)		
Severe apathy	1/1[5] (1F)		
Supine position	1/1[5] (1F)		
Respiratory symptoms	1/1[5] (1F)		
Diarrhea			2/3[4-8] (2M)
Passage of thin feces	1/1[2] (1F)		
Increased salivation			1/1[10] (1F)
<u>During administration:</u>			
Vomiting	1/1[1] (1M)		
Substance vomited	1/1[10] (1M)		
<u>9-30 minutes after administration:</u>			
Slight apathy	1/1[3] (1F)	1/1[4] (1F)	1/1[3] (1M)
Moderate apathy			2/6[1-5] (2F)
Severe apathy	1/1[4] (1F)		3/7[1-5] (2M,1F)
Loss of consciousness			2/2[1] (2M)
Lateral position	1/2[3-4] (1F)		1/2[2-3] (1M)
Eyelid closure			1/1[1] (1M)
Vomiting		1/1[4] (1M)	
Slightly increased lacrimation			1/1[2] (1M)
Severely increased lacrimation			1/1[1] (1M)
Increased salivation			4/5[1-10] (2M,2F)
<u>45 minutes to 2 3/4 hours after administration:</u>			
Slight apathy	1/1[2] (1F)	2/2[14] (2F)	4/15[2-14] (2M,2F)
Moderate apathy		1/4[8-13] (1F)	3/6[1-12] (1M,2F)
Severe apathy			2/4[5-13] (2M)
Lateral position	1/1[2] (1F)		3/5[4-13] (2M,1F)
Supine position			1/1[7] (1M)
Vomiting			1/1[4] (1F)
Severely increased lacrimation			1/1[10] (1M)
Increased salivation		1/1[5] (1F)	3/3[1-10] (2M,1F)
Eyelid closure			1/1[1] (1F)

Body weights:



Body weights decreased greatest in the high dose group

Food consumption:



Food consumption was lowest in the high dose group

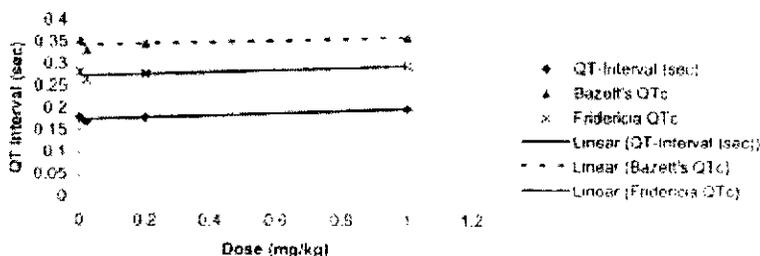
Ophthalmoscopy: no treatment related findings

EKG:

Dose, Heart Rate, QT interval and QTc interval Spreadsheet

Animal	Drug Name	Dose (mg/kg)	Dosing Period	Heart Rate (BPM)	QT-Interval (sec)	R-R Interval	Bazett's QTc	Fridericia QTc
Monkey	iloprost	0		234	0.18	0.256	0.355	0.283
Monkey	iloprost	0.02		228	0.17	0.263	0.331	0.265
Monkey	iloprost	0.2		222	0.18	0.270	0.348	0.278
Monkey	iloprost	1		196	0.2	0.306	0.361	0.297
Monkey	iloprost					#DIV/0!	#DIV/0!	#DIV/0!
						#DIV/0!	#DIV/0!	#DIV/0!
						#DIV/0!	#DIV/0!	#DIV/0!
						#DIV/0!	#DIV/0!	#DIV/0!
						#DIV/0!	#DIV/0!	#DIV/0!
						#DIV/0!	#DIV/0!	#DIV/0!

QT interval-Dose Relationship



Hematology: a decrease in monocyte count was seen at the high dose

Clinical chemistry:

Urinalysis: only change seen was all the females in the 0.2 and 1.0 mg/kg/day groups were menstruating, since iloprost does have reproductive effects, this could be a consequence of treatment.

Gross pathology: no treatment related changes were noted

Organ weights (specify organs weighed if not in histopath table): no treatment related changes were noted.

Histopathology: Adequate Battery: yes (X), no ()—explain
Peer review: yes (), no (X)

Toxicokinetics:

Table 5: Plasma levels of ZK 36.374 (ng/ml)

Dose mg/kg	Animal No.	Day of treatment		
		1	8	12
0	(controls)			
	31M	0		0
	362M	0	0	0
	3519F	0	0	0
	3536F	0	0	
0.02	3693F	- *		
	357M			
	202M			
	3600F ^{a)}		-	-
0.2	66F			
	200M		- ***	
	85F			
	240M			
1.0 (on day 1 2.0 mg/kg, except animal no. 448M)	37F			
	158M ^{b)}		-	-
	138F			
	304M			
	448M			-

* faulty determination

** RIA 017

*** sample lost

a) animal No. 3600F died on day 5 of treatment

b) animal No. 158M died on first day of treatment

c) determination on treatment day 7

Other:

Study title: Systemic tolerance study of ZK 36.374 in monkey (*Macaca fascicularis*) after continuous intravenous infusion using the — osmotic-pump over a period of four weeks.

Key study findings: Cynomolgus monkeys were given 0.02, 0.17, or 1.41 mg/kg/day of iloprost. All the animals in the high or mid dose group animals died in the first or second week of the study. Most likely the cause of death was hypotensive shock due to the vasodilatation. The 0.02 mg/kg/day dose was well tolerated for the 4 week study.

Study no.: 5482

Volume #, and page #: 5.8, pp.205-313

Conducting laboratory and location: Schering, Germany

Date of study initiation: July 30, 1982

GLP compliance: no

QA report: yes () no (x)

Drug, lot #, and % purity: ZK 36.374, batch # 68-AA-1/ISC .Fr .3-17

Methods

Doses: 0, 0.02, 0.17, 1.41 mg/kg/dy

Species/strain: *Macaca fascicularis*

Number/sex/group or time point (main study): 4 groups each with 2 males and 2 females

Route, formulation, volume, and infusion rate: IV using the — osmotic-pump, vehicle was 37.21 mg trometamol, water to 1 ml, HCl to adjust pH to 8.2

Satellite groups used for toxicokinetics or recovery: no

Age: unknown

Weight (nonrodents only): 1.7-2.0 kg

Unique study design or methodology (if any):

Observation times and results

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Mortality:

All animals at the mean dose levels of 0.17 and 1.41 mg/kg (groups 3 and 4) died within the first or second week of treatment. Distribution of deaths according to day of treatment is as follows:

Group 3 (X 0.17 mg/kg/day)			Group 4 (X 1.41 mg/kg/day)		
Animal No.	Sex	day	Animal no.	Sex	day
29.4	male	14	342.4	male	4
180.4	male	7	476.4	male	2
49.4	female	7	456.4	female	9
190.4	female	7	459.3	female	4

Clinical signs:

Table 2: Signs and behaviour after treatment with ZK 36,374

Signs	Group 1 (control)		Group 2 (0.02 mg/kg/day)				Group 3 (0.17 mg/kg/day)				Group 4 (1.41 mg/kg/day)					
	male	female	male	female	male	female	male	female	male	female	male	female				
	N/S	F/L	N/S	F/L	N/S	F/L	N/S	F/L	N/S	F/L	N/S	F/L				
slight apathy				1/1	1				2/5	3/6	2/3	1/3	2/8	1/4	2/9	1/6
moderate apathy									2/9	2/11	2/4	2/4	1/1	2	1/2	2/3
severe apathy									2/15	3/13	2/8	3/6	2/8	1/7	2/9	3/9
unconsciousness											2/2	6				
lateral position								2/2	7/13							
prone position											2/4	6	2/4	3/7	2/2	4/9
supine position													1/1	7		
squatting position	1/1	1	1/1	1					2/25	2/13	2/16	1/5	2/11	1/5	2/16	1/8
disequilibrium								1/3	4/3				1/1	3	2/3	2/6
irritability													2/8	1/4	1/7	3/6
croaking													1/1	3		
gnashing of teeth								1/1	4	1/2	1/3					
wailing								1/6	4/8							
moderate salivation													1/1	3		
diarrhea															1/1	2

Legend: N/S: Number of animals with the signs/sum of the observations
 F/L: First day/last day of observation

Body weights: no effect was noted on animals receiving 0.02 mg/kg/day compared to controls. With the high and mid dose animals, most died prior to weighing, in the few remaining animals there was a significant weight loss compared to controls.

Food consumption: no effect was noted on animals receiving 0.02 mg/kg/day compared to controls. With the high and mid dose animals, most died prior to weighing, in the few remaining animals there was a significant decrease in food consumption compared to controls.

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Ophthalmoscopy: no treatment related effects were reported

EKG: no treatment related effects were reported, however, the high and mid dose animals were not evaluated.

Hematology: Little information was collected on the high and mid dose group animals due to premature death. Controls and 0.02 mg/kg/day iloprost treatment were not significantly different

Clinical chemistry:

Table 4: Findings of blood chemistry

Group	Mean dose level mg/kg/day	Number of animals examined /with findings	Findings	time of examination
2	0.02	2M,2F/2M,2F	increased albumin-globulin quotient (P < 0.05)	week 5
		2M,2F/2M,2F	slight increase in serum calcium (P < 0.01)	week 5
3	0.17	2M, 1F/1M (No. 1804)	high urea nitrogen (128 mg/100 ml)	day 7
		1M, 1F/1F (No. 1904)	slight increase in serum creatinine (1.3 mg/100 ml)	day 4
		2M, 1F/1M (No. 294)	high SGPT value (176 mg/ml)	day 10
		2M, 1F/2M, 1F	slight decrease in γ -globulin	week 1 or 2
4	1.41	1M,2F/1M,2F	high serum urea nitrogen (193,214,121 mg/100 ml)	week 1 or 2
		2F/2F (No. 4564, 4593)	marked increase in serum creatinine (3.5, 2.2 mg/100ml)	day 9 and 4
		1M,2F/1F (No. 4564)	low total cholesterol (33 mg/100 ml)	day 9
		1M,2F/1F (No. 4564)	low serum glucose (19 mg/100 ml)	day 9
		1M,2F/1M, 1F (No. 4764, 4564)	low serum calcium (5.5, 5.8 mg/100 ml)	day 7 and 3
		1M,2F/1F (No. 4564)	raised serum potassium (3.9 mMol/l)	day 9
		1M,2F/1M,2F	slight decrease in γ -globulin	week 1 or 2

Urinalysis: only control and low dose animals were evaluated with no treatment related effects reported

Gross pathology: In the animals that died prematurely in the study, 3 of 4 had redness of the gastric mucosa, and 2 of 4 animals had gastric hemorrhagic erosions. One male in the

high dose group had a yellow small focus on the liver. No other treatment emergent effects were noted.

Organ weights (specify organs weighed if not in histopath table): absolute liver weights were increased and pituitary weights were decreased.

Histopathology: Adequate Battery: yes (), no ()—explain

Peer review: yes (), no ()

Histological findings were consistent with the premature deaths being due to hypotensive shock, with hyperemia of the liver (3 of 4 animals), adrenal cortex (2 of 4 animals), and pancreas (2 of 4 animals).

Toxicokinetics: not done

Other:

Study title: Systemic tolerance study in beagle dogs after continuous sub-cutaneous infusion with the — osmotic pump over 26 weeks.

Key study findings: Dogs received 0, 0.024, 0.048, or 0.096 mg/kg/day of iloprost. The low dose had very little toxicity associated with it, however, the higher doses were associated with high levels of diarrhea in the mid and high dose groups, with one female in the high dose group being sacrificed due to premature death with diarrhea, emesis, and severe decrease in body weight due to lack of ability to eat.

Study no.: 7949

Volume #, and page #: 5.12, pp.1-399

Conducting laboratory and location: Schering, Germany

Date of study initiation: March 24, 1987

GLP compliance: yes

QA report: yes () no ()

Drug, lot #, and % purity: ZK 36.374, batch # 68-AA-11 Teil II

Methods

Doses: 0, 0.024, 0.048, 0.096 mg/kg/day

Species/strain: beagle dogs

Number/sex/group or time point (main study): 4 males and 4 females per dose group

Route, formulation, volume, and infusion rate: subcutaneous infusion with the alzet osmotic pump, flow rate of 2.72 or 2.59 microl/hr

Satellite groups used for toxicokinetics or recovery: no

Age: not given

Weight (nonrodents only): males: 8.2-11.7 kg, females: 6.9-10.1 kg

Unique study design or methodology (if any):

Observation times and results

Mortality: Although no animal died in the study, one female in the high dose group was sacrificed moribund on day 9 of the study after severe diarrhea, vomiting and inability to eat.

Clinical signs: although diarrhea and GI tract problems are mentioned, there is no listing of observations. One male in the high dose group had a prolapsed rectum that was surgically repaired

Body weights: only the high dose females did not add weight in the study

Food consumption: no treatment related effects were noted

Ophthalmoscopy: no treatment related effects were noted

EKG: no treatment related effects were noted

Hematology: no treatment related effects were noted

Clinical chemistry:

Parameter	↑ Increase ↓ Decrease	Group	Sex	Week	Statistical significance
glutamic oxaloacetic trans-aminase	↑	2, 4	M	4	p < 0.05, p < 0.01
	↓	3, 4	M	1	p < 0.01, p < 0.05
	↓	4	F	1	p < 0.05
alkaline phosphatase	↑	3	M	12	p < 0.01
creatinine	↑	4	M	4	p < 0.05
total bilirubin	↓	2, 3	M	4	p < 0.05
albumin (relative)	↓	2	F	26	p < 0.05
α ₁ -globulin (relative)	↓	4	F	26	p < 0.05
total α-globulin (absolute)	↑	2	F	4	p < 0.05
β ₁ -globulin (relative)	↑	3	F	4	p < 0.01
β ₁ -globulin (rel. and abs.)	↑	2	F	26	p < 0.05
total β-globulin (relative)	↑	2	F	26	p < 0.05
β ₁ -globulin (absolute)	↑	3, 4	F	4	p < 0.01, p < 0.05
albumin/globulin quotient	↓	2	F	26	p < 0.05
potassium	↑	4	M	4	p < 0.05

Urinalysis: no treatment related effects were noted

Gross pathology: no treatment related effects were noted

Organ weights (specify organs weighed if not in histopath table): In group 2, 2 females had decreases thyroid weights, and 2 males had decreased epididymal weights.

Histopathology: Adequate Battery: yes (x), no ()—explain

Peer review: yes (), no (x)

Toxicokinetics:

Table 3: Plasma levels of iloprost (in ng/ml) after continuous s.c. infusion of 2K 36.374 in Beagle dogs over a period of 26 weeks (11/89)

Group	Planned dose (mg SH 36.374/kg/day)	Number of animals and sex	Weeks of treatment								Mean over the total implantation period (first and last implantation)
			1	2	3	4	21	22	23	24	
2	0.031	4 M	0.4/0.1	0.4/0.0	0.3/0.1	0.4/0.1	0.4/0.1	0.4/0.1	0.3/0.1	0.6/0.1	0.4/0.2
		4 F	0.4/0.1	0.5/0.1	0.3/0.1	0.5/0.2	0.3/0.1	0.2/0.1	0.3/0.2	0.4/0.1	0.4/0.2
3	0.081	4 M	0.9/0.2	1.1/0.2	0.7/0.1	1.2/0.2	1.0/0.2	0.6/0.1	0.9/0.5	1.1/0.1	0.9/0.4
		4 F	1.2/0.2	0.9/0.1	0.9/0.0	1.1/0.4	0.9/0.2	0.6/0.2	0.5/0.2	1.1/0.5	0.9/0.1
4	0.115	4 M	1.2/0.1	2.1/0.9	2.6/2.0	1.2/2.4	2.0/0.5	1.7/0.2	1.8/0.2	2.4/0.2	2.1/1.2
		4 F	1.5/0.1	1.8/0.2	1.8/2.1	1.9/0.5	1.5/0.3	1.2/0.4	1.1/0.4	2.0/0.3	1.6/0.6

* Animal no. 6138 F showed plasma levels of 1.7 ng/ml on day 7 and 2.1 ng/ml on day 9 after pump implantation and was replaced by animal no. 6555 F on day 10.

Other:

Study title: Systemic tolerance study (including local tolerance) in beagle dogs after daily oral administration of retard formulation of iloprost (iloprost clathrate, SH K 529 I/M) over 53 weeks (2 applications per day at a 6-hour interval)

Key study findings: Controls, 2x 25 microg/day or 2x 75 microg/day were the dosages tested. Dogs receiving the high dose had diarrhea for 8 weeks, one animal died of intestinal torsion. At the low dose, there were few findings in the animals.

Study no.: A706

Volume #, and page #: 5.13, pp. 1-480

Conducting laboratory and location: Schering, Germany

Date of study initiation: November, 1991

GLP compliance: yes

QA report: yes (x) no ()

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Drug, lot #, and % purity: ZK 96.944, batch # 106 760

Methods

Doses: Control (no treatment), Control (placebo capsules), 2x 25 microg/day, 2x 75 microg/day.

Species/strain: Beagle dogs

Number/sex/group or time point (main study): 4 male and 4 female beagle dogs/group

Route, formulation, volume, and infusion rate: oral, capsules,

Satellite groups used for toxicokinetics or recovery: no

Age: not given

Weight (nonrodents only): males 8-15.2 kg; females 7.9-13 kg

Unique study design or methodology (if any):

Observation times and results

Mortality: 1 female in the high dose group died of volvulus (intestinal torsion) in week 7, and one female in the low dose group was sacrificed for humanitarian reasons in week #44 due to necrotizing stomatitis. Although the sponsor did not consider the second animals problems to be compound related, due to the GI tract being the primary target organ in the high dose group, and the small sample size, I would have to consider this event compound related.

Clinical signs: In the high dose group, diarrhea lasting 8 weeks occurred in the animals, followed by mucous in the stools for the remainder of the study. This probably indicates the primary target organ is the GI tract. In the low dose group, one female developed necrotizing stomatitis in week 10 leading to a humane sacrifice at week 44.

Body weights: no treatment related effects were reported by the sponsor

Food consumption: no treatment related effects were reported by the sponsor

Ophthalmoscopy: no treatment related effects were reported by the sponsor

EKG: no treatment related effects were reported by the sponsor, however, ECG changes were only reported in animals receiving iloprost, raising concerns

Hematology: no treatment related effects were reported by the sponsor, changes in the treatment groups, although statistically significant ($p < .05$), were within the normally reported reference ranges.

Clinical chemistry: in the high dose females, significant decreases in serum protein and calcium were found, as well as an increase in blood glucose levels in high dose group males and females.

Urinalysis: no treatment related effects were reported by the sponsor

Gross pathology: no treatment related effects were reported by the sponsor, except in the high dose female that died of intestinal torsion.

Organ weights (specify organs weighed if not in histopath table): The only significant change was in adrenal weights in the group 3 females that increased significantly.

Histopathology: Adequate Battery: yes (x), no ()—explain

Peer review: yes (), no (x)

no treatment related effects were reported by the sponsor

Toxicokinetics:

Table 6: Pharmacokinetic data of iloprost after twice daily p.o. application (at a 6-hour interval) of iloprost retard formulation SH K 529 I/M in Beagle dogs (Mean/SD, n=4)

Week of study	Pharmacokinetic parameter	Group 3 (2x25 µg iloprost/kg/day)		Group 4 (2x75 µg iloprost/kg/day)	
		Male	Female	Male	Female
1	C _{max} (pg/ml)				
	- after 1. application	— (n=1)	506/100	1283/248 (n=3)	1547/217 (n=3)
	- after 2. application	— (n=1)	138/43	— (n=1)	488/134 (n=3)
	AUC 0-24 hours (pg x hour/ml)	— (n=1)	2322/1181	— (n=1)	9973/1324 (n=3)
4	C _{max} (pg/ml)				
	- after 1. application	487/238	542/292	651/234	106/128
	- after 2. application	181/153	104/36	287/36	475/90
	AUC 0-24 hours (pg x hour/ml)	3707/556 (n=3)	2836/1275	5069/1005	3645/946
12	C _{max} (pg/ml)				
	- after 1. application	371/99	446/245	845/36	1115/540 (n=3)
	- after 2. application	301/167	254/152	446/63	428/4 (n=2)
	AUC 0-24 hours (pg x hour/ml)	3466/1469	2257/1062	5934/1405	7732/52 (n=2)
25	C _{max} (pg/ml)				
	- after 1. application	454/224	597/326	520/125	741/158 (n=3)
	- after 2. application	186/88	565/643 (n=2)	326/120	302/62 (n=3)
	AUC 0-24 hours (pg x h/ml)	2633/694	3276/1486	4939/411	4777/468 (n=3)
51	C _{max} (pg/ml)				
	- after 1. application	349/66	696/221 (n=3)	958/153	407/227 (n=3)
	- after 2. application	212/168	132/63 (n=3)	255/71	331/66 (n=3)
	AUC 0-24 hours (pg x hour/ml)	2491/1247	3154/1767 (n=3)	5528/1295	6092/167 (n=3)

Other:

2.6.6.4 Genetic toxicology

Study title: Evaluation of ZK 36.374 in the Ames Salmonella/microsome mutagenicity test

Key findings: Iloprost was non-toxic at up to 5 mg/plate and showed no signs of mutagenicity. Positive and negative controls were consistent with a valid assay system.

Study no: 5169

Study type (if not reflected in title):

Volume #, and page #: 5.18, pp.193-209

Conducting laboratory and location: Schering, Germany

Date of study initiation: April 15, 1982

GLP compliance: no

QA reports: yes () no (x)

Drug, lot #, radiolabel, and % purity: not given

Formulation/vehicle: 0.1 M phosphate buffer

Methods:

Strains/species/cell line: *S. typhimurium* strains: TA1535, TA1537, TA98, and TA100

Dose selection criteria: none given

Basis of dose selection:

Range finding studies:

Test agent stability: solutions made fresh immediately prior to use

Metabolic activation system: rat hepatic post-mitochondrial fraction – S9-fraction

Controls:

Vehicle: phosphate buffer

Negative controls: vehicle

Positive controls: 2-aminoanthracene, 2-nitrofluorene, 1-methyl-3-nitro-1-nitrosoguanidine, cyclophosphamide and benzo[a]pyrene

Comments:

Exposure conditions:

Incubation and sampling times: Plates were incubated at 37C for 3 days prior to scoring.

Doses used in definitive study: .01, .025, .1, .25, 1, and 5 mg/plate

Study design:

Analysis:

No. of replicates: for the solvent controls, 3 plates per group; for the treated groups, 3 plates per group; for the positive controls, 3 plates per group.

Counting method: visual scoring of revertant colonies.

Criteria for positive results: section 3.2.7.- "Where the data for any treatment level show a response ≥ 2 times the concurrent vehicle control value (TA98, TA100), or ≥ 3 times the concurrent vehicle control value (TA1535 and TA1537), in conjunction with a dose-related response, the result is considered positive"

Summary of individual study findings:

Study validity: positive and negative controls indicated assay was valid.
Study outcome: study results were negative for Iloprost.

Study title: Evaluation of ZK 36.374 in the Ames Salmonella/microsome mutagenicity test with preincubation

Key findings: Iloprost was non-toxic at up to 5 mg/plate and showed no signs of mutagenicity. Positive and negative controls were consistent with a valid assay system.

Study no: 7466

Study type (if not reflected in title):

Volume #, and page #: 5.18, pp.210-227

Conducting laboratory and location: Schering, Germany

Date of study initiation: December 12, 1986

GLP compliance: yes

QA reports: yes () no (x)

Drug, lot #, radiolabel, and % purity: not given

Formulation/vehicle: DMSO

Methods:

Strains/species/cell line: *S. typhimurium* strains: TA1535, TA1537, TA98, and TA100

Dose selection criteria: none given

Basis of dose selection:

Range finding studies:

Test agent stability: solutions made fresh immediately prior to use

Metabolic activation system: rat hepatic post-mitochondrial fraction -- S9-fraction

Controls:

Vehicle: phosphate buffer

Negative controls: vehicle

Positive controls: 2-aminoanthracene, 2-nitrofluorene, N-nitrosodimethylamine, cyclophosphamide, sodium azide, 9-aminoacridine and benzo[a]pyrene

Comments:

Exposure conditions:

Incubation and sampling times: Plates were incubated at 37C for 3 days prior to scoring with a 60 minutes at 37C preincubation period prior to plating

Doses used in definitive study: .05, .1, .25, .5, 1, 2.5 and 5 mg/plate

Study design:**Analysis:**

No. of replicates: for the solvent controls, 3 plates per group; for the treated groups, 3 plates per group; for the positive controls, 3 plates per group.

Counting method: visual scoring of revertant colonies.

Criteria for positive results: section 3.2.7. - "Where the data for any treatment level show a response ≥ 2 times the concurrent vehicle control value (TA98, TA100), or ≥ 3 times the concurrent vehicle control value (TA1535 and TA1537), in conjunction with a dose-related response, the result is considered positive"

Summary of individual study findings:

Study validity: positive and negative controls indicated assay was valid.

Study outcome: study results were negative for Iloprost.

Study title: Mutagenicity evaluation of ZK 36.374 in the reverse mutation assay with E.coli strain WP2uvrA.

Key findings: Iloprost was non-toxic at up to 5 mg/plate and showed no signs of mutagenicity. Positive and negative controls were consistent with a valid assay system.

Study no: 7548

Study type (if not reflected in title):

Volume #, and page #: 5.18, pp.228-249

Conducting laboratory and location: [

]

Date of study initiation: January 7, 1987

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, radiolabel, and % purity: Zk 36.374, batch # 68-AA-11 Teil II

Formulation/vehicle: DMSO

Methods:

Strains/species/cell line: *S. typhimurium* strains: TA1535, TA1537, TA98, and TA100

Dose selection criteria: none given

Basis of dose selection:

Range finding studies:

Test agent stability: solutions made fresh immediately prior to use

Metabolic activation system: rat hepatic post-mitochondrial fraction – S9-fraction

Controls:

Vehicle: phosphate buffer

Negative controls: vehicle

Positive controls: 2-aminoanthracene and Ethylmethanesulfonate

Comments:

Exposure conditions:

Incubation and sampling times: Plates were incubated at 37C for 3 days prior to scoring.

Doses used in definitive study: .001, .01, .1, .5, 1, 2.5 and 5 mg/plate

Study design:

Analysis:

No. of replicates: for the solvent controls, 3 plates per group; for the treated groups, 3 plates per group; for the positive controls, 3 plates per group.

Counting method: visual scoring of revertant colonies.

Criteria for positive results: section 3.2.7. - "Where the data for any treatment level show a response ≥ 2 times the concurrent vehicle control value (TA98, TA100), or ≥ 3 times the concurrent vehicle control value (TA1535 and TA1537), in conjunction with a dose-related response, the result is considered positive"

Summary of individual study findings:

Study validity: positive and negative controls indicated assay was valid.

Study outcome: study results were negative for Iloprost.

Study title: Test report of study LMP 261: ZK 36.374 – detection of gene mutations in somatic mammalian cells in culture: HGPRT-test with V79 cells

Key findings: Iloprost appeared to be mutagenic in this assay, with a statistically significant increase in mutants over the controls with S9 increasing mutagenicity further. Although the sponsor contends the data falls within historical negative control range, the most important factor is the internal consistency in the assay. Second, the dose levels used were not high enough, with only a mild indication of cell mortality at the highest dose used. Generally sponsors present these assays with dose titrations up to high levels of mortality. Therefore, although weak, the study should be considered positive. Prostacyclin receptors are found on CHO cells and may indicate a cAMP dependent mechanism.

Study no: 7429

Study type (if not reflected in title):

Volume #, and page #: 5.18, pp.250-278

Conducting laboratory and location: Schering, Berlin, Germany

Date of study initiation: October 27, 1986

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, radiolabel, and % purity: ZK 36.374, batch # 68-AA-8 Teil 1

Formulation/vehicle: DMSO

Methods:

Strains/species/cell line: Chinese Hamster Ovary V79 cells

Dose selection criteria: none given

Basis of dose selection:

Range finding studies:

Test agent stability: unknown, therefore solutions were made fresh every day

Metabolic activation system: rat liver post-mitochondrial fraction (S9)

Controls:

Vehicle: DMSO

Negative controls:

Positive controls: Methyl methanesulphonate and 9,10-dimethyl-1,2-benzanthracene.

Comments:

Exposure conditions:

Incubation and sampling times: not given

Doses used in definitive study: -S9: .025, .1, .15, .25 mg; +S9: .0154, .0475, .1, .3, .475 mg

Study design:

Treatment scheme:

Day 1: Subculturing of a log-phase culture

a) About 500 cells in 5 ml medium/25 cm²-plastic-flask for plating efficiency; in duplicate per experimental point

b) 8×10^5 cells in 30 ml medium/175 cm²-plastic-flask for the mutagenicity test, 1 flask per experimental point

2: Treatment of a) and b);

5: Subculturing of b) in 175 cm²-plastic-flasks

8: Fixation and staining of colonies in a)-flasks
= plating efficiency and dose relationship

9: Subculturing of b) in five 80 cm²-plastic-flasks containing selective medium: mutant selection (about $4 - 6 \times 10^5$ cells/flask); subculturing of b) in two 25 cm²-flasks for plating efficiency (about 500 cells/flask)

16: Fixation and staining of colonies in b) - derived flasks seeded on day 9.

All incubations were done at 37° C.

Staining was done with 10 % methylene blue in 0.01 n-KOH solution
[]

The stained colonies with more than 50 cells were counted with a preparation microscope []

Criteria for positive results: both duplicate plates must show a > 1.5 fold increase over the vehicle controls to be positive.

Summary of individual study findings:

Study validity: negative and positive controls demonstrated the validity of the assay.

Study outcome: The Mann-Whitney U test results showed a statistically significant difference between solvent controls and the highest dose of iloprost tested. Although the result was within the historical vehicle control range, the internal consistency of the assay is the more important criteria.

Study title: Evaluation of the clastogenic potential in the human lymphocyte test

Key findings: No evidence of chromosomal aberrations was seen in the assay. Assay went to a significant level of mitotic inhibition and was valid. Mitotic inhibition was probably due to the interaction of iloprost with native prostacyclin receptors.

Study no: 7160

Study type (if not reflected in title):

Volume #, and page #: 5.18, pp.279-297

Conducting laboratory and location: Schering, Germany

Date of study initiation: November 21, 1985

GLP compliance: Yes

QA reports: yes () no (x)

Drug, lot #, radiolabel, and % purity: ZK 36.374, batch # 68-AA-8 Teil 2

Formulation/vehicle: DMSO

Methods:

Strains/species/cell line: human lymphocytes

Dose selection criteria:

Basis of dose selection: based on mitotic inhibition

Range finding studies:

Test agent stability: from section 3.1: made fresh to avoid stability issues?

Metabolic activation system: +S9

Controls:

Vehicle: DMSO

Negative controls: vehicle

Positive controls: cyclophosphamide and triaziquone

Exposure conditions:

Incubation and sampling times:

Doses used in definitive study: .0025, .005, .01, .025, .05, .1, .25, .5, & 1 mg/ml

Study design: f
Analysis:
No. of replicates:
Counting method:
Criteria for positive results:

Summary of individual study findings:

Study validity: Positive and negative controls responded reasonably, indicating a valid assay system
Study outcome: Iloprost was not shown to cause chromosomal aberrations in this assay system.

Study title: Studies on the mutagenic potential of ZK 36.374 in the mouse micronucleus test.

Key findings: No sign of an increased potential for mutagenicity of iloprost was found in this assay system.

Study no: 5360

Study type (if not reflected in title):

Volume #, and page #: 5.18, pp. 298-315

Conducting laboratory and location: Schering, Berlin, Germany

Date of study initiation: April 5, 1982

GLP compliance: no

QA reports: yes () no (x)

Drug, lot #, radiolabel, and % purity: ZK 36.374, batch # 03741/81

Formulation/vehicle: physiological saline

Methods:

Strains/species/cell line: NMRI/SPF mice, 9-10 weeks old

Dose selection criteria:

Basis of dose selection:

Range finding studies:

Test agent stability:

Metabolic activation system: none

Controls:

Vehicle: physiological saline

Negative controls: vehicle

Positive controls: triaziquone

Comments:

Exposure conditions:

Incubation and sampling times:

Doses used in definitive study: animals were given control (10ml/kg physiological saline), 0.2 mg/kg iloprost (in 10 ml/kg physiological saline), or 0.125 mg/kg triaziquone (single i.p. treatment)

Study design: 5 males and 5 females per group

Analysis:

No. of replicates:

Counting method: micronucleated cells per 1000 polychromatic erythrocytes and per 1000 normochromatic erythrocytes was done.

Criteria for positive results: statistically significant differences from the negative controls ($p < .05$)

Summary of individual study findings:

Study validity: positive and negative controls were consistent with a valid assay.

Study outcome: Study did not show iloprost increased levels of micronucleation in erythrocytes. Although there were differences in some of the readings between the two examiners, their conclusions did not differ.

Genetic toxicology summary and conclusions: Except for the CHO V79 chromosomal aberration assay, none of the mutagenicity assays were positive. The V79 chromosomal aberration assay was only weakly positive, and CHO cells do have prostacyclin receptors, indicating the possibility of the assay being due to a physiological response. Prostacyclins do have internalized, translocating receptors similar to estrogen that bind to DNA. Therefore, the weakness of the response, with no collaborating positive responses, indicates the need to keep an eye on Iloprost, but probably not enough to warrant a change in direction and the carcinogenicity studies do not show a significant tumorigenicity potential for iloprost.

Labeling recommendations: It could be indicated that Iloprost \square however, result was very equivocal.

2.6.6.5 Carcinogenicity

Study title: Iloprost calthrate: tumorigenicity study in mice after daily dietary administration of the ground extended release formulation SH K 529 over approximately 96 weeks

Key study findings: In this 95-97 week study, there was no noted treatment related neoplastic or non-neoplastic lesions found in the mice.

Adequacy of the carcinogenicity study and appropriateness of the test model: The use of iloprost clathrate limits the utility of this assay. Iloprost is only ~13% of the iloprost clathrate molecule, making the 10% iloprost clathrate dose only 1.3% iloprost, the important component. The adequacy of this trial is questionable.

Evaluation of tumor findings: no trends were noted of an increase in tumors in iloprost clathrate treated animals.

Study no.: AW35

Volume #, and page #: 5.19 – 5.21, all pp.

Conducting laboratory and location: Schering, Germany

Date of study initiation: September 1994

GLP compliance: no

QA report: yes () no (x)

Drug, lot #, and % purity: iloprost clathrate, batch # 108920

CAC concurrence:

Methods

Doses:

Basis of dose selection (MTD, MFD, AUC etc.):

Species/strain:

Number/sex/group (main study):

Route, formulation, volume:

Frequency of dosing:

Satellite groups used for toxicokinetics or special groups:

Age:

Animal housing:

Restriction paradigm for dietary restriction studies:

Drug stability/homogeneity:

Dual controls employed:

Interim sacrifices:

Deviations from original study protocol:

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Summary of the report

55-70 male and 55-70 female Han: NMRI (SPF) mice per group received the ground test article mixed with the diet, ground placebo pellets mixed with the diet, or blank diet according to the following treatment scheme:

Treatment scheme

Group	Number of animals for assessment of tumorigenicity	Number of animals for determination of serum levels	Control / test article	Concentration	Dose (mg iloprost/kg/day)			
					nominal calculated on the basis of 30 g body weight and 6 g/day food intake		actual mean values calculated on the basis of actual food consumption and actual body weight over the whole study period	
					iloprost	iloprost dihydrate	iloprost	'iloprost' dihydrate
1	50M/50F	5M/5F ¹⁾	Blank diet (control)	--	--	--	--	--
2	50M/50F	5M/5F ¹⁾	Ground placebo retard pellets (placebo control)	10% (w/w)	--	--	--	--
3	50M/50F	20M/20F ²⁾	Ground iloprost dihydrate retard pellets	3% (w/w)	4.7	35.7	males: 3.1 females: 2.7	males: 23.6 females: 23.1
4	50M/50F	20M/20F ²⁾	Ground iloprost dihydrate retard pellets	10% (w/w)	15.4	117.0	males: 10.5 females: 13.3	males: 78.3 females: 101.1

- 1) 5 male and 5 female animals were sacrificed in week 4
- 2) 5 male and 5 female animals were sacrificed in weeks 4, 25, 52 and 80

The dose selection was based on an orientating mouse study (Schering report A040) over 29 days with dietary administration of the ground test article (iloprost dihydrate retard pellets) in a 10% concentration. In this study mean serum levels of the active ingredient iloprost were achieved at different time-points of blood sampling which ranged between a multiple of 14-27 of those mean levels obtained after therapeutic application in humans. On an extrapolated AUC_{0-12 hours}-basis the multiples of the human exposure ranged between 17-21. The administration of higher concentrations (> 10 %) did not seem advisable as this would impair the balanced nutrition of the animals. As even after the high dose of a 10 % concentration in the diet only slight systemic effects were expected, the testing of two dose levels below the 10% concentration seemed not to increase the information concerning dose-dependent effects. Thus, only one low dose was selected and set at a 3 % concentration in the diet. Assuming a linear dose-effect ratio, this concentration was expected to lead to serum levels in mice which correspond to 4-8 times the therapeutic plasma levels in humans. Therefore, 3 and 10 % dietary concentrations seemed to be sufficient to obtain a reasonable dose graduation

Animals were housed individually under standard conditions. The pulverized diet and water were offered ad libitum. Until termination of the study general observations, mortality, food and

water consumption, body weights, and serum levels of iloprost were determined at regular intervals. At the end of the 95-97-week treatment period hematological examinations, necropsy findings, organ weights and histological examinations were used for the assessment of tumorigenicity as well as systemic toxicity.

The study was terminated after treatment for 95-97 weeks due to high mortality (ca. 70%) in female animals in all groups incl. the control group.

Statistical analysis was performed for parameters obtained during the in-life phase by the Dunnett-test and for parameters related to tumor incidence by using the trend test according to Peto et al. (1980)

The following information could be obtained from the study data.

The serum level monitoring revealed a constant and dose-dependent systemic exposure [based on AUC_{0-9h}-values approximately 6 times (group 3) and up to 57 times (group 4) higher than in humans after high therapeutic doses]

Observation times

Mortality: animals were checked 2x a day Monday-Friday, and 1x a day on weekends

Clinical signs: animals were checked 2x a day Monday-Friday, and 1x a day on weekends

Body weights: body weight was recorded weekly for the first 13 weeks, then monthly through the end of the study

Food consumption: was monitored on a weekly basis for 13-14 weeks, then one week per month until the end of the study

Histopathology: Peer review: yes (), no (x)

Toxicokinetics: were checked in 5 males and 5 females per group after 4, 26, 52, and 95 weeks in the study.

Results

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Mortality:

Text Table 5 Mortality rates and assumed causes of death in control and treatment groups after dietary administration of ground placebo or ground iloprost clathrate extended release pellets over approximately 96 weeks to mice in comparison to control animals which received the blank diet

Group	1 blank diet control		2 ground placebo pellets control		3 ground iloprost clathrate extended release pellets		4 ground iloprost clathrate extended release pellets	
	M	F	M	F	M	F	M	F
Dose, mg iloprost/kg/day: nominal	0		0		4.7		15.4	
actual	0		0		3.1	3.7	10.3	13.3
No. of animals for assessment of tumorigenicity	50	50	50	50	50	50	50	50
Survivors / premature deaths	31/19	16/34	31/19	15/35	35/14	20/30	32/18	15/35
Mortality, %	38	68	38	70	28	60	36	70
Kind of death:								
- found dead	19	24	17	28	11	26	17	31
- killed moribund or for humane reasons	-	9	2	6	3	4	1	3
- death by accident	-	1	-	1	-	-	-	1
Cause of death:								
- not evident	5	3	9	7	5	4	8	5
- non-neoplastic changes	6	6	1	6	1	3	1	3
- neoplastic changes (malignant lymphoma)	8 (6)	25 (20)	9 (9)	22 (19)	8 (6)	23 (20)	9 (7)	27 (24)
No. of satellite animals for serum level determination	5 ¹⁾	5 ¹⁾	5 ¹⁾	5 ¹⁾	20 ²⁾	20 ²⁾	20 ²⁾	20 ²⁾
Survivors to interim sacrifice / premature deaths	5/0	5/0	5/0	5/0	18/2	16/4	19/1	17/3

M = males F = females

¹⁾ = interim sacrifice week 4

²⁾ = interim sacrifice week 4, 26, 52 and 95

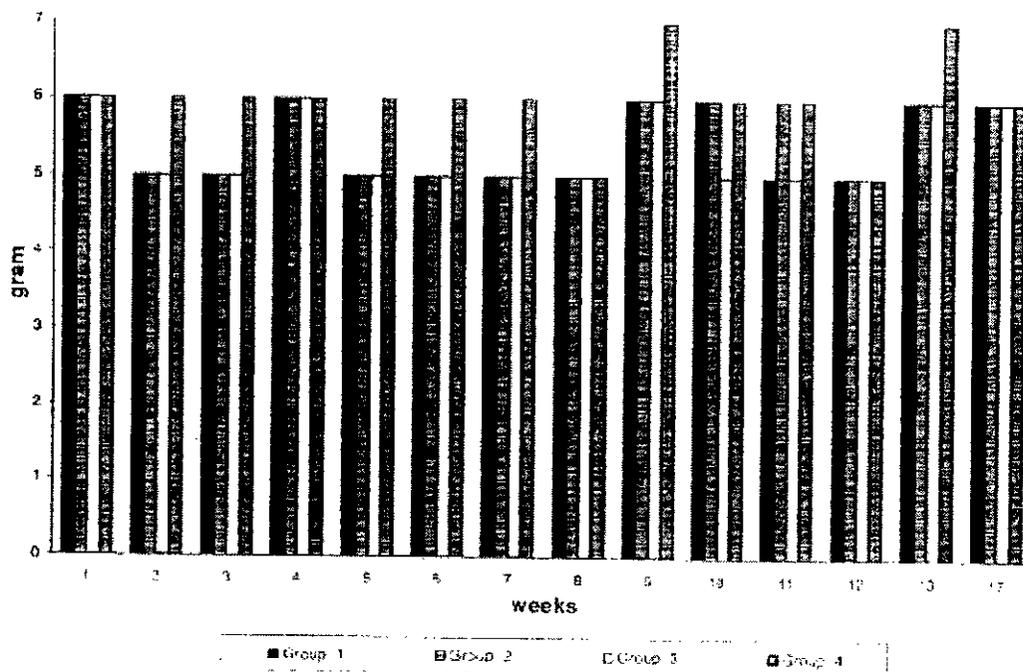
Clinical signs: No treatment related findings were reported by the sponsor

Body weights: No treatment related findings were reported by the sponsor

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Food consumption: there was an increase in food consumption in group 4 females in the first 13 weeks. This effect resolved after that, there was no effect noted in body weights.

Text Figure 1 Daily food consumption in female animals (week 1 to 17)



Gross pathology: Although there were lesions in the animals that died, as with the mortality, there was no discernible pattern due to treatment

Histopathology:

Non-neoplastic: various inflammatory, degenerative and hyperplastic lesions were seen, however, they were generally related to aging and were not correlatable to treatment.

Neoplastic: the most prominent finding was malignant lymphomas and in the lungs adenomas and carcinomas. However, there was no indication of an increase in incidence in treated versus control mice.

Toxicokinetics:

Serum levels of iloprost declined over the period of the study. Through week 4 there appeared to be gender differences, however, this effect resolved over the course of the study.

Group	gender	dose	week 4 serum level(ng/ml)	week 26 serum level(ng/ml)	week 52 serum level(ng/ml)	week 95 serum level(ng/ml)
3	M	2.93 mg/kg	2.7	2.7	1.3	0.9
3	F	3.54 mg/kg	2	1.3	1.3	1.1
4	M	9.855 mg/kg	4.7	4.4	4.4	2.6
4	F	12.59 mg/kg	9.3	4.6	3.7	3.9

Study title: Iloprost clathrate. Tumorigenicity study in rats after daily dietary administration of the ground extended release formulation SH K 529 over approximately 2 years.

Key study findings: No treatment related increase in tumors was seen over the course of the two-year study.

Adequacy of the carcinogenicity study and appropriateness of the test model: The use of iloprost clathrate limits the utility of this assay. Iloprost is only ~13% of the iloprost clathrate molecule, making the 10% iloprost clathrate dose only 1.3% iloprost, the important component. The adequacy of this trial is questionable.

Evaluation of tumor findings: The only finding was in placebo and 10% iloprost clathrate treated animals with a small increase in pancreatic islet tumors.

Study no.: AP21

Volume #, and page #: 5.22 to 5.24, all pp.

Conducting laboratory and location: Schering, Germany

Date of study initiation: January 1994

GLP compliance: no

QA report: yes () no (x)

Drug, lot #, and % purity: iloprost clathrate, batch #'s 106760, 107510, and 107550

CAC concurrence: ?

Methods

Doses:

Basis of dose selection (MTD, MFD, AUC etc.):

Species/strain:

Number/sex/group (main study):

Route, formulation, volume:

Frequency of dosing:

Satellite groups used for toxicokinetics or special groups:

Age:

Animal housing:

Restriction paradigm for dietary restriction studies:

Drug stability/homogeneity:

Dual controls employed:

Interim sacrifices:

Deviations from original study protocol:

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Summary of the report

50 male and 50 female Han: WIST (SPF) rats per group received the test article iloprost clathrate admixed as ground extended release pellets to the diet, ground placebo pellets in the diet or blank diet over 104-108 weeks according to the following treatment scheme:

Treatment scheme

Group	Number of animals for tumorigenicity	Test article	Concentration	Dose (mg/kg/day)			
				nominal		actual	
				iloprost	iloprost clathrate	iloprost	iloprost clathrate
1	50M/50F	Blank diet (control)	--	--	--	--	--
2	50M/50F	Ground placebo pellets (placebo control)	10% (w/w)	--	--	--	--
3	50M/50F	Ground iloprost clathrate pellets	3% (w/w)	1.8	13.7	1.3 (M)* 1.6 (F)*	9.9 (M)* 12.2 (F)*
4	50M/50F	Ground iloprost clathrate pellets	10% (w/w)	6.0	45.6	4.2 (M)* 5.3 (F)*	31.9 (M)* 40.3 (F)*

* = mean value calculated over the whole study period

M = males

F = females

The test article selection for this tumorigenicity study was based on the need to mimic the exposure situation in human patients as far as possible. According to a comparative toxicokinetic study with iloprost, iloprost clathrate and ground iloprost clathrate extended release pellets (Schering report AA52) and a feasibility study in rats (Schering report AC83) it became obvious that only the dietary administration of the drug substance (iloprost clathrate) or the ground drug product (iloprost clathrate extended release pellets) could result in long-persisting plasma levels of iloprost which are representative of therapy in humans. Selection of the drug substance iloprost clathrate as test article would not allow a complete evaluation of the final drug product, i.e. the effects of the excipients and their possible interactions with the drug could not be assessed. Therefore, the ground drug product (iloprost clathrate extended release pellets) was selected as test article. The dose selection for this study was based on results of systemic tolerance studies performed in rats with this test article after 1, 3 and 10% (w/w) dietary administration for treatment periods of up to 28 weeks. In these studies long-lasting plasma levels of iloprost were obtained which reached a multiple of up to 31 times the plasma levels (Schering reports AC52 and AB90) achieved after oral therapeutic application of

the test article in human patients. The administration of a higher dose by increasing the dietary concentration (> 10%) did not seem advisable, as this would impair the balanced nutrition of the animals. As even after the high concentration of 10% in the diet only slight systemic effects were expected, the testing of two dose levels (concentrations) below the 10% concentration seemed unlikely to increase the information concerning dose-dependent effects. Thus, only one low dose was selected and set at a 3% dietary concentration.

A placebo control group was used to find out effects not related to the active principle ioprost.

The systemic exposure was monitored by determinations of plasma drug concentrations in weeks 26, 52 and 104.

Animals were housed individually under standard conditions. The pulverized diet and water were offered ad libitum. Until termination of the study general observations, mortality, food and water consumption, body weights were determined at regular intervals. At the end of the two-year treatment period hematological and bone marrow examinations, necropsy findings, organ weights and histological examinations were used for the assessment of tumorigenicity as well as systemic toxicity.

Statistical analysis was performed for parameters obtained during the in-life phase by the DUNNETT-test (group 1 (blank diet) vs. group 2 (placebo) and group 1 vs. groups 3 (3%) and 4 (10%)) and for parameters related to tumor incidence by using the trend test according to Peto et al. (1980) (group 1 vs. groups 2, 3 and 4).

Observation times

Mortality:

Clinical signs:

Body weights:

Food consumption:

Histopathology: Peer review: yes (), no ()

Toxicokinetics:

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3.2. Observations, clinical and laboratory studies

3.2.1. General observations

All signs of weak health or reactions to treatment were recorded twice daily and on weekends once daily. Detailed clinical inspections including palpation of the animals were performed once weekly. From week 91 onwards the recording of findings from clinical examinations was reduced from twice daily to once weekly during the clinical inspections, if no severe impairment of health status occurred.

3.2.2. Mortality

Time of premature death or scheduled sacrifice was recorded.

3.2.3. Food consumption

The individual food consumption was determined in all animals per group and sex at weekly intervals up to week 13 and for one week in every 4-week period thereafter until week 103.

3.2.4. Water consumption

The amount of water consumed was determined in 20 animals per group and sex (when available) at weekly intervals up to week 13 and for one week in every 3-month period thereafter until week 103.

3.2.5. Body weight

Body weight was determined once per week in all surviving animals of all groups in the first 13 weeks of the study and once per month thereafter. Body weight gain was calculated for all surviving animals over the period of 1-13 and 1-104 weeks.

3.2.6. Hematology

Hematological investigations were performed in weeks 104-105 for all surviving animals per group for determination of the following parameters:

erythrocyte count, leucocyte count, hemoglobin, hematocrit (packed cell volume), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and platelet count. Where possible, hematology was also performed prior to necropsy for all animals sacrificed in a moribund state or for humane reasons.

3.2.7. Bone marrow

At termination of the study in weeks 105-109 the number of nucleated cells per milligram bone marrow collected from the femur after exsanguination was determined and bone marrow smears were prepared from all animals. The bone marrow smears were not evaluated because the histological examination of femoral bone marrow did not give any evidence which would require detailed examination by a myelogram.

Where possible, the number of nucleated cells per milligram bone marrow was also determined for all animals sacrificed in a moribund state or for humane reasons.

3.2.8. Plasma concentration of iloprost

Citrate plasma samples were collected between 7 and 8 a.m. in weeks 26, 52 and 104 from 10 male and 10 female fed animals of each group and were stored frozen at ca. -20°C until analysis by a radioimmunoassay at the Department of Pharmacokinetics, Schering AG, Berlin.

The blood collection was restricted to one time-point (7-8 a.m.). This was based upon the results of previous studies (Schering reports AA52 and AB90) which showed that iloprost plasma levels remain almost constant in the range of 0.5 and 1.5 ng/ml in rats following dietary intake ranging between 0.69 and 3.9 mg iloprost/kg during the night covering a time period (uptake of food) of at least 12 hours. The maximal plasma levels were seen at 12 and 16 hours after access to food following single dietary administration over 24 hours and according to the feed intake behavior (the animals eat most of their food during the night). The collecting time point between 7 and 8 a.m. seemed to be suited for sufficient plasma level monitoring especially because the duration of half-maximal plasma levels (half-value duration²) was > 12 hours at dietary administration as compared to less than 0.5 hours after corresponding i.g. administration (for further information see Schering report AA52).

3.2.9. Necropsy

A full post-mortem examination was carried out in all animals of the study. All macroscopic findings were recorded.

3.2.10. Organ weights

From all animals the following organs were removed, dissected free of fat and surrounding connective tissue and weighed, the paired organs being weighed together:

liver, kidneys, heart, spleen, pituitary, thyroid with parathyroids, adrenals, testes, prostate, epididymides, seminal vesicles, ovaries, uterus, salivary glands (submand./subling.).

For weight analysis only the organs of terminally sacrificed exsanguinated animals were used. Weights of animals which were found dead or sacrificed prematurely were only recorded when possible. When macroscopic alterations, which could bias the evaluation of absolute and/or relative weights, e.g. tumors, cysts, abscesses etc., were observed the weight of the corresponding organ was only recorded, but not used for statistical analysis.

3.2.11. Microscopic examinations

From all animals of all groups the following organs/tissues or representative samples thereof were fixed in Lillie's neutral buffered formalin:

- liver
- kidneys
- urinary bladder
- brain (cerebrum, cerebellum, medulla oblongata)
- spinal cord (cervical, thoracal,

² long half value duration is an indication of a slow drug release

- heart
- aorta thoracalis
- vena cava caudalis
- trachea
- lung
- pituitary
- thyroid, parathyroid
- adrenals
- ovaries
- uterus (horns, corpus, cervix)
- vagina
- clitoral gland/preputial gland
- skin
- mammary gland
- testes
- epididymides
- prostate
- seminal vesicle
- thymus
- spleen
- mesenteric lymph node
- mandibular lymph nodes
- bone (femur with bone marrow)
- lumbar)
- peripheral nerve (N. saphenus)
- tongue
- salivary gland (submand./subling.)
- esophagus
- stomach (non-gland./gland.)
- duodenum:
- jejunum
- ileum
- cecum
- colon
- rectum
- pancreas
- Harderian gland
- eyes
- any other tissue with macroscopic alteration, if necessary for diagnostic reasons

For microscopic examination, all collected tissues from all animals of all groups of the study were processed for histological examination, which was performed in hematoxylin-eosin stained 4-6 μ m paraffin sections. Histological examination and statistical analysis were performed by

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Results

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Mortality:

Text Table 3. Mortality rates and assumed causes of death in control and treatment groups after dietary administration of ground placebo or ground iloprost clathrate extended release pellets over 2 years to rats in comparison to control animals which received the blank diet

Compound	Control	Placebo	Ground iloprost clathrate extended release pellets	Ground iloprost clathrate extended release pellets
Dose (mg iloprost/kg/day, nominal)	0	0	1.8 (3% diet admixture)	6.0 (10% diet admixture)
Group	1	2	3	4
No. of animals	50M, 50F	50M, 50F	50M, 50F	50M, 50F
Survivors/decedents				
- males	36/14	34/16	30/20	39/11
- females	37/13	37/13	38/12	36/14
Kind of death				
- found dead	12M, 9F	13M, 12F	16M, 9F	9M, 10F
- killed moribund or for humane reasons	2M, 4F	3M, 1F	4M, 3F	1M, 3F
- death by accident (e.g. at blood sampling)	0	0	0	1M, 1F
Mortality (%)	28 (M), 26 (F)	32 (M), 26 (F)	40 (M), 24 (F)	22 (M), 28 (F)
Cause of death				
Cause of death not evident	1M, 1F	2M	2M	2M
Pituitary gland - adenoma	12M, 9F	10M, 7F	14M, 8F	6M, 8F
Other causes				
- neoplastic change	3F	4M, 6F	3M, 4F	1M, 5F
- non-neoplastic change	1M		1M	2M, 1F

M = males

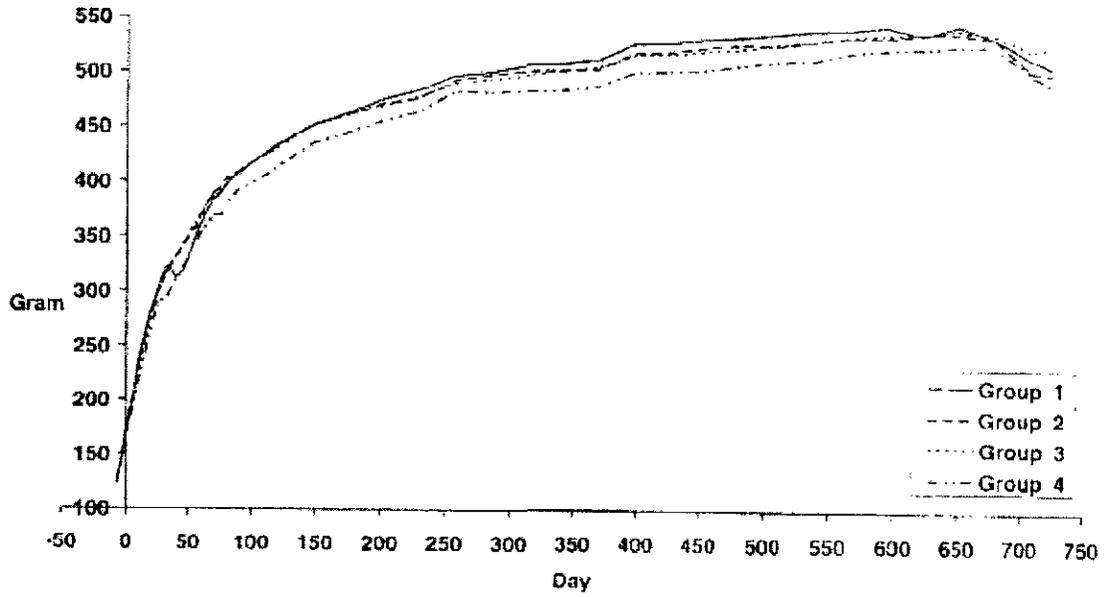
F = females

The aforementioned Text Table 3 demonstrates that the mortality rate was not influenced by the treatment with the test article in comparison to the corresponding control groups.

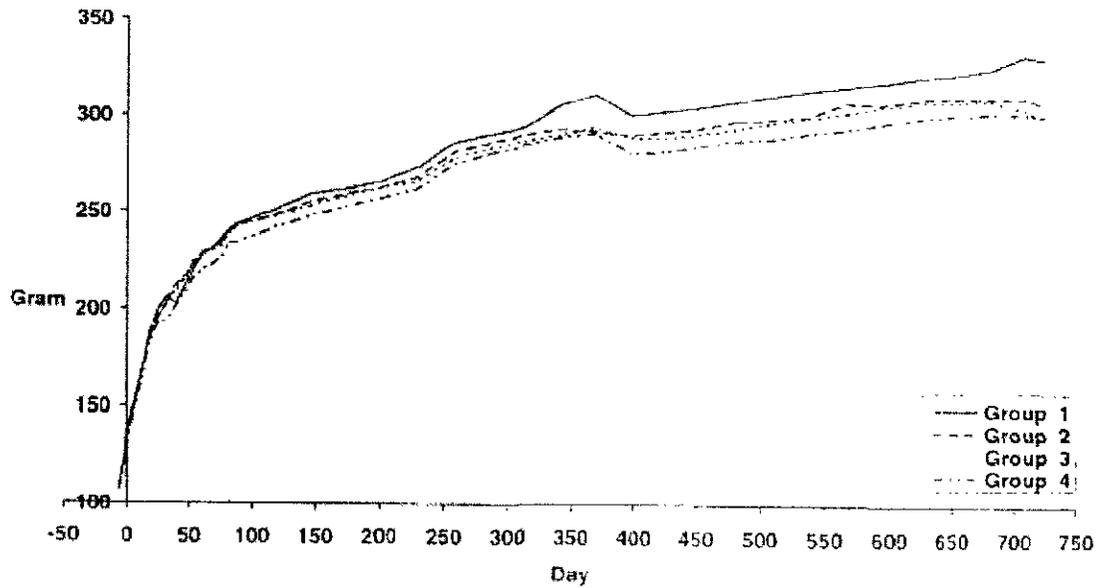
Clinical signs: no treatment related effects were reported by the sponsor

Body weights:

Text Figure 4: Body weight of male animals



Text Figure 5: Body weight of female animals



Food consumption:

Text Table 4: Compilation of mean food consumption per day of male and female rats after daily dietary administration of ground isoprost clathrate retard pellets over 103 weeks (in g; mean values/standard deviation)

Group	Dietary concentration of ground isoprost clathrate (in %)	Sex	Time interval of determination (weeks)							
			1-14	17-26	29-38	41-50	53-62	65-77	80-89	92-103
1	0 (diet control)	Males	24/2	22/2	23/2	22/1	23/2	21/1	20/2	21/3
		Females	17/2	17/1	17/1	17/1	16/1	17/2	17/2	17/2
2	0 (placebo control)	Males	24/1	23/1	23/1	22/2	23/2	22/2	21/3	21/3
		Females	17/2	17/1	17/2	17/1	18/1	17/2	16/2	16/2
3	3	Males	23/2	23/2	23/2	22/2	22/2	21/2	21/3	21/2
		Females	17/1	17/1	17/1	17/1	17/1	17/2	16/2	17/2
4	10	Males	22/1**	22/2	22/2	22/2	22/1	21/2	21/2	21/2
		Females	17/1	17/1	17/1	17/2	18/1	17/2	17/2	17/2

** = p < 0.01

A slight decrease in food consumption was observed in males of group 4 in weeks 1-14 (p < 0.01). Food consumption in female animals was not affected.

Gross pathology: no finding distinguished treated from untreated animals

Histopathology:

Non-neoplastic: no finding distinguished treated from untreated animals

Neoplastic: no finding distinguished treated from untreated animals

Text Table 8: Compilation of neoplastic lesions in pancreas and ovaries

Organ/Tumor	Group 1 (Diet control)		Group 2 (Placebo control)		Group 3 (3% diet admixture)		Group 4 (10% diet admixture)	
	Male	Female	Male	Female	Male	Female	Male	Female
Ovary	--		--		--		--	
- granulosa-theca cell tumor (benign)		2 (= 4%)		2 (= 4%)		4 (= 8%)		5 (= 10%)
- thecoma (malignant)								1 (= 2%)
Σ	--	2 (= 4%)	--	2 (= 4%)	--	4 (= 8%)	--	6 (= 12%)
Pancreas								
- islet cell adenoma	2 (= 4%)	1 (= 2%)	4 (= 8%)	0	1 (= 2%)	2 (= 4%)	6 (= 12%) ^a	0
- islet cell carcinoma							1 (= 2%)	
Σ	2 (= 4%)	1 (= 2%)	4 (= 8%)	0	1 (= 2%)	2 (= 4%)	7 (= 14%) ^a	0

^a = p < 0.05 in comparison to group 1, but not significant in comparison to group 2

^b = not tested statistically

Text Table 9: Incidence data for granulosa-theca cell tumors and islet cell tumors in Wistar rats

	Incidence from historical reference data ^{*)}	Incidence from the literature (5)	Incidence observed in this study
Granulosa-theca cell tumor (benign)	0 - 4%	0 - 8.3%	Diet control : 4% Placebo control: 4% Low dose : 8% High dose: 10%
Granulosa-theca cell tumor (malignant)	0%	0-2%	Diet control : 0% Placebo control: 0% Low dose : 0% High dose: 2%
Islet cell adenoma	Males: 4%	Males: 0-2.1%	Males (diet control): 4% Males (placebo): 8% Males (low dose): 2% Males (high dose): 12%
Islet cell carcinoma	Males: 0%	Males: 0-2.0%	Diet control : 0% Placebo control: 0% Low dose : 0% High dose: 2%

^{*)} = data from 5 studies with 50 control animals per sex each

Toxicokinetics:

Text Table 7: Iloprost plasma concentration after daily dietary administration of ground iloprost clathrate extended release pellets to rats over 104 weeks

Concentration of ground iloprost clathrate extended release pellets in food (in %)	Doses in mg iloprost/kg body weight/day, calculated on the basis of body weight and food intake in the week of blood sampling				Iloprost plasma concentration ng/ml (M/SD)		
	Week	25-26	52-53	103-104	Week 26	Week 52	Week 104
					(n = 10)	(n = 10)	(n = 10)
3	Males	1.1	1.1	0.9	0.62/0.13	0.41/0.11	0.39/0.18
	Females	1.5	1.5	1.2	0.52/0.24	0.52/0.15	0.46/0.14
10	Males	3.6	3.8	3.0	2.0/0.57	1.2/0.33	1.2/0.33
	Females	5.2	5.0	4.1	2.0/0.93	1.5/0.7*	1.5/0.57

Study title: Carcinogenicity Study of Iloprost Clathrate in Mice

Key study findings: Mice received 0, 5, 50, or 125 mg/kg/day of iloprost via gastric gavage for 104 weeks. No evidence of carcinogenicity emerged from this study.

Adequacy of the carcinogenicity study and appropriateness of the test model:

Executive CAC

May 13, 1997

Committee: Joseph DeGeorge, PhD, HFD-24, Chair

Joseph Contrera, PhD, HFD-900, Member

Robert Osterberg, PhD, HFD-520, Rotating Member

Jasti Choudary, PhD, Division Team Leader

Lillian Patrician, MS, MBA, HFD-024, Project Manager

Tanveer Ahmad, PhD, Presenting Reviewer

IND: — (Ahmad/Choudary;

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Dose Selection for Rodent Carcinogenicity Studies

Mouse Carcinogenicity Study: The sponsor found lethality at a HD of 250 mg/kg, and subsequently proposed dose selection of 5, 50, and 125 mg/kg based on MTD. The Division agreed with this. The Committee expressed some concern that the HD of 125 may exceed the MTD. However, both the LD and MD are viable. There is good kinetic data reported. The AUC spanned a useful range.

The CAC concluded that the proposed doses of 5, 50, and 125 mg/kg/day are acceptable.

Rat Carcinogenicity Study: This was the first gavage study conducted. In the 13-week oral (gavage) dose ranging study, doses of 5, 50, 125 and 250 mg/kg/day were used. No significant effect on body weight gains and food intake were seen in males treated with 5 mg/kg/day, as well as in females of all dose groups, when compared to their respective control. Body weight gains were decreased by 18.6%, 27.8% and 25.7% in males treated with 50, 125 and 250 mg/kg/day dose levels. In males, decreases in body weight gains at 125 and 250 mg/kg/day dose levels were accompanied with reduction in food consumptions (13-140/0). No significant effects on food intake were seen in males treated with 50 mg/kg/day. The platelet count also decreased in treated males (24-42%) and females (15-33%). The reviewer recommended 50 mg/kg/day as the MTD for both sexes.

However, based on the above data, the Committee concluded that 50 mg/kg/day is the MTD in males and doses of 5, 25 and 50 mg/kg/day should be used in males. Based on the reduction of platelet count in treated females, the Committee concluded that 125 mg/kg/day is the MTD in females, and doses of 5, 50 and 125 mg/kg/day should be used.

Joseph DeGeorge, PhD

Chair, CAC

cc: IND —

HFD- Div. File

HFD- Pharm/Ahmad

HFD- PTL/Choudary

HFD- CSO/

Evaluation of tumor findings:

The most frequently observed gross lesions were in the lungs and liver which corresponded to bronchiolar-alveolar neoplasms in the lungs and hepatocellular neoplasms in the liver. There was no evidence of a test article effect in the frequency of these neoplasms.

Study no.: BC60

Volume #, and page #: eCTD

Conducting laboratory and location: []

Date of study initiation: May 24, 1996

GLP compliance: yes

QA report: yes (X) no ()

Drug, lot #, and % purity: The test material, iloprost clathrate (also identified as iloprost clathrat, iloprost-@-cyclodextrin, ZK 96.944, and/or iloprost-@-cyclodextrin-clathrat), was received from Schering AG, Berlin, Germany, in several shipments as follows:

Description	Lot No.	Date Received	Weeks Used
White Powder	117800	July 6, 1996	1-76
White Powder	117880	July 27, 1996	76-81
White Powder	117810	July 27, 1996	82-105

These materials were stored frozen — protected from light. The purity was assumed to be — %.

CAC concurrence: CAC concurred on October 26, 2004 that there was no indication of treatment related carcinogenicity.

Methods

Doses: 0, 5, 50, 125 mg/kg/day

Basis of dose selection (MTD, MFD, AUC etc.): see Adequacy of the carcinogenicity study and appropriateness of the test model:

Species/strain: A total of 644 (322/sex), approximately 4-week-old, Crl:CD1 (ICR)BR albino mice was received on June 25, 1996, []

Number/sex/group (main study):

Dosage Level	Concentration		Number of Animals		Animal Numbers	
	mg/kg/day	mg/mL	Male	Female	Male	Female
Main Study						
1 (Control)	vehicle	-	70	70	A62549- A62618	A62619- A62688
2 (Low)	5	0.5	70	70	A62689- A62758	A62759- A62828
3 (Mid)	50	5	70	70	A62829- A62898	A62899- A62968
4 (High)	125	12.5	70	70	A62969- A63038	A63039- A63108
Satellite Study						
2 (Low)	5	0.5	5	5	A63109- A63113	A63114- A63118
3 (Mid)	50	5	5	5	A63119- A63123	A63124- A63128
4 (High)	125	12.5	5	5	A63129- A63133	A63119- A63123

* Satellite animals were used for collection of blood samples 15 minutes after dosing once during Week 53 (for determination of plasma iloprost levels). Since these mice were not used for toxicologic assessment, food consumption, clinical pathology, detailed clinical observations, and gross and microscopic pathology data were not collected on these

animals. Satellite animals were weighed at the same intervals as the Main Study animals, but only for the purpose of dose adjustments. After the collection of blood during Week 53, all Satellite animals were sacrificed and discarded without necropsy. Satellite animals that died on treatment or were sacrificed in a moribund condition were discarded without necropsy.

7.1 Dose Preparation

Dosing formulations were prepared once weekly. For the purpose of dosage calculations, iloprost clathrate and the — base were assumed to be 100% pure. To prepare the Tris buffer vehicle/control, the appropriate amount of — base was transferred to a precalibrated beaker containing nine-tenths of the total volume of deionized water. The formulation was mixed on a magnetic stirrer for 5 minutes while the pH range was monitored. HCl and/or NaOH was used to adjust the pH to 8.3. Additional deionized water was added to bring the solution to the desired volume. The formulations were mixed on a magnetic stirrer for an additional 10 minutes, and the pH was remeasured and adjusted (if necessary). For each test material dosing formulation, the appropriate amount of iloprost clathrate was transferred to a precalibrated beaker containing two-thirds the total volume of Tris buffer solution. The formulation was mixed on a magnetic stirrer for 2 minutes and brought close to the final volume with additional vehicle. The pH of the solution was checked and adjusted to the range of to 8.4 with HCl and/or NaOH. The formulation was brought to total volume with additional vehicle and mixed on a magnetic stirrer for 5 minutes while the pH range was monitored. The formulations were transferred to amber jars.

7.2 Method of Administration

Mice were given the appropriate dosing formulation via oral gavage once daily, 7 days per week, for at least 104 weeks; dosing continued through the day prior to scheduled necropsy. Each animal was held securely to immobilize the head and administered the appropriate volume of test solution or control/vehicle material via a laboratory gavage needle. Individual dose volume was based on the most recently recorded body weight and the dose volume of 10 mL/kg/day. All dosing formulations were administered at approximately the same time each day (1033 to 1433 hours), removed from the refrigerator approximately one-half hour before dosing, and stored refrigerated between use.

The test material was administered orally because this is the intended route of human exposure.

Route, formulation, volume:

Frequency of dosing:

Satellite groups used for toxicokinetics or special groups:

Age:

Animal housing:

5.2 Housing

Upon receipt, mice of the same sex were housed two/cage in stainless-steel, hanging, wiremesh cages measuring 24.4 x 10.5 x 13.2 cm (d x w x h). Following assignment to study, each animal was individually housed.

Restriction paradigm for dietary restriction studies:

Drug stability/homogeneity:

Dual controls employed: no

Interim sacrifices:

Deviations from original study protocol:

Protocol Deviations

The following protocol deviations were noted:

On November 1, 1996, the second (p.m.) mortality and moribundity check was inadvertently not performed.

On December 20, 1996, the postdose observations for Group 3 animals were performed late due to a temporary computer systems downtime. In addition, a backwash of the test material occurred in three animals, leading to an incomplete administration of the test material. Animal No. A62850 was estimated to have received 0.30 mL, and animal Nos. A62830 and A62897 were estimated as receiving 0.01 mL each. Due to the computer systems malfunction, animal No. A62874 was inadvertently under dosed by 0.04 mL and A62876 was overdosed by 0.04 mL.

On August 8, 1997, the second (p.m.) mortality and moribundity check was inadvertently not performed.

On June 29, 1997, the time for the Group 4 males postdose observations was inadvertently not recorded.

The reserve samples taken on July 8, 1996, could not be located; therefore, the samples were not archived.

Complete blood count (CBC) was inadvertently performed on the following moribund animals:

A63042 (F4) A63056 (F4) A62839 (M3) A62934 (F3) A62728 (M2)

A62610 (M1) A62589 (M1) A62742 (M2) A62878 (M3) A62845 (M3)

A62683 (F1) A62557 (M1) A62875 (M3) A62590 (M1) A62897 (M3)

A62725 (M2) A62891 (M3) A62609 (F4) A62698 (M2)

A62781 (F2) A62577 (M1) A63105 (F4) A62640 (F1)

A62804 (F2) A63084 (F4) A62931 (F3) A62806 (F2)

Retention samples taken for Weeks 1, 2, 20, 43, 48, 53, 57, 67, 98, and 105 could not be located; therefore, the samples were not disposed.

Retention samples were not taken for the Week 104 dose preparation.

Dosing formulations were prepared more than once weekly during Weeks 14, 24, 30, and 45

On October 8, 1996, December 17, 1996, January 31, 1997, and May 16, 1997, the test material had to be remixed causing more than one preparation per week.

At the request of the Sponsor, the mailing address was changed

Several tissues were missing and not microscopically examined or only unilaterally examined due to technical errors, sectioning difficulties, or other reasons.

Observation times

8.1 Observation of Animals

8.1.1 Clinical Observations

All study mice were observed twice daily (a.m. and p.m., at least 6 hours between observations) for evidence of mortality and moribundity.

Twice daily (one performed in the morning, the other performed within 30 minutes postdose), cageside observations for obvious indications of a toxic and/or pharmacologic effect were recorded as they were observed for all Main Study animals, noting only those mice for which an observation was made.

Once prior to initiation of treatment and weekly thereafter, each Main Study animal was removed from its cage and examined for grossly visible or palpable masses, signs of poor health, and indications of abnormal behavior. Findings (including time of onset, location, size, appearance, and progression of grossly visible or palpable masses) were recorded for each animal.

8.1.2 Special Procedures

Several mice were treated, as directed by a staff veterinarian, with hydrogen peroxide for skin lesions. Hydrogen peroxide was applied to lesions two to three times daily with sterile gauze for 7 to 10 days, and lesions were reevaluated by a staff veterinarian.

8.1.3 Body Weights

Individual body weights were recorded prior to treatment, weekly for Weeks 1-14 and every fourth week thereafter, and at Week 105.

8.1.4 Food Consumption

Food consumption was measured weekly for Weeks 1-13 and once every fourth week thereafter for all Main Study animals. When obvious spillage or wastage was recorded for an animal during the detailed physical examination, the estimate of the food consumed by the animal was excluded from the group mean calculation for that particular interval.

8.2 Toxicokinetic Analyses

Blood samples were collected for determination of iloprost in plasma. During Week 53, blood samples were collected from each of the surviving Satellite mice in the 5, 50, and 125 mg/kg/day dose groups (maximum of five mice/sex/group) at 15 minutes postdose. At least 1 mL of blood (if possible) was collected from the vena cava using sodium pentobarbital as anesthesia. Sodium citrate solution (one part sodium citrate solution to nine parts blood) was used as the anticoagulant. Mice were not fasted prior to sampling. After collection, blood samples were centrifuged at approximately 2000 x g for approximately 15 minutes to obtain plasma. The approximate volume of plasma collected was determined (to the nearest 0.1 mL) and recorded. The resulting plasma fractions were separated into two subsamples (0.5 mL was aliquoted as the first subsample and the remainder reserved as the second subsample). If a total of less than 0.5 mL of plasma was collected, the total volume of plasma was designated as the first subsample, and a second subsample for that animal was not prepared. The first set of samples was frozen [] and shipped on dry ice to the Sponsor (or designee) on July 15, 1997. The second set of subsamples served as the reserve set and remains frozen [] at []. Drug analyses may be conducted by the Sponsor or designee. The GLP compliance of the conduct, reporting, and record retention of these data is the responsibility of the Sponsor.

8.3 Clinical Pathology

Blood samples for hematology evaluations were obtained from 10 mice/sex/group (the same animals at each interval, if possible) during Week 52 and at termination via venous sinus (orbital), using carbon dioxide/oxygen inhalation for anesthesia. The anticoagulant for the hematology samples was ethylenediaminetetraacetate (EDTA). The following parameters were determined:

Hematology

differential leukocyte and cell morphology

erythrocyte count

leukocyte count

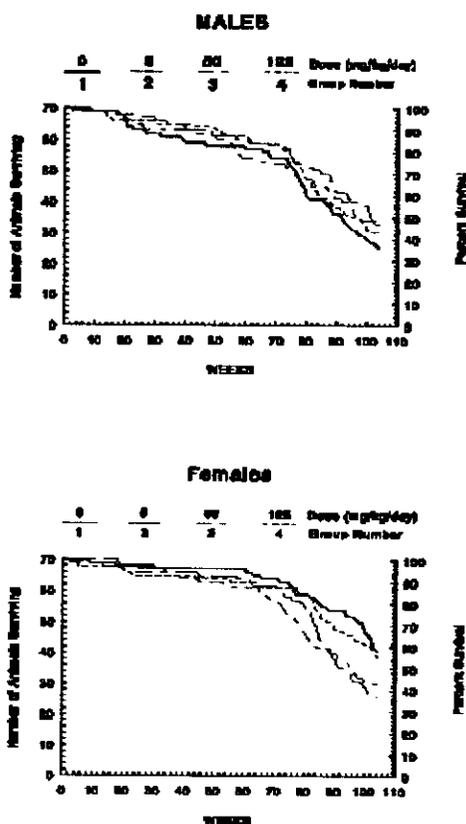
Clinical hematology analyses were performed using a Coulter Counter [] A differential leukocyte count and cell morphology were performed manually from peripheral blood smears for the 125 mg/g/day (high-dose) and control mice.

If clinical observations suggested a deterioration in health of the mice during the study (e.g., moribund condition), a differential leukocyte count and cell morphology evaluation was performed on the affected mice as soon as possible.

Results

Mortality: In male mice, there was no significant difference in mortality by dose group. In female mice, interestingly the low and mid dose groups had higher mortality than the control and high dose group. This elevation in mortality in female mice in the low and mid dose groups was not associated with an increase in neoplasms, in the low dose group there was an increase in abscess/septicemia resulting in death.

Text Figure 1: Adjusted Percent Survival

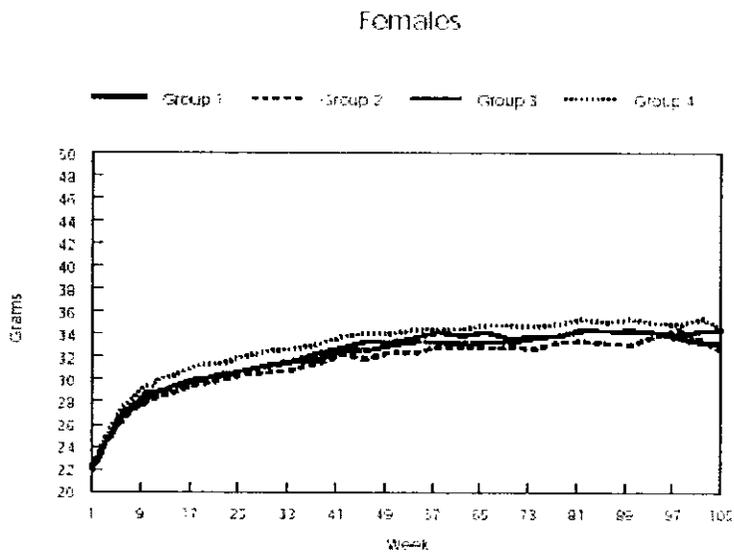
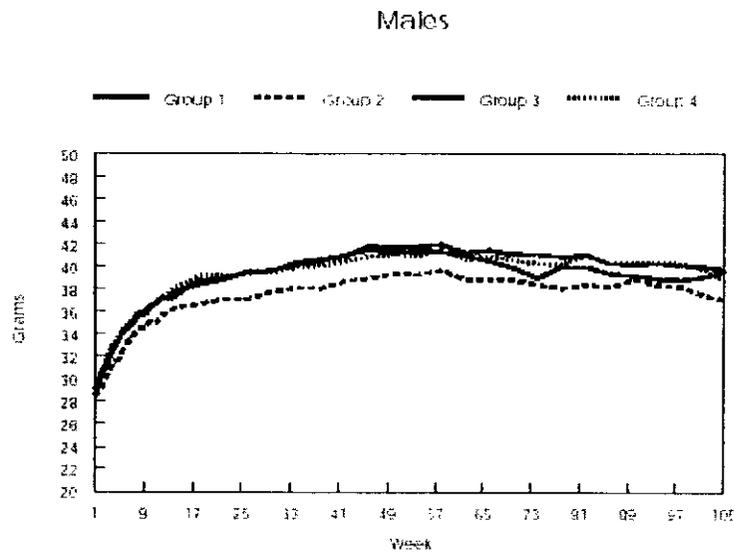


Clinical signs:

Body weights: Mean body weights were significantly lower for the 5 mg/kg/day males and higher for the 125 mg/kg/day females throughout most of the study, although terminal body weights were essentially comparable in the control and treated groups. There was no evidence of a test article effect in the body weight data.

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Text Figure 2: Mean Body Weights

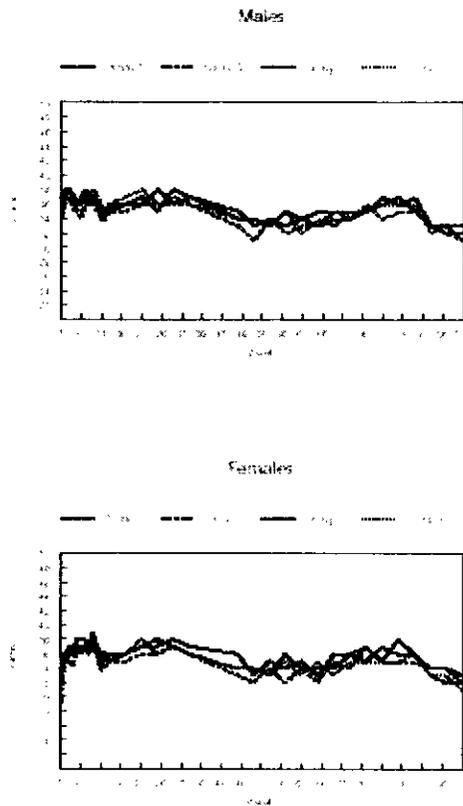


Food consumption:

Although weekly mean food consumption of treatment animals were occasionally significantly different than those of control animals, there were no obvious treatment-related differences in mean weekly food consumption values. There were no significant differences in the mean total

food consumption value in either sex.

Text Figure 3: Mean Food Consumption



Gross pathology: There were no treatment related gross pathology findings and/or microscopic findings; specifically there were no treatment-related increases in the incidence of neoplasms.

Histopathology:

Non-neoplastic: Test article-related histomorphologic changes were not observed in the tissues examined.

Neoplastic: A variety of other spontaneous disease lesions, including neoplasms in the lungs and liver, hematopoietic neoplasia, and degenerative cardiomyopathy, occurred without relationship to treatment, and were of the expected type and severity for mice of this age and strain.

Toxicokinetics: not done

Study title: Carcinogenicity Study of Iloprost Clathrate in Rats

Key study findings: Rats were treated with 0, 5, 50, or 125/100 mg/kg/day for 104 weeks. As predicted, the 125 mg/kg/day group dose was above the MTD and was reduced during the study to 100 mg/kg/day. Although higher mortality was found in the high dose group, and some signs of toxicity in the mid dose group (50 mg/kg/day), there were no treatment related neoplastic findings noted.

Adequacy of the carcinogenicity study and appropriateness of the test model:

Executive CAC
May 13, 1997

Committee: Joseph DeGeorge, PhD, HFD-24, Chair
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Jasti Choudary, PhD, Division Team Leader
Lillian Patrician, MS, MBA, HFD-024, Project Manager
Tanveer Ahmad, PhD, Presenting Reviewer
IND: — (Ahmad/Choudary;)

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Dose Selection for Rodent Carcinogenicity Studies

Mouse Carcinogenicity Study: The sponsor found lethality at a HD of 250 mg/kg, and subsequently proposed dose selection of 5, 50, and 125 mg/kg based on MTD. The Division agreed with this. The Committee expressed some concern that the HD of 125 may exceed the MTD. However, both the LD and MD are viable. There is good kinetic data reported. The AUC spanned a useful range.

The CAC concluded that the proposed doses of 5, 50, and 125 mg/kg/day are acceptable.

Rat Carcinogenicity Study: This was the first gavage study conducted. In the 13-week oral (gavage) dose ranging study, doses of 5, 50, 125 and 250 mg/kg/day were used. No significant effect on body weight gains and food intake were seen in males treated with 5 mg/kg/day, as well as in females of all dose groups, when compared to their respective control. Body weight gains were decreased by 18.6%, 27.8% and 25.7% in males treated with 50, 125 and 250 mg/kg/day dose levels. In males, decreases in body weight gains at 125 and 250 mg/kg/day dose levels were accompanied with reduction in food consumptions (13-140/0). No significant effects on food intake were seen in males treated with 50 mg/kg/day. The platelet count also decreased in treated males (24-42%) and females (15-33%). The reviewer recommended 50 mg/kg/day as the MTD for both sexes.

However, based on the above data, the Committee concluded that 50 mg/kg/day is the MTD in males and doses of 5, 25 and 50 mg/kg/day should be used in males. Based on the reduction of platelet count in treated females, the Committee concluded that 125 mg/kg/day

is the MTD in females, and doses of 5, 50 and 125 mg/kg/day should be used.

Joseph DeGeorge, PhD
Chair, CAC

cc: IND

HFD: Div. File

HFD: Pharm/Ahmad

HFD: PTL/Choudary

HFD: CSO/

Evaluation of tumor findings:

There was a significant decrease in the 125/100 mg/kg/day male Adrenal Medulla Pheochromocytoma and in the 125 mg/kg/day Female Mammary Carcinoma over their respective controls. There were no other statistically remarkable findings in the incidence data of this study.

Study no.: BC61

Volume #, and page #: eCTD

Conducting laboratory and location: []

Date of study initiation: May 24, 1996

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity:

Description	Lot No.	Date Received	Weeks Used
White Powder	117860	July 27, 1996	1-16
White Powder	117820	July 27, 1996	16-45
White Powder	117880	July 27, 1996	45-78
White Powder	117810	July 27, 1996	78-105

CAC concurrence: CAC concurred on October 26, 2004 that there was no indication of treatment related carcinogenicity.

Methods

Doses:

Group	Dosage Level mg/kg/day	Concentration mg/mL	Number of Animals		Animal Numbers	
			Male	Female	Male	Female
Main Study						
1 (Control)	vehicle	-	70	70	B79980-B80049	B80050-B80119
2 (Low)	5	0.5	70	70	B80120-B80189	B80195-B80264
3 (Mid)	50	5.0	70	70	B80270-B80339	B80345-B80414
4 (High - Male) ^a	125/100	10.0/12.5	70	-	B80420-B80489	-
4 (High - Female)	125	12.5	-	70	-	B80495-B80564
Satellite Study^a						
2 (Low)	5	0.5	5	5	B80190-B80194	B80265-B80269
3 (Mid)	50	5.0	5	5	B80340-B80344	B80415-B80419
4 (High - Male) ^b	125/100	10.0/12.5	5	-	B80490-B80494	-
4 (High - Female)	125	12.5	-	5	-	B80565-B80569

^a Satellite rats were used for the collection of blood samples 15 minutes after dosing once during Week 53 (for determination of plasma iloprost levels). Since these rats were not used for toxicologic assessment, food consumption, clinical pathology, detailed clinical observations, and pathology evaluations were not performed. Satellite animals were weighed at the same intervals as the Main Study rats, but only for the purpose of dose adjustments. After the collection of blood during Week 53, all Satellite animals were sacrificed and discarded without necropsy. One Group 4 male Satellite was sacrificed in a moribund condition and discarded without necropsy.

^b Beginning on January 20, 1997, the dose level of Group 4 (high dose) males was lowered to 100 mg/kg/day from 125 mg/kg/day due to a higher-than-expected mortality rate.

Basis of dose selection (MTD, MFD, AUC etc.): see Adequacy of the carcinogenicity study and appropriateness of the test model:

Species/strain: Six hundred sixty-nine (334 males and 335 females), approximately 4-week-old, Sprague-Dawley Cri:CD@BR rats were received on July 16, 1996. C

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Route, formulation, volume & Frequency of dosing::

Rats were given the appropriate dosing formulation via oral gavage once daily, 7 days per week, for at least 104 weeks; dosing continued through the day prior to the scheduled necropsy. Due to the high mortality rate in high-dose males (Group 4, 125/100 mg/kg/day), the dosing of the 125/100 mg/kg/day males continued through July 21, 1998 (Day 723), when the number of high-dose males surviving reached 18. Dosing was discontinued for the 125/100 mg/kg/day males; however, the animals remained on study until scheduled termination.

Satellite groups used for toxicokinetics or special groups:

Age: approximately 4 weeks old

Animal housing:

Upon receipt, animals of the same sex were housed two/cage in stainless-steel, hanging, wiremesh cages measuring 24.2 x 22.0 x 17.3 cm (d x w x h). Following assignment to study, each animal was individually housed. Some animals were placed in polycarbonate cages during the study when health problems dictated.

Restriction paradigm for dietary restriction studies:

Drug stability/homogeneity:

Dual controls employed: No

Interim sacrifices:

Deviations from original study protocol:

Protocol Deviations

The following protocol deviations were noted:

On July 16, 1996, cage side observations were not documented as being performed (day of animal receipt).

On August 17, 1996, the post-dose time was not recorded for the 125 mg/kg/day (Group 4) females.

Morning cageside observations were not documented on August 28, 1996.

On September 6, 1996, post-dose observations were not performed within the 30 minute timeframe for vehicle control (Group 1) animals due to computer systems downtime.

On September 10, 1996, the 50 mg/kg/day (Group 3) male postdose observations were not performed within the 30 minute timeframe from the first animal dosed in the 50 mg/kg/day males. Animals were not dosed within the 1000-1400 hours timeframe on October 7, 1996. Dose formulations were not received until 1430.

On October 14, 1996, the p.m. mortality and moribundity check was not performed.

Morning cageside observations were not performed on November 7, 1996.

Vehicle control (Group 1) males were dosed after 1400 on June 1, 1997, due to a needed remix of the vehicle/control material. Dosing started at 1015 and was not completed until 1345.

Postdose

observations were therefore not performed within the 30 minute timeframe from the start of dosing for the vehicle control males.

Morning cageside observations were not documented on November 3, 1997.

Postdose observation were not within 30 minutes from the first animal dosed in the 50 mg/kg/day (Group 3) males on January 7 and February 6, 1998.

On January 27, 1998, the postdose observation time for the 125/100 mg/kg/day (Group 4) males was not within 30 minutes from the first animal dosed within the group.

On February 24, 1998, the postdose observation time was not recorded for the 50 mg/kg/day (Group 3) females.

Although the protocol specified that a differential leukocyte and cell morphology would be performed, a complete blood count was performed on the following animals (moribund sacrifice): B80081 (1F), B80110 (1F), B80133 (2M), B80214 (2F), and B80351 (3F).

Reserve samples taken on April 21 and December 15, 1997, could not be located, therefore, the samples were not archived.

Retention samples taken for Weeks 11, 27, and 46 could not be located; therefore, the samples were not disposed.

The pH was taken but not recorded for the 125/100 mg/kg/day (Group 4) males and females during the Week 75 dose preparation mix.

A reserve sample of iloprost clathrate, Lot No. 117820, was not taken as required.

Dose preparation mixes for Weeks 6 and 7 were prepared more than once per week (as was required by protocol).

On November 4, 1996, animals were dosed earlier than the 1000-1400 hours specified dose time. The following Satellite animal blood samples were collected late (ranging from 2-6 minutes) during the Week 53 plasma sampling collection: Group 2 (5 mg/kg/day) males B80192, B80193, and

B80194, and Group 2 (5 mg/kg/day) females B80265, B80266, B80267, B80268, and B80269.

Some tissues were missing and not microscopically examined or only unilaterally examined due to technical errors, sectioning difficulties, or other reasons.

Observation times

Histopathology: Peer review: yes (), no ()

8.1 Observation of Animals

8.1.1 Clinical Observations

All study rats were observed twice daily (a.m. and p.m., at least 6 hours between observations) for evidence of mortality and moribundity.

Twice daily (once at the a.m. mortality and moribundity check, and again within approximately one-half hour following dosing), cageside observations for obvious indications of a toxic and/or pharmacologic effect were recorded as they were observed for all Main Study animals, noting only those animals for which an observation was made.

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Once prior to initiation of treatment and weekly thereafter, each Main Study animal was removed from its cage and examined for grossly visible or palpable masses, signs of poor health, and indications of abnormal behavior. Findings (including time of onset, location, size, appearance, and progression of grossly visible or palpable masses) were recorded for each animal.

8.1.2 Special Procedures

At the request of the Study Director, several animals were placed into polycarbonate cages containing bedding due to the presence of sores and swellings on the feet. The feet were cleaned daily with a 10% ┌ (chlorhexidine diacetate). If the feet had fully healed, animals were returned to their original cages.

8.1.3 Body Weights

Individual body weights were recorded prior to treatment, weekly for Weeks 1-14 and every fourth week thereafter, at Week 105, and at termination.

8.1.4 Food Consumption

Food consumption was measured weekly for Weeks 1-13 and once every fourth week thereafter for all Main Study animals. When obvious spillage or wastage was recorded for an animal during the detailed physical examination, the estimate of the food consumed by the animal was excluded from the group mean calculation for that particular interval.

8.2 Toxicokinetic Analyses

Blood samples were collected for determination of iloprost in plasma. During Week 53, blood samples were collected from each of the surviving Satellite rats in the 5, 50, 125/100, and 125 mg/kg/day dose groups (maximum of five rats/sex/group) at 15 minutes postdose. At least 2 mL of whole blood (if possible) was collected from the jugular vein. Prior to returning the animal to its cage after collection, attempts were made to ensure clotting at the sample collection site was achieved. Sodium citrate solution (one part sodium citrate solution to nine parts blood) was used as the anticoagulant. Rats were not fasted prior to sampling.

After collection, blood samples were centrifuged at approximately 3000 rpm for approximately 10 minutes to obtain plasma. The approximate volume of plasma collected was determined (to the nearest 0.1 mL) and recorded. The resulting plasma fractions were separated into two subsamples (0.5 mL was aliquoted as the first subsample and the remainder reserved as the second subsample); the second set of subsamples served as the reserve set and will remain frozen (approximately ┌

— and shipped on dry ice to the Sponsor (or designee). Drug analyses may be conducted

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by the Sponsor or designee. The GLP compliance of the conduct, reporting, and record retention of these data are the responsibility of the Sponsor.

8.3 Clinical Pathology

Prior to each clinical sampling, selected Main Study animals were fasted overnight with water available. Blood samples for hematology evaluations were obtained from the first 10 surviving animals/sex/group (collection was from the same animals at each interval, if possible) during Week 52 and at terminal sacrifice via orbital plexus, using carbon dioxide/oxygen inhalation for anesthesia. The anticoagulant for the hematology samples was ethylenediaminetetraacetic acid (EDTA). The following parameters were determined:

Hematology

differential leukocyte and cell morphology

erythrocyte count

leukocyte count

Clinical hematology analyses were performed using a Coulter counter ┌] A

differential leukocyte count and cell morphology evaluation were performed manually from peripheral blood smears for high-dose and control rats.

If clinical observations suggested a deterioration in health of the rats during the study (e.g., moribund condition), a differential leukocyte count and cell morphology evaluation were performed on the affected rats as soon as possible.

Results

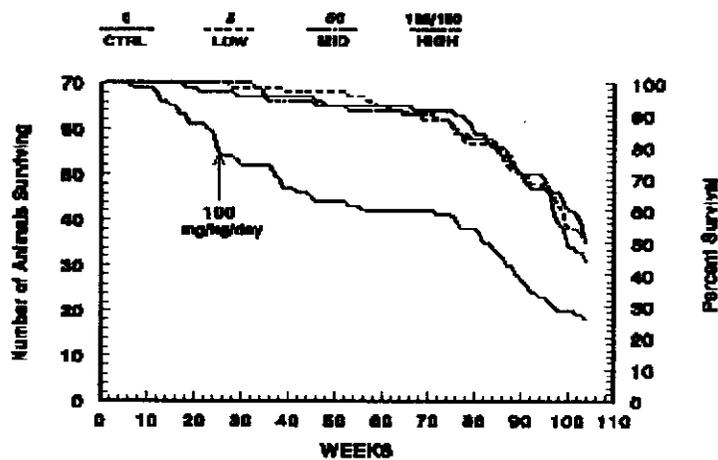
Mortality:

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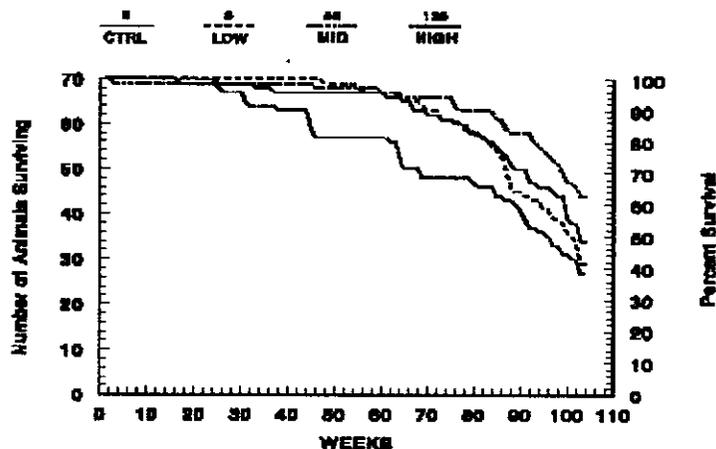
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Text Figure 1: Adjusted Percent Survival

Males



Females



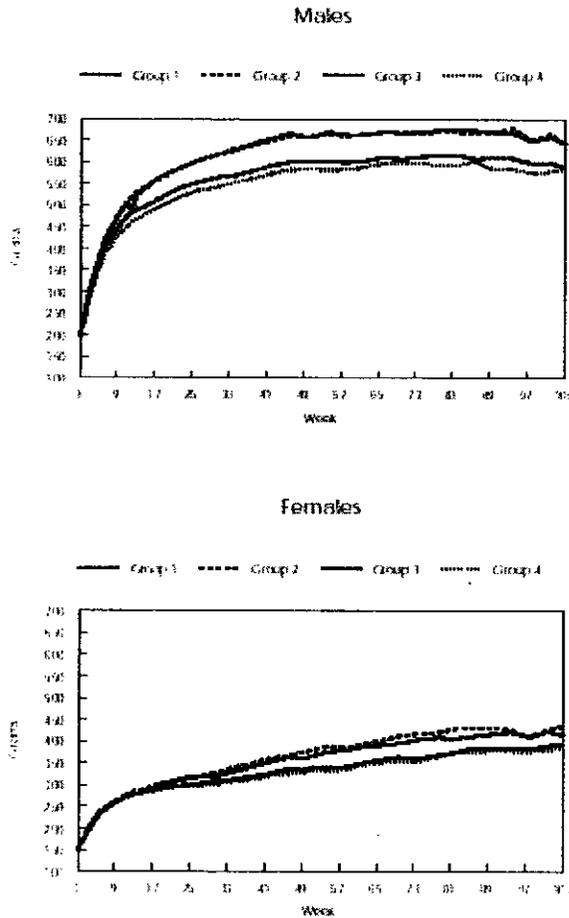
Clinical signs:

Treatment-related postdose clinical observations were noted at the mid- and high-dose levels (dose levels of ≈ 50 mg/kg/day). At the 30-minute postdose interval, high-dose (125/100 mg/kg/day males and 125 mg/kg/day females) animals were noted throughout the study as appearing hypoactive. In addition, their extremities (tail, nose, ears, and feet) were

noted as red. The mid-dose (50 mg/kg/day) animals were also noted as hypoactive and exhibited redness on their extremities. These findings occurred more frequently for males than for females and less frequently at the mid-dose level than at the high-dose level. Salivation was present most notably in the 50 mg/kg/day (mid-dose) and 125/100 mg/kg/day males and 125 mg/kg/day females (high-dose). Other clinical observations were generally of the type and frequency noted in this strain of laboratory rat at this facility and are not treatment related.

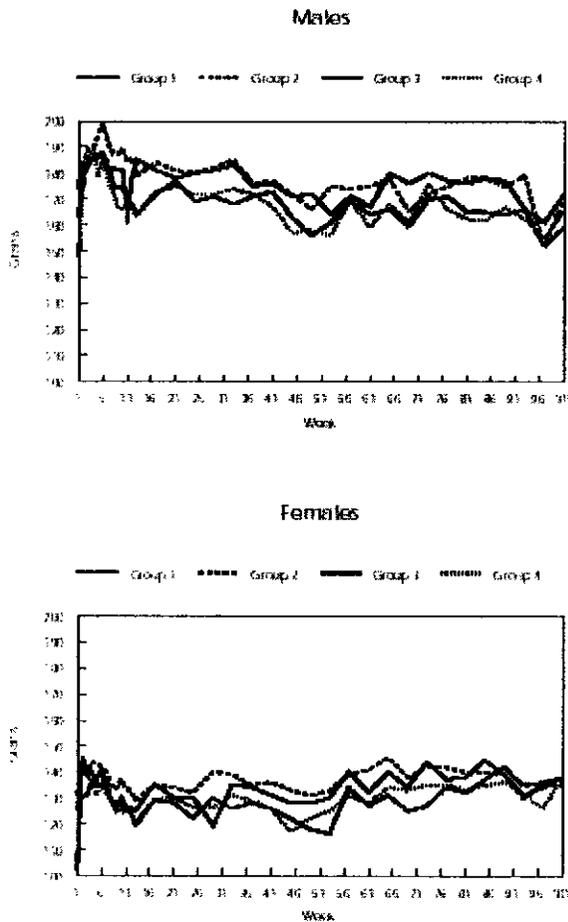
Body weights:

Text Figure 2: Mean Body Weights



Food consumption:

Text Figure 3: Mean Food Consumption



Gross pathology:

Histopathology:

Non-neoplastic:

There were no consistent gross findings with corresponding histologic correlates indicative of a direct treatment-related effect in the scheduled and unscheduled deaths. The livers were observed to be enlarged with greater frequency in the 125/100 mg/kg/day (high-dose) males, specifically in the unscheduled deaths. Overall, there were fewer pituitary observations in the 50 mg/kg/day animals, the 125/100 mg/kg/day males and the 125 mg/kg/day females. Treatment-related histomorphologic alterations were observed in the pancreas of animals which received the mid- and high-doses. Pancreatic change consisted of an increased incidence and severity of parenchymal degeneration and was characterized microscopically by atrophy or degeneration of the acinar cells and interstitial fibrosis with residual ducts and acini. This histologic alteration varied in severity from a minimal change, in which isolated focal areas of degeneration were observed, to a moderately severe change, in which whole lobes of pancreas

were involved. The morphology of this alteration was similar to that observed in the pancreas of aging rats, as observed in the control animals, and differed only in the incidence and severity. The incidences of pancreatic degeneration for 50 mg/kg/day males and 50 and 125 mg/kg/day females were statistically greater than those of the respective controls. The incidence in the 125/100 mg/kg/day males showed an increase over that of the control animals, although it was not statistically significant.

An increase in the incidence and severity of acute inflammation in the lungs was observed in the males given 125/100 mg/kg/day, exclusively in the unscheduled deaths. In the majority of the males dying or sacrificed on or before Week 26, pulmonary changes that were characterized by neutrophil infiltration and often deposition of fibrin in the alveolar spaces were observed. In about half of these males, the cause of death was designated as acute pulmonary inflammation. The occurrence of this histomorphologic alteration was dramatically reduced after the dosage was adjusted to a lower level. There were significant increases in the acute inflammation of the lungs of the 125/100 (males) and 125 (females) mg/kg/day animals that may be related to test material influx in the tissue, which probably occurred when animals became hypoactive and recumbent as a result of the administration of the test material.

Neoplastic: No evidence of an increase in the incidence of neoplasms occurred as a result of treatment. The most frequent causes of death, when determined, were pituitary neoplasms, nephropathy in males, and mammary neoplasms in females.

Toxicokinetics: Not done

2.6.6.5 Reproductive and developmental toxicology

Note: The involvement of prostacyclin, and prostaglandins in general, in reproductive cycles indicate there will be effects of iloprost on fertility and embryonic development.

Fertility and early embryonic development

Study title: ZK 36.374 – Study of the fertility and general reproductive performance in the rat after continuous intravenous infusion with the — Osmotic-Pump in female animals for 14 days before start of mating to day 7 of gestation.

Key study findings :

Study no.: 7569

Volume #, and page #: 5.14, pp.2-104

Conducting laboratory and location: Schering, Germany

Date of study initiation: October 21, 1985

GLP compliance: yes

QA reports: yes () no (x)

Drug, lot #, and % purity: ZK 36.3745, batch # 68-AA-8 Teil 1; U-No. 4108/84

Methods

Group	Number of evaluated animals ⁺	Period of continuous infusion from day 1 p.m.p. to day 7 p.c. (range of days)	Concentration of ZK 36.374 ng/ml	Planned dose of ZK 36.374 (ng/kg/24h)	Administered dose of ZK 36.374 (ng/kg/24h)	
					Mean/SD per group	Range
1	20	22-27	0	0	--	--
2	24	22-27	0.04	0.01	0.010;0.001	0.009-0.012
3	19	22-27	0.4	0.1	0.10;0.01	0.09-0.11
4	23	22-28	4.0	1.0	1.03;0.07	0.91-1.18

⁺ only those animals are considered, which showed intact catheter-pump connection on day 7 p.c. as well as catheter positioned inside the permeable vein and functioning pumps on day 21 p.c.

Species/strain: Female wistar-han rats,

Route, formulation, volume, and infusion rate: — Osmotic-Pump, Aqueous solution, 1.0 ml, 2.58 microl/h

Satellite groups used for toxicokinetics:

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Study design:

Group	Number of evaluated animals ⁺	Period of continuous infusion from day 1 p.p. to day 7 p.c. (range of days)	Concentration of ZK 36.374 (ng/ml)	Planned dose of ZK 36.374 (ng/kg/24h)	Administered dose of ZK 36.374 (ng/kg/24h)	
					Mean/SD per group	Range
1	20	22-27	0	0	--	--
2	24	22-27	0.04	0.01	0.010;0.001	0.009-0.012
3	19	22-27	0.4	0.1	0.10;0.01	0.09-0.11
4	23	22-28	4.0	1.0	1.03;0.07	0.91-1.18

⁺ only those animals are considered, which showed intact catheter-pump connection on day 7 p.c. as well as catheter positioned inside the peritoneic vein and functioning pumps on day 21 p.c.

Parameters and endpoints evaluated: The effect of the test compound was assessed in the females during the pre-mating, mating, and gestation period as well as in the fetuses on day 21 of gestation (Sponsor material, vol 5.14, p. 14-4).

Results

Mortality: in the intermediate dose group 4 female rats died during the pre-mating period – 3 had seroma at the pump site; 1 female rat died in the high dose group on day 10 post conception with pulmonary edema

Clinical signs:

Body weight: there was a slight decrease in body weights in the intermediate and high dose groups, however, it was not statistically significant

Food consumption: no data

Toxicokinetics: i.v. study, only dosage data given

Necropsy: thickened fibrous capsule and/or seroma at the pump site was a common finding

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

		Group #1	Group #2	Group #3	Group #4
Dose	Mg/ml	0	.04	.4	4
# Females at start		30	30	30	30
# females for data		20	24	19	23
Mated		30	30	26	30
Non-pregnant		4	0	7	2
Fertility index		0.87	1.00	0.96	0.93
Animals with living fetuses		20	24	17	23
Animals with fetal death		0	0	2	0
Died or sacrificed		0	0	0	1
Corpora lutea (total)		251	344	269	298
Preimplantational loss		30	56	45	43
Implantations		221	288	224	255
Living fetuses		211	271	203	235
Resorptions (all early)		10	17	21	19
Sex of living fetuses (Male/Female)		106/105	126/145	102/101	104/131
Weight of living fetuses	Grams	51.7	54.5	56.6	51.2
Fetuses w/minor anomalies		1	1	3	4
Fetuses w/major anomalies		0	0	0	0
Fetuses w/visceral variations	%	38.3	29.3	22.8	40
Fetuses w/rib variations - 14 th rib	%	30.8	51.4	48	43.5
Fetuses w/sternum variations	%	57.7	50.7	55.9	47
Fetuses w/foot bone variations	%	63.5	68.8	77.5	60

Study title: ZK 36.374 – study of the fertility and general reproductive performance in the rat after treatment of males during the pre-mating and mating period by continuous intravenous infusion.

Key study findings:

Study no.: 7778

Volume #, and page #: 5.14, pp105-354

Conducting laboratory and location: Schering, Germany

Date of study initiation: November 18, 1985

GLP compliance: yes

QA reports: yes () no (x)

Drug, lot #, and % purity: ZK 36.374, batch # 68-AA-8 part 2

Methods

Group	Number of evaluated males	28 day-periods of continuous infusion (no. of period)	ZK 36.374	
			Planned dose (mg/kg/24h)	Administered* dose (Mean/SD)
1	23	I - III	--	--
2	23	I II + III	0.01	0.007/0 0.009/0
3	27	I II + III	0.1	0.07/0.01 0.09/0.01
4	21	I II + III	1.0	0.7 /0.1 0.9 /0.1

* According to analytical results obtained with the formulations at various concentrations after storage in osmotic pumps for 4 weeks under in vivo conditions, the actual dosages were expected to be at least about 0.003, 0.06 and 0.8 mg/kg/24 h in the low, intermediate and high dose group respectively.

Species/strain: wistar-han rats

Route, formulation, volume, and infusion rate: — -Osmotic-Pump, Aqueous solution, 1.0 ml, 2.58 microl/h

Satellite groups used for toxicokinetics:

Study design:

Group	Number of evaluated males	28 day-periods of continuous infusion (no. of period)	ZK 36.374	
			Planned dose (mg/kg/24h)	Administered* dose (Mean/SD)
1	23	I - III	--	--
2	23	I II + III	0.01	0.007/0 0.009/0
3	27	I II + III	0.1	0.07/0.01 0.09/0.01
4	21	I II + III	1.0	0.7 /0.1 0.9 /0.1

* According to analytical results obtained with the formulations at various concentrations after storage in osmotic pumps for 4 weeks under in vivo conditions, the actual dosages were expected to be at least about 0.003, 0.06 and 0.8 mg/kg/24 h in the low, intermediate and high dose group respectively.

Parameters and endpoints evaluated: The effect of the test compound was assessed in the males during the pre-mating, mating, and gestation period as well as in the fetuses on day 21 of gestation (Sponsor material, vol 5.14, p. 14-4).

Results

Mortality: 4,4,3, and 8 males from groups 1,2,3, and 4 died or had to be sacrificed moribund during the study. Most of the deaths occurred shortly after surgery, and were not considered treatment (iloprost) related.

Clinical signs: none noted

Body weight: was not affected by the drug treatment except for a brief effect in week 1 in the high dose group

Food consumption: no data

Toxicokinetics: no data

Necropsy: no drug related changes noted, osmotic pump did develop a fibrotic capsule

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): no significant changes noted through the F2 generation

Study title: Combined study of fertility and embryonic development and pre- and postnatal development, including maternal function, of iloprost clathrate in female rats

Key study findings:

Study no.: 97004

Volume #, and page #: 5.15, pp. 1-514

Conducting laboratory and location: τ

1

Date of study initiation: April 16, 1996

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Iloprost clathrate, lot # 115020 & 115050, assumed —

Methods

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Group Assignment and Dosage Levels

F₁ females were initially accepted for the randomization pool based upon physical examinations. Animals which exhibited clinical signs were eliminated from the randomization pool. Two hundred forty female rats (approximately 8 weeks old) were randomly assigned to study by eliminating the animals with extreme body weights and selecting the random assignment which produced homogeneity of body weight variance and means by Bartlett's Test (1937) and One-Way Analysis of Variance (ANOVA). At the time of randomization, the weight variation of the females selected did not exceed ± 2 standard deviations of the mean body weight and the mean body weight for each group was not statistically different. F₁ females were assigned to groups as follows:

Group	Dosage Level mg/kg/day	Concentration ng/mL	Number of Females	Animal Numbers
1 (Control)	0	0	60	B76167-B76226
2 (Low)	5	0.5	60	B76227-B76286
3 (Mid)	50	5	60	B76287-B76346
4 (High)	250	25	60	B76347-B76406

- 15 -

Doses:

Species/strain: Sprague-Dawley rats

Number/sex/group:

Upon confirmation, females were assigned to one of three phases: 15 females/group were assigned to gestation Day 13 uterine examinations; 20 females/group were assigned to gestation Day 20 cesarean sections (teratology phase); and 25 females/group were allowed to deliver and raise their young until lactation Day 21. Females that did not confirm to mate were automatically assigned to the natural delivery phase of this study.

Route, formulation, volume, and infusion rate: oral gavage

Satellite groups used for toxicokinetics:

Study design:

Parameters and endpoints evaluated: F0 and F1 observations, mating procedures, estrous cycle, day 13 & 20 Cesarean section, Fetal exams from day 20 Cesarean sections, and sacrifice of females assigned to lactation phase.

ResultsMortality: Clinical signs:

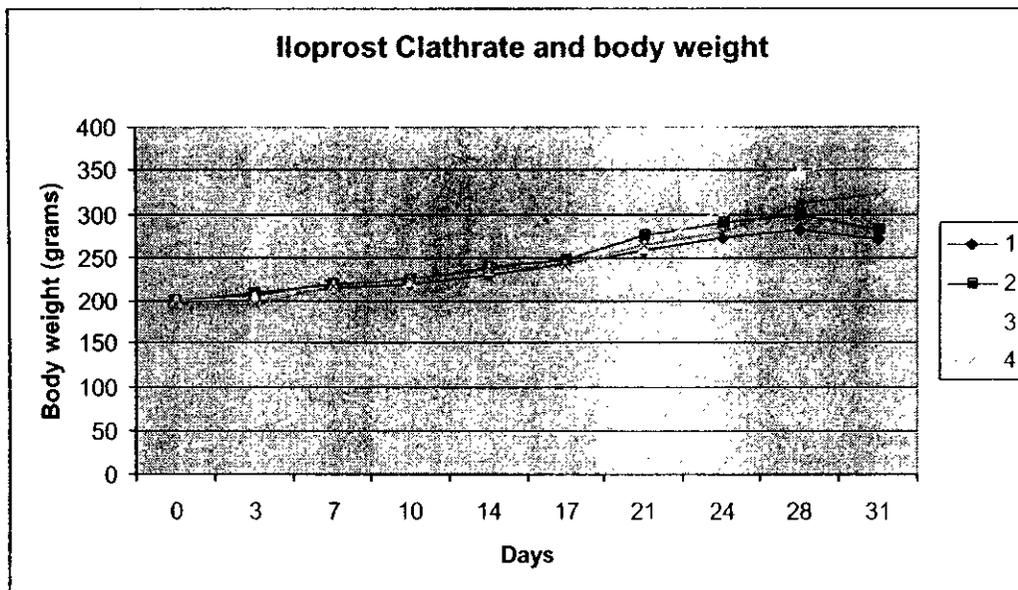
TABLE 2A
COMBINED STUDY OF FERTILITY AND EMBRYONIC DEVELOPMENT AND PRE- AND POSTNATAL DEVELOPMENT,
INCLUDING MATERNAL FUNCTION, OF ILOPROSI CLATHRATE IN FEMALE RATS
SUMMARY INCIDENCE OF CLINICAL OBSERVATIONS

		DAY : 0 3 7 10 14 17 21 24 28 31 35 38 42													
NUMBER OF ANIMALS EXAMINED															
GROUP 1	0 MG/KG/DAY	60	60	60	60	60	37	7	5	4	1	0	0	0	
GROUP 2	5 MG/KG/DAY	60	60	60	60	60	37	7	7	6	1	0	0	0	
GROUP 3	50 MG/KG/DAY	60	60	60	60	60	31	7	3	1	1	1	1	0	
GROUP 4	250 MG/KG/DAY	60	60	60	60	60	32	10	4	3	1	1	1	1	
NORMAL															
NO SIGNIFICANT CLINICAL OBSERVATIONS		1	60	60	59	60	60	37	7	5	4	1	-	-	-
		2	60	60	59	60	60	37	7	7	6	1	-	-	-
		3	60	59	59	59	30	7	3	1	1	1	1	1	0
		4	60	60	60	59	60	31	6	4	3	1	1	1	0
DEAD															
FOUND DEAD		1	0	0	0	0	0	0	0	0	0	3	-	-	-
		2	0	0	0	0	0	0	0	0	0	3	-	-	-
		3	0	0	0	0	0	0	0	0	0	0	0	0	-
		4	0	0	0	0	0	0	0	0	0	0	0	0	1
APPEARANCE															
1 HOUR PDO - REDDENED EXTREMITIES		1	0	0	0	0	0	0	0	0	0	-	-	-	-
		2	0	0	0	0	0	0	0	0	0	-	-	-	-
		3	0	0	0	0	6	0	0	0	0	0	0	0	-
		4	34	36	16	9	24	4	3	1	1	0	0	0	0
ACTIVITY															
1 HOUR PDO - HYPOACTIVITY		1	0	0	0	0	0	0	0	0	0	-	-	-	-
		2	0	0	0	0	0	0	0	0	0	-	-	-	-
		3	0	0	0	0	0	0	0	0	0	0	0	0	-
		4	0	0	0	0	6	0	0	0	0	0	0	0	0
EYE(S)															
CHROMODACRYORRHEA		1	0	0	0	0	0	0	0	0	0	0	-	-	-
		2	0	0	1	0	0	0	0	0	0	0	0	0	-
		3	0	1	1	1	1	0	0	0	0	0	0	0	-
		4	0	0	0	0	0	0	0	0	0	0	0	0	0
LACRIMATION		1	0	0	1	0	0	0	0	0	0	-	-	-	-
		2	0	0	0	0	0	0	0	0	0	-	-	-	-
		3	0	0	0	0	0	0	0	0	0	0	0	0	-
		4	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 2A
COMBINED STUDY OF FERTILITY AND EMBRYONIC DEVELOPMENT AND PRE- AND POSTNATAL DEVELOPMENT,
INCLUDING MATERNAL FUNCTION, OF ILOPROSI CLATHRATE IN FEMALE RATS
SUMMARY INCIDENCE OF CLINICAL OBSERVATIONS

		DAY : 0 3 7 10 14 17 21 24 28 31 35 38 42													
NUMBER OF ANIMALS EXAMINED															
GROUP 1	0 MG/KG/DAY	60	60	60	60	60	37	7	5	4	1	0	0	0	
GROUP 2	5 MG/KG/DAY	60	60	60	60	60	37	7	7	6	1	0	0	0	
GROUP 3	50 MG/KG/DAY	60	60	60	60	60	31	7	3	1	1	1	1	0	
GROUP 4	250 MG/KG/DAY	60	60	60	60	60	32	10	4	3	1	1	1	1	
MOUTH															
TEETH CUT		1	0	0	0	0	0	0	0	0	0	-	-	-	-
		2	0	0	0	0	0	0	0	0	0	-	-	-	-
		3	0	0	1	0	1	0	0	0	0	0	0	0	-
		4	0	0	0	0	0	0	0	0	0	0	0	0	0
MALOCCLUSION		1	0	0	0	0	0	0	0	0	0	-	-	-	-
		2	0	0	0	0	0	0	0	0	0	-	-	-	-
		3	0	0	0	1	0	0	0	0	0	0	0	0	-
		4	0	0	0	0	0	0	0	0	0	0	0	0	0

Body weight:



Food consumption:

TABLE 9
COMBINED STUDY OF FERTILITY AND EMBRYONIC DEVELOPMENT AND PRE- AND POSTNATAL DEVELOPMENT,
INCLUDING MATERNAL FUNCTION, OF ILOPROST CLATHRATE IN FEMALE RATS
MEAN MATERNAL FOOD CONSUMPTION DURING GESTATION -- SUMMARY

DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 50 MG/KG/DAY	GROUP 4 250 MG/KG/DAY
MATERNAL FOOD CONSUMPTION -- grams/ANIMAL/DAY				
DAYS 0 TO 3				
MEAN	21.6	22.1	21.9	20.6
S.D.	2.5	3.0	2.4	2.1
N	55	59	56	58
SPILLED	2	1	1	0
DAYS 3 TO 7				
MEAN	22.1	23.0	22.0	22.6
S.D.	2.4	1.9	2.1	2.2
N	57	58	57	57
SPILLED	0	2	0	1
DAYS 7 TO 10				
MEAN	23.8	24.9	24.6	24.0
S.D.	3.1	2.9	2.3	2.8
N	55	60	56	58
SPILLED	2	0	1	0
DAYS 10 TO 13				
MEAN	24.1	25.7**	25.1	25.0
S.D.	2.8	2.2	2.3	2.4
N	57	60	57	56
SPILLED	0	0	0	2
DAYS 13 TO 17				
MEAN	25.0	27.1*	26.5	26.0
S.D.	2.1	2.3	2.5	2.5
N	42	45	42	43
SPILLED	0	0	0	0
DAYS 17 TO 20				
MEAN	26.6	27.9	27.0	25.7
S.D.	2.4	1.3	4.3	2.3
N	42	44	42	43
SPILLED	0	1	0	0

SIGNIFICANTLY DIFFERENT FROM CONTROL. * - P<0.05, ** - P<0.01

TABLE 10
COMBINED STUDY OF FERTILITY AND EMBRYONIC DEVELOPMENT AND PRE- AND POSTNATAL DEVELOPMENT,
INCLUDING MATERNAL FUNCTION, OF ILOPROST CLATHRATE IN FEMALE RATS
MEAN MATERNAL FOOD CONSUMPTION DURING LACTATION -- SUMMARY

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 50 MG/KG/DAY	GROUP 4 250 MG/KG/DAY
MATERNAL FOOD CONSUMPTION -- grams/ANIMAL/DAY DAYS 0 TO 4	MEAN	39.8	40.6	42.1	37.2
	S.D.	7.5	8.2	11.0	8.4
	N	21	24	14	21
DAYS 4 TO 7 RT	SPILLED	1	1	8	2
	MEAN	44.4	46.7	49.9	45.1
	S.D.	3.8	5.0	11.5	10.8
DAYS 7 TO 10	N	22	25	21	22
	SPILLED	0	0	1	1
	MEAN	54.3	55.3	54.1	49.4*
DAYS 10 TO 14	S.D.	5.8	5.0	4.3	7.0
	N	22	25	22	21
	SPILLED	0	0	0	1
	MEAN	59.4	57.7	55.1	53.1
	S.D.	4.7	5.5	11.8	8.0
	N	18	13	13	17
	SPILLED	4	12	9	5

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P < 0.05; ** = P < 0.01

RT = DATA ANALYZED FOLLOWING RANK TRANSFORMATION

Toxicokinetics: no data

Necropsy:

TABLE 21
COMBINED STUDY OF FERTILITY AND EMBRYONIC DEVELOPMENT AND PRE- AND POSTNATAL DEVELOPMENT,
INCLUDING MATERNAL FUNCTION, OF ILOPROST CLATHRATE IN FEMALE RATS
SUMMARY OF PARENTAL NECROPSY OBSERVATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 50 MG/KG/DAY	GROUP 4 250 MG/KG/DAY
FEMALES	N	45	45	45	45
LYMPH NODE(S)-ENLARGED	N	0	0	1	0
LUNGS-DARK	N	0	0	0	1
SPLEEN-DARK	N	0	0	1	3
LIVER-DARK	N	0	0	1	0
LIVER-NOTTLED	N	0	0	1	0
LIVER-ENLARGED	N	0	0	1	0
STOMACH-DISTENDED	N	0	0	1	0
STOMACH-DARK AREAS	N	0	0	1	0
KIDNEY(S)-DILATED PLOISIS(ES)	N	1	0	1	0
INTESTINES-DISTENDED	N	0	0	1	0
UTERUS-RETAINED FETUSES/PUPS	N	0	0	0	1
UTERUS-DRAVID*	N	0	0	2	1
UTERUS-HYDROMETRA	N	0	0	0	1

N = Number

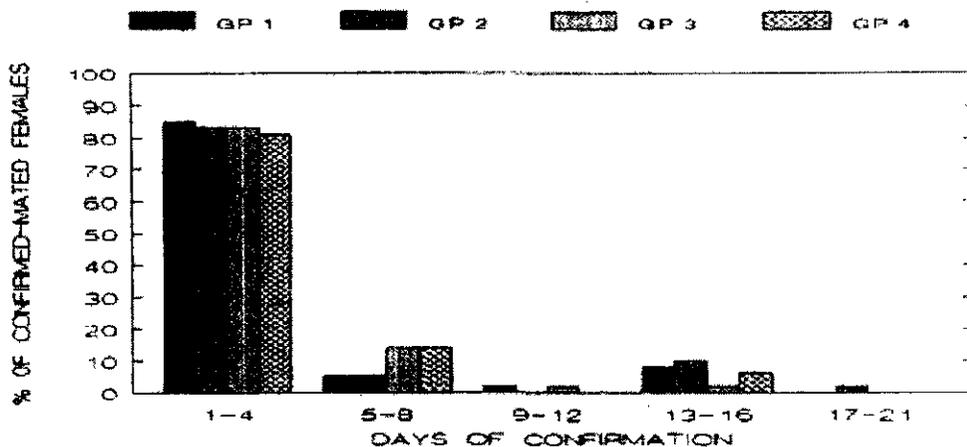
* Description of uterine contents for all unscheduled deaths.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Text Table 1
Reproductive Indices

Dose Level (mg/kg/day)	0	5	50	250
No. of females paired with at least one male	60	60	50	60
No. (%) of females mated (Female Copulation Index)	60 (100)	60 (100)	60 (100)	60 (100)
No. (%) of females successfully mated (Female Fertility)	57 (95)	60 (100)	58 (97)	59 (98)

Figure 3
Precoital Histogram



Embryofetal development

Study title: Commentary on the report of [] dated June 5, 1989 (— Project # 14/45): ZK 36.374 – Embryotoxicity including teratogenicity study in the monkey (Cynomolgus) after continuous intravenous infusion from day 20 to 50 of gestation

Key study findings: sponsor reported that no treatment related maternal or fetal effects were found in the study. The study did have a high spontaneous abortion rate in the controls (7/12 – historical controls ranged from 0-40%) potentially indicating the monkeys feeling highly stressed. The low and intermediate dose animals had a similar rate of spontaneous abortions (7/12 & 9/12, respectively) while the high dose animals had a much lower rate (2/12).

Study no.: 8493

Volume #, and page #: 5.17, pp.1-213

Conducting laboratory and location: []

Date of study initiation: May 27, 1988

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: ZK 36.374, Batch # 68-AA-12 part 1

Methods

Four groups of female Cynomolgus monkeys (*Macaca fascicularis*) were used for this experiment. In each dose group, twelve pregnant animals received the test compound ZK 36.374 as an aqueous solution at nominal dose levels of 0.004 mg/kg/24h, 0.012 mg/kg/24h, or 0.040 mg/kg/24h by continuous intravenous infusion via an osmotic pump from day 20 to 50 post-coitum. Based on the measurement of the pumped volume (filled volume on day 20 minus removed volume on day 50) and on the mean of the body weight of day 20 and 48 of gestation in each animal, mean actual doses of 0.0075, 0.017 and 0.043 mg/kg/24h, respectively, were calculated in the three dose groups. A control group of twelve pregnant animals received the vehicle only on the same days of pregnancy and by the same method of administration.

The pregnancies of the animals were terminated on day 100 +/- 1 post-coitum by cesarean section and the fetuses were examined for external, visceral and skeletal anomalies.

Results

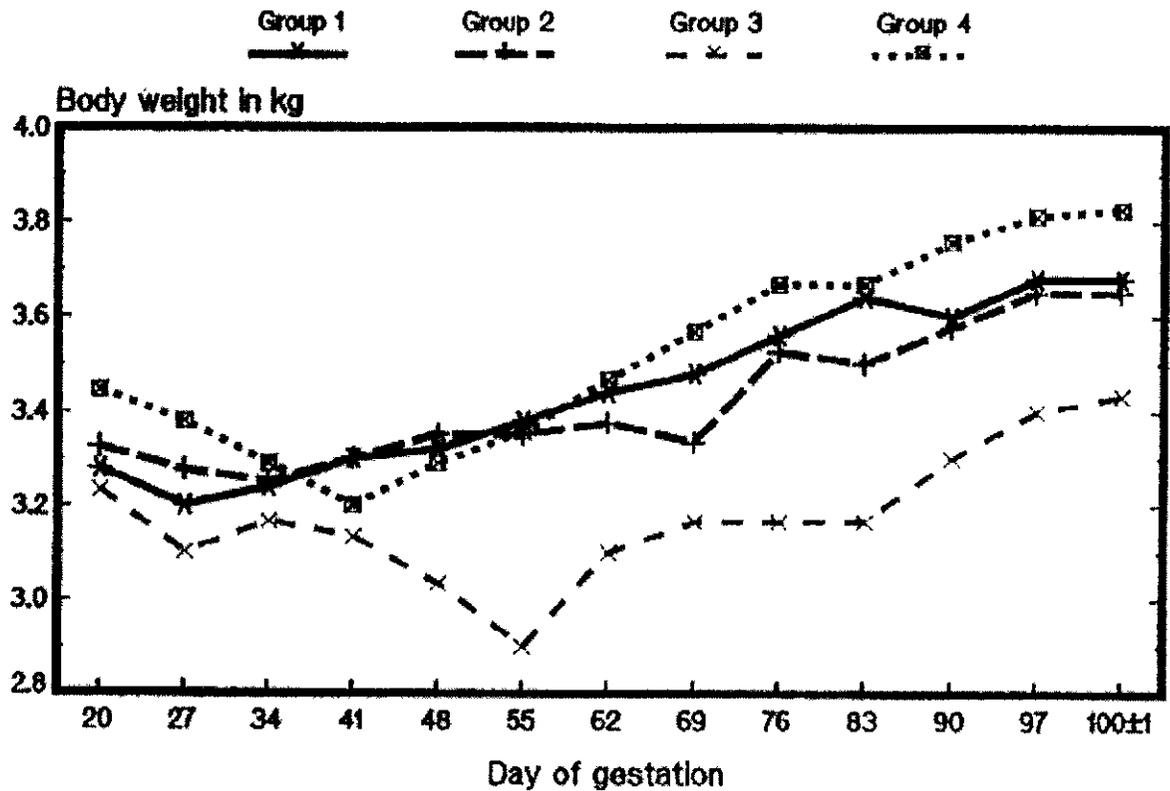
Mortality (dams): none

Clinical signs (dams): The primary clinical signs were a decrease in food intake in all groups, and the other signs were related to bleeding and the spontaneous abortions observed in groups 1, 2 and 3.

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Body weight (dams):

Group Mean Body Weight (kg)



Food consumption (dams): no data provided, only a statement in tables that “food consumption decreased” for many of the animals

Toxicokinetics: no data, textual info:

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The plasma samples taken from the monkeys on the days 27, 34, 41 and 48 of gestation were analysed for the content of ZK 36.374 by [] The mean values of all animals and time points per group were 0.35 ng/ml after 0.004 mg/kg/24 hours (= ca. 3 ng/kg/min), 0.93 ng/ml after 0.012 mg/kg/24 hours (= ca. 8 ng/kg/min) and 1.33 ng/ml after 0.04 mg/kg/24 hours (= ca. 28 ng/kg/min). These values showed a clear-cut dose-dependent increase although the individual values in each dose group revealed a moderate inter- and intraindividual variation.

It is worth mentioning that the order of magnitude of these plasma levels in monkeys were commensurate with those measured during 26/28-week tolerance studies after comparable doses in rats (< 1.1 ng/ml after the low dose of ca. 35 ng/kg/min and 1.2-5.4 ng/ml after the mid dose of ca. 70 ng/kg/min; [] Report No. 7442 of January 14, 1987) and in dogs (0.4 ng/ml after the low dose of ca. 17 ng/kg/min and 0.9 ng/ml after the mid dose of ca. 33 ng/kg/min; [] Report no. 7949 of June 2, 1988).

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Group I (Control):

Group 1 - 0.000 mg/kg/24h

In two animals (females nos. 433 and 238), the pregnancy test was considered as false positive.

Seven of twelve pregnant animals (females nos. 351, 289, 226, 368, 138, 445, and 416) aborted.

Two animals (females nos. 138 and 445) which aborted on days 63 and 66 post-coitum or on days 60 and 61 post-coitum, respectively, showed bad physical condition on some days prior to abortion. In one of these animals (female no. 138), an incorrect position of the catheter was detected on day 50 post-coitum when the catheter was removed.

Group 2 - 0.004 mg/kg/24h

In two animals (females nos. 132 and 496), the pregnancy test was considered as false positive.

Seven of twelve pregnant animals (females nos. 374, 418, 448, 453, 522, 487, and 139) aborted.

Group 3 - 0.012 mg/kg/24h

In two animals (females nos. 243 and 471), the pregnancy test was considered as false positive.

Nine of twelve pregnant animals (females nos. 184, 147, 218, 117, 34, 524, 447, 379, and 355) aborted.

Group 4 - 0.040 mg/kg/24h

In two animals (females nos. 349 and 477), the pregnancy test was considered as false positive.

Two of twelve pregnant animals (females nos. 397 and 347) aborted when heavy bleeding was observed on days 21 and 24 to 26 of gestation or on day 28 of gestation, respectively.

Offspring (malformations, variations, etc.):

Group 1 - 0.000 mg/kg/24h (cont.)

In five females (animals nos. 129, 154, 124, 428, and 273), live fetuses were removed by cesarean section.

External findings as bent tail end or small tissue ball at the tail end were observed in three fetuses (animals nos. 124, 428, and 273).

Two fetuses (animals nos. 428 and 273) showed visceral findings as scattered punctiform hemorrhages at cardia.

Skeletal findings as incompletely and/or not ossified sternbrae and/or zygo-style asymmetrically ossified and/or 12th rib on the left side shortened were observed in all fetuses.

Group 2 - 0.004 mg/kg/24h

In four animals (females nos. 510, 324, 181, and 153), live fetuses were removed by cesarean section and in one female (animal no. 384), a dead fetus was removed.

Skeletal findings as incompletely and/or not ossified sternbrae and/or extra rib on the left side were observed in all fetuses. Neither external nor visceral findings were detected.

No external, visceral, or skeletal malformations were observed in these fetuses.

The size and stage of development of the dead fetus (female no. 384) removed by cesarean section was according to day 97 to 100 of pregnancy. Abdominal organs were autolytic. No external abnormalities were detected.

The mean fetal weight, fetal body measurements, and placental weight were comparable with the control group.

The mean fetal thymus weight was slightly lower than in the concurrent control group. This finding is considered to be incidental, because individual values were within the normal range.

All other mean fetal organ weights were generally comparable with the control group.

Group 3 - 0.012 mg/kg/24h

In three animals (females nos. 485, 42, and 171), live fetuses were removed by cesarean section. One fetus (animal no. 171) showed externally the prepuce not patent and one further fetus (animal no. 485) showed visceral findings as scattered punctiform hemorrhages at cardia.

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Skeletal findings as incompletely and/or not ossified sternebrae were observed in all three fetuses.

No external, visceral, or skeletal malformations were observed in the fetuses.

The mean fetal weight and mean fetal body measurements were comparable with the control group.

The mean placental weight was slightly lower than in the concurrent control group but individual values were within the normal range.

The mean fetal organ weights were generally comparable with the control group.

The mean fetal eye weights were slightly lower but individual values were within the normal range.

The mean body weight gain of the pregnant animals was decreased from day 41 to 55 post-coitum. Due to the fact that only three animals were available for evaluation, and one of these showed a marked weight loss from day 41 to 55 post-coitum, this finding is considered to be incidental.

Group 4 - 0.040 mg/kg/24h

In nine animals (females nos. 406, 315, 187, 284, 473, 467, 161, 537, and 538), live fetuses were removed by cesarean section and in one female (animal no. 431) a dead fetus was removed. The size and stage of development of this dead fetus was according to day 100 of gestation.

Five live fetuses (females nos. 406, 187, 473, 537, and 538) showed external findings as small tissue ball at the tail end and/or prepuce not patent or bent tail end.

Five live fetuses (animals nos. 315, 187, 284, 161, and 537) showed visceral findings as scattered punctiform hemorrhages at cardia or hemorrhages at cardia.

Skeletal findings as 12th pair of ribs shortened and/or incompletely and/or not ossified sternbrae and/or zygostyle asymmetrically ossified were observed in all live fetuses.

The dead fetus could also be examined and showed externally a small tissue ball at tail end and prepuce not patent, visceraally an enlarged left adrenal, and skeletally incompletely and not ossified sternbrae.

No external, visceral, or skeletal malformation were observed in the fetuses.

The mean fetal weight and mean fetal body measurements were comparable with the control group.

The mean fetal thymus weight was slightly lower than in the control but individual values were within the normal range.

Study title: Oral development toxicity study of iloprost clathrate in rabbits

Key study findings: see Appendix for prior review

Study no.: 97005

Volume #, and page #: 5.17, pp.214-435

Conducting laboratory and location: 1

Date of study initiation: 4/16/96

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: ZK 96.944, Batch # 115050

Prenatal and postnatal development

Study title: ZK 36.374 – peri- and postnatal study in the rat after continuous intravenous infusion with the — Osmotic pump from day 15 of gestation to day 22 post partum.

Key study findings: Han-Wistar rats seem especially susceptible to skeletal variations in the digits and sternum.

Study no.: 7780

Volume #, and page #: 5.18, pp. 1-191

Conducting laboratory and location: Schering, Germany

Date of study initiation: February 17, 1986

GLP compliance: yes

QA reports: yes () no (x)

Drug, lot #, and % purity: ZK 36.374, batch # 68-AA-8 part 2

Methods

2.3.1 Survey of the study design

Four groups of inseminated female rats were treated from day 15 p.c. through day 22 p.p. by continuous intravenous infusion of the vehicle or aqueous solutions of the test compound according to the following scheme:

Group	Number of animals	Period of continuous infusion (no. of days)	ZK 36.374	
			Planned dose	Administered dose (Mean;SD) ^{a)}
			(mg/kg/24h)	
1	30	28 ± 1	0	--
2	33	28 ± 1	0.03	0.009; 0.001
3	31	28/29	0.1	0.09; 0.01
4	31	29 ± 1	1.0	0.9; 0.1

- a) According to analytical results obtained with the formulations at various concentrations after storage in osmotic pumps for 4 weeks under in vivo conditions, the actual dosages were expected to be at least about 0.003, 0.06 and 0.9 mg/kg/24 h in the low, intermediate and high dose group respectively.

Doses:

Species/strain: Han-Wistar rats

Number/sex/group:

Route, formulation, volume, and infusion rate:

Intravenous

Formulation Type: aqueous solution

Contents of formulation		vehicle	group 2	group 3	group 4
ZK 36.374; 100 %	(mg)	--	0.040	0.400	4.000
Trometamol	(mg)	9.696	0.097	0.97	9.696
dist. water ad	(ml)	1.0	1.0	1.0	1.0
HCl (1N)	(ml)	0.036	--	--	--

pH adjusted to 8.3

Continuous intravenous infusion over the period of about 28 days was achieved by means of an infusion system based on Osmotic pumps. The pump attached to a catheter was surgically implanted on day 15 p.c. into the subcutis of the back, and the free end of the catheter was inserted at a length of about 15 mm into the external jugular vein and fixed. On day 22 p.p., the pump was removed during necropsy of the dams of the P-generation. The surgical procedure for the implantation of the pumps was performed during the morning of the respective days.

Satellite groups used for toxicokinetics: no

Parameters and endpoints evaluated:

The dams of the P-generation were allowed to litter and raise their offspring. The parental females were examined during gestation and lactation period and sacrificed after weaning (day 22 p.p.). In the untreated F1-animals, physical, functional, and behavioral development was recorded and tested during the lactation and the growth period. At the age of about 90 days, the selected F1-animals were mated within each study group on a 1:1 basis for a period of maximally 14 days, or until copulation had been confirmed by the presence of sperm in the vaginal smears. One male and one female from each litter (as far as possible) of the F1-animals, which had already been selected for the postweaning functional and behavioral tests, were used for mating. The inseminated F1-females were sacrificed on day 21 p.c. in order to examine their reproductive performance and to examine the F2-fetuses for anomalies. The corresponding male mating partners as well as all further F1-animals were necropsied after autopsy of the F1-dams had been finished.

Results

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F₀ in-life: Only issue was seroma at the site of the infusion pump

F₀ necropsy: only findings were associated with the pump having a thickened fibrous capsule around the infusion pump in some of the animals.

F₁ physical development: There was a higher rate of pup mortality in the lactation period in the high dose group (21.8% versus 8.6% in the controls).

Formular-Gr. 207.1
S C H E P I R G Table No. 6 Group survey of postnatal loss Appendix I/page 8
Exp. Toxicology of young Study No. ITR 55.164
F - Generation

		Group No. 1	Group No. 2	Group No. 3	Group No. 4	Group No.
Loss from day 1 to 3 pp	(n)	5	18	3	22	
% of living young at day 3 pp	(%)	2.0	6.6*	1.1	9.6*	
Loss from day 5 to 6 pp	(n)	2	0	5	2	
% of living young at day 4 pp	(%)	0.9	0	2.5	1.0	
Loss from day 9 to 15 pp	(n)	29	12	21	22	
% of living young at day 8 pp	(%)	5.5	4.6	9.1	10.7*	
Loss from day 16 to 22 pp	(n)	2	3	3	4	
% of living young at day 15 pp	(%)	0.9	1.2	1.3	2.2	
Loss from day 1 to 22 pp	(n)	22	33	33	50	
% of living young at day 1 pp	(%)	9.6	11.9	12.3	21.8*	
w per mother animal	(Mean/SD)	9.6/21.9	14.3/26.7	12.6/16.6	11.8/13.1	
Viability Index: All young at day 1 pp	(n/100%)	256	282	275	233	
Living young at day 4 pp	(n/%)	250/97.7	262/92.9*	263/98.1	207/89.8*	
Lactation Index: Living young at day 4 pp	(n/100%)	250	262	265	207	
Living young at day 22 pp	(n/%)	233/91.2	247/94.3	235/88.7	173/86.5*	
Mother animals with living young at day 1 pp	(n/100%)	27	26	29	27	
Mother animals with living young at day 22 pp	(n/%)	27/100.0	26/92.9	29/100.0	25/92.6	
Number of litters at ca. day 90 pp	(n/%)	27/100.0	26/92.9	29/100.0	25/92.6	
Loss from day 22 pp to ca. day 90 pp	(n)	9	14	8	8	
% of living young at day 22 pp	(%)	3.3	5.2	3.4	4.5	

Explanations: * p < 0.05 Chi-Square / Fisher-Test

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Skeletal abnormalities occurred in the treated animals particularly in the digits with crooked or malformed digits along with the sternum in the groups receiving

iloprost.

Formular-Wr. 226.1

Table No. 10.2.1 : Group incidences of young with external anomalies (day 1 to 22 pp)

Appendix I/page 11

Study No.: 91 85.164

Exp. Toxicology

2 / F1 - Generation

		Group No. 1	Group No. 2	Group No. 3	Group No. 4	Group No.
MINOR ANOMALIES						
Examined young	(n)	256	282	270	233	
Young with anomalies	(n)	1	1	0	1	
% per group	(%)	0.4	0.4	0	0.4	
% per litter	(Mean/SD)	0.4/1.1	0.3/1.7	0	0.3/1.4	
Examined litters	(n)	27	28	29	27	
Litters with abnormal young	(n)	1/4.7	1/3.6	0	1/3.7	
MAJOR ANOMALIES						
Examined young	(n)	256	282	270	233	
young with anomalies	(n)	3	6	0	5	
% per group	(%)	0	2.1 *1	0	2.1 *1	
% per litter	(Mean/SD)	0	1.7/7.4	0	1.0/7.4	
Examined litters	(n)	27	28	29	27	
Litters with abnormal young	(n)	0	2/7.1	0	3/11.1	

Explanation: *1 p = 0.05 Chi-Square / Fisher-Test
 *2 p = 0.05 Kruskal-Wallis-Test

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Formular-Wr. 225.1

Table No. 10.2.1 : Individual external anomalies in young (day 1 to 22 pp)

Appendix II/page 40

Study No.: 91 85.164

Exp. Toxicology

2 / F1 - Generation

Group No. 1

Mother animal no.	Examined young Day 1 pp	Young no.	Findings	Classification	Young with external anomalies
					Num. Ex. Num. h Num. s
21	10	all	anf		10 0 0 0 0
22	10	all	anf		10 0 0 0 0
23	11	5	opaqueless of right cornea	1 x 2 s1	11 1 0 1 0
24	12	all	anf		12 0 0 0 0
25	13	2/3/5	phalanges shortened (left forelimb)	3 x 2 s2	
		6	phalanges shortened (forelimbs)	1 x 2 s2	
		8	phalanges severely shortened and flexed (forelimbs)	1 x 2 s2	13 0 0 0 5
27	9	all	anf		9 0 0 0 0
28	8	8	exophthalmos (day 22 pp) (not classified as anomaly)		8 0 0 0 0
29	9	all	anf		9 0 0 0 0
30	12	all	anf		12 0 0 0 0
31	9	all	anf		9 0 0 0 0
32	13	all	anf		13 0 0 0 0
33	12	all	anf		10 0 0 0 0
34	13	13	slight flexion of hindlimbs; head shaped longish	1 x 2 s2	13 0 0 1 7.7
35	10	all	anf		10 0 0 0 0
36	5	all	anf		5 0 0 0 0
37	12	all	anf		12 0 0 0 0
38	7	all	anf		7 0 0 0 0
39	11	all	anf		11 0 0 0 0
40	11	all	anf		11 0 0 0 0
41	11	all	anf		11 0 0 0 0
42	9	all	anf		9 0 0 0 0
43	12	all	anf		12 0 0 0 0
44	10	all	anf		10 0 0 0 0

Formular-Nr. 225.1

Table No. 10.4 : Individual external anomalies in Appendix 11/page 51

Exp. Toxicology: young (Day 1 to 22 ppt) Stud; No.: TR 85.104

F / F1 - Generation Group No.: 4

Pocher no.	Examined young Day 1 DP	Young no.	Findings	Classifi- cation	Young with external anomalies			
					Num. nr.	%	Num.	%
91	6	all	nat	6	0	0	0	0
92	10	all	nat	10	0	0	0	0
93	11	all	nat	11	0	0	0	0
94	11	all	nat	11	0	0	0	0
95	11	all	nat	11	0	0	0	0
97	9	all	nat	9	0	0	0	0
98	7	all	nat	7	0	0	0	0
99	11	all	nat	11	0	0	0	0
100	4	all	nat	4	0	0	0	0
101	15	all	nat	15	0	0	0	0
102	6	all	nat	6	0	0	0	0
104	10	all	nat	10	0	0	0	0
105	5	all	nat	5	0	0	0	0
106	9	all	nat	9	0	0	0	0
107	14	11	opacities of right cornea	I & E nr	14	1	7.1	0
108	12	5	hydrocephalus	I & E nr	12	0	0	1 #Z
109	9	9	phalanges severely shortened (left forelimb)	I & E nr	9	0	0	0
110	5	7; 8	phalanges severely shortened (forelimbs)	I & E nr	5	0	0	1 37.5
111	11	all	nat	11	0	0	0	0
112	5	all	nat	5	0	0	0	0
114	6	all	nat	6	0	0	0	0
115	4	all	nat	4	0	0	0	0
116	9	all	nat	9	0	0	0	0
118	9	all	nat	9	0	0	0	0
119	9	all	nat	9	0	0	0	0
120	12	12	phalanges severely shortened (left forelimb)	I & E nr	12	0	0	1 8.3
121	12	all	nat	12	0	0	0	0
Settings					27			
Sum					213	1	---	---
%					---	0.4	---	2.1
Mean					---	---	0.2	---
SD					---	---	1.4	---

Abbreviations: nat = no abnormal findings; E = External; nr = major; # = minor

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F₁ behavioral evaluation: only in the very first water maze did a difference emerge, with the high dose animals performing poorly, however, after the initial test, there were no differences found between groups.

F₁ reproduction: The only differences observed were a slight increase in wavy ribs in the intermediate and high dose groups. Otherwise, no effect of iloprost treatment was seen in the reproductive performance or prenatal development in F1 dams or F2 fetuses.

F₂ findings: The only differences observed were a slight increase in wavy ribs in the intermediate and high dose groups. Otherwise, no effect of iloprost treatment was seen in the reproductive performance or prenatal development in F1 dams or F2 fetuses.

2.6.6.7 Local tolerance

2.6.6.8 Special toxicology studies

Study title: Evaluation of the report entitled: Antigenicity study of iloprost in guinea pigs.

Key study findings: Unconjugated iloprost showed no ability to elicit an antibody response in Guinea pigs, while the BSA-conjugated iloprost elicited a strong antibody response as well as a strong anaphylactic response.

Study no.: 886165

Volume #, and page #: 5.24, pp.24-419 to 24-453

Conducting laboratory and location: □

1

Date of study initiation: July 26, 1988

GLP compliance: yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Iloprost/ no lot #, batch # H8726/purity= —

Formulation/vehicle: 5 mg/ml iloprost, 2.79 mg/ml trometamol, appropriate amounts of HCl and distilled water for injection

Methods

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Summary**SCIENTIFIC/METHODOLOGICAL BACKGROUND**

The potential of iloprost to exert antigenic properties in guinea-pigs was investigated according to the guidelines for antigenicity studies drafted by [] which have been principally established for testing of drugs which probably bind tightly with proteins, in order to evaluate the risk of drug-induced hypersensitivity reactions.

The test systems applied were:

1. Generalized systemic anaphylactic (≅ active systemic anaphylactic (ASA)) reaction (GSA)

The assay principle is based mainly on the investigation/observation of clinical symptoms in specifically sensitized animals (IgE-mediated) after specific provocation with the test compound.

2. Passive cutaneous anaphylactic reaction (PCA)

The assay principle is based on the indirect detection of released vasoactive substances in formerly unsensitized animals to which drug-specific antiserum (containing specific IgE), derived from sensitized animals has been transferred (passive sensitization). Specific provocation with the test compound has been performed in these animals.

3. Investigation of specific anti-iloprost antibody formation

The assay principle based on the determination of specific antibody formation (total immunoglobulins including IgG, IgM, IgA and IgE) in animals which have been immunized with the test compound including the use of the immunostimulant Freund's complete adjuvant.

In systems 1 and 2, the immune response to iloprost has been tested on the basis of specific IgE formation which represents the immunoglobulin mainly associated with immediate hypersensitivity (type I) reactions.

In system 3 the immune response to iloprost has been tested on the basis of specific total immunoglobulin (IgG, IgM, IgA and IgE) formation, which represents the immunoglobulins associated in all antigen-antibody-mediated immune reactions.

4. In addition in order to investigate a potential immunomodulatory capacity of iloprost, the antibody response of animals to bovine serum albumin (BSA) was investigated in the presence and absence of co-administered iloprost. The rationale for this particular investigation is based on an apparent modulatory activity of prostaglandins on cell-mediated immunity (1).

EXPERIMENTAL PROCEDURES

Four groups of male guinea-pigs (Hartley strain) were dosed according to the following scheme, in order to induce an immunological reaction and to sensitize or immunize the animals with the respective compounds:

Group no.	Number of animals	Test compound administered	First application (s.c.) Day 1		Second application (s.c.) Day 15	
			Dosage of test compound	Additional compounds	Dosage of test compound	Additional compounds supplemented
1	10	Iloprost	0.25 mg/animal	FCA BSA 0.25 mg/animal	0.05 mg/animal	BSA 0.25 mg/animal
2	10	Iloprost-BSA conjugate	0.25 mg/animal ¹⁾	FCA	0.25 mg/animal ¹⁾	--
3	10	Saline	--	FCA BSA 0.25 mg/animal	--	BSA 0.25 mg/animal
4	5	Egg albumin (EA)	0.25 mg/animal	FCA	0.25 mg/animal	--

FCA = Freund's complete adjuvant

BSA = bovine serum albumin

EA = egg albumin

Iloprost-BSA in form of covalently linked hapten-carrier complex

¹⁾ expressed in terms of respective protein assay value

Results:**2.6.6.9 Discussion and Conclusions**

The apparent results of this study are that iloprost is not immunogenic by itself, but it does become immunogenic when conjugated to BSA (Bovine Serum Albumin). Protein binding of iloprost to serum proteins is not discussed although it is important to developing an understanding of the immunologic potential of iloprost. Sponsor has included a critique of the study suggesting a number of problems and indicating that there are sufficient questions about the study to make it difficult to interpret or analyze.

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2.6.6.10 Tables and Figures

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Table 2 Results of Active Systemic Anaphylaxis Test*

Group/Treatment	Elicitor									
	Iloprost-EA conjugate (0.25 mg/animal)					EA (0.25 mg/animal)				
	Anaphylactic Grading**									
	-	±	+	++	+++	-	±	+	++	+++
Group 1 Iloprost with adjuvant	9	0	0	0	0	not tested				
Group 2 Iloprost-BSA conjugate with adjuvant	0	0	4***	6	0	not tested				
Group 3 Saline with adjuvant	7***	0	3****	0	0	not tested				
Group 4 EA with adjuvant	0	0	2	0	0	0	0	2	1	0

*Number of animals responding.

**Anaphylactic reactions graded as follows:

- : no reaction, ± : pilo-erection and/or nose rubbing.

+ : sneezing, cough, dyspnea, vomiting, wheezing and/or weakness

++ : convulsion and/or collapse, +++ : death

***This figure contains one animal that was administered 0.1 mg/animal of elicitor.

****The dose of elicitor was 0.5 or 1 mg/animal.

Table 3 Results of Passive Cutaneous Anaphylaxis Test*

Group/Treatment	Antibody Titer**				
	0	-10	-10 ²	-10 ³	>10 ³
Group 1 Iloprost with adjuvant	9	0	0	0	0
Group 2 Iloprost-BSA conjugate with adjuvant	0	0	0	1	9
Group 3 Saline with adjuvant	10	0	0	0	0
Group 4 EA with adjuvant	1	0	0	4	0

*Number of animals responding.

**Antibody titer expressed as the reciprocal of the maximum dilution giving a positive skin reaction. 0 means no reaction.

Table 4 Results of ELISA*

Group/Treatment	Antibody Titer**				
	0	-10	-10 ²	-10 ³	>10 ³
Group 1					
Iloprost with adjuvant	9	0	0	0	0
Group 2					
Iloprost-BSA conjugate with adjuvant	0	0	0	0	10
Group 4					
EA with adjuvant	0	0	0	0	5

*Number of animals responding.

**Antibody titer expressed as the reciprocal of the maximum dilution giving a positive reaction in ELISA: 0 means negative reaction.

Table 5 Results of ELISA Test

Group/Treatment	Number of animals showing positive reaction/Total
Group 2	
Iloprost - BSA conjugate with adjuvant	10/10
Group 4	
EA with adjuvant	0/5

Table 6 Detection of Antibodies against BSA in Animals Administered Iloprost or Saline*

Group/Treatment	Antibody Titer**			
	2 ³	2 ⁴	2 ⁵	2 ⁶ (x10 ⁴)
Group 1				
Iloprost with adjuvant	1	5	1	2
Group 3				
Saline with adjuvant	1	2	0	7

*Number of animals responding.

**Antibody titer expressed as the reciprocal of the maximum dilution giving a positive reaction in ELISA.

2.6.6.9 Discussion and Conclusions

2.6.6.10 Tables and Figures

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2.6.7 TOXICOLOGY TABULATED SUMMARY

Table TT4 Lethal doses of iloprost after single administration

	LD ₅₀ (mg/kg body weight)			
	oral		intravenous	
Mouse	> 100	Report 4300	~ 200	Reports 5359 / 5389
Rat	> 100	Report 4301	119	Report 5362
Rabbit	nd*	—	9.8	Report 5326

* Not determined.

Table TT3 Pharmacokinetics of iloprost in rats after daily inhalation*†

Actual dose →	Daily inhalation for 135 minutes over 28 days					
	0.9 µg/kg		9.25 µg/kg		22.6 µg/kg	
	Day 1	Day 26	Day 1	Day 26	Day 1	Day 26
C _{max} (pg/mL)‡	0	0	617	453	766	677
T _{max} (h)¶	n/a	n/a	1.5	0.25	2.25	2.25
AUC _{0-last} (pg×h/mL)§	n/a	n/a	961	910	1309	1409
Actual dose →	Daily inhalation for 135 or 240 minutes over 182 days					
	3.6 µg/kg		24.6 µg/kg		43.7 µg/kg	
	Day 1	Day 182	Day 1	Day 182	Day 1	Day 182
C _{max} (pg/mL)‡	497	712	1929	1954	1966	2233
T _{max} (h)¶	1.6	1.0	1.9	1.9	1.0	2.5
AUC _{0-last} (pg×h/mL)§	783	1083	3317	2983	4900	7500

* Pharmacokinetic variables derived from mean serum level time courses.

† Data for males and females combined (2 male and 2 female animals per group).

‡ Maximum serum concentration.

¶ Time to reach C_{max}.

§ Area under the concentration-versus-time curve from dosing time to the last time point.

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Table TT6 Chronic inhalation toxicity study in rats: Study design and dose regimen*

Number of animals per group / sex	Iloprost concentration in nebulizer (µg/mL)	Aerosol concentration of iloprost (Mean/SD) (µg/L air)	Mass Median Aerodynamic Diameter (MMAD) / Geometric Standard Deviation (µm)	Achieved dose levels of iloprost (µg/kg/d)	Achieved dose levels of iloprost (µg/kg/min)	Duration of daily treatment (min)
	(Control)	—	—	0	0	135
	(Control)	—	—	0	0	240
20M, 20F	20 µg/mL (low dose)	0.051 / 0.012	1.24-1.73 / 1.65-2.72	3.55	0.026	135
	20 µg/mL (medium dose)	0.402 / 0.060	0.92-2.14 / 1.83-2.37	27.8	0.206	135
	20 µg/mL (high dose)	0.396 / 0.065	1.40-1.82 / 1.88-2.49	48.7	0.203	240

* Systemic tolerance test with daily inhalative administration of iloprost over 26 weeks.

Table TT8 Genetic toxicity studies of iloprost

Study type	Test system (strain/cells)	Evaluation	Report
In vitro bacteria	<i>Salmonella typhimurium</i> (TA1535, TA100, TA1537, TA1538, TA98), with/without S9 mix; direct plate incorporation	Not mutagenic	5169
	<i>Salmonella typhimurium</i> (TA1535, TA100, TA1537, TA1538, TA98), with/without S9 mix; pre-incubation	Not mutagenic	7166
	<i>Escherichia coli</i> (WP2uvrA), with/without S9 mix	Not mutagenic	7548
In vitro mammalian cells	HGPRT test (V79 cells), with/without S9 mix	Not mutagenic up to cytotoxic concentrations	7429
	Chromosomal aberrations (human lymphocytes), with/without S9 mix	Not clastogenic up to cytotoxic concentrations	7160
In vivo mouse	Micronucleus test (NMRI/SPF mouse), single i.v. administration; sampling (5M, 5F) times at 24, 48 and 72 h after application	Not clastogenic up to highest dose (40 mg/kg)	5360

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Table TT10 Comparison of systemic iloprost exposure — Humans versus animals*

Species	Type of study	Dose	Average		Approximate multiples of the intended human			(Reference) or (Report)
			steady-state plasma levels (ng/mL)	estimated AUC (ng·h/mL)	dose	steady state plasma levels	AUC	
Parenteral administration								
Human	i.v. infusion, ≤ 28 days	1-3.3 mg/kg/min† 4-6 h/d	0.039-0.275	0.253-1.056 (0-24h)	1 (1.3 mg/kg/min)	1 (0.175 mg/min)	1 (0.25 mg/min)	[29]
Rat	i.v. infusion, 28 weeks	147 ug/kg/min† 24 h/d	8.4	154 (0-24h)	2105	223	2146	[288]
Dog	s.c. infusion, 26 weeks	57 mg/kg/min† 24 h/d	1.9	46 (0-24h)	220	27	224	[292]
Oral administration								
Human	capsules, single administration	2-6 µg/kg/d‡	0.3-0.3 5-8 h/d	1-2 (0-24h)	1 (6 µg/kg/d**)	1 (0.01 mg/min)	1 (2 mg/min)	[325]
Mouse	dietary intake, 28-29 days	16.3 mg/kg/d§	2-4 9-12 h/d	28-34 (0-12 h)	22700	3-40	14-34	[A936]
Rat	dietary intake, 8 days	6 mg/kg/d§	1-4 28 h/d	39-25 (0-8 h)	21000	5-40	10-21	[9940]
Rat	dietary intake, 26 weeks	≤ 1.8 mg/kg/d††	1-1.7 28 h/d	10 (0-8 h)	309	3.3-17	1-10	[A352]
Dog	Capsules, 53 weeks	≤ 2.75 µg/kg/d‡	≤ 0.1-1.5 24 h/d	3.2-10 (0-24 h)	211	≤ 0.1-15	1.8-10	[A706]

* All doses and other levels are presented as iloprost equivalents. † Maximum reasonably applicable dosages
 ‡ Maximum tolerated dosages. § Maximum applied dosage
 †† On the basis of no estimated body weight of 50 kg. ** A rate of application per day might be possible to extend exposure period.

Note: For the safety assessment, partial AUCs in animals (calculated from plasma level from drug over a limited period of time) were compared with total AUC in humans. The total AUC in animals is higher, yielding a greater safety margin.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Iloprost in high concentrations was able to cause death by vascular collapse after the induction of severe hypotension. Reproductive toxicity was low, however, although rabbits and monkeys showed no large issues, in the Han-Wistar rats there were problems with drug exposure linked to crooked digits, and a hint of such problems in the cynomolgus monkeys. This is an issue that may require further study by the sponsor. Genotoxicity was low, with one assay, the V79 chinese hamster ovary cells giving a weak positive result. This is thought to be due to the existence of prostacyclin receptors in this cell line.

This submission suffers from an “unevenness” in the presentation of non-clinical results. Interchangeable use of iloprost and iloprost clathrate is problematic, since iloprost clathrate is only 13% iloprost. Also, some of the submitted data from the late 1970’s and not all studies were done using GLP standards nor quality assurance.

Overall, the conclusion is that it is safe to cautiously proceed with the protocol presented.

Unresolved toxicology issues (if any): Further evaluation of the “crooked digits” in the reproductive toxicity assays may be needed.

Recommendations:

Suggested labeling:

Changes to the Ventavis labeling:

Pregnancy

[]

Original Wording:

Pregnancy

[]

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

Appendix I: PHARMACOLOGIST'S REVIEW OF IND —
(Amendment)

Appendix II: PHARMACOLOGIST'S REVIEW OF IND —
(Amendment)

Appendix III: IND Number: —
Review Number: Original review (or Review #1)
Sequence No./Date/Submission
000/ January 30, 2002/

Appendix IV: PHARMACOLOGIST'S REVIEW OF IND —
(Amendment) Dated , —
Amendment #

Appendix V: Statisticians Review of Carcinogenicity studies

Appendix VI: Executive CAC Minutes

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PHARMACOLOGIST'S REVIEW OF IND

Sponsor & Address: []

Reviewer: Timothy W. Robison, Ph.D.
Pharmacologist, []

Date of Submission: []

Date of HFD- Receipt: []

Date of Review: []

Drug: Iloprost β -cyclodextrin Clathrate []

Category: []

Submission Contents:

1. Toxicokinetic study in pregnant female Sprague-Dawley rats.
2. Toxicokinetic study in pregnant female New Zealand White rabbits.
3. Combined Segment I fertility and reproductive performance and Segment II teratology study in female Sprague-Dawley rats.
4. Segment II teratology study in New Zealand White rabbits.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION:

Absorption

Plasma Levels and Pharmacokinetics of Iloprost after Daily Repeated Administration of Iloprost Clathrate at 5, 50, 125, 250, and 500 mg/kg/day by Gavage as part of a Pilot Fertility Study in Female Sprague Dawley Rats (Research Report AM96).

Methods: As part of a dose range finding fertility study, plasma iloprost levels were measured in female rats that received iloprost clathrate by oral gavage at doses of 5, 50, 125, 250, and 500 mg/kg/day. Doses are expressed in terms of iloprost clathrate. Iloprost clathrate was administered from 14 days prior to mating, through the mating period, and through to day 16 of gestation. There were 18 female rats per dose group. On days 6 and 16 of gestation, blood for determination of plasma iloprost levels was collected at 0.083,

0.25, 1, 4, 8, and 24 hr after dosing. For each time point, blood was collected from 3 rats/dose. Plasma iloprost levels were quantified by radioimmunoassay.

Results: Plasma AUC values for iloprost were similar on days 6 and 16 of gestation for pregnant female rats. AUC values for iloprost were generally proportional to dose from 5 to 125 mg/kg/day; however, at doses ≥ 250 mg/kg/day, deviation from linearity occurred. Plasma clearance values for iloprost at all doses (1217-2988 mL/min/kg) significantly exceeded renal plasma flow (21.3 mL/min/kg) or hepatic plasma flow (31.9 mL/min/kg), (Pharmaceutical Research 10:1093-1095, 1993) suggesting a rapid metabolic clearance of iloprost.

Toxicokinetic parameters for plasma iloprost levels on days 6 or 16 of gestation for pregnant female rats that received iloprost clathrate at doses of 5, 50, 125, 250, and 500 mg/kg/day from 14 days prior to mating, through the mating period, and through day 16 of gestation.

Dose, mg/kg/day	C _{max} , ng/mL		T _{max} , hr		AUC _{0-24hr} , ng*hr/mL		MRT, hr		Cl/f mL/min/kg	
	Day 6	Day 16	Day 6	Day 16	Day 6	Day 16	Day 6	Day 16	Day 6	Day 16
5	2.3	3.0	0.25	0.083	9.5	8.9	4.7	5.4	1217	1292
50	-	38.3	-	0.083	68.4	72.7	6.2	5.7	1679	1579
125	136	65.7	0.083	0.083	227.2	201.3	4.9	5.9	1264	1426
250	-	114.5	-	0.25	192.1	269.6	7.7	5.0	2988	2130
500	-	171.4	-	0.25	539.5	694.7	4.0	2.8	2128	1653

Plasma Levels and Pharmacokinetics of Iloprost After Repeated Once-A-Day Administration of Iloprost Clathrate at 10, 40, 80, and 120 mg/kg/day by Gavage from Days 6 to 18 of Gestation in Pregnant Rabbits (Research Report AM31).

Methods: As part of a dose range finding fertility study, plasma iloprost levels were measured in pregnant female rabbits that received iloprost clathrate by oral gavage at doses of 10, 40, 80, and 120 mg/kg/day. Doses are expressed in terms of iloprost clathrate. Iloprost clathrate was administered from days 6 through 18 of gestation. There were 4 satellite female rabbits per dose group. On days 6 and 18 of gestation, blood for determination of plasma iloprost levels was collected at 0.083, 0.25, 1, 4, 8, and 24 hr after dosing. Plasma iloprost levels were quantified by radioimmunoassay.

Results: Increases in plasma AUC values for iloprost were proportional to increasing dose at ≥ 40 mg/kg/day. Clearance values for plasma iloprost decreased with increasing dose, particularly on day 6. The increased clearance observed at 10 mg/kg/day was most likely responsible for the lower than expected plasma AUC value at this dose. Clearance values (451-1141 mL/min/kg) significantly exceeded liver plasma flow (47.2 mL/min/kg) or renal plasma flow (21.3 mL/min/kg) (Pharmaceutical Research 10:1093-1095, 1993) suggesting a rapid metabolic clearance of iloprost.

Toxicokinetic parameters for plasma iloprost in pregnant female rabbits that received iloprost clathrate at doses of 10, 40, 80, and 120 mg/kg/day on days 6 and 18 of gestation.

Dose, mg/kg/day	C _{max} , ng/mL		AUC _{0-24hr} , ng*hr/mL		MRT, hr		Cl/f, ML/min/kg	
	Day 6	Day 18	Day 6	Day 18	Day 6	Day 18	Day 6	Day 18
10	10.9	18.6	21.0	31.1	2.7	2.6	1141	779
40	35.9	46.9	175	151	4.8	3.0	650	686
80	44.8	88.7	400	N.C.	7.4	N.C.	512	N.C.
120	86.0	N.C.	615	N.C.	4.7	N.C.	451	N.C.

N.C. = not calculated because of high mortality at 80 mg/kg/day or total mortality at 120 mg/kg/day before day 18.

TOXICOLOGY:

Reproductive Toxicology

Combined Study of Fertility and Embryonic Development and Pre- and Postnatal Development Including Maternal Function of Iloprost Clathrate in Female Rats (Report number 97004).

Testing Laboratory: □

1

Date Started: April 16, 1996

Date Completed: February 25, 1998

GLP Compliance: Statements of compliance with GLP Regulations and the Quality Assurance Unit were included.

Animals: Female Sprague Dawley CrI:CD®BR rats □

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Drug Batch: Iloprost clathrate, Lot numbers 115050 and 115020.

Methods: In accordance with Guideline for Industry entitled "Detection of Toxicity to Reproduction for Medicinal Products, ICH-S5A September 1994", in a combined Segment I/Segment II study, the effects of iloprost clathrate were assessed on fertility and reproductive performance in F₀ female rats, on implantation (uterine evaluation on day 13 of gestation), on morphological development of the conceptus (uterine evaluation on day 20 of gestation), on natural delivery and the lactation phase of F₀ females, and on viability and growth of F₁ offspring during the weaning phase. Female rats received iloprost clathrate by oral gavage at doses of 0, 5, 50, and 250 mg/kg/day. Doses are expressed in terms of iloprost clathrate. Control animals received the vehicle, Tris buffer (adjusted

to pH 8.2-8.4 with HCl or NaOH). The dose volume was 10 mL/kg. Iloprost clathrate was administered daily for 14 days prior to mating and through day 13 of gestation, day 20 of gestation, or day 21 of lactation. Dose selection was based upon a dose range finding study (Report number 96030), in which female rats received iloprost clathrate by oral gavage at doses of 5, 50, 125, 250, and 500 mg/kg/day from 14 days prior to mating, through the mating period, and through to day 19 of gestation or day 6 of lactation. There were 10 female rats/groups. On day 20 of gestation, 4 to 5 pregnant female rats/dose were sacrificed and examined for fertility and reproductive performance as well as external malformations and variations. Remaining rats were allowed to spontaneously deliver their offspring and were retained until day 6 of lactation. Observed effects from 1 to 4 hr after dosing at 50 to 500 mg/kg/day included reddening of the ears, nose, and/or feet. Hypoactivity was observed at doses ≥ 250 mg/kg/day. Two female rats at 500 mg/kg/day were sacrificed in a moribund condition during the first week of treatment. Clinical signs for these two animals included cyanosis, hunched posture, and tremors. Macroscopic findings for 1 animal included a pale spleen and distended intestines. Mating and fertility indexes were unaffected by treatment. Body weight gains for dams at 500 mg/kg/day during the gestation and lactation periods were reduced to 85.1 and 62% of the control, respectively. Total resorptions at doses of 250 and 500 mg/kg/day were increased to 15.6 and 10.5%, respectively, as compared to 5.6% for the control. Post-implantation losses at doses of 250 and 500 mg/kg/day were increased to 17.0 and 10.5%, respectively, as compared to 5.6% for the control. For dams allowed to spontaneously deliver their offspring, the number of liveborn pups/dam at 500 mg/kg/day was reduced to 10.5 as compared to 14 for the control. There were no treatment-related external malformations or variations. In the present study, there were 60 female rats/group. During the mating period, daily vaginal smears were collected to assess the stage of estrus. Estrus cycle determination continued until confirmation of mating or the mating period ended. Following the mating period with untreated male rats, female rats were assigned to one of the three phases: 15 female rats/group were assigned to gestation day 13 uterine examination; 20 female rats/group were assigned to gestation day 20 cesarean sections (teratology phase); and 25 female rats/group were allowed to deliver and raise their young until lactation day 21. Rats were observed for clinical signs of toxicity at 1 hr after dosing and for moribundity/ mortality twice daily. Animals were weighed twice weekly during the pre-mating phase and during mating. Mated female rats were weighed on days 0, 3, 7, 10, 13, 17, and 20. Female rats that were allowed to deliver their litters were weighed on days 0, 4, 7, 10, 14, 17, and 21 of lactation. Food consumption was measured for mated female rats on days 0-3, 3-7, 7-10, 10-13, 13-17, and 17-20 of gestation and for female rats that were allowed to deliver their offspring on days 0-4, 4-7, 7-10, and 10-14 of lactation. A physical examination was performed at each weighing interval. During gestation, females were observed for signs of abortion, premature delivery, or difficult and prolonged parturition.

On day 13 of gestation, selected female rats were sacrificed and the uterus from each female was excised, weighed, and examined for the number and placement of implantation sites, live and dead embryos, early resorptions, and any abnormalities of the uterus or embryonic sac. The ovaries were examined for the number of corpora lutea. On day 20 of gestation, selected female rats were sacrificed and the uterus from each female was excised, weighed, and examined for the number and placement of implantation sites, live and dead fetuses, early and late resorptions, and any abnormalities of the uterus or

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embryonic sacs. The ovaries were examined for the number of corpora lutea. Each fetus

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was sexed, weighed, examined for external abnormalities, and sacrificed. Approximately one-half of all the fetuses from each litter were randomly selected and processed for visceral examination. Remaining fetuses were eviscerated and processed for skeletal examination. Selected pregnant female rats were allowed to deliver and raise their young to day 21 postpartum. Pups were sexed, weighed, and examined for external abnormalities. On days 0, 4 (precull), 7, 14, and 21 of lactation, the number of live pups of each sex per litter, body weights of all live pups, and remarkable clinical conditions were recorded. On day 4 of lactation, all litters with >8 pups were culled to produce litters containing 4 pups/sex, if possible. During the lactation period, fetuses in each litter were examined for clinical signs of toxicity and moribundity/mortality. Pups that died were examined for visceral abnormalities and preserved. Following weaning on day 21 of lactation, all surviving F₁ pups were sacrificed and subjected to a gross examination of viscera. F₀ female rats allowed to deliver their offspring were sacrificed on day 21 postpartum, subjected to a gross examination, and the ovaries, uterus, and any abnormal viscera were preserved.

Results:

1. Observed Effects: For F₀ female rats at 50 mg/kg/day on day 14, 6 animals were observed with reddened extremities within 1 hr after dosing. For F₀ dams at 250 mg/kg/day from days 0 to 28, between 1 and 34 dams/day were observed with reddened extremities within 1 hr after dosing. For dams at 250 mg/kg/day during gestation, 2-8 animals/day were observed with reddened extremities within 1 hr after dosing. For dams at 250 mg/kg/day during lactation, 4-7 animals/day were observed with reddened extremities within 1 hr after dosing.

2. Mortality: One female rat at 250 mg/kg/day died during parturition. Two female rats at 50 mg/kg/day died during lactation (days 3 and 15). Gross pathological findings for these two female rats included darkened tissues (lung, liver, and/or spleen) and/or distended tissues (intestines or stomach). One female rat at 250 mg/kg/day was sacrificed on day 9 of lactation following death of all fetuses in her litter.

3. Body Weight and Food Consumption: Body weight gains of treatment groups during the 14-day treatment period prior to mating and during the gestation and lactation periods were unaffected.

4. Day 13 Uterine Examination: Fertility and reproductive performance were unaffected for F₀ female rats that received doses ≤250 mg/kg/day. Corpora lutea/dam, implantation sites/dam, pre-implantation loss, live fetuses/dam, resorptions/dam, and post-implantation loss were unaffected by treatment at doses ≤250 mg/kg/day.

Fertility and reproductive performance for F₀ female rats that were sacrificed on day 13 of gestation.

Parameter	0 mg/kg/day	5 mg/kg/day	50 mg/kg/day	250 mg/kg/day
Pregnant rats	15	15	15	15
Pregnant rats with viable fetuses	14	15	15	15
Dams with no viable fetuses	1	0	0	0
Corpora lutea/dam	16.5 (247/15)	19.1 (287/15)	19.1 (286/15)	19.5 (292/15)
Implantation sites/dam	14.3 (215/15)	17.0 (255/15)	17.4 (261/15)	17.5 (263/15)
Pre-implantation loss, %	16.1 (32/247)	10.4 (32/287)	8.7 (25/286)	9.4 (29/29.2)
Live fetuses/dam	12.8 (192/15)	16.3 (245/15)	16.3 (244/15)	14.5 (218/15)
Resorptions/dam				
-Total	1.5 (22/15)	0.7 (10/15)	1.1 (17/15)	3.0 (45/15)
-Early	1.5 (22/15)	0.7 (10/15)	1.1 (17/15)	3.0 (45/15)
Post-Implantation loss, %	17.5	3.7	6.7	17.6

5. Day 20 Teratology Examination: After correction for gravid uterus weight, body weight gain for dams at 250 mg/kg/day was reduced to 86% of the control (74.62 g). Live births/dam were decreased at 250 mg/kg/day. In conjunction with this decrease in live births/dam, early resorptions and post-implantation loss were increased at 250 mg/kg/day. Corpora lutea/dam, implantation sites/dam, and pre-implantation loss were unaffected by treatment. No treatment-related external, visceral, or skeletal malformations or variations were observed.

Fertility and reproductive performance for F₀ female rats that were sacrificed on day 20 of gestation.

Parameter	0 mg/kg/day	5 mg/kg/day	50 mg/kg/day	250 mg/kg/day
Pregnant rats	20	20	20	20
Pregnant rats with viable fetuses	20	20	19	20
Dams with no viable fetuses	0	0	1	0
Corpora lutea/dam	18.2 (364/20)	18.1 (363/20)	19.1 (383/20)	19.6 (393/20)
Implantation sites/dam	16.1 (323/20)	16.0 (320/16)	17.0 (339/20)	17.5 (350/20)
Pre-implantation loss, %	10.7% (41/364)	11.4% (43/363)	11.5% (44/383)	10.6% (43/393)
Live F ₁ fetuses				
-per dam	15.4 (309/20)	15.5 (310/20)	16.0 (320/20)	14.9 (297/20)
-%	96.1%	97%	92.1%	85.0*
F ₁ Male to Female Ratio	50:50 (153:156)	51:49 (157:153)	53:47 (168:152)	48:52 (143:154)
F ₁ Fetal body weight				
-male	3.49	3.54	3.60	3.43
-female	3.34	3.39	3.41	3.25
Resorptions/dam				
-Total	0.7= 14/20	0.5=10/20	0.9=19/20	2.7=53/20
-Early	0.7= 14/20	0.5=10/20	0.9=19/20	2.5=51/20
-Late	0	0	0	0.1=2/20
Resorptions, %				
-Total, %	3.9% (~14/323)	3% (~10/320)	7.9% (~19/339)	15% (~53/350)
-Early, %	3.9% (~14/323)	3% (~10/320)	7.9% (~19/339)	14.5% (~51/350)
-Late, %	0	0	0	0.5% (~2/350)
Post-Implantation loss, %	3.9%	3.0%	7.9%	15.0%*

p ≤ 0.05

F₁ fetal visceral malformations and variations at doses of 0, 5, 50, and 250 mg/kg/day. Data expressed as number of fetuses (%) / number of litters (%).

Parameter	0 mg/kg/day	5 mg/kg/day	50 mg/kg/day	250 mg/kg/day
Fetuses/Litters examined	151/20	152/20	158/19	145/20
Microphthalmia (M)	0	0	0	1 (0.7%) / 1 (5.0%)
Increased renal pelvic cavitation (V)	0	5 (3.3%) / 3 (15%)	2 (1.3%) / 2 (11%)	3 (1.4%) / 2 (10%)

F₁ fetal skeletal malformations and variations at doses of 0, 5, 50, and 250 mg/kg/day. Data expressed as number of fetuses (%) / number of litters (%).

Parameter	0 mg/kg/day	5 mg/kg/day	50 mg/kg/day	250 mg/kg/day
Fetuses/Litters examined	158/20	158/20	162/19	152/20
Major fusion of sternbrae (M)	1 (0.6%) / 1 (5.0%)	0	0	0
Incomplete ossification of skull (M)	5 (3.2%) / 4 (20%)	11 (7.0%) / 7 (35%)	9 (5.6%) / 7 (37%)	9 (5.9%) / 7 (35%)
<4 sacral vertebrae ossified	0	0	1 (0.6%) / 1 (0.3%)	0
6 th Sternebra unossified	1 (0.6%) / 1 (5%)	2 (1.3%) / 2 (10%)	2 (1.2%) / 2 (11%)	6 (3.9%) / 4 (20%)
Other Sternebra(e) unossified	4 (2.5%) / 3 (15%)	2 (1.3%) / 2 (10%)	3 (1.9%) / 3 (16%)	7 (4.6%) / 4 (20%)
Sternebra(e) asymmetrically ossified	3 (1.9%) / 3 (15%)	5 (3.2%) / 4 (20%)	1 (0.6%) / 1 (5.3%)	6 (3.3%) / 7 (4 (20%))
7 th Cervical ribs	0	0	0	1 (0.7%) / 1 (5.0%)
Incomplete ossification of rib(s)	0	0	2 (1.2%) / 2 (11)	0
Wavy/bent ribs	0	0	1 (0.6%) / 1 (5.3%)	1 (0.7%) / 1 (5.0%)
<3 Metacarpals ossified	0	0	1 (0.6%) / 1 (5.3%)	0
<4 Metacarpals ossified	0	0	1 (0.6%) / 1 (5.3%)	1 (0.7%) / 1 (5.0%)

6. Examinations of F₀ Dams Allowed to Deliver and their F₁ Offspring: The duration of gestation was increased for dams at 250 mg/kg/day. The numbers of F₀ dams with stillborn pups was increased at doses of 50 and 250 mg/kg/day. The number of stillborn F₁ pups was increased at doses of 50 and 250 mg/kg/day. The live birth index was decreased at a dose of 250 mg/kg/day. The number of F₁ pup dying, killed, missing, and/or cannibalized from days 0-4 was increased at doses of 50 and 250 mg/kg/day. The pup viability index at day 4 was reduced at a dose of 250 mg/kg/day. Implantation sites/ dam, the male to female F₁ pup ratio, and F₁ pup weight (days 0 to 21) were unaffected. Three control dams, 2 dams at 50 mg/kg/day, and 1 dam at 250 mg/kg/day were not pregnant.

Natural delivery parameters for F₀ dams that received doses of 0, 5, 50, and 250 mg/kg/day and viability and body parameters for F₁ pups (n = 25 F₀ dams/group).

Parameter	0 mg/kg/day	5 mg/kg/day	50 mg/kg/day	250 mg/kg/day
F ₀ Pregnant rats	22	25	23	24
F ₀ Dams Delivering	22	25	23	23
F ₀ Dams with liveborn pups	22	25	23	23
F ₀ Dams with stillborn pups	1	1	4	9
Duration of Gestation (days)	21.9	22.1	22.2	22.6*
Implantation Sites/dam	15.45 (340/22)	16.60 (415/25)	15.13 (348/23)	17.39 (400/23)
F ₁ pups delivered/dam	13.64 (300/22)	14.96 (374/25)	14.87 (342/23)	14.22 (327/23)
Liveborn pups/dam	13.59 (299/22)	14.92 (373/25)	14.56 (335/23)	13.17 (303/23)
F ₁ pups				
-liveborn	299	373	335	303
-stillborn	1	1	6	22
-uncertain	0	0	1	2
Live birth index	100	100	97	92
F ₁ pups dying, killed, missing, and/or cannibalized				
-days 0-4	6	9	19	28
-days 5-21	1	0	8	6
Viability Index, %	98	98	95	90
F ₁ pups alive day 4 (precull)/ number liveborn				
Weaning Index, %	99	100	95	94
Number alive at weaning/ number alive day 4 (postcull)				
% Males				
-day 0	56 (168)	50 (185)	52 (171)	51 (157)
-day 4 (precull)	56 (164)	50 (180)	52 (161)	50 (138)
-day 21	51 (89)	50 (99)	50 (84)	47 (80)
Pup Weight, M/F				
-day 0	6.54/6.20	6.66/6.27	6.49/6.16	6.13/5.78
-day 4 (precull)	10.42/9.81	10.59/10.00	10.05/9.56	9.31/8.87
-day 4 (postcull)	10.47/9.86	10.61/10.08	10.16/9.64	9.79/8.97
-day 7	17.14/16.08	17.41/16.52	16.60/15.95	14.31/13.61
-day 14	34.83/33.43	35.05/33.70	32.55/31.52	28.67/27.27
-day 21	57.79/55.15	59.29/56.75	54.88/52.52	49.24/46.37

In combined Segment I/Segment II studies, the effects of iloprost clathrate were assessed on fertility and reproductive performance in F₀ female rats, on implantation (uterine evaluation on day 13 of gestation), on morphological development of the conceptus (uterine evaluation on day 20 of gestation), on natural delivery and the lactation phase of F₀ females, and on viability and growth of F₁ offspring during the weaning phase. Female rats were treated with iloprost clathrate at doses of 0, 5, 50, and 250 mg/kg/day. Iloprost clathrate was administered daily for 14 days prior to mating and through day 13 of gestation, day 20 of gestation, or day 21 of lactation. For female rats treated through day 13 of gestation, iloprost clathrate at doses ≤250 mg/kg/day had no effect on fertility and reproductive performance. Iloprost clathrate at doses ≤250 mg/kg/day had no effect on implantation. For female rats treated through day 20 of gestation, iloprost clathrate at

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doses \leq 250 mg/kg/day was not teratogenic. Maternal toxicity was evident at doses of 50 and 250 mg/kg/day. One female dam at 250 mg/kg/day died during parturition. Two dam at 50 mg/kg/day died during lactation. One dam at 250 mg/kg/day was sacrificed on day 9 of lactation following death of all fetuses in her litter. Live births/dam were decreased at 250 mg/kg/day. Post-implantation loss at 250 mg/kg/day was increased to 15% as compared to 3.9% for the control. For F₀ dams allowed to deliver their F₁ offspring, the duration of gestation for dams at 250 mg/kg/day was increased to 22.6 days as compared to 21.9 days for the control. The numbers of F₀ dams with stillborn pups was increased at doses of 50 and 250 mg/kg/day. The number of stillborn F₁ pups was increased at doses of 50 and 250 mg/kg/day. The live birth index at a dose of 250 mg/kg/day was decreased to 92% as compared to 100% for the control. The number of F₁ pup dying, killed, missing, and/or cannibalized from days 0-4 was increased at doses of 50 and 250 mg/kg/day. The pup viability index on day 4 at a dose of 250 mg/kg/day was reduced to 90% as compared to 98% for the control.

Rabbit

Segment II Oral Developmental Toxicity Study of Iloprost Clathrate in Rabbits (Report No. 97005).

Testing Laboratory:

Date Started: April 16, 1996

Date Completed: February 25, 1998

GLP Compliance: Statements of compliance with GLP regulations and the Quality Assurance Unit were included.

Animals: Pregnant female (NZW)SPF rabbits were used in this study. On day 0 of gestation, animals were approximately 5½ months old and body weights ranged from 3031 to 4242 g.

Drug Batch: Iloprost Clathrate, Batch No. 115050.

Methods: In a Segment II teratology study, pregnant female rabbits received iloprost clathrate by oral gavage at doses of 0, 10, 20, and 40 mg/kg/day from days 7 to 20 of gestation. Doses are expressed in terms of iloprost clathrate. Animals in the control group received the vehicle, Tris (Trometamol) buffer solution (Base in deionized water) with the pH adjusted to 8.3 using HCl and/or NaOH. Dose selection was based upon a Segment II dose range finding study in which pregnant female rabbits received iloprost clathrate by oral gavage at doses of 10, 40, 80, and 120 mg/kg/day from days 6 to 18 of gestation (Report No. 96031). There were 5 dams/group and surviving animals

were sacrificed on day 29 of gestation. Death or moribund sacrifice occurred at doses of 40 (20%, 1/5), 80 (60%, 3/5), and 120 (100%, 5/5) mg/kg/day. Observed effects included reddened ears and rapid respiration within 1 hr after dosing for all treatment groups and persisting for several hours in the 80 and 120 mg/kg/day groups. Body weights on day 19 for the 40 and 80 mg/kg/day groups were reduced by 3.2 and 18.1%, respectively, as compared to body weights on day 6. Food consumption for the 40 and 80 mg/kg/day groups were reduced to 70 and 7.7% of the control (159 g/animal/day), respectively, between days 6 and 19 of gestation. Clinical chemistry values were assessed on day 19 as follows: creatinine levels for the 40 and 80 mg/kg/day groups were elevated to 145.4 and 181.8% of the control (1.1 mg/dL), respectively; albumin levels for the 40 and 80 mg/kg/day groups were decreased to 84.1 and 81.8% of the control (4.4 g/dL), respectively; the albumin to globulin ratio for the 40 and 80 mg/kg/day groups were decreased to 83.5 and 70.7% of the control (2.42), respectively; and total cholesterol levels for the 80 mg/kg/day group were increased to 293% (85 ± 89.8 mg/dL) of the control (29 ± 9 mg/dL). Early resorptions for the 40 and 80 mg/kg/day groups were increased to 27.8 and 50%, respectively, as compared to 9.5% for the control. Post-implantation losses for the 40 and 80 mg/kg/day groups were increased to 31.3 and 50.0%, respectively, as compared to 9.5% for the control. Live fetuses/dam for the 40 and 80 mg/kg/day groups were decreased to 5.8 (23/4) and 4.0 (8/2), respectively, as compared to 7.6 (38/5) for the control. One of the two surviving dams at 80 mg/kg/day had no viable fetuses. No external malformations or variations were evident in any of the fetuses. In the present study, there were 20 pregnant female rabbits/group. The dose volume was 3 mL/kg. Animals were observed for mortality/moribundity twice per day. Animals were monitored for clinical signs of toxicity daily at approximately 1 hr after dosing. Body weights were measured on days 0, 4, 7, 9, 11, 15, 18, 21, 24, and 29. A physical examination was conducted at each weighing interval. Food consumption was measured at body weight intervals starting on day 4 of gestation. All dams which died prior to scheduled cesarean section (including abortions) were subjected to gross examinations. On day 29 of gestation, all surviving dams were sacrificed and subjected to a gross examination. The uterus from each gravid female rabbit was removed, weighed, and examined for number and placement of implantation sites, live and dead fetuses, early and late resorptions, and any abnormalities of the uterus or embryonic sacs. The ovaries were examined for the number of corpora lutea. Each fetus was weighed, examined for external malformations and variations, and sacrificed. Fetuses were examined soft tissue malformations and variations, during which the sex was determined. Fetuses were subsequently eviscerated, processed, and examined for skeletal malformations and variations.

Results:

1. Observed Effects: Observed effects at doses of 20 mg/kg/day (3 to 15 animals/day) and 40 mg/kg/day (9 to 18 animals/day) included reddening of the ears and rapid respiration within 1 hr after dosing from days 7 through 20. Few feces were observed during the study period with following incidence: 2 dams in the control group, 1 dam at

10 mg/kg/day, 7 dams at 20 mg/kg/day, and 9 dams at 40 mg/kg/day.

2. Mortality: At the high dose of 40 mg/kg/day, 4 dams died between days 9 to 15 of gestation (1 each on days 9, 11, 12, and 15). Ataxia and tremors were observed in one dam on day 15 shortly before death. One dam at 20 mg/kg/day (day 22) and 2 dams at 40 mg/kg/day (1 on day 25 and 1 on day 29) were sacrificed following the development of signs of abortion. For the six unscheduled deaths in the 40 mg/kg/day, each dam was found with a gravid uterus.

3. Body Weight and Food Consumption: Body weight gains for dams at 40 mg/kg/day were decreased during the treatment period from days 7 to 21. Mean body weights for control dams on day 7 and 21 were 3720 and 3924.8 g, respectively. For the control, 10 mg/kg/day, and 20 mg/kg/day groups, mean body weights on day 21 were increased by 5.5, 3.0, and 3.7% of mean body weights on day 7, respectively. For the 40 mg/kg/day group, the mean body weight value on day 7 was decreased by 5.7% of the mean body weight value on day 7. Food consumption for dams at 10, 20, and 40 mg/kg/day appeared to be decreased to 89.2, 82, and 47.6% of the calculated mean for control dams (181.8 g/animal/day) from days 7 to 21, respectively.

4. Gross Pathology: The mean gravid uterus weight for the 40 mg/kg/day groups was decreased to 75.8% of the control (504.42 g).

5. Embryo-Fetal Development: Three dams at 40 mg/kg/day had no viable fetuses. Total resorptions, consisting primarily of early resorptions, were increased to 30.5% for the 40 mg/kg/day group as compared to 6.9% for the control group. Post-implantation loss (i.e., total resorptions) for the 40 mg/kg/day group were increased to 30.5% as compared to 6.9% for the control group. The percent live fetuses/dam for the 40 mg/kg/day was decreased to 69.5% as compared to 93.1% for the control group. There were no treatment-related external, visceral, or skeletal malformations. The incidence of vertebral anomaly with/without associated rib anomaly were increased for the 20 and 40 mg/kg/day groups; although, there was no dose response relationship. The incidences of variations of major vessels (i.e., left carotid arises from innominate) and small gall bladder was increased for the 40 mg/kg/day group. The incidence of the skeletal variations, angulated hyoid wings, incomplete/unossified hyoid wings, incomplete ossification of other sternebra(e), and incomplete ossification of ribs, were increased for the 20 and 40 mg/kg/day groups. The incidence of unossified 5th sternebra and unossified talus(I) (i.e., the highest of the tarsal bones and the one which articulates with the tibia and fibula to form the ankle joint) were increased for all iloprost clathrate treatment groups. The incidence of bipartite other sternebra(e) were increased the 40 mg/kg/day group.

Cesarean Section Data for dams that received iloprost clathrate by the oral gavage at doses of 0, 10, 20, and 40 mg/kg/day from days 7 to 20 of gestation.

Parameter	0 mg/kg/day	10 mg/kg/day	20 mg/kg/day	40 mg/kg/day
Females mated	20	20	20	20
Pregnant dams	19	20	19	20
Aborted	0	0	1	2
Died	0	0	0	4
Pregnant at C-section	19	20	18	14
Dams with viable fetuses	19	20	18	11
Dams with no viable fetuses	0	0	0	3
Corpora lutea/dam	9.3 (176/19)	9.1 (181/20)	9.4 (170/18)	9.6 (135/14)
Implantation sites/dam	8.3 (158/19)	8.6 (171/20)	8.8 (159/18)	8.8 (123/14)
Mean pre-implantation loss, %	10.4% (18/176)	5.6% (10/181)	6.3% (11/170)	8.6% (13/135)
Total Resorptions -resorptions/dam -percentage	0.6 (11/19) 6.9%	0.4 (8/20) 5.1%	0.2 (3/18) 1.6%	2.8 (39/14) 30.5%
Early Resorptions -resorptions/dam -percentage	0.3 (6/19) 4.2% (6/158)	0.3 (5/20) 3.5% (5/171)	0.1 (2/18) 1.0% (2/159)	2.7 (38/14) 29.6% (38/123)
Late Resorptions -resorptions/dam -percentage	0.2 (5/19) 2.7% (5/158)	0.2 (3/20) 1.6% (3/171)	0.1 (1/18) 0.6% (1/159)	0.1 (1/14) 0.9% (1/123)
Mean post-implantation loss, %	6.9%	5.1%	1.6%	30.5%
Live fetuses/dam, mean	7.7 (147/19)	8.1 (163/20)	8.7 (156/18)	6.0 (84/14)
Live fetuses/dam, %	93.1%	94.9%	98.4%	69.5%
Sex ratio, M:F	53: 47	51: 49	60: 40	68: 32
Fetal body (viable),g				
-male	44.24	43.85	43.51	41.08
-female	42.15	43.43	43.22	40.89

External malformations and variations for F₁ fetuses of F₀ dams that received iloprost clathrate at doses of 0, 10, 20, and 40 mg/kg/day from days 7 to 20 of gestation. Data expressed as number of fetuses (%)/number of litters (%).

Parameter	0 mg/kg/day	10 mg/kg/day	20 mg/kg/day	40 mg/kg/day
Fetuses/Litters Evaluated	147/19	163/20	156/18	84/11
Malrotated limbs	0	0	1 (0.6%)/ 1 (5.6%)	0

Soft tissue (i.e., visceral) malformations and variations for F₁ fetuses of F₀ dams that received iloprost clathrate at doses of 0, 10, 20, and 40 mg/kg/day from days 7 to 20 of gestation. Data expressed as number of fetuses (%)/number of litters (%).

Parameter	0 mg/kg/day	10 mg/kg/day	20 mg/kg/day	40 mg/kg/day
Fetuses/Litters Evaluated	147/19	163/20	156/18	84/11
Kidneys malpositioned (M)	0	0	1 (0.6%)/ 1 (5.6%)	0
Variations of the major vessels (i.e., left carotid arises from innominate) (V)	1 (0.7%)/ 1 (5.3%)	1 (0.6%)/ 1 (5.0%)	0	3 (3.6%)/ 2 (18%)
Gall bladder, small (V)	2 (1.4%)/ 1 (5.3%)	1 (0.6%)/ 1 (5.0%)	1 (0.6%)/ 1 (5.6%)	5 (6.0%)/ 4 (36%)
Gall bladder, enlarged (V)	5 (3.4%)/ 3 (16%)	10 (6.1%)/ 6 (30%)	17 (11%)/ 5 (28%)	3 (3.6%)/ 1 (9.1%)

Skeletal malformations and variations for F₁ fetuses of F₀ dams that received iloprost clathrate at doses of 0, 10, 20, and 40 mg/kg/day from days 7 to 20 of gestation. Data expressed as number of fetuses (%)/number of litters (%).

Parameter	0 mg/kg/day	10 mg/kg/day	20 mg/kg/day	40 mg/kg/day
Fetuses/Litters Evaluated	147/19	163/20	156/18	84/11
Vertebral anomaly with/without associated rib anomaly ^a (M)	0	0	2 (1.3%)/ 2 (11%)	1 (1.2%)/ 1 (9.1%)
Angulated hyoid wings (V)	4 (2.7%)/ 3 (16%)	1 (0.6%)/ 1 (5.0%)	5 (3.2%)/ 4 (22%)	10 (12%)/ 4 (36%)
Incomplete/unossified hyoid wings (V)	0	0	1 (0.6%)/ 1 (5.6%)	1 (1.2%)/ 1 (9.1%)
26 presacral vertebrae (V)	18 (12%)/ 7 (37%)	14 (8.6%)/ 8 (40%)	18 (12%)/ 7 (39%)	23 (27%)/ 7 (64%)
<16 caudal vertebrae ossified (V)	2 (1.4%)/ 2 (11%)	6 (3.7%)/ 3 (15%)	7 (4.5%)/ 4 (22%)	3 (3.6%)/ 3 (27%)
5 th /6 th sternebra(e), incomplete ossification (V)	39 (27%)/ 15 (79%)	51 (31%)/ 18 (90%)	58 (37%)/ 16 (89%)	17 (20%)/ 9 (82%)
Sternebrae, extra ossification sites (V)	0	1 (0.6%)/ 1 (5%)	1 (0.6%)/ 1 (5.6%)	0
5 th Sternebra, unossified (V)	15 (10%)/ 9 (47%)	27 (17%)/ 12 (60%)	34 (22%)/ 15 (83%)	29 (35%)/ 7 (64%)
Other sternebra(e), incomplete ossification (V)	1 (0.7%)/ (5.3%)	1 (0.6%)/ 1 (5.0%)	2 (1.3%)/ 1 (5.6%)	2 (2.4%)/ 2 (18%)
6 th Sternebra, unossified (V)	9 (6.1%)/ 3 (16%)	8 (4.9%)/ 6 (30%)	6 (3.8%)/ 4 (22%)	10 (12%)/ 5 (45%)
Other sternebra(e), bipartite (V)	0	0	0	1 (1.2%)/ 1 (9.1%)
5 th /6 th Sternebra(e), bipartite (V)	0	1 (0.6%)/ 1 (5.0%)	1 (0.6%)/ 1 (5.6%)	0
13 th Full ribs (V)	57 (39%)/ 16 (84%)	47 (29%)/ 15 (75%)	51 (33%)/ 12 (67%)	45 (54%)/ 11 (100%)
13 th Rudimentary rib(s) (V)	29 (20)/ 15 (79)	35 (21%)/ 15 (75%)	21 (13%)/ 12 (67%)	11 (13%)/ 7 (64%)
Incomplete ossification of ribs (V)	0	0	1 (0.6%)/ 1 (5.6%)	1 (1.2%)/ 1 (9.1%)
Unossified talus(I), (V)	0	1 (0.6%)/ 1 (5.0%)	1 (0.6%)/ 1 (5.6%)	1 (1.2%)/ 1 (9.1%)

a. No further description of vertebral anomaly with/without associated rib anomaly was

given.

In a Segment II teratology study, pregnant female rabbits received iloprost clathrate by oral gavage at doses of 0, 10, 20, and 40 mg/kg/day from days 7 to 20 of gestation. No teratogenic effects were observed with iloprost clathrate at oral doses ≤ 40 mg/kg/day. Maternal mortality occurred at 40 mg/kg/day. Aborted fetuses were observed at oral doses ≥ 20 mg/kg/day. Three dams at 40 mg/kg/day had no viable fetuses. Total resorptions, consisting primarily of early resorptions, were increased to 30.5% for the 40 mg/kg/day group as compared to 6.9% for the control group. Post-implantation loss (i.e., total resorptions) for the 40 mg/kg/day group were increased to 30.5% as compared to 6.9% for the control group. The percent live fetuses/dam for the 40 mg/kg/day was decreased to 69.5% as compared to 93.1% for the control group. There were no treatment-related external, visceral, or skeletal malformations. The incidence of vertebral anomaly with/without associated rib anomaly were increased for the 20 and 40 mg/kg/day groups; although, there was no dose response relationship. The incidence of variations of major vessels (i.e., left carotid arises from innominate) and small gall bladder was increased for the 40 mg/kg/day group. The incidence of the skeletal variations, angulated hyoid wings, incomplete/unossified hyoid wings, incomplete ossification of other sternebra(e), and incomplete ossification of ribs, were increased for the 20 and 40 mg/kg/day groups. The incidence of unossified 5th sternebra and unossified talus(I) were increased for all iloprost clathrate treatment groups. The incidence of bipartite other sternebra(e) were increased in the 40 mg/kg/day group.

SUMMARY AND EVALUATION:

[] In the present amendment, the sponsor has submitted reproductive toxicology studies, conducted using the oral route of administration, consisting of toxicokinetic studies in pregnant female rats and rabbits, a combined Segment I fertility and performance study and Segment II teratology study in female rats, and a Segment II teratology study in pregnant female rabbits.

In a toxicokinetic study with pregnant female Sprague-Dawley rats, iloprost clathrate was administered by oral gavage at doses of 5, 50, 125, 250, and 500 mg/kg/day. Iloprost clathrate was administered from 14 days prior to mating, through the mating period, and through to day 16 of gestation. Plasma AUC values for iloprost on days 6 at doses of 5, 50, 125, 250, and 500 mg/kg/day were similar to values observed on day 16. AUC values for iloprost were generally proportional to dose from 5 to 125 mg/kg/day; however, at doses ≥ 250 mg/kg/day, deviation from linearity occurred. Plasma clearance values for iloprost at all doses (1217-2988 mL/min/kg) significantly exceeded renal or hepatic plasma flow (21.3 and 31.9 mL/min/kg, respectively) (Pharmaceutical Research 10:1093-1095, 1993) suggesting a rapid metabolic clearance of iloprost. In a toxicokinetic study with pregnant female rabbits, iloprost clathrate was administered by oral gavage at doses of 10, 40, 80, and 120 mg/kg/day from days 6 through 18 of gestation. On days 6 and 16, increases in plasma AUC values for iloprost were proportional to dose at ≥ 40 mg/kg/day. Clearance values for plasma iloprost decreased with increasing dose, particularly on day

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increased clearance observed at 10 mg/kg/day was most likely responsible for the lower than expected plasma AUC value at this dose. Clearance values (451-1141 mL/min/kg) significantly exceeded liver plasma flow (47.2 mL/min/kg) or renal plasma flow (21.3 mL/min/kg) (Pharmaceutical Research 10:1093-1095, 1993) suggesting a rapid metabolic clearance of iloprost.

In combined Segment I/Segment II studies, the effects of iloprost clathrate were assessed on fertility and reproductive performance in F₀ female rats, on implantation (uterine evaluation on day 13 of gestation), on morphological development of the conceptus (uterine evaluation on day 20 of gestation), on natural delivery and the lactation phase of F₀ females, and on viability and growth of F₁ offspring during the weaning phase. Female rats were treated with iloprost clathrate at doses of 0, 5, 50, and 250 mg/kg/day. Iloprost clathrate was administered daily for 14 days prior to mating and through day 13 of gestation, day 20 of gestation, or day 21 of lactation. For female rats treated through day 13 of gestation, iloprost clathrate at doses \leq 250 mg/kg/day had no effect on fertility and reproductive performance. Iloprost clathrate at doses \leq 250 mg/kg/day had no effect on implantation. For female rats treated through day 20 of gestation, iloprost clathrate at doses \leq 250 mg/kg/day was not teratogenic. Maternal toxicity was evident at doses of 50 and 250 mg/kg/day. One female dam at 250 mg/kg/day died during parturition. Two dam at 50 mg/kg/day died during lactation. One dam at 250 mg/kg/day was sacrificed on day 9 of lactation following death of all fetuses in her litter. Live births/dam were decreased at 250 mg/kg/day. Post-implantation loss at 250 mg/kg/day was increased to 15% as compared to 3.9% for the control. For F₀ dams allowed to deliver their F₁ offspring, the duration of gestation for dams at 250 mg/kg/day was increased to 22.6 days as compared to 21.9 days for the control. The numbers of F₀ dams with stillborn pups was increased at doses of 50 and 250 mg/kg/day. The number of stillborn F₁ pups was increased at doses of 50 and 250 mg/kg/day. The live birth index at a dose of 250 mg/kg/day was decreased to 92% as compared to 100% for the control. The number of F₁ pup dying, killed, missing, and/or cannibalized from days 0-4 was increased at doses of 50 and 250 mg/kg/day. The pup viability index on day 4 at a dose of 250 mg/kg/day was reduced to 90% as compared to 98% for the control.

In a Segment II teratology study, pregnant female rabbits received iloprost clathrate by oral gavage at doses of 0, 10, 20, and 40 mg/kg/day from days 7 to 20 of gestation. No teratogenic effects were observed with iloprost clathrate at oral doses \leq 40 mg/kg/day. Maternal mortality occurred at 40 mg/kg/day. Aborted fetuses were observed at oral doses \geq 20 mg/kg/day. Three dams at 40 mg/kg/day had no viable fetuses. Total resorptions, consisting primarily of early resorptions, were increased to 30.5% for the 40 mg/kg/day group as compared to 6.9% for the control group. Post-implantation loss (i.e., total resorptions) for the 40 mg/kg/day group were increased to 30.5% as compared to 6.9% for the control group. The percent live fetuses/dam for the 40 mg/kg/day was decreased to 69.5% as compared to 93.1% for the control group. There were no treatment-related external, visceral, or skeletal malformations.

τ, the sponsor submitted reproductive toxicology studies in pregnant female Wistar rats consisting of a Segment II teratology study and a Segment III perinatal and postnatal development study in which malformations consisting of shortened and/or crooked digits were observed following administration of iloprost β-cyclodextrin clathrate by continuous intravenous infusion at doses ≤1 mg/kg/day. Digit defects were attributed to the pharmacological action of the drug, which produced a reduction of uteroplacental blood flow and resulted in hypoxia in the affected structures (Toxicology Letters 78: 223-224, 1995). This suggests that observed digit defects have a direct relationship to drug treatment. Further, the digit defects observed in fetuses from pregnant female Wistar rats treated with iloprost clathrate, while related to the to the pharmacological mechanism of drug action, suggest that the drug may pose a significant risk to the human fetus in utero. In the present amendment, the sponsor has submitted a Segment II study in pregnant female Sprague-Dawley rats, in which no teratogenic effects were observed at doses ≤250 mg/kg/day. Given the use of different rat strains in these studies, the findings of the oral Segment II study in pregnant female Sprague-Dawley rats do not negate the findings of the continuous intravenous infusion Segment II and Segment III studies in pregnant female Wistar rats.

RECOMMENDATION:

Information should be communicated to the sponsor as described below.

1. In reproductive toxicology studies with pregnant female Wistar rats consisting of a Segment II teratology study and a Segment III perinatal and postnatal development study, malformations consisting of shortened and/or crooked digits were observed following administration of iloprost β-cyclodextrin clathrate by continuous intravenous infusion at doses ≤1 mg/kg/day. In the present amendment, the sponsor has submitted a Segment II study in pregnant female Sprague-Dawley rats, in which no teratogenic effects were observed at doses ≤250 mg/kg/day. Given the use of different rat strains in these studies, the findings of the oral Segment II study in pregnant female Sprague-Dawley rats cannot negate the findings of the continuous intravenous infusion Segment II and Segment III studies in pregnant female Wistar rats.

2. With regard to skeletal malformations observed in the Segment II study with rabbits (— Report No. 97005), could the sponsor describe in greater detail the vertebral anomaly with/without associated rib anomaly observed in fetuses from the mid and high dose groups.

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Timothy W. Robison, Ph.D.

Date

cc:

Orig IND —

HFD-

HFD- /CSO

HFD- Dr. Choudary

HFD- Dr. Robison

R/D Init.: J. Choudary 6/14/99

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IND

PHARMACOLOGIST'S REVIEW OF IND

Sponsor (or agent):

Manufacturer for drug substance: Same

Reviewer Name: Timothy W. Robison, Ph.D.

Division Name:

HFD#

IND number:

Serial number/date/type of submission:

Date of HFD- Receipt:

Review Completion Date:

Information to sponsor: Yes () No (X)

Drug: Iloprost β -cyclodextrin Clathrate

Relevant INDs/NDAs/DMFs:

Drug Class: Prostaglandin analogue

Indication:

Route of administration: Oral

Previous clinical experience: Iloprost β -cyclodextrin

Disclaimer: The sponsor's material has been incorporated in parts of this review.

Introduction and drug history:

the sponsor provided a study report of a Segment II teratology study in rabbits. Pregnant rabbits received iloprost clathrate by oral gavage at doses of 0, 10, 20, and 40 mg/kg/day from days 7 to 20. Vertebral anomaly with or without associated rib anomaly, was reported for 2 of 156 (1.3%) fetuses at 20 mg/kg/day and 1 of 84 (1.2%) fetuses at 40 mg/kg/day. In the present amendment, the sponsor has responded to a Division question regarding this observed finding. The Division question is listed below, followed by the sponsor's response within quotations, and an evaluation of this response.

Studies reviewed within this submission:

1. Correspondence.

CORRESPONDENCE:

1. With regard to the Segment II teratology study entitled "Report 97005: Oral development toxicity study of iloprost clathrate in rabbits", please describe in greater detail, the "vertebral anomaly with and without associated rib anomaly", observed in fetuses from the mid and high dose groups.

Sponsor's Response:

Response:

In this study (Report 97005), there were three fetuses that were reported with this condition; two Group 3 fetuses (20/mg/kg/day; fetus number 5 from litter E54307 and fetus number 2 from litter E54328) and one Group 4 fetus (40 mg/kg/day; fetus number 9 from litter E54292). The expanded description of the malformations associated with these fetuses can be found in the table below as well as in Appendix 10 of the Final Report, on pages 186, 189, and 194, respectively.

GROUP	FETUS #	Litter	EXPANDED FINDING	Page
20 mg/kg/day	5	E54307	Vertebral Anomaly with/without associated rib anomaly 14 thoracic vertebral arches on left side 13 thoracic vertebral arches on right side 13 thoracic centra present T-11 through L-2 centra misaligned 6 right lumbar vertebral arches present 5 left lumbar vertebral arches present L-1 hemicentrum right	186
	2	E54328	Vertebral Anomaly with/without associated rib anomaly 13 thoracic arches on both sides 7 th right thoracic arch. Small ossification site 13 thoracic centra present 13 ribs on left side 12 ribs on right side 6 th right rib forked proximal T-8 through T-11 centra misaligned T-9 centrum fused to T-10 centrum T-10 centrum also bipartite	189
40 mg/kg/day	9	E54292	Vertebral Anomaly with/without associated rib anomaly 11 th left thoracic arch small 11 th through 13 th thoracic arches misaligned T-11 through T-13 centra misaligned T-12 and T-13 centra fused	194

Evaluation: The term "vertebral anomaly with and without associated rib anomaly" was used in a general manner. This term encompassed findings for 2 fetuses from the mid dose and 1 fetus from the high dose that were different for each F₁ offspring. The sponsor's response appears adequate.

OVERALL SUMMARY AND EVALUATION

⌊

In the present amendment, the sponsor has responded to a

IND

Page 3

Division question regarding a Segment II teratology study in rabbits provided in Amendment

In a Segment II teratology study, pregnant rabbits received iloprost clathrate by oral gavage at doses of 0, 10, 20, and 40 mg/kg/day from days 7 to 20. Vertebral anomaly with or without associated rib anomaly, was observed for 2 of 156 (1.3%) fetuses at 20 mg/kg/day and 1 of 84 (1.2%) fetuses at 40 mg/kg/day. The term "vertebral anomaly with and without associated rib anomaly" was used in a general manner. This term encompassed findings for 2 fetuses from the mid dose and 1 fetus from the high dose that were different for each F₁ offspring. The incidence of these findings appeared to have no treatment relationship.

RECOMMENDATIONS: None.

/S/

Timothy W. Robison, Ph.D.
Pharmacologist,

Date

Comments:

/S/

Jasti B. Choudary, B.V.Sc., Ph.D.
Supervisory Pharmacologist,

Date

cc:

Orig IND

HFD-

HFD- /CSO

HFD- /Dr. Choudary

HFD- /Dr. Robison

R/D Init. J. Choudary 1-15-01

/s/

Timothy Robison
1/22/01 08:46:04 AM
PHARMACOLOGIST

Jasti Choudary
1/22/01 10:58:58 AM
PHARMACOLOGIST

PHARMACOLOGY / TOXICOLOGY REVIEW COVER SHEET

Application Information:

IND Number: _____
Review Number: Original review (or Review #1)
Sequence No./Date/Submission Type: 000/ January 30, 2002/ Original submission, Received on 2/1/02
Information to the Sponsor: None
Sponsor/or Agency: _____

Review Information:

Reviewer Name: Luqi Pei, Ph.D.
Division Name: Pulmonary and Allergy Drug Products
Division Code: HFD-570
Review Completion Date: February 26, 2002

Drug:

Trade Name: None
Generic Name: Iloprost Clathrate
Code Name: None
Chemical Name: _____

CAS Registry Number: N/A
Molecular Formula/Weight: $C_{22}H_{32}O_4 \cdot 2C_{42}H_{70}O_{35} \cdot XH_2O$ /2630.5 (Iloprost $C_{22}H_{32}O_4$, 360.49)
Structure: Omitted
Manufacturer for the Drug: _____
Drug Class: Prostaglandins, _____

Route of Administration:

Relevant INDs/NDAs: IND _____

Clinical Formulation:

Not submitted. But the following information is present in Dr. T. Ahmad's review dated (IND _____)

[

]

Proposed Clinical Use:

[

Previous Human Experience:

[

]

Introduction and Drug History:

[

]

The submission contained no nonclinical data, [

] The current review conducted the safety evaluation based on nonclinical information available in IND

Studies Reviewed within This Submission: None.

Studies Not Reviewed within This Submission: None.

DETAILED CONCLUSION AND RECOMMENDATIONS:

Conclusion:

[

]

General Toxicology Issues:

There are no general toxicity issues in this application.

[

]

The following is a summary of the information that is most relevant to the current application. Oral toxicity of iloprost has been evaluated in rats, dogs and monkeys. The treatment duration was up to 6 months in rats (dietary), 1 year in dogs (capsule), and 2 weeks in monkeys, respectively.

In a 6-month oral dietary study, rats were fed diet containing 1%, 3% and 10% (W/W) of ground iloprost clathrate retard. The estimated dose levels were 0.50 – 0.59, 1.53 – 1.73, and 4.97 – 5.68 mg/kg/day. The high dose group showed increases in urinary sodium, calcium and chloride excretion; and enlargement of secretory end-pieces of submandibular salivary gland. Plasma iloprost levels (day 90) were 2.7 and 3.7 ng/ml in males and females, respectively.

In a one-year oral (capsule) study in dogs, iloprost doses were 50 and 150 µg/kg/day (given in equally divided doses, bid). One high dose female (of 4 per group) died of jejuno-ileal-cecal torsion of the gut (volvulus). The death was considered treatment-related. Other findings in the high dose group were increases in serum glucose levels (16 – 22%) in both sexes, a decrease in serum protein (10%) calcium levels (13%) in females. The dose of 50 µg/kg/day was considered the NOAEL. Plasma iloprost levels and AUC 0-24 hr were 0.1 – 0.7 ng/ml and 2.2 – 3.7 ng.hr/ml, respectively.

In a two-week oral study, monkeys were given oral gavage of iloprost at doses of 160 and 800 µg/kg/day (given in 8 equal doses with a dosing interval of one hr between two consecutive doses, 5 day a week). The drug was well tolerated. Observed abnormalities were apathy, abnormal posture and an increase in serum total beta-globulin (31%). However, in another oral toxicity studies, all the tested doses (20, 200, and 2000/1000 µg/kg/day) was lethal. Hypotension was considered the cause of death.

Toxicity of iloprost was also evaluated in mice, rats, guinea pigs, rabbits, dogs and monkeys by other route of administration (oral, intravenous, or subcutaneous). The treatment duration ranged from acute to six months. The data for these studies are not summarized here.

The target of organs of iloprost toxicity include the gastrointestinal tract, liver, thymus and adrenals

Carcinogenicity Issues:

The evaluation of carcinogenic potential of iloprost is in progress. ☐

☐ The CDER's Carcinogenicity Assessment Committee has reviewed protocols for the carcinogenicity assays. The committee accepted the mouse protocol during the meeting on May 13, 1997, and the rat protocol on June 8, 1998 (see Dr. Robinson's review). The results of these studies are unavailable at present time.

Genotoxicity:

Iloprost is non-genotoxic based on the results from the following four assays: bacterial gene mutation assay, Chinese hamster lung cell (V79/HGPRT) forward gene mutation assay, chromosomal aberration assay in human lymphocytes, and mouse micronucleus assay (See Dr. T Ahmad review dated May 28, 1997).

Pregnancy and Fertility Issues:

Iloprost is neither teratogenic, nor does it affect fertility, delivery and lactation. Effects of iloprost on pregnancy and fertility were evaluated in rats and rabbits (See Dr. T. Robinson's reviews dated June 16, 1999 and January 22, 2001). Iloprost doses were 5, 50, and 250 mg/kg/day rats and 10, 20 and 40 mg/kg/day in rabbits, respectively. Maternal toxicity was observed in the highest doses in both species. The highest dose was considered the maximum tolerated dose (MTD) in either species. Iloprost is non-teratogenic at the MTD. Thus, iloprost does not affect fertility, implantation and delivery in rats and rabbits.

Recommendation:

Clinically, the sponsor states that humans tolerated well at iloprost doses up to 150 µg, bid for six to 12 months (6 µg/kg/day). Preclinically, a sufficient margin of safety exists between the one-year oral NOAEL value of 50 µg/kg/day in dogs and the proposed high dose in humans. The dog is the most sensitive animal species. The trial does not have any significant safety concerns and should be allowed to proceed.

Luqi Pei, Ph.D.
Pharmacologist

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

/s/

Luqi Pei
2/26/02 02:24:41 PM
PHARMACOLOGIST

Joseph Sun
2/26/02 03:54:45 PM
PHARMACOLOGIST
I concur.

IND ...

**PHARMACOLOGIST'S REVIEW OF IND
(Amendment
Amendment**

Sponsor & Address: ☐

☐

Reviewer: Timothy W. Robison, Ph.D.
Pharmacologist,

Date of Review: July 14, 1998

Date of Submission: Amendment
Amendment

Date of HFD- Receipt: Amendment
Amendment ;

Drug: Iloprost Clathrate ☐

☐

Category: ☐

☐

Submission Contents:

1. Pharmacology.
2. Investigation of the Biotransformation of Iloprost and Its Diastereoisomers in Dog During a Six-Hour Intravenous Infusion of Each Compound.
3. Pharmacokinetics of Iloprost and Its Diastereoisomers in Rats After Intravenous Infusion of 0.3 µg/kg/min and of 3 µg/kg/min ZK 91404 Over 30 Min.
4. Acute Toxicity of Iloprost (diastereoisomer-mixture), and Its Diastereoisomers, 4S-form and 4R-form, in Male and Female Rats After a Single Intragastric or Intravenous Application.
5. Iloprost Clathrate (ZK 96944), ZK 153485 and ZK 155534 Comparative Systemic Tolerance Study in Rats After Daily Intragastric Administration Over 28 to 38 Days.

PHARMACOLOGY:

Effects of Iloprost and the E(4S) and E(4R) Diastereoisomers of Iloprost, ZK 95302 and ZK 91404, on ADP and Collagen-Induced Aggregation of Human and Rat Platelets (Report No. AH35).

The effects of iloprost and its diastereoisomers, E(4S) and E(4R), on human and rat platelet aggregation were evaluated. Platelets were obtained from healthy human volunteers or male Wistar rats. Prior to induction of aggregation with ADP, platelets were pre-incubated with the appropriate test article for 1, 5, or 15 min. However, with collagen stimulation, platelets were incubated with the test article for only 1 min. Human platelets were incubated with iloprost at concentrations from 0.08 to 6 nM, E-4R at concentrations of 0.6-60 nM, or E-4S at concentrations of 0.06 to 4 nM. Rat platelets were incubated with iloprost at concentrations of 2 to 400 nM, E-4R at concentrations of 10 to 2000 nM, or E-4S at concentrations of 1 to 200 nM. All three compounds produced concentration-dependent inhibition of human or rat platelet aggregation induced by either ADP or collagen. E-4S was more potent than E-4R with regard to inhibition of human or rat platelet aggregation induced by ADP or collagen. Longer incubation of iloprost, E-4R, or E-4S with human platelets prior to stimulation with ADP led to decreasing IC₅₀ values; however, this phenomenon was not observed with rat platelets. Human platelet aggregation induced by ADP or collagen was more sensitive to inhibitory actions of iloprost and its diastereoisomers than rat platelets.

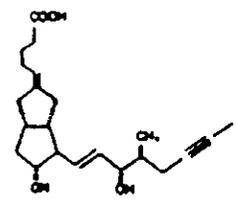
IC₅₀ (nM) values at three different incubation times for iloprost and its diastereoisomers, E-4R and E-4S, with human and rat platelet aggregation induced by ADP.

Incubation time (min)	Human Platelets			Rat Platelets		
	Iloprost	E-4R	E-4S	Iloprost	E-4R	E-4S
1	1.07	4.55	0.43	31.62	225.5	17.25
5	0.57	2.94	0.28	61.83	349.27	26.63
15	0.44	1.87	0.15	87.20	536.66	41.30

IC₅₀ (nM) values for iloprost and its diastereoisomers, E-4R and E-4S, with human and rat platelet aggregation induced by Collagen.

Compound	Human Platelets	Rat Platelets
Iloprost	1.82	13.2
E-4R	10.37	80.53
E-4S	0.97	6.5

Structures of Iloprost and its 4S- and 4R-diastereoisomers:

Compound(s) Studied
Chemical Name 5-[(E)-(1S, 5S, 6R, 7R)-7-hydroxy-6-[(E)-(3S, 4R)-3-hydroxy-4-methyl-1-octen-8-ynyl]-bicyclo[3.3.0]oct-3-ylidene]-pentanoic acid
Compound Name E-4RS Iloprost
Compound No ZK-36374
 <p>The structure shows a bicyclo[3.3.0]octane core with a carboxylic acid group at position 5 and a bicyclic side chain at position 6. The side chain includes a double bond with E-configuration, a hydroxyl group, and a methyl group, all on the same carbon. The terminal part of the side chain is an 1-octen-8-ynyl group.</p>
ZK 36 374 Structural Formula
Chemical Name
 <p>A thick black diagonal line redacts the chemical structure in this section.</p>
Structural Formula
Chemical Name
 <p>A thick black diagonal line redacts the chemical structure in this section.</p>
Structural Formula

Interaction Between Platelet Receptors and Iloprost Isomers (Biochimica et Biophysica Acta 942: 220-226, 1988).

Biological activity and platelet receptor binding characteristics were compared between the two diastereoisomers of iloprost. The authors used a different nomenclature for numbering the compound; the 4(S) isomer is equivalent to the 16(S) isomer and the 4(R) isomer is equivalent to the 16(R) isomer. Diastereoisomers of a mixture of iloprost and ³H-iloprost were separated by reverse phase HPLC. Platelet rich plasma was prepared from venous blood. Membrane fractions were prepared from isolated platelets for determination of receptor binding characteristics. Inhibition of collagen-induced platelet aggregation was significantly greater for the 16(S) isomer with an IC₅₀ value at 3.5 nM as compared to 65 nM for the 16(R) isomer. The 16(S) isomer had a much higher affinity for the prostacyclin receptor as compared to the 16(R) isomer. Binding capacity was relatively similar between the 16(S) and 16(R) isomers. Association rates were significantly different between the 16(S) and 16(R) isomers. The IC₅₀ values for inhibition of collagen-induced platelet aggregation as well as dissociation constants and association rates for the racemic mixture and the 16(S) isomer agreed closely. Thus, the potency of iloprost is attributable to the 16(S) isomer, which constitutes approximately 40% of the molarity. The 16(R) isomer does not appear to interfere with the binding of the 16(S) isomer. The decreased binding affinity of the 16(R) isomer results from hindered accessibility, rather than a fast dissociation of the ligand from its receptor. Limited flexibility resulting from the 16(S) methyl group facilitates binding of the molecule and permits close interaction between the active functional groups and the binding domain of the receptor. In contrast, the 16(R) methyl group hinders fitting of the molecule to the receptor due to an abnormal orientation, thus the association rate is reduced and the binding affinity is reduced.

Biological activity and platelet receptor binding characteristics for the two diastereoisomers of iloprost. The authors used a different nomenclature for numbering the compound; the 4(S) isomer is equivalent to the 16(S) isomer and the 4(R) isomer is equivalent to the 16(R) isomer (Biochimica et Biophysica Acta 942: 220-226, 1988).

Compound	Iloprost 16(R,S)	16(S) Isomer	16(R) Isomer
IC ₅₀ (nM) for inhibition of collagen-induced platelet aggregation	5.5	3.5	65
Dissociation constant, K _d , nM	16.5	13.4	228
Binding Capacity, fmol/mg membrane	743	665	425
Association rate, sec ⁻¹	0.024	0.036	0.001

Iloprost, ZK 91403, ZK 91404, ZK 95302, ZK 115891. Evaluation of the report entitled "Vasodilatory action, hypotensive action and inhibition of platelet aggregation of iloprost and its isomers (Report No. AF18).

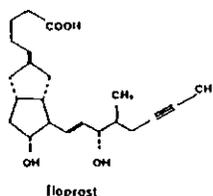
Pharmacological effects of iloprost, its E(4S) and E(4R) diastereoisomers (ZK 95302 and ZK 91404, respectively) and the 4S and 4R diastereoisomers of the Z-configuration of iloprost (ZK 115891 and ZK 91403) were determined with regard to platelet aggregation *in vitro*, vasodilatory activity on isolated vascular tissue (rabbit mesenteric artery), and blood pressure in the anesthetized rat. Iloprost has a methyl group at C-4 in its structure and, therefore, two isomers, 4S and 4R. Further, there are cis-trans isomers, E and Z, of Iloprost. Altogether, iloprost has 4 isomers as follows: E-4S, E-4R, Z-4S, and Z-4R (see structures below). For studies with isolated vascular tissue, maximal contraction was produced by treatment with 10^{-5} M L-phenylephrine prior to addition of iloprost or its isomers. For studies with anesthetized rats, changes of blood pressure in the aorta as well as changes in systolic and diastolic pressure were monitored in response to treatment with iloprost and its E(4S) and E(4R) diastereoisomers at concentrations of 0.1, 0.3, 1, 3, and 10 $\mu\text{g}/\text{kg}$ or the 4S and 4R diastereoisomers of the Z-configuration of iloprost at concentrations of 3, 10, 30, and 100 $\mu\text{g}/\text{kg}$ (Note: the sponsor stated that there were significant omissions and errors in these studies with anesthetized rats). There were significant typographical errors in this section of the report. It was not clear, if each rat received all doses or whether one rat was used for each dose. Platelet aggregation was induced by addition of collagen, ADP, or thrombin and the anti-aggregatory actions of iloprost and its E(4S) and E(4R) diastereoisomers or the 4S and 4R diastereoisomers of the Z-configuration of iloprost were examined. Iloprost and its isomers, E-4S, E-4R, and Z-4S, produced a dose-dependent reduction of mean blood pressure. The hypotensive action of Iloprost and the E-4S isomer was greater for diastolic pressure than systolic pressure. Iloprost and its isomers produced a dose-dependent inhibition of agonist-induced platelet aggregation. The 4S and 4R diastereoisomers of the Z-configuration of iloprost were significantly less potent than iloprost with regard to inhibition of phenylephrine-induced contraction of mesenteric artery, vasodilation of mesenteric artery, and inhibition of agonist-induced platelet aggregation. The E-4S isomer is more potent than iloprost with regard to inhibition of phenylephrine-induced contraction of mesenteric artery and agonist-induced platelet aggregation. Further, the E-4S isomer has a more potent vasodilatory effect on mesenteric artery than iloprost. The E-4R isomer was less potent than either iloprost or the E-4S isomer. The order of potency of iloprost and its isomers was as follows: E-4S > Iloprost > E-4R > Z-4S > Z-4R.

Effects of Iloprost and its isomers on phenylephrine-induced contraction of mesenteric artery, mesenteric artery vasodilation, and agonist-induced platelet aggregation.

Preparation	Iloprost	E-4S	E-4R	Z-4S	Z-4R
ED ₅₀ (-log M) on phenylephrine-induced contraction of mesenteric artery	6.74	7.21	6.13	5.42	4.32
Vasodilatory effects, µg/kg					
-ED ₂₀ (A)	0.80	0.4	30	30	-
-ED ₁₀ (B)	0.25	0.15	1	10	-
Anti-aggregatory activity, µM ^C					
-collagen	0.8	0.5	2.1	26.3	0.9
-ADP	1.7	1.1	7.8	62.1	1.6
-thrombin	0.4	0.3	1.5	14.4	0.8

- A. dosage required to lower mean blood pressure by 20 mm Hg.
- B. dosage required to lower mean blood pressure by 10 mm Hg.
- C. This was listed as nM in the table, but the sponsor stated that the actual units were µM.

Structures of Iloprost and its isomers.



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Fig. 1 Structures of Iloprost and its isomer Iloprost

Iloprost chemical name: (E)-3aS,4R,5R,6aS)-hexahydro-6-hydroxy-4-[(E)-3S,4RS)-3-hydroxy-4-methyl-1-octen-6-ynyl]-Δ^{2(11H)}-pentalenevateric acid

Effects of Intravenous Infusion of Iloprost and the E(4S) and E(4R) Diastereoisomers of Iloprost, ZK95302, and ZK91404, on Blood Pressure and Heart Rate in the Anesthetized Rat (Report No. AG23).

The effects of iloprost and its diastereoisomers, ZK 91404 (E-4R) and ZK 95302 (E-4S), on blood pressure was examined in the anesthetized rats. Arterial blood pressure and heart rate were monitored following administration of the test article by continuous intravenous infusion over a period of 30 min. Iloprost and the E-4S isomer were examined at doses of 0.03, 0.10, 0.30, or 1.00 µg/0.1 mL/kg/min. The E-4R isomer was examined at doses of 0.30, 1, 3, or 10 µg/0.1 mL/kg/min. Each rat received only one compound at one dose level. Control animals received the vehicle, 0.9% NaCl solution containing 0.3% ethanol, at 0.1 mL/kg/min. In parallel experiments to determine plasma test article concentrations, four groups of four animals received either iloprost at 0.30 µg/kg/min, E-4R at 0.30 or 3.00 µg/kg/min, or E-4S at 0.30 µg/kg/min, respectively. These results are reported in ADME section below. All three compounds induced a dose-dependent reduction in arterial pressure accompanied by a reflex increase in heart rate. The order of potency was as follows: E-4S > iloprost > E-4R.

ED₂₀ values for Iloprost and its isomers, E-4R and E-4S, for lowering arterial blood pressure by 20 mm Hg.

Compound	Raw Curves, µg/kg/min	Fitted Curves, µg/kg/min
Iloprost	0.33	0.28
ZK 91404 (E-4R)	1.52	1.44
ZK 95302 (E-4S)	0.14	0.14

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION:

Absorption

Investigation of the Biotransformation of Iloprost and Its Diastereoisomers in Dog During a Six-Hour Intravenous Infusion of Each Compound (Report No. 8994).

Methods: The stereoselective pharmacokinetics of iloprost diastereoisomers (E-4RS, E-4R, or E-4S iloprost) in dogs were followed in 3 mongrel female dogs administered the test material by intravenous infusion at a dose of 100 ng/kg/min. There was a minimum 1 week washout period between treatments with E-4RS, E-4R, or E-4S iloprost. Blood for determination of plasma E-4R and/or E-4S levels was collected at 0 (pre-dose), 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, and 6 hr during infusion and 0.5 hr post-infusion. The total concentration of iloprost (sum of diastereoisomers) in each plasma sample was determined using a GC/MS method, which was highly accurate for iloprost levels but nonspecific for diastereoisomer separation. Determination of the E-4R and E-4S ratios in plasma was performed using an HPLC-fluorescence iloprost diastereoisomer plasma assay that could quantify levels down to ~ 0.01 µg/mL using a

1.0 mL aliquot of plasma. All plasma samples from dogs receiving E-4RS iloprost were assayed by both the HPLC and GC/MS procedures. For dogs that received either E-4R or E-4S iloprost by infusion, plasma samples collected at 2, 4, and 6 hr were also analyzed using the HPLC assay.

Results: Iloprost diastereoisomer ratios from 0 to 6.5 hr after the start of the infusion with E-4RS iloprost ranged from 0.84 to 1.16 (%E-4R/%E-4S). This data illustrates that the diastereoisomer ratio did not change versus infusion time. The mean E-4R to E-4S iloprost ratio found in vivo in all dogs was 1.03, which closely matched the starting infusion solution diastereoisomer ratio of 1.1. No interconversion of iloprost diastereoisomers was found. During the 6 hr infusion of E-4R iloprost, no E-4S iloprost was detected. Conversely, during the 6 hr infusion of E-4S iloprost, no E-4R iloprost was detected. Following infusion of either E-4RS iloprost, E-4S iloprost, or E-4R iloprost, AUC values were 21.7, 10.6, and 25.3 ng*hr/mL, respectively. Following infusion of E-4S iloprost, the AUC value was significantly lower as compared to the AUC value following infusion of E-4R iloprost. Potentially, the clearance of E-4S iloprost may be more rapid in the absence of E-4R iloprost.

Pharmacokinetics of Iloprost and Its Diastereoisomers in Rats After Intravenous Infusion of 0.3 µg/kg/min and of 3 µg/kg/min ZK 91404 Over 30 Min (Report No. AG42).

Methods: In parallel with experiments that determined the effects of iloprost and its diastereoisomers on blood pressure in anesthetized rats, plasma test article concentrations were determined in groups of rats that received either iloprost at 0.30 µg/kg/min, E-4R at 0.30 or 3.00 µg/kg/min, or E-4S at 0.30 µg/kg/min, respectively. Each test article was administered by continuous intravenous infusion for a 30 min period. Each group consisted on 4 male rats. Blood samples for determination of plasma test article concentrations were determined at 0, 15, 30, 35, 40, 45, 60, 90, 120, and 180 min after the start of the infusion. At each time point, 2 to 3 animals were sampled and each individual rat was used for 5 to 6 sampling time points. Plasma levels of iloprost and its diastereoisomers, E-4S and E-4R, were determined by radioimmunoassays.

Results: For E-4R at doses of 0.3 and 3 µg/kg/min, AUC values were proportional to dose. Total clearance for all compounds significantly exceeded hepatic plasma flow (31.9 mL/min/kg), suggestive of a rapid metabolic clearance. The clearance of E-4S was 1.5 times that of E-4R. The terminal half-lives for all compounds were <0.26 hr, further suggestive of rapid elimination. The volume of distribution at steady state exceeded blood volume (0.054 L/kg), suggestive of extensive distribution into tissues. The volume of distribution of E-4S was more than twice that of E-4R. E-4S is more extensively distributed than E-4R; however, it is more rapidly eliminated. Half-lives and mean residence times for the two diastereoisomers were similar.

Compound	Iloprost	E-4R		E-4S
Infusion rate $\mu\text{g}/\text{kg}/\text{min}$	0.3	0.3	3	0.3
C_{ss} , ng/mL	5.5	5.6	39.8	3.7
$T_{1/2} \lambda_1$, hr	0.002	0.004	0.02	0.06
$T_{1/2} \lambda_2$, hr	0.20	0.21	0.21	0.26
k_{el} , hr^{-1}	0.08	0.03	0.007	0.003
AUC, $\text{ng}\cdot\text{hr}/\text{mL}$	3.1	3.1	21.5	2.0
Cl_T , mL/min/kg	49.1	48.7	69.6	75.0
MRT, hr	0.35	0.34	0.35	0.38
Vd_c , L/kg	0.01	0.02	0.17	0.42
Vd_{ss} , L/kg	0.29	0.26	0.40	0.59

$T_{1/2} \lambda_1$ = half-life of distribution phase; $T_{1/2} \lambda_2$ = half-life for elimination; MRT = mean residence time; Vd_c = volume of distribution in the central compartment; and Vd_{ss} = volume of distribution during steady state.

TOXICOLOGY:

Acute Toxicity

Acute Toxicity of Iloprost (diastereoisomer-mixture), and Its Diastereoisomers, 4S-form and 4R-form, in Male and Female Rats After a Single Intragastric or Intravenous Application (Report numbers AL61 and AL26).

Testing Laboratory: Schering AG
Berlin, Germany

Study Started: March 21, 1995 (Intragastric administration)
July 12, 1995 (Intravenous administration)

Study Completed: February 9, 1996 (Intragastric administration)
September 3, 1996 (Intravenous administration)

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Male and female Han: WIST (SPF) rats were used in this study. For studies using the intragastric route of administration, body weight ranges were 92-123 g for male rats and 92-110 g for female rats. For studies using the intravenous route of administration, body weight ranges were 95-128 g for male rats and 84-116 g for female rats.

Drug Batch: For studies using the intragastric route, drug batches were as follows: iloprost clathrate (diastereoisomer mixture), batch number 112960; 4S-form clathrate, batch number FB3384; and 4R-form clathrate, batch number FB3388. For studies using the intravenous route, drug batches were as follows: iloprost (diastereoisomer mixture), batch number GB 1151; 4S-form, batch number FB 3407; and 4R-form, batch number FB 3382

Methods: The acute toxicity of iloprost (diastereoisomer mixture) and its diastereoisomers, 4S- and 4R-forms, were examined in male and female Han: WIST (SPF) rats following administration by the intragastric or intravenous routes. For studies by the intragastric route of administration, iloprost and its diastereoisomers were complexed with clathrate. For studies using the intragastric route, the 4S-form was administered at doses of 500, 1000, or 2000 mg/kg, while iloprost and the 4R-form were administered at doses of 1000, 2000, or 4000 mg/kg. There were 5 rats/sex/group. For studies using the intravenous route, iloprost and its diastereoisomers were administered at doses of 0, 50, 125, or 200 mg/kg. For the 0, 50, and 200 mg/kg groups, there were 5 rats/sex/group; however, for the 125 mg/kg group, there were 10 rats/sex/group. Clinical signs of toxicity were observed continuously immediately prior to and after drug administration, four times on the day of treatment, and daily thereafter. Animals were monitored twice daily for mortality. Observation periods for the intragastric and intravenous toxicity studies were 14 and 23 days, respectively. Following death during the treatment period or at scheduled termination, animals were subjected to a gross examination.

Results: Following intragastric administration, the acute toxicity of the 4S-form was significantly greater than iloprost or the 4R-form. In contrast, there were no significant differences in toxicity between iloprost, the 4-S form, and the 4-R form following intravenous administration. The sponsor provided no explanation for lack of difference in toxicity between the two stereoisomers following intravenous administration as opposed to intragastric administration, where the toxicity of the 4S-form was significantly greater. Apathy was observed in an approximate dose-dependent manner for iloprost and its diastereoisomers following either intragastric or intravenous administration. Observed effects for iloprost and its diastereoisomers following intragastric administration were similar and included disturbances in gait, incomplete eyelid closure, red-colored dacryorrhea, skin reddening, extended abdomen, altered posture, tremors at higher doses, and bluish discoloration of the skin at higher doses. Gross examination following intragastric treatment with iloprost or its diastereoisomers revealed dose-dependent reddening of the stomach mucosa, meteorism of stomach and intestines, and decreased spleen size. For animals that died during the treatment period, death was attributed to circulatory and/or irritative alterations. Observed effects for iloprost and its diastereoisomers following intravenous administration were similar and included disturbances in gait, skin reddening, and eyelid closure at 50 mg/kg. Unconsciousness, lateral position (conscious), disturbances in respiration, vocalization,

and changes in color of the tail leading to necrosis were observed at 125 mg/kg. Body weight gain and food consumption were decreased at 125 mg/kg (principally female rats). Bilateral decreases in the sizes of the testes were observed for iloprost and the 4S-form at 125 mg/kg.

Acute toxicity of iloprost and its diastereoisomers, 4-S and 4-R, following administration by the intragastric or intravenous routes to rats.

Compd.	Route	Dose, mg/kg	rats/sex /group	Maximum nonlethal dose, mg/kg		Minimum lethal dose, mg/kg		LD ₅₀ , mg/kg M + F	Time to death M + F
				Male	Female	Male	Female		
Iloprost	I.G.	1000	5	1000	1000	2000	2000	1864	2-5 days
		2000	5						
		4000	5						
4-S form	I.G.	500	5	1000	500	2000	1000	1073	3-5 days
		1000	5						
		2000	5						
4-R form	I.G.	1000	5	2000	2000	4000	4000	3820	2-3 days
		2000	5						
		4000	5						
Iloprost	I.V.	50	5	50	50	125	125	130	1 day
		125	10						
		200	5						
4-S form	I.V.	50	5	50	50	125	125	128	1-5 days
		125	10						
		200	5						
4-R form	I.V.	50	5	50	50	125	125	126	1 day
		125	10						
		200	5						

The acute toxicity of iloprost and its diastereoisomers were evaluated in rats following intragastric or intravenous administration. Following intragastric administration, the acute toxicity of the 4S-form (LD₅₀ = 1073 mg/kg) was significantly greater than iloprost (LD₅₀ = 1864 mg/kg) or the 4R-form (LD₅₀ = 3820 mg/kg). With intravenous administration, there were no significant differences in toxicity between iloprost (LD₅₀ = 130 mg/kg), the 4-S form (LD₅₀ = 128 mg/kg), and the 4-R form (LD₅₀ = 126 mg/kg). For animals that died during the treatment period, death was attributed to circulatory and/or irritative alterations.

Subacute Toxicity

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Iloprost Clathrate (ZK 96944), ZK 153485 and ZK 155534 Comparative Systemic Tolerance Study in Rats After Daily Intra-gastric Administration Over 28 to 38 Days (Report No. AN54).

Testing Laboratory: Schering AG
Berlin, Germany

Study Started: April 20, 1995

Study Completed: February 6, 1998

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Han: WIST (SPF) rats were used in this study. Body weight ranges on the first day of administration were 171-229 g for male rats and 148-185 g for female rats.

Drug Batch: Iloprost clathrate (diastereoisomer mixture), batch number 112960; 4S-form of Iloprost clathrate, batch number FB3384; and 4R-form of Iloprost clathrate, batch number FB3388.

Methods: Han: WIST (SPF) rats received either iloprost clathrate, the 4(S)-diastereoisomer of iloprost clathrate, or the 4(R)-diastereoisomer of iloprost clathrate by the intra-gastric route of administration at doses of 5, 50, or 500 mg/kg/day for a period of 28 to 38 days. The control group received the vehicle, 0.9% NaCl at pH 8.3. Amendment contained a summary of this study. Amendment contained the actual data for this study. There were 10 rats/sex/group. The dosing volume was 10 mL/kg. Animals were observed for clinical signs of toxicity and mortality three times per day, once prior to dosing, once at 5 to 10 min after dosing, and once in the afternoon. Body weight was measured weekly until day 28 of the study. Food and water consumption were recorded weekly until day 28 of the study. Ophthalmic examinations were performed on days 22-23 for 5 rats/sex/group. Blood for determination of hematological, biochemical, and coagulation parameters was collected on days 24-25 from 4-5 male rats and 5 female rats per group. Urine for analysis was collected from 4-5 male rats and 3-5 female rats per group over an 18-hr period from days 22 to 23. Blood for determination of 4RS-iloprost, 4S-iloprost, and 4R-iloprost was collected from 3 rats/sex/group on day 29, immediately prior to treatment and at 1 hr after dosing, and on day 30, at 0.25 and 4 hr after dosing. Following a treatment period ranging from 28 to 38 days, animals were sacrificed and subjected to a gross examination. Necropsy for each animal occurred on the last day of treatment. The number of nucleated cells per mg bone marrow was determined from bone marrow smears prepared from the femurs of all animals at necropsy on days 30-37. Myelograms were initially performed for the control and high dose groups. Later, myelograms were also performed for rats that received the 4R-form at 5 and 50 mg/kg/day. Absolute and relative organ weights were determined for the liver, kidneys,

heart, lung, pituitary gland, thyroid with parathyroid glands, adrenal glands, ovaries, uterus including both horns and cervix, testes, seminal vesicles, prostate, thymus, spleen, iliac lymph nodes, cerebrum, cerebellum, medulla oblongata, submandibular salivary glands, and pancreas. Organs and tissues were collected and fixed as follows: liver, kidneys, urinary bladder, heart, heart atrium, aorta thoracalis, vena cava caudalis, trachea, lung, pituitary gland, thyroid with parathyroid glands, adrenal glands, ovaries, uterus including both horns and cervix, vagina, mammary glands, skin, testes, epididymides, prostate, seminal vesicles, spleen, thymus, iliac lymph nodes, mandibular lymph nodes, bone including femur and sternum, brain including cerebrum, cerebellum, and medulla oblongata, spinal cord (cervical), nervus saphenus, eyes, lacrimal glands, tongue, submandibular salivary glands, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, skeletal muscle [M. gastrocnemius], and all organs/tissues with macroscopic findings. Histological analysis was performed for only the control and high dose groups; however, the heart atrium, aorta, vena cava caudalis, iliac lymph nodes, peripheral nerve, skeletal muscle, and epididymides were not further processed. The heart, submandibular salivary glands, pancreas, and adrenal glands were examined for the control and all treatment groups. The prostate was also examined for groups treated with iloprost or the 4S-form at 5 and 50 mg/kg/day. The testes and seminal vesicles were also examined in groups treated with the 4S-form at 5 and 50 mg/kg/day.

Results:

1. Observed Effects: Observed effects of iloprost consisted of slight apathy, stimulated vocalization, diminished muscular tone, slight to marked sialorrhea, and slight to moderate skin reddening at 50 mg/kg/day; and moderate to severe apathy, curved back, disturbances in gait, slight dacryorrhea, ruffled fur, wet matted fur of the inguinal region, diarrhea, and severe skin reddening at 500 mg/kg/day. Observed effects for the 4S-form of iloprost included diminished muscular tone, slight sialorrhea, and slight to moderate skin reddening at 5 mg/kg/day; slight to moderate apathy, stimulated vocalization, disturbances in gait, drawn-in flanks, ruffled fur, slight to marked sialorrhea, and severe skin reddening at 50 mg/kg/day; and severe apathy, curved back, spasmodic twitches, slight to red colored dacryorrhea, slight to moderate emaciation, wet matted fur of the inguinal region, skin cyanosis of the tip of the tail, and the snout was red-colored, encrusted, and dried at 500 mg/kg/day. Observed effects for the 4R-form of iloprost included slight sialorrhea at 50 mg/kg/day; and slight apathy, ruffled fur (1 animal), and slight to moderate skin reddening at 500 mg/kg/day. For iloprost and the 4R-form of iloprost, there were no clinical signs at 5 mg/kg/day.

2. Mortality: There were 3 treatment-related deaths. The sponsor suggested that these deaths were due to the exaggerated pharmacological properties of iloprost and its 4S-form (i.e., prolonged decrease in blood pressure). One male (#68) treated with

iloprost at 500 mg/kg/day died on day 26 of treatment. Observed effects included skin reddening 1 day prior to death. Necropsy findings included a reddish discoloration of all lung lobes. One female (#138) and one male (#123) treated with the 4S-form at 500 mg/kg/day died on days 6 and 22, respectively. Observed effects on the day prior to death for the female rat (#138) included ataxic gait, ruffled fur, and moderate skin reddening. Observed effects for the male rat (#123) included moderate apathy, curved back, ruffled fur, wet matted fur of the inguinal region, moderate emaciation, disturbances in gait, diminished muscular tone, exsiccosis, skin cyanosis of the tip of the tail, and moderate skin reddening. Microscopic findings included focal myocardial fibrosis, severe congestion with hemorrhage in the lungs, moderate hemorrhagic erosions in the stomach, and slight mucosal hemorrhage in the duodenum and jejunum. One female (#178) treated with 50 mg/kg/day 4R-iloprost died on day 29 due a blood sampling accident. The chest cavity was found to contain blood and blood clots.

3. Body Weight and Food and Water Consumption: Body weight gains were impaired by >10% for male rats that received iloprost at 500 mg/kg/day, male rats that received the 4S-form at 50 mg/kg/day, male and female rats that received the 4S-form at 500 mg/kg/day, and male rats that received the 4R-form at 500 mg/kg/day. Food consumption for male and female rats that received iloprost at 500 mg/kg/day was reduced to 77.3 and 82.35% of respective control values (22 and 17 g/day/rat). Food consumption for male and female rats that received the 4S-form at 500 mg/kg/day was reduced to 54.5 and 70.6% of respective control values (22 and 17 g/day/rat). Water consumption was generally increased for treatment groups. Increased water consumption appeared to be dose-related for rats that received iloprost; however, with the 4S- and 4R-forms, dose response relationships were either not present or relatively flat.

Body weight gains for rats that received treatment with iloprost, 4S-iloprost, or 4R-iloprost by the intragastric route at 5, 50, or 500 mg/kg/day. Asterisks denote where body weight gain was impaired by >10%.

Compound	Sex	Body weight gain, % of control ^A		
		5 mg/kg/day	50 mg/kg/day	500 mg/kg/day
Iloprost	Male	100.2	97.5	67.8*
	Female	108.9	96.6	103.8
4S-Iloprost	Male	96.3	86.3*	23.5*
	Female	107.3	92.8	80*
4R-Iloprost	Male	99.8	93.2	89.0*
	Female	96.6	92.8	105.4

A. Body weights for the male control rats on days 1 and 28 were 188 and 298 g, respectively. Body weights for the female control rats on days 1 and 28 were 165 and 210 g, respectively.

Water consumption for rats that received treatment with iloprost, 4S-iloprost, or 4R-iloprost by the intragastric route at 5, 50, or 500 mg/kg/day. Asterisks denote where body weight gain was impaired by >10%.

Compound	Sex	Water consumption, % of control ^A
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		5 mg/kg/day	50 mg/kg/day	500 mg/kg/day
Iloprost	Male	113.8	120.7	127.6
	Female	103.7	122.2	140.7
4S-Iloprost	Male	113.8	137.9	100
	Female	111.1	111.1	114.8
4R-Iloprost	Male	110.3	113.8	120.7
	Female	114.8	111.1	133.3

A. Water consumption for male and female control rats were 29 and 27 mL/day/rat, respectively.

4. Hematology Bone Marrow, and Blood Coagulation:

Hematology: White blood cell and lymphocyte counts on day 24-25 for male rats that received the 4S-form at 500 mg/kg/day were decreased to 59.4 and 50.9% of the control (9600 and 8844 cell/mm³), respectively. Lymphocyte counts on days 24-25 for female rats that received the 4S-form at 500 mg/kg/day were decreased to 72.8% of the control (4578 cells/mm³). Neutrophil counts on days 24-25 for female rats that received the 4S-form at 5, 50, and 500 mg/kg/day were decreased to 57.4, 29.8, and 61.6% of the control (768 cells/mm³), respectively. Neutrophil counts on days 24-25 for male rats that received the 4R-form at 500 mg/kg/day were increased to 203.5% of the control (592 cells/mm³). Platelet counts on days 24-25 for male rats that received iloprost at 500 mg/kg/day, the 4S-form at 50 mg/kg/day, and the 4S-form at 500 mg/kg/day were decreased to 62.3, 81.0, and 59.2% of the control (9.34 x 10⁵/mm³), respectively. Platelet counts on days 24-25 for female rats that received the 4S-form at 50 and 500 mg/kg/day were decreased to 80.9 and 68.9% of the control (9.49 x 10⁵/mm³), respectively.

Bone Marrow: There were no significant alteration of bone marrow cell parameters following treatment with iloprost or its stereoisomers.

Blood Coagulation: There were no treatment-related changes of thrombin time, partial thromboplastin time, activated partial thromboplastin time, or fibrinogen levels.

5. Blood Biochemistry and Urinalysis:

Blood Biochemistry: Alkaline phosphatase activity on day 2 for female rats that received the 4S-form at 500 mg/kg/day was increased to 153.9% of the control (228 U/L). Serum cholesterol levels on day 24-25 for male and female rats that received the 4S-form at 500 mg/kg/day were increased to 141.7 and 147.5% of the control (60 and 59 mg/100 mL). Serum glucose levels on day 24-25 for male rats that received the 4S-form at 500 mg/kg/day were elevated to 143.4% of the control (76 mg/100 mL). Serum glucose levels on day 24-25 for female rats that received iloprost at 500 mg/kg/day were elevated to 125% of the control (76 mg/100 mL). Alterations in serum levels of total protein, albumin, absolute and relative α -globulin, absolute and relative α_1 -

globulin, absolute and relative α_2 -globulin, relative and absolute γ -globulin were reported in response to treatment with iloprost and its stereoisomers and ranged from 10 to 40% in magnitude. However, the biological significance of these alterations was questionable, because changes in serum levels of total protein, albumin, and total α -globulin were <10 to 20%, normal variations in levels of these proteins for untreated rats is fairly wide, and no histopathological changes were observed for the liver where these proteins are synthesized. Further, these changes were primarily confined to the high dose, 500 mg/ kg/day, for iloprost and its diastereoisomers.

Urinalysis: Urinary pH values on day 22-23 for male and female rats that received the 4S-form at 500 mg/kg/day were increased to 8.8 and 7.4 as compared with respective control values of 6.8 and 5.8. Urinary sodium excretion on day 22-23 for male and female rats that received iloprost at 500 mg/kg/day were increased to 241 and 375.5% of control values (192 μ mole/18 hr for male and female control rats), respectively. Urinary potassium excretion on day 22-23 for female rats that received the 4R-form at 500 mg/kg/day was increased to 175.7% of the control (371 μ mole/18 hr). Urinary calcium excretion on day 22-23 for female rats that received the 4S-form at 500g/kg/day was increased to 322.2% of the control (9 μ mole/18 hr). Urinary chloride excretion values for female rats that received iloprost at 5, 50, or 500 mg/kg/day were increased to 137.8, 195.5, and 212.2% of the control (222 μ mole/18 hr), respectively; although, only the value at 500 mg/kg/day was significantly different. Urinary chloride excretion values for female rats that received the 4S-form at 5, 50, or 500 mg/kg/day were increased to 174.3, 234.2, and 358.1% of the control (222 μ mole/18 hr), respectively; although, only the value at 500 mg/kg/day was significantly different. Urinary chloride excretion values for female rats that received the 4R-form at 5, 50, or 500 mg/kg/day were increased to 136, 209.9, and 239.6% of the control (222 μ mole/18 hr), respectively; although, only the value at 500 mg/kg/day was significantly different. It should be noted that no alterations of serum electrolyte levels were observed for any treatment group.

6. Ophthalmic Examination: No treatment-related ophthalmic changes were found on day 22-23.

7. Organ Weights: Changes in absolute and relative weights for several organs were evident; however, significant histopathological changes were confined to the heart and pancreas. Histopathological changes were also evident in the prostate and seminal vesicles for male rats that received the 4S-form at 500 mg/kg/day.

Changes in absolute and relative weights for heart, pancreas, prostate, and seminal vesicles for male and female rats that received treatment with iloprost, 4S-iloprost, or 4R-iloprost.

Compd	Dose, mg/kg/day	Sex	% of Control

			Heart ^A		Pancreas ^B		Prostate ^C		Seminal Vesicles ^D	
			Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
Iloprost	50	M	-	-	-	-	-	-	-	-
		F	-	-	126.7	126.1				
	500	M	-	119.2	134.3	147.2	-	-	-	-
		F	113.6	-	162.8	157.1				
4S-form	50	M	-	-	128.7	133.3	-	-	-	-
		F	-	-	130.2	131				
	500	M	-	130.8	130.6	169.4	75.4	82.6	38.2	47.8
		F	-	113.8	166.3	173.8				
4R-form	500	M	-	115.4	127.8	133.3	-	-	-	-
		F	115.3	-	131.4	128.6				

- A. Heart: Male Control (0.79g and 0.26%) and Female Control (0.59 g and 0.29%).
 B. Pancreas: Male Control (1.08g and 0.36%) and Female Control (0.86 g and 0.42%).
 C. Prostate: Male Control (0.69g and 0.23%).
 D. Seminal Vesicles: Male Control (0.68g and 0.23%).

8. **Gross Pathology:** Gross pathological changes were not reported.

9. **Histopathology:** The target organs of toxicity appeared to be the pancreas and heart. Changes for the pancreas consisted of acinar cell hypertrophy, acinar cell basophilia, and focal acinar cell atrophy. These pancreatic histopathological findings occurred for rats that received iloprost or the 4S-form at 50 and 500 mg/kg/day; however, for the 4R-form, they were only found at 500 mg/kg/day. Changes for the heart consisted of focal fibrosis of the myocardium and were confined to the dose at 500 mg/kg/day for rats treated with iloprost, the 4S-form, or the 4R-form. For male rats that received the 4S-form at 500 mg/kg/day, histopathological changes included spermatogenic cell degeneration for the testes, reduced secretion content for the prostate, and decreased secretion for the seminal vesicles; however, these changes were not found with iloprost or the 4R-form.

Histopathological findings for rats that received either iloprost, the 4S-form of iloprost, or the 4R-form of iloprost at 5, 50, or 500 mg/kg/day for 28 days. Control rats received saline. There were 10 rats/sex/group.

Organ/Tissue	Saline		Iloprost						4S-Iloprost						4R-Iloprost						
	0		5		50		500		5		50		500		5		50		500		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	

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Heart -focal fibrosis of myocardium	0	0	0	0	0	0	6	5	0	0	0	0	7	4	0	0	0	0	1	0
Pancreas -acinar hypertrophy	0	0	0	0	0	3	5	5	0	0	3	3	4	5	0	0	0	0	3	4
-acinar cell basophilia	0	0	0	0	0	1	1	2	0	0	1	2	1	2	0	0	0	0	2	1
-focal acinar atrophy	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Testes -spermatogenic cell degeneration	0	-	0	-	0	-	0	-	0	-	0	-	5	-	0	-	0	-	0	-
-decreased sperm contents in epididymides	0	-	0	-	0	-	0	-	0	-	0	-	1	-	0	-	0	-	0	-
Prostate -reduced secretion content	0	-	0	-	0	-	4	-	0	-	0	-	7	-	0	-	0	-	0	-
-epithelial atrophy	0	-	0	-	0	-	0	-	0	-	0	-	2	-	0	-	0	-	0	-
Seminal vesicles -decreased secretion	0	-	0	-	0	-	0	-	0	-	0	-	7	-	0	-	0	-	0	-
-epithelial atrophy	0	-	0	-	0	-	0	-	0	-	0	-	2	-	0	-	0	-	0	-
Lungs -perivascular edema	0	0	0	0	0	0	2	0	0	0	0	0	1	1	0	0	0	0	0	0
-alveolar edema	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
-foam cell aggregation	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Adrenal Gland -Incr. Cort. L. vacuoles	0	0	0	0	1	0	2	0	0	0	0	0	2	0	0	0	0	0	0	0
-cortical hemorrhage and focal necrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Stomach -erosion	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
-mucosal congestion	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0
-focal forestomach inflammation	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0
Liver -single cell necrosis	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2

10. Serum Drug Levels: Serum C_{max} , AUC_{0-24hr} and AUC_{0-4hr} values for iloprost and its diastereoisomers increased as the dose was increased. In many cases, AUC values for iloprost and its diastereoisomers at a dose of 5 mg/kg/day were larger than expected based upon values at doses of 50 and 500 mg/kg/day. There were no significant differences in serum C_{max} , AUC_{0-24hr} and AUC_{0-4hr} values between male and

female rats. Serum C_{max} , AUC_{0-24hr} and AUC_{0-4hr} values between 4S-iloprost and 4R-iloprost were similar.

Serum C_{max} , AUC_{0-24hr} , and AUC_{0-4hr} on day 29 for rats treated with either iloprost, 4S-iloprost, or 4R-iloprost by oral gavage at doses of 5, 50, or 500 mg/kg/day.

Compound	Dose mg/kg/day	C_{max} , ng/mL		AUC_{0-24hr} , ng*hr/mL		AUC_{0-4hr} , ng*hr/mL	
		Male	Female	Male	Female	Male	Female
Iloprost	5	3.4	2.0	17.1	14.8	5.1	4.3
	50	24.7	16.5	67.2	78.5	27.5	27.8
	500	132	283	771	718	227	268
4S-Iloprost	5	6.5		48.1	73.0	11.5	11.2
	50	13.8	21.7	143	140	35.9	34.6
	500	125	391	1020	952	233	398
4R-Iloprost	5	6.9	7.2	24.3	23.8	9.4	8.6
	50	19.0	22.0	142	98.8	48.1	43.7
	500	211	436	978	1140	316	536

Han: WIST (SPF) rats received either iloprost clathrate, the 4(S)-diastereoisomer of iloprost clathrate, or the 4(R)-diastereoisomer of iloprost clathrate by the intragastric route of administration at doses of 5, 50, or 500 mg/kg/day for a period of 28 to 38 days. No effect doses were 50 mg/kg/day for the 4R-form of iloprost and 5 mg/kg/day for iloprost and the 4S-form of iloprost. Treatment-related mortality occurred for iloprost and the 4S-form of iloprost at 500 mg/kg/day. The target organs of toxicity appeared to be the pancreas and heart. Changes for the pancreas consisted of acinar cell hypertrophy, acinar cell basophilia, and focal acinar cell atrophy. These pancreatic histopathological findings occurred for rats that received iloprost or the 4S-form at 50 and 500 mg/kg/day; however, for the 4R-form, they were only found at 500 mg/kg/day. Changes for the heart consisted of focal fibrosis of the myocardium and were confined to the dose at 500 mg/kg/day for rats treated with iloprost, the 4S-form, or the 4R-form.

SUMMARY AND EVALUATION

Iloprost is a synthetic, stable analogue of prostacyclin (PGI_2). \square

Iloprost is composed of a mixture of two diastereoisomers (4R-iloprost and 4S-iloprost). In a meeting with the sponsor, the Division raised questions regarding the development of iloprost as a diastereoisomeric mixture rather than as a single isomer. In response to these questions, the sponsor submitted a series of studies in the present amendment, which examine the pharmacodynamic effects, pharmacokinetics, and toxicity of 4R-iloprost, 4S-iloprost, and 4R,S-iloprost (racemic mixture). Studies included within this amendment were as follows: pharmacology; ADME in rats and dogs; acute toxicity

studies in rats after intragastric or intravenous administration; and a subacute toxicity study in rats following intragastric administration for 28 to 38 days.

Iloprost (0.08-6 nM), 4S-iloprost (0.6-4 nM), and 4R-iloprost (0.6-60 nM) produced concentration-dependent inhibition of human or rat platelet aggregation induced by either ADP or collagen. With regard to inhibition of human or rat platelet aggregation induced by ADP, the 4S-form (maximal IC_{50} values of 0.15 and 17.25 nM, respectively) was more potent than E-4R (maximal IC_{50} values of 1.87 and 225.5 nM, respectively). With regard to inhibition of human or rat platelet aggregation induced by collagen, the 4S-form (IC_{50} values of 0.97 and 6.5 nM, respectively) was more potent than E-4R (IC_{50} values of 10.37 and 80.53 nM, respectively). The 4S-isomer ($K_d = 13.4$ nM) was found to have a much higher affinity for the prostacyclin receptor as compared to the 4R isomer ($K_d = 228$ nM). Association rates for the 4S- and 4R-isomers of 0.036 and 0.001 sec^{-1} , respectively, were significantly different. The IC_{50} values for inhibition of collagen-induced platelet aggregation as well as dissociation constants and association rates for the racemic mixture of iloprost and the 4S-stereoisomer agreed closely. Thus, the potency of iloprost is attributable to the 4S-stereoisomer, which constitutes approximately 40% of the molarity. Iloprost and its isomers, E-4S, E-4R, and Z-4S, produced a dose-dependent reduction of mean blood pressure. The hypotensive action of iloprost and the E-4S isomer was greater for diastolic pressure than systolic pressure. Iloprost and its isomers produced a dose-dependent inhibition of agonist-induced platelet aggregation. The 4S and 4R diastereoisomers of the Z-configuration of iloprost were significantly less potent than iloprost with regard to inhibition of phenylephrine-induced contraction of mesenteric artery, vasodilation of mesenteric artery, and inhibition of agonist-induced platelet aggregation. The E-4S isomer is more potent than iloprost with regard to inhibition of phenylephrine-induced contraction of mesenteric artery and agonist-induced platelet aggregation. Further, the E-4S isomer has a more potent vasodilatory effect on mesenteric artery than iloprost. The E-4R isomer was less potent than either iloprost or the E-4S isomer. The order of potency of iloprost and its isomers was as follows: E-4S > Iloprost > E-4R > Z-4S > Z-4R. Iloprost and its diastereoisomers, E-4R and E-4S, induced a dose-dependent reduction in arterial pressure accompanied by a reflex increase in heart rate in anesthetized rats. The order of potency was as follows: E-4S > iloprost > E-4R.

The stereoselective pharmacokinetics of iloprost diastereoisomers (E-4RS, E-4R, or E-4S iloprost) were followed in 3 mongrel female dogs administered the test material by intravenous infusion at a dose of 100 ng/kg/min. Iloprost diastereoisomer ratios from 0 to 6.5 hr after the start of the infusion with E-4RS iloprost ranged from 0.84 to 1.16 (%E-4R/%E-4S). This data illustrates that the diastereoisomer ratio did not change versus infusion time. The mean E-4R to E-4S iloprost ratio found *in vivo* in all dogs was 1.03, which closely matched the starting infusion solution diastereoisomer ratio of 1.1. No interconversion of iloprost diastereoisomers was found. During the 6 hr infusion of E-4R iloprost, no E-4S iloprost was detected. Conversely, during the 6 hr infusion of E-4S iloprost, no E-4R iloprost was detected. Anesthetized rats received

either iloprost at 0.30 $\mu\text{g}/\text{kg}/\text{min}$, E-4R at 0.30 or 3.00 $\mu\text{g}/\text{kg}/\text{min}$, or E-4S at 0.30 $\mu\text{g}/\text{kg}/\text{min}$. Each test article was administered by continuous intravenous infusion for a 30 min period. For E-4R at doses of 0.3 and 3 $\mu\text{g}/\text{kg}/\text{min}$, AUC values were proportional to dose. Total clearance for all compounds significantly exceeded hepatic plasma flow (31.9 mL/min/kg), suggestive of a rapid metabolic clearance. The clearance of E-4S was 1.5 times that of E-4R. The terminal half-lives for all compounds were <0.26 hr, further suggestive of rapid elimination. The volume of distribution at steady state exceeded blood volume (0.054 L/kg), suggestive of extensive distribution into tissues. The volume of distribution of E-4S was more than twice that of E-4R. E-4S is more extensively distributed than E-4R; however, it is more rapidly eliminated. Half-lives and mean residence times for the two diastereoisomers were similar.

The acute toxicity of iloprost and its diastereoisomers were evaluated in rats following intragastric or intravenous administration. Following intragastric administration, the acute toxicity of the 4S-form ($\text{LD}_{50} = 1073 \text{ mg}/\text{kg}$) was significantly greater than iloprost ($\text{LD}_{50} = 1864 \text{ mg}/\text{kg}$) or the 4R-form ($\text{LD}_{50} = 3820 \text{ mg}/\text{kg}$). In contrast with intravenous administration, there were no significant differences in toxicity between iloprost ($\text{LD}_{50} = 130 \text{ mg}/\text{kg}$), the 4-S form ($\text{LD}_{50} = 128 \text{ mg}/\text{kg}$), and the 4-R form ($\text{LD}_{50} = 126 \text{ mg}/\text{kg}$). For animals that died during the treatment period, death was attributed to circulatory and/or irritative alterations. The sponsor provided no explanation for lack of difference in toxicity between the two stereoisomers following intravenous administration as opposed to intragastric administration, where the toxicity of the 4S-form was significantly greater.

Han: WIST (SPF) rats received either iloprost clathrate, the 4(S)-diastereoisomer of iloprost clathrate, or the 4(R)-diastereoisomer of iloprost clathrate by the intragastric route of administration at doses of 5, 50, or 500 mg/kg/day for a period of 28 to 38 days. No effect doses were 50 mg/kg/day for the 4R-form of iloprost and 5 mg/kg/day for iloprost and the 4S-form of iloprost. Treatment-related mortality occurred for iloprost and the 4S-form of iloprost at 500 mg/kg/day. The target organs of toxicity appeared to be the pancreas and heart. Changes for the pancreas consisted of acinar cell hypertrophy, acinar cell basophilia, and focal acinar cell atrophy. These pancreatic histopathological findings occurred for rats that received iloprost or the 4S-form at 50 and 500 mg/kg/day; however, for the 4R-form, they were only found at 500 mg/kg/day. Changes for the heart consisted of focal fibrosis of the myocardium and were confined to the dose at 500 mg/kg/day for rats treated with iloprost, the 4S-form, or the 4R-form. For male rats that received the 4S-form at 500 mg/kg/day, histopathological changes included spermatogenic cell degeneration for the testes, reduced secretion content for the prostate, and decreased secretion for the seminal vesicles; however, these changes were not found with iloprost or the 4R-form. Serum C_{max} , $\text{AUC}_{0-24\text{hr}}$ and $\text{AUC}_{0-4\text{hr}}$ values for iloprost and its diastereoisomers increased as the dose was increased. In many cases, AUC values for iloprost and its diastereoisomers at a dose of 5 mg/kg/day were larger than expected based upon values at doses of 50 and 500 mg/kg/day. There were no significant differences in

serum C_{max} , AUC_{0-24hr} and AUC_{0-4hr} values between male and female rats. Serum C_{max} , AUC_{0-24hr} and AUC_{0-4hr} values between 4S-iloprost and 4R-iloprost were similar.

Based upon the results of these studies, the sponsor has requested that development of iloprost as a diastereoisomeric mixture be allowed to continue. Based upon the FDA's Policy Statement For The Development of New Stereoisomeric Drugs published on May 1, 1992 and revised on January 3, 1997, development of iloprost as a diastereoisomeric mixture appears to be justified. This judgement is based upon the following rationale. (1) No interconversion of isomers was found to occur in vivo in studies with dogs. (2) Pharmacological activity exists with both isomers; although, the pharmacological properties of iloprost appear to predominantly reside with the 4S-diastereoisomer. (3) The 4S-diastereoisomer exhibits more potent pharmacological and toxicological properties than the 4R-diastereoisomer; however, toxicity associated with the 4S-diastereoisomer and the 4R,S-diastereoisomeric mixture were not different.

RECOMMENDATION: Information should be communicated to the sponsor as follows:

The sponsor has requested that development of iloprost as a diastereoisomeric mixture be allowed to continue. Based upon the FDA's Policy Statement For The Development of New Stereoisomeric Drugs published on May 1, 1992 and revised on January 3, 1997, development of iloprost as a diastereoisomeric mixture appears to be justified. This judgement is based upon the following rationale. (1) No interconversion of isomers was found to occur in vivo in studies with dogs. (2) Pharmacological activity exists with both isomers; although, the pharmacological properties of iloprost appear to predominantly reside with the 4S-diastereoisomer. (3) The 4S-diastereoisomer exhibits more potent pharmacological and toxicological properties than the 4R-diastereoisomer; however, toxicity associated with the 4S-diastereoisomer and the 4R,S-diastereoisomeric mixture were not different.

/s/

Timothy W. Robison, Ph.D.

Date

cc:

Orig IND

HFD-

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HFD- Dr. Choudary

HFD- Dr. Robison

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Statistical Review and Evaluation
Review of Carcinogenicity Studies

NDA#: 21,779

APPLICANT:

NAME OF DRUG: Iloprost clathrate

STUDIES REVIEWED: Two-Year Study in Rats and Two-Year Study in Mice

PHARMACOLOGY REVIEWER: James Willard, Ph.D. (HFD-110)

STATISTICAL REVIEWER: Jasmine Choi, M.S. (HFD-710)

This review consists of 4 pages of text and another 16 pages of graphs and tables.

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1. Introduction

This NDA was submitted to evaluate the carcinogenic potential of iloprost clathrate when administered once daily by oral gavage. A statistical review was done for two carcinogenicity studies: a two-year mouse study (Study BC 60) and a two-year rat study (Study BC 61).

2. Two-Year Study in Mice (BC 60)

2.1 Study Design

A total of 560 Crl:CD-1 (ICR) BR albino mice per gender (70 mice/sex/group) were assigned to the vehicle group, the 5 mg/kg/day, the 50 mg/kg/day, or the 125 mg/kg/day dose groups. The animals in the vehicle controls were administered a Tris buffer solution at a volume of 10 mL/kg/day. The treatment was administered once daily by gavage. At the end of the treatment period all surviving animals were euthanized and necropsied.

2.2 Sponsor's Analysis Methods and Results

Cumulative survival data were analyzed using Kaplan-Meier product limit estimates, Cox-Tarone binary regression, and generalized Gehan-Breslow nonparametric score tests. The trend analysis of survival was evaluated at the 5% one-tailed probability level. There was a significant decrease in the survival for the 5 and 50 mg/kg/day females when compared with the control group. However, the sponsor stated that it was probably due to chance since the decrease in survival did not occur in a dose-related manner. No significant difference was noted in the high-dose females or among the males when compared with the concurrent control group.

Different statistical methods were used for incidental and fatal tumors. For incidental tumors, logistic prevalence tests were performed for trend and for control versus treated group comparisons. Cox-Tarone binary regression method was used for the analysis of fatal tumors. There was no significant positive trend or increase in any neoplastic incidence in either of the two sexes.

2.3 Reviewer's Analysis Methods and Results

This reviewer performed dose-mortality trend tests and homogeneity tests as survival analysis. Survival of the females in the low and the mid dose groups was decreased compared to the control group and the homogeneity test showed that the difference was statistically significant. However, the survival of the high dose group was similar to the one of the control group and the dose-mortality trend test showed no statistically significant trend. The survival of the males was similar across all groups (Appendices 1-4). The test results are summarized in the following Table 1.

Table 1: Survival Analysis of Mice

Gender	Tests	P-values	
		Cox	Kruskal-Wallis
Male	Dose-Mortality Trend	0.9369	0.9094
	Homogeneity	0.4495	0.4782
Female	Dose-Mortality Trend	0.4614	0.5813
	Homogeneity	0.0128	0.012

Tumor findings were analyzed by an exact permutation trend test. The following standard approach was used in the analyses: for rare tumors (defined as incidence rate $\leq 1\%$ usually based on concurrent controls) the significance level was 0.025 while for common tumors, the significance level was 0.005. Fatal and incidental tumors were analyzed separately using the death rate and the prevalence methods, respectively. The asymptotic test was used when fatal and incidental tumors occurred in the same time interval if the number of tumors was not small. The analysis of tumor formation showed no statistically significant positive trend among males or females.

2.4 Validity of Mice Study

Since there was no statistically significant dose related tumor trend in mice, the validity of the study was evaluated for each gender. The study needs to show that a sufficient number of animals is exposed for an adequate length of time to allow for late developing tumors and that the high dose is close to the maximum tolerated dose (MTD). Since the pharmacologist determined that the study was valid from a dose-selection standpoint, this reviewer evaluated only the adequacy of the number of animals at risk for late developing tumors.

The proportion surviving at weeks 80-90 was examined. If more than 50% of the usual 50 initial animals in the high dose group were alive between weeks 80-90, it would be considered a sufficient number and adequate length of exposure. The survival rates for all groups of male and female mice at weeks 80-90 were over 50% (Appendices 3 & 4), therefore, it was concluded that a sufficient number of animals were at risk for a sufficient length of time.

3. Two-Year Study in Rats (BC 61)

3.1 Study Design

A total of 560 Sprague-Dawley Crl:CD BR rats per gender (70 mice/sex/group) were assigned to the vehicle control, the 5 mg/kg/day, the 50 mg/kg/day, or the 125 mg/kg/day dose groups. The dose level for the males in the high dose group was lowered to 100 mg/kg/day at the beginning of Week 26 due to a higher-than-expected rate of mortality. The animals in the control group were administered a Tris buffer solution at a volume of 10 mL/kg/day. The treatment was administered once daily by oral gavage. At the end of the treatment period all surviving animals were sacrificed.

3.2 Sponsor's Analysis Methods and Results

The same survival methods were used as described in section 2.2. A statistically significant increase of the mortality was seen in the high dose group compared to the control group among males ($p=0.0002$, and $p=0.0000$). The survival of the other two treated groups was similar to the control group. In the females, the Cox-Tarone test showed a borderline (statistically significant) mortality increase in the high dose group compared to the control group ($p=0.0573$) and the Gehan-Breslow test showed a statistically significant difference between the high dose and the control group ($p=0.0189$).

The trend test on tumor formation was analyzed by the method as described in section 2.2. The tumor analysis showed no statistically significant positive trend in either females or males.

3.3 Reviewer's Analysis Methods and Results

For the survival analysis, dose-mortality trend tests and homogeneity tests were performed. As shown in Appendices 7-10, the survival of the high dose group decreased in both males and females. The tests showed that there was a statistically significant dose-mortality trend and heterogeneity among males, and a statistically significant heterogeneity among females. The test results are shown in the table below.

Table 2: Survival Analysis of Rats

Gender	Tests	P-values	
		Cox	Kruskal-Wallis
Male	Dose-Mortality Trend	<0.0001	<0.0001
	Homogeneity	<0.0001	<0.0001
Female	Dose-Mortality Trend	0.1615	0.051
	Homogeneity	0.007	0.0022

The exact permutation trend test was performed to analyze the positive linear trend of any tumor type. The analysis showed no statistically significant positive tumor trend in males or females.

3.4 Validity of the Rats Study

The validity of the rat studies (males and females) was evaluated because no statistically significant tumor trend was found. Because the pharmacologist confirmed that the high dose reached the MTD, this reviewer evaluated only whether enough animals were at risk for a sufficient length of time. The survival rate of both males and females at week 80 was over 50% (Appendix 9 and 10) and therefore, it was concluded that sufficient numbers of animals were at risk for a sufficient length of time.

4. Summary

4.1 Mouse Study

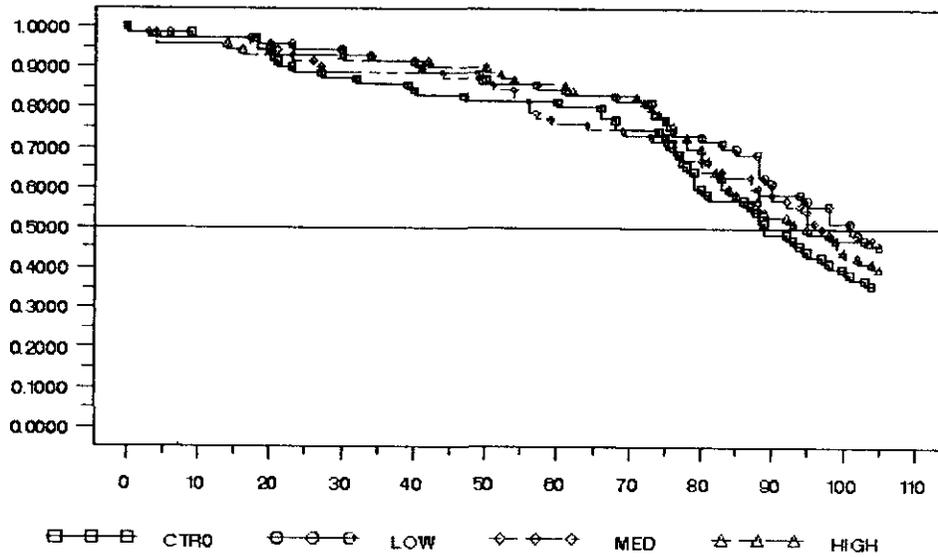
Seventy mice per group/sex received the drug at levels of 0 (vehicle), 5, 50, 125 mg/kg/day via gavage for two years. The low and the mid dosed females had decreased survival compared to the controls. The high dose group of the females showed a survival similar to the control group and the dose-mortality trend test showed no statistically significant trend, but the homogeneity test showed a statistically significant difference among the groups. The survival of the males was similar across the groups. The exact permutation trend test on tumor incidences showed no statistically significant positive tumor trends among both the males and the females. These findings agree with the sponsor's. The evaluation of the validity of the study showed that sufficient numbers of animals were at risk for a sufficient length of time. The pharmacologist determined that the high dose reached the MTD.

4.2 Rat Study

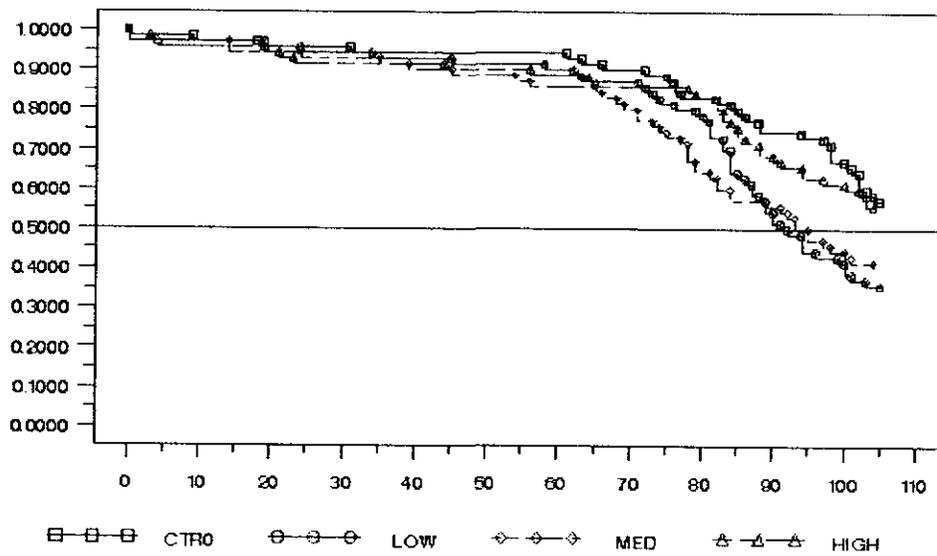
Seventy rats per group/sex were studied for tumor formation, and those rats received the drug at levels of 0 (vehicle), 5, 50, and 125 mg/kg/day via gavage. The dose level of the male high dose group was lowered to 100 mg/kg/day at the beginning of Week 26 because of the high mortality rate. Survival of the high dose groups in both males and females was decreased. The dose-mortality trend and the homogeneity test for the males as well as the homogeneity test for the females reached the statistical significance. The trend test on tumor incidences showed no statistically significant positive trend in either male or female rats. These findings were consistent between the sponsor and the reviewer. This reviewer's evaluation of the validity showed that sufficient numbers of rats lived long enough to present late developing tumors. The pharmacologist determined that the high dose reached MTD.

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Appendix 1: Survival Graph of Male Mouse



Appendix 2: Survival Graph of Female Mouse



Appendix 3: Mortality of Male Mice

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTRO	0-52	70	12	58	82.9	17.1
	53-78	58	12	46	65.7	34.3
	79-91	46	10	36	51.4	48.6
	92-104	36	11	25	35.7	64.3
	FINALKILL105-105	25	25	0		
LOW	0-52	70	9	61	87.1	12.9
	53-78	61	9	52	74.3	25.7
	79-91	52	9	43	61.4	38.6
	92-104	43	10	33	47.1	52.9
	FINALKILL105-105	33	33	0		
MED	0-52	70	10	60	85.7	14.3
	53-78	60	12	48	68.6	31.4
	79-91	48	7	41	58.6	41.4
	92-104	41	8	33	47.1	52.9
	FINALKILL105-105	33	33	0		
HIGH	0-52	70	8	62	88.6	11.4
	53-78	62	11	51	72.9	27.1
	79-91	51	13	38	54.3	45.7
	92-104	38	9	29	41.4	58.6
	FINALKILL105-105	29	29	0		

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Appendix 4: Mortality of Female Mice

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTRO	0-52	70	3	67	95.7	4.3
	53-80	67	8	59	84.3	15.7
	81-91	59	5	54	77.1	22.9
	92-104	54	13	41	58.6	41.4
	FINALKILL105 -105	41	41	0		
LOW	0-52	70	5	65	92.9	7.1
	53-80	65	10	55	78.6	21.4
	81-91	55	19	36	51.4	48.6
	92-104	36	10	26	37.1	62.9
	FINALKILL105 -105	26	26	0		
MED	0-52	70	7	63	90	10
	53-80	63	16	47	67.1	32.9
	81-91	47	8	39	55.7	44.3
	92-104	39	10	29	41.4	58.6
	FINALKILL105 -105	29	29	0		
HIGH	0-52	70	6	64	91.4	8.6
	53-80	64	5	59	84.3	15.7
	81-91	59	12	47	67.1	32.9
	92-104	47	8	39	55.7	44.3
	FINALKILL105 -105	39	39	0		

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Appendix 5: Tumor Trend Test of Male Mice

				LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
AC	ADRENAL, CORTEX	<u>238</u>	B-ADENOMA, SUBCAPSULA R CELL	1	0	0	1	0.8571
AM	ADRENAL, MEDULLA	<u>141</u>	M- MALIGNANT PHEOCHROM OCYTOMA	0	1	0	0.676	0.8066
AM	ADRENAL, MEDULLA	<u>358</u>	B- PHEOCHROM OCYTOMA	0	1	0	0.7105	0.7693
BR	BRAIN W/STEM	<u>344</u>	B- HEMANGIOM A	1	0	0	1	0.824
GB	GALLBLADDE R	<u>298</u>	B-ADENOMA	1	0	0	1	0.8529
HG	HARDERIAN GLAND	<u>211</u>	B-ADENOMA	7	2	2	0.4907	0.4816
HG	HARDERIAN GLAND	<u>281</u>	M- CARCINOMA	0	2	0	0.5074	0.5965
HN	HEMATO NEOPLASIA	<u>1</u>	M- MALIGNANT LYMPHOMA, LYMPHO	7	3	2	0.993	0.9898
HN	HEMATO NEOPLASIA	<u>137</u>	M-LEUKEMIA, GRANULOCY TIC	1	0	1	0.7671	0.7767
HN	HEMATO NEOPLASIA	<u>2</u>	M- HISTIOCYTIC SARCOMA	1	2	0	0.4907	0.5311
HN	HEMATO NEOPLASIA	<u>228</u>	M-LEUKEMIA, MAST CELL	0	0	0	0.4268	0.1303
LI	LIVER	<u>184</u>	M- CARCINOMA, HEPATOCELL ULAR	7	7	1	0.9625	0.9568
LI	LIVER	<u>246</u>	B-ADENOMA, HEPATOCELL ULAR	2	7	3	0.4419	0.4485
LI	LIVER	<u>297</u>	M- HEMANGIOSA RCOMA	6	2	0	0.9718	0.9652
LI	LIVER	<u>347</u>	B- HEMANGIOM A	0	1	1	0.6375	0.7002
LU	LUNG	<u>200</u>	B-ADENOMA, BRONCHIOLA R-ALVEO	14	11	7	0.6882	0.6897
LU	LUNG	<u>69</u>	M- CARCINOMA, BRONCHIOLA R-ALV	8	9	7	0.5632	0.5679

PC	CAVITY, ABDOM	<u>268</u>	M-NEUROFIBROSARCOMA	0	0	0	1	0.125	0.0074
SP	SPLEEN	<u>307</u>	M-HEMANGIOSARCOMA	2	0	0	1	0.8202	0.6897
SP	SPLEEN	<u>340</u>	B-HEMANGIOMA	1	0	0	0	1	0.8025
SQ	SUBCUTANEOUS TIS	<u>199</u>	M-FIBROSARCOMA	2	1	0	0	1	0.9382
SU	STOMACH, NONGL	<u>303</u>	B-SQUAMOUS CELL PAPILLOMA	0	0	1	0	0.5227	0.4713
SU	STOMACH, NONGL	<u>314</u>	M-SQUAMOUS CELL CARCINOMA	0	0	1	0	0.5227	0.4713
TE	TESTIS	<u>241</u>	M-INTERSTITIAL CELL TUMOR	1	0	0	0	1	0.7985
TE	TESTIS	<u>249</u>	B-INTERSTITIAL CELL TUMOR	1	1	0	1	0.6624	0.7002
TE	TESTIS	<u>334</u>	M-SERTOLI CELL TUMOR	1	0	0	0	1	0.8318
TY	THYROID	<u>339</u>	B-FOLLICULAR CELL ADENOMA	1	0	0	0	1	0.8571

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Appendix 6: Tumor Trend Test of Female Mice

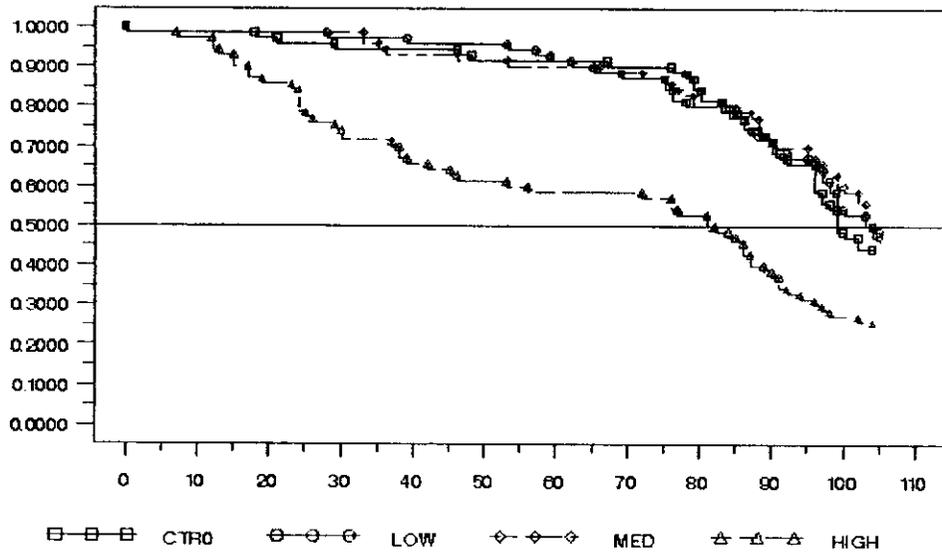
Tumor Name	Organ	Total	Tumor Type	Control	Dose			P-Value (Exact Method)	P-Value (Asymptotic Method)
					LOW	MED	HIGH		
AM	ADRENAL, MEDULLA	<u>141</u>	M-MALIGNANT PHEOCHROMOCYTOMA	0	1	0	0	0.6829	0.7594
BR	BRAIN W/STEM	<u>406</u>	M-EPENDYMOMA	0	0	0	1	0.3198	0.0842
CE	CECUM	<u>450</u>	M-LEIOMYOSARCOMA	0	1	0	0	0.6829	0.7594
CV	UTERUS, CERVIX	<u>387</u>	M-LEIOMYOSARCOMA	1	1	0	0	0.7617	0.9086
CV	UTERUS, CERVIX	<u>390</u>	B-GRANULAR CELL TUMOR	0	0	0	1	0.2051	0.0377
CV	UTERUS, CERVIX	<u>418</u>	B-LEIOMYOMA	0	1	0	1	0.2348	0.2068
CV	UTERUS, CERVIX	<u>446</u>	M-CARCINOMA, SQUAMOUS CELL	0	0	0	1	0.481	0.1523
DU	DUODENUM	<u>455</u>	M-CARCINOMA	0	1	0	0	0.6683	0.7867
FM	MARROW, FEMUR	<u>441</u>	M-HEMANGIOSARCOMA	1	0	0	0	1	0.8348
GB	GALLBLADDER	<u>298</u>	B-ADENOMA	1	0	0	0	1	0.8348
HG	HARDERIAN GLAND	<u>211</u>	B-ADENOMA	4	4	1	5	0.5815	0.611
HG	HARDERIAN GLAND	<u>281</u>	M-CARCINOMA	1	1	1	0	0.7615	0.8151
HN	HEMATO NEOPLASIA	<u>1</u>	M-MALIGNANT LYMPHOMA, LYMPHO	8	5	12	12	0.3088	0.309
HN	HEMATO NEOPLASIA	<u>2</u>	M-HISTIOCYTIC SARCOMA	7	3	3	2	0.9572	0.9531
LI	LIVER	<u>184</u>	M-CARCINOMA, HEPATOCELLULAR	1	1	1	0	0.7951	0.8083
LI	LIVER	<u>246</u>	B-ADENOMA, HEPATOCELLULAR	2	0	1	0	0.8469	0.8094
LI	LIVER	<u>297</u>	M-HEMANGIOSARCOMA	0	0	1	0	0.4939	0.4785
LU	LUNG	<u>200</u>	B-ADENOMA, BRONCHIOLAR-ALVEOLAR	10	7	15	8	0.5193	0.5217

LU	LUNG	<u>69</u>	M-CARCINOMA, BRONCHIOLA R-ALV	12	6	9	12	0.2677	0.2671
MF	MAMMARY, FEMALE	<u>367</u>	M-CARCINOMA	2	1	2	1	0.6034	0.584
OV	OVARY	<u>364</u>	B-ADENOMA	3	1	1	1	0.8022	0.7819
OV	OVARY	<u>379</u>	B-GRANULOSA/THECA CELL TUMOR	2	0	2	1	0.5251	0.4907
OV	OVARY	<u>398</u>	M-GRANULOSA/THECA CELL TUMOR	1	0	0	0	1	0.8227
OV	OVARY	<u>416</u>	B-LUTEOMA	1	0	1	0	0.7596	0.7391
OV	OVARY	<u>424</u>	M-HEMANGIOSARCOMA	1	1	0	0	0.9152	0.8864
OV	OVARY	<u>428</u>	B-HEMANGIOMA	0	0	0	1	0.3	0.0774
PA	PANCREAS	<u>394</u>	B-ADENOMA, ISLET CELL	2	0	1	0	0.8581	0.8637
PA	PANCREAS	<u>444</u>	B-ADENOMA, ACINAR CELL	0	0	0	1	0.4699	0.1478
PI	PITUITARY	<u>373</u>	B-ADENOMA	3	0	0	1	0.8796	0.7911
SP	SPLEEN	<u>307</u>	M-HEMANGIOSARCOMA	1	1	0	0	0.9477	0.8927
SP	SPLEEN	<u>340</u>	B-HEMANGIOMA	1	0	0	0	1	0.8329
SQ	SUBCUTANEOUS TIS	<u>199</u>	M-FIBROSARCOMA	2	0	0	2	0.1	0.0537
SU	STOMACH, NONGL	<u>303</u>	B-SQUAMOUS CELL PAPILOMA	0	0	0	1	0.2727	0.0628
SU	STOMACH, NONGL	<u>314</u>	M-SQUAMOUS CELL CARCINOMA	1	0	0	1	0.716	0.4732
TY	THYROID	<u>339</u>	B-FOLLICULAR CELL ADENOMA	1	0	1	1	0.4585	0.4661
TY	THYROID	<u>415</u>	M-FOLLICULAR CELL CARCINOMA	1	0	0	0	1	0.8348
UT	UTERUS	<u>378</u>	B-ENDOMETRIAL STROMAL POLYP	8	9	1	4	0.9754	0.9707

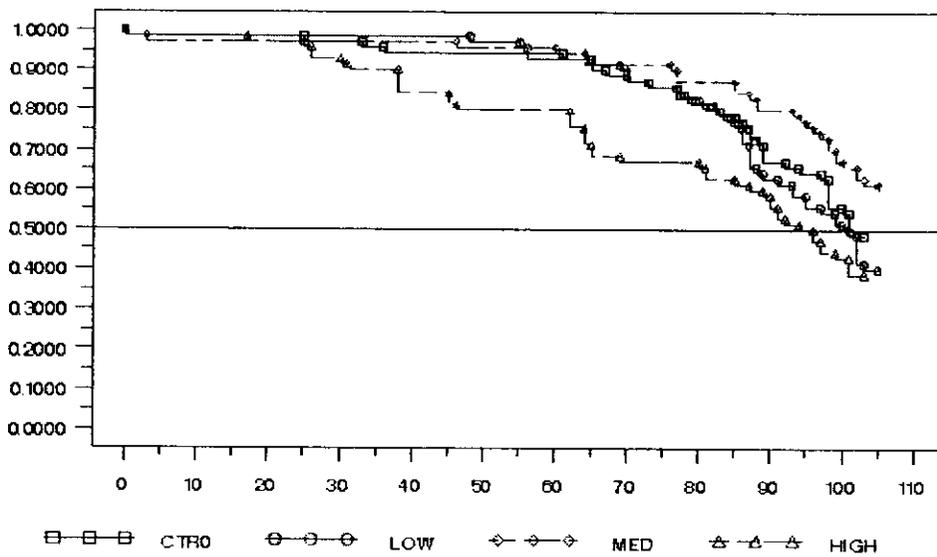
UT	UTERUS	<u>386</u>	M- LEIOMYOSAR COMA	0	0	0	1	0.312	0.0827
UT	UTERUS	<u>392</u>	M- CARCINOMA	3	1	0	0	0.99	0.9626
UT	UTERUS	<u>412</u>	B- LEIOMYOMA	1	1	1	0	0.8222	0.8475
UT	UTERUS	<u>413</u>	M- ENDOMETRIA L STROMAL SARCOM	1	0	0	0	1	0.8234
UT	UTERUS	<u>417</u>	B- HEMANGIOM A	1	2	0	0	0.9148	0.9348
UT	UTERUS	<u>419</u>	M- HEMANGIOSA RCOMA	1	1	0	1	0.5771	0.558
VA	VAGINA	<u>453</u>	M- LEIOMYOSAR COMA	0	1	0	0	0.8108	0.7989

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Appendix 7: Survival Graph of Male Rats



Appendix 8: Survival Graph of Female Rats



Appendix 9: Mortality of Male Rats

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR0	0-52	70	5	65	92.9	7.1
	53-80	65	6	59	84.3	15.7
	81-91	59	11	48	68.6	31.4
	92-104	48	17	31	44.3	55.7
	FINALKILL105-106	31	31	0		
LOW	0-52	70	2	68	97.1	2.9
	53-80	68	11	57	81.4	18.6
	81-91	57	7	50	71.4	28.6
	92-104	50	15	35	50	50
	FINALKILL105-106	35	35	0		
MED	0-52	70	5	65	92.9	7.1
	53-80	65	7	58	82.9	17.1
	81-91	58	8	50	71.4	28.6
	92-104	50	15	35	50	50
	FINALKILL105-106	35	35	0		
HIGH	0-52	70	26	44	62.9	37.1
	53-80	44	6	38	54.3	45.7
	81-91	38	12	26	37.1	62.9
	92-104	26	8	18	25.7	74.3
	FINALKILL105-106	18	18	0		

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Appendix 10: Mortality of Female Rats

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTRO	0-52	70	3	67	95.7	4.3
	53-80	67	9	58	82.9	17.1
	81-91	58	8	50	71.4	28.6
	92-104	50	16	34	48.6	51.4
	FINALKILL105-106	34	34	0		
LOW	0-52	70	1	69	98.6	1.4
	53-80	69	11	58	82.9	17.1
	81-91	58	14	44	62.9	37.1
	92-104	44	15	29	41.4	58.6
	FINALKILL105-106	29	29	0		
MED	0-52	70	2	68	97.1	2.9
	53-80	68	5	63	90	10
	81-91	63	5	58	82.9	17.1
	92-104	58	14	44	62.9	37.1
	FINALKILL105-106	44	44	0		
HIGH	0-52	70	13	57	81.4	18.6
	53-80	57	10	47	67.1	32.9
	81-91	47	8	39	55.7	44.3
	92-104	39	12	27	38.6	61.4
	FINALKILL105-106	27	27	0		

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Appendix 11: Tumor Trend Test of Male Rats

Organ	Organ Name	Tumor Code	Tumor Name	Obs	LOW	Med	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
AC	ADRENAL, CORTEX	<u>172</u>	B-ADENOMA	3	0	2	0	0.845	0.8224
AM	ADRENAL, MEDULLA	<u>328</u>	M-MALIGNANT PHEOCHROM OCYTOMA	0	1	0	0	0.4364	0.7529
AM	ADRENAL, MEDULLA	<u>76</u>	B-PHEOCHROM OCYTOMA	7	4	1	0	0.9973	0.9925
BR	BRAIN W/STEM	<u>122</u>	M- ASTROCYTO MA	2	3	2	1	0.7152	0.7326
BR	BRAIN W/STEM	<u>323</u>	B-GRANULAR CELL TUMOR	0	0	0	1	0.3103	0.0764
BR	BRAIN W/STEM	<u>65</u>	M- OSTEOSARC OMA, MENINGES	1	0	0	0	1	0.7928
CE	CECUM	<u>320</u>	B-POLYP	1	0	0	0	1	0.782
CO	COLON	<u>301</u>	M- CARCINOMA	1	0	0	0	1	0.782
EY	EYE	<u>338</u>	M-SARCOMA NOS, RETROORBIT AL	0	0	1	0	0.3396	0.4444
HN	HEMATO NEOPLASIA	<u>126</u>	M- MALIGNANT FIBROUS HISTIOCY	2	1	0	1	0.6326	0.6474
HN	HEMATO NEOPLASIA	<u>158</u>	M- MALIGNANT LYMPHOMA, UNDIFF	0	1	0	0	0.6039	0.7598
HN	HEMATO NEOPLASIA	<u>272</u>	M- MALIGNANT LYMPHOMA, LYMPHO	0	3	2	0	0.8477	0.7928
HT	HEART	<u>299</u>	M- ENDOCARDIA L SCHWANNOM A	1	0	0	0	1	0.7794
KD	KIDNEY	<u>176</u>	B-ADENOMA, TUBULAR CELL	0	0	0	1	0.3158	0.0849
KD	KIDNEY	<u>332</u>	B-LIPOMA	0	1	0	0	0.7395	0.76
KD	KIDNEY	<u>341</u>	M-RENAL MESENCHYM AL TUMOR	0	0	1	0	0.4454	0.3696

LI	LIVER	<u>212</u>	M-CARCINOMA, HEPATOCELLULAR	0	1	1	2	0.0448	0.0271
LI	LIVER	<u>346</u>	B-ADENOMA, HEPATOCELLULAR	0	1	0	0	0.7395	0.76
LU	LUNG	<u>291</u>	B-ADENOMA, BRONCHOLAR-ALVEOLAR	1	0	0	0	1	0.7944
MM	MAMMARY, MALE	<u>319</u>	M-CARCINOMA	1	0	0	0	1	0.818
MM	MAMMARY, MALE	<u>349</u>	B-FIBROADENOMA	0	1	0	0	0.75	0.7904
PA	PANCREAS	<u>204</u>	B-ADENOMA, ISLET CELL	4	9	2	4	0.6093	0.6136
PA	PANCREAS	<u>211</u>	M-CARCINOMA, ISLET CELL	1	2	1	2	0.2303	0.2259
PI	PITUITARY	<u>33</u>	B-ADENOMA	38	25	25	17	0.9499	0.9479
SQ	SUBCUTANEOUS TIS	<u>210</u>	B-FIBROMA	2	1	4	3	0.0952	0.0876
SQ	SUBCUTANEOUS TIS	<u>280</u>	M-FIBROSARCOMA	0	0	1	0	0.5	0.4211
SQ	SUBCUTANEOUS TIS	<u>289</u>	M-OSTEOSARCOMA	1	0	0	0	1	0.8021
SQ	SUBCUTANEOUS TIS	<u>304</u>	M-CHONDROSARCOMA	1	0	0	0	1	0.8693
SQ	SUBCUTANEOUS TIS	<u>336</u>	M-MYXOSARCOMA	0	0	1	0	0.5	0.1645
SQ	SUBCUTANEOUS TIS	<u>99</u>	M-UNDIFFERENTIATED SARCOMA	1	0	0	0	1	0.8021
SS	SKIN, OTHER	<u>119</u>	B-KERATOACANTHOMA	5	2	3	3	0.3836	0.3816
SS	SKIN, OTHER	<u>170</u>	B-BASAL CELL ADENOMA	1	0	0	0	1	0.834
SS	SKIN, OTHER	<u>263</u>	M-SQUAMOUS CELL CARCINOMA	0	1	0	0	0.7	0.7521
SS	SKIN, OTHER	<u>275</u>	B-SQUAMOUS CELL PAPILOMA	2	0	1	1	0.2598	0.2101
SU	STOMACH, NONGL	<u>274</u>	B-LEIOMYOMA	0	0	1	0	0.4182	0.3526
TE	TESTIS	<u>141</u>	B-INTERSTITIAL CELL TUMOR	1	4	3	1	0.6465	0.6438

TE	TESTIS	<u>310</u>	M- MESOTHELIO MA	0	0	0	2	0.0759	0.0136
TH	THYMUS	<u>202</u>	M-THYMOMA	0	1	0	0	0.7222	0.8062
TY	THYROID	<u>130</u>	M-"C" CELL CARCINOMA	0	2	0	0	0.6704	0.8347
TY	THYROID	<u>208</u>	M- FOLLICULAR CELL CARCINOMA	0	3	0	0	0.8297	0.8971
TY	THYROID	<u>288</u>	B- FOLLICULAR CELL ADENOMA	4	2	0	1	0.8509	0.8746
TY	THYROID	<u>84</u>	B-"C" CELL ADENOMA	4	3	2	2	0.7428	0.7499

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Appendix 12: Tumor Trend Test of Female Rats

Organ Code	Organ Name	Tumor Count	Tumor Name	CTR	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
AC	ADRENAL, CORTEX	<u>172</u>	B-ADENOMA	4	4	2	3	0.6212	0.6334
AM	ADRENAL, MEDULLA	<u>328</u>	M- MALIGNANT PHEOCHROM OCYTOMA	1	0	0	0	1	0.8129
AM	ADRENAL, MEDULLA	<u>76</u>	B- PHEOCHROM OCYTOMA	3	0	1	2	0.2974	0.2682
CV	UTERUS, CERVIX	<u>225</u>	M-SARCOMA, ENDOMETRIA L STROM	1	0	0	0	1	0.8017
CV	UTERUS, CERVIX	<u>334</u>	B-GRANULAR CELL TUMOR	1	0	0	0	1	0.8145
HN	HEMATO NEOPLASIA	<u>126</u>	M- MALIGNANT FIBROUS HISTIOCY	1	0	0	0	1	0.8057
HN	HEMATO NEOPLASIA	<u>158</u>	M- MALIGNANT LYMPHOMA, UNDIFF	0	0	0	1	0.4098	0.1179
HN	HEMATO NEOPLASIA	<u>272</u>	M- MALIGNANT LYMPHOMA, LYMPHO	1	0	1	0	0.6653	0.7363
KD	KIDNEY	<u>276</u>	B- PAPILLOMA, TRANSITIONA L CE	0	0	1	0	0.4561	0.4211
KD	KIDNEY	<u>332</u>	B-LIPOMA	1	0	1	0	0.7808	0.7084
KD	KIDNEY	<u>352</u>	M- CARCINOMA, TRANSITIONA L CE	0	0	1	1	0.1727	0.0864
LI	LIVER	<u>346</u>	B-ADENOMA, HEPATOCELL ULAR	0	1	1	0	0.613	0.6817
MF	MAMMARY, FEMALE	<u>103</u>	B- FIBROADENO MA	14	18	19	9	0.9539	0.9518
MF	MAMMARY, FEMALE	<u>53</u>	M- CARCINOMA	15	9	14	6	0.9551	0.9525
OV	OVARY	<u>190</u>	B- GRANULOSA/ THECA CELL TUMOR	1	0	0	1	0.4361	0.2883
PA	PANCREAS	<u>204</u>	B-ADENOMA, ISLET CELL	2	1	3	3	0.1562	0.1359

PA	PANCREAS	<u>211</u>	M-CARCINOMA, ISLET CELL	1	1	1	0	0.8015	0.7907
PI	PITUITARY	<u>33</u>	B-ADENOMA	56	57	47	40	0.9893	0.9882
PI	PITUITARY	<u>57</u>	M-CARCINOMA	1	0	0	1	0.4339	0.3015
SP	SPLEEN	<u>161</u>	M-HEMANGIOSARCOMA	0	0	1	0	0.4337	0.4621
SQ	SUBCUTANEOUS TIS	<u>210</u>	B-FIBROMA	0	2	1	1	0.7	0.8115
SQ	SUBCUTANEOUS TIS	<u>356</u>	B-LIPOMA	0	0	0	1	0.2	0.033
SS	SKIN, OTHER	<u>170</u>	B-BASAL CELL ADENOMA	0	1	0	0	0.913	0.8808
SU	STOMACH, NONGL	<u>364</u>	B-SQUAMOUS CELL PAPILLOMA	0	1	0	0	0.7193	0.769
TY	THYROID	<u>130</u>	M-"C" CELL CARCINOMA	4	0	3	0	0.8625	0.8736
TY	THYROID	<u>208</u>	M-FOLLICULAR CELL CARCINOMA	1	0	0	0	1	0.7994
TY	THYROID	<u>84</u>	B-"C" CELL ADENOMA	7	1	1	4	0.6137	0.6028
UT	UTERUS	<u>105</u>	M-ENDOMETRIAL STROMAL SARCOM	0	0	1	1	0.1609	0.1236
UT	UTERUS	<u>330</u>	B-ENDOMETRIAL STROMAL POLYP	3	0	3	1	0.6939	0.7104
UT	UTERUS	<u>355</u>	M-LEIOMYOSARCOMA	1	0	0	0	1	0.7951

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/s/

Jasmine Choi
11/9/04 11:46:52 AM
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Roswitha Kelly
11/9/04 02:28:35 PM
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Kooros Mahjoob
11/15/04 04:52:01 PM
BIOMETRICS

Executive CAC

Date of Meeting: October 26, 2004

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-024, Member
Chuck Resnick, Ph.D., HFD-110, Team Leader

Presenting Reviewer and Author of Draft: Jim Willard, Ph.D., HFD-110

NDA #: 21779

Drug Name: Ventavis

Sponsor: CoTherix, Inc.

Background: Ventavis is iloprost, a synthetic prostacyclin derivative being proposed for the treatment of pulmonary arterial hypertension. In 1994, Schering, the developer of iloprost, performed 2 year carcinogenicity studies in both rats and mice with iloprost clathrate in the diet at concentrations of 3 and 10%. ☐

☐ conducted additional 2 year studies, gavage studies, in rats and mice after consulting with the Center's ExecCAC (in 1997) on dose selection. It is not at all clear why the additional studies were done. (The original studies were found by the sponsor and by the reviewer of the CoTherix NDA to be negative and adequately performed. Those studies were not, however, taken to the Center's statisticians or the Exec CAC.) The doses used in the more recent studies ranged from 5-125 mg/kg/day for both rats and mice, while the proposed human dose is 5 µg, up to 9 doses per day for a maximum of 45 µg, or 0.0008 mg/kg/day, for a 60 kg human. It is these studies that are being brought today to the attention of the ExecCAC. CoTherix, the sponsor for Ventavis NDA, is licensed by Schering to develop iloprost for pulmonary arterial hypertension.

Mouse Carcinogenicity Study

The mouse study was an oral gavage study of 104 weeks duration with CD1(ICR)BR albino mice.

Treatment	# males	# females
Control	70	70
5 mg/kg/day	70	70
50 mg/kg/day	70	70
125 mg/kg/day	70	70

Survival rates were sufficient for a valid assay system. At least 25 mice/sex/group survived to 104 months. Statistical analyses by CDER and the sponsor showed no positive trends for survival or tumor incidence.

Rat Carcinogenicity Study

The rat study was an oral gavage dosing study of 104 weeks duration with Sprague-Dawley rats.

Treatment	# males	# females
Control	70	70
5 mg/kg/day	70	70
50 mg/kg/day	70	70
125/100 mg/kg/day	70	70

The ExecCAC had recommended lower doses for males (5, 25, and 50 mg/kg/day) due to reduced body weights and food intake seen at 125 mg/kg/day in a preliminary study. Approximately 8 months into the carcinogenicity study, the conducting laboratory reduced the high dose from 125 mg/kg/day to 100 mg/kg/day due to high mortality. However, the mortality rate remained elevated, indicating that the reduced dose was probably still above the MTD. Nevertheless, survival was sufficient to allow for evaluation and validation of the assay (at least 18 rats/sex/group survived to 104 weeks). Statistical analyses showed no positive trends for tumor incidence.

Executive CAC Recommendations and Conclusions

Rat: The Committee found the study to be acceptable and concluded that there were no drug related tumor findings.

Mouse: The Committee found the study to be acceptable and concluded that there were no drug related tumor findings.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc: NDA 21779
/Division File, HFD-110
Al Defelice/Team leader, HFD-110
Jim Willard/Reviewer, HFD-110
Melissa Robb/CSO/PM, HFD-110
/ASeifried, HFD-024

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/s/

James Willard
12/8/04 10:28:50 AM
PHARMACOLOGIST

Albert Defelice
12/14/04 12:03:23 PM
PHARMACOLOGIST