

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-484

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: **22-484**

SERIAL NUMBER: 1

DATE RECEIVED BY CENTER: 03-31-2009

PRODUCT: TRADENAME (**itraconazole**) **Film-coated Tablets, 200mg**

INTENDED CLINICAL POPULATION: Onychomycosis subjects above ^(b)₍₄₎ years of age

SPONSOR: Stiefel Laboratories, Inc., 20 T.W. Alexander Drive, P.O.Box 14910

Research Triangle Park, NC 27709

REVIEW DIVISION: **Division of Dermatology and Dental Products (HFD-540)**

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EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability: Approvable
- B. Recommendation for nonclinical studies: None
- C. Recommendations on labeling: The old draft for Sporanox (100mg) re-submitted by the sponsor has been corrected and updated.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings:

The non-clinical safety profile of the proposed tablet formulation is essentially based on studies conducted to support the safety of SPORANOX (200mg) tablets (NDA 20-083 approved in 1992) for blastomycosis and histoplasmosis in AIDS patients, and SPORANOX solution for oropharyngeal candidiasis (NDA 20-657 approved in 1997). Both drugs were reviewed and approved by the Division of Antiviral Drug Products. Most of these studies were conducted prior to ICH guidelines in early 1990s. At that time the importance of Safety Pharmacology in the drug development and approval process was not much recognized, therefore, no such studies were specifically planned for itraconazole (ITR) formulations.

No non-clinical studies were ever conducted with the proposed clinical formulation or for that matter with any ITR formulations ever evaluated in HFD-540. In animals as well as in humans, ITR has been proved to be one of the most toxic antifungal imidazoles known.

The oral sub-chronic and chronic studies were conducted in rats (5-160mg/kg/day via gavage or dietary admix) and dogs (5-80mg/kg/day in gelatin capsules). Of two species, rats were much more sensitive to ITR to the extent that no clear cut NOAEL was established in 3-12 month studies. In rats, the adverse effects at the mid- and high-dose levels (20-160mg/kg/day) were severe and life threatening. The lesions/adverse effects included significantly decreased erythrocytes, neutrophils, eosinophils, hematocrit, increased lymphocytes, increased weights of liver, kidneys, heart, thymus, lungs, adrenals and spleen; and increased levels of cholesterol and phospholipids, bone fractures, reduced growth plate (ribs and tibia), reduced thickness (tibia), and hypocellular pulp in the teeth.

In dogs, adverse effects were restricted to hematologic (decreased erythrocytes and thrombocytes), occult blood in the urine, and the irreversible adrenal hypertrophy at the mid- and high-dose levels (10-80mg/kg/day). The NOAEL of 2.5mg/kg established in the 3-month study was extended to 5mg/kg in the 12-month study. The dosing regimen for the proposed itraconazole tablets is 12 weeks.

Over 17 years of its clinical use, ITR had been associated with a spectrum of modest to severe adverse effects. The drug is tolerated at or below 200mg/day level; however,

significant gastric intolerance had been reported at or above 400mg/day level. Less common effects have included hypertriglyceridemia, edema, urticaria, anaphylaxis, erythema multiforme, neuropathy, hypertension, leucopenia, and nephritic syndrome. A few rare cases of hepatic injury and fulminant hepatotoxicity have also been reported. The use of ITR in patients with a history of heart failure and liver disease is contraindicated.

Taking into account a NOAEL of 5mg/kg in dogs, the margin of safety for a 200mg/day clinical dose will be 1.5 times in terms of body weight, and 0.8 times in terms of body surface area. Apparently, the test species (rat, dog) are much more sensitive to ITR than humans, resulting in a very low margin of safety. However, irrespective of the non-clinical safety limits, based on the extensive long-term clinical experience with 200mg ITR twice a day, the treatment for three months with a single daily dose of 200mg is not expected to produce any significant undesirable effects.

During Phase 3 trials, a thermal stress organic impurity (b) (4) at a maximum level of (b) (4) was found in the clinical formulation. To meet the requirement of quantitative impurity specification under ICH Q3B (R2), the sponsor had conducted one short-term animal and two genotoxicity studies. The potential systemic toxicity of (b) (4) was evaluated in a two-week oral rat study (0, 10, and 40mg ITR/kg/day with or without 0.6% impurity). Absolutely, no impurity related systemic toxicity was observed, and the NOAEL for ITR was 40mg/kg with or without the impurity. Assuming 100% absorption, a subject will receive a maximum of (b) (4), providing a safety margin of 927 times of the maximum recommended human dose (MRHD) in terms of body surface area (mg/m^2).

ITR itself tested non-genotoxic in multiple *in vivo* and *in vitro* tests. Also, the drug with or without (b) (4) tested non-mutagenic and non-clastogenic in Ames assay and mouse micronucleus test, respectively.

ITR did not exhibit any carcinogenic potential in mice receiving oral doses up to 80mg/kg/day (2 times MRHD) for 23 months. A slightly increased incidence of soft tissue sarcoma was observed in male rats administered 25mg/kg/day (1.3 times MRHD) for 24 months. These tumors may have been related to hypercholesterolemia caused by chronic treatment with itraconazole in rats; hypercholesterolemia is not observed with such treatment in dogs or humans. Compared to untreated controls, female rats receiving 50mg/kg/day (2.5 times MRHD) had a statistically insignificant increase in squamous cell carcinoma in lungs (2/50), an uncommon tumor in rats.

Itraconazole produced a significant dose-related increase in maternal toxicity, embryotoxicity, and teratogenicity in rats at dose levels of 40-160mg/kg/day (2-10 times MRHD), and in mice at 80mg/kg/day (2 times MRHD). Teratogenic changes in rats included major skeletal defects; encephalocele and/or macroglossia developed in mice.

Itraconazole did not affect fertility in male or female rats treated with oral doses up to 40mg/kg/day (3 times MRHD); however, parental toxicity occurred at this dosage. More severe parental toxicity was observed at 160mg/kg/day (10 times MRHD).

B. Pharmacologic activity: The primary target of ITR is the cell membrane in the fungus. The triazole moiety of ITR binds to the heme iron of the fungal cytochrome P-450 enzymes blocking the oxidation caused by these enzymes. The drug specifically inhibits 14 α -demethylase, which is responsible for the oxidative removal of C-14 methyl group from lanosterol or 24-methylene-dihydrolanosterol. It results in accumulation of lanosterol and some other 14-methylated sterols. These sterols are precursors of ergosterol, an architectural membrane component. Lanosterol and its analog methyl sterols due to their protruding methyl groups do not properly fit in the mosaic of membrane causing it to become permeable to protons and eventual burst. The cumulative damaging ITR effects include changes at the ultra-structural level and inhibition of hyphal outgrowth in *C. albicans*.

The very high selectivity and specificity of ITR for fungal cytochrome P-450s as opposed to those present in the mammalian cells is due to the long tail structure attached to the asymmetric carbon in the triazole moiety that is common to all azole antifungals. The lower host toxicity of triazoles compared to imidazoles has correlated with their lower affinity for mammalian CYP-450 enzymes and lesser propensity to inhibit mammalian sterol synthesis.

The fungal mitochondria also contain high concentration of ergosterol, and its deficiency may also lead to a dysfunctional membrane. In addition, depletion of ergosterol may also interfere with the regulation of chitin synthesis, another integral component of the fungal cell wall.

C. Nonclinical safety issues relevant to clinical use: None

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY:

Itraconazole (ITR) a synthetic broad spectrum antimycotic agent is most commonly used against *Candida* and other yeasts, dermatophytes, and pathogenic fungi. It is also widely employed for the treatment of local and systemic fungal infections. ITR is also a drug of choice for subcutaneous chromoblastomycosis. Its oral formulation has also been used to treat *tinea capitis*. The drug is also effective in the immunocompromised patients. In comparison to other azoles (e.g. ketoconazole), ITR has a long half-life (30-40 hours) with a MIC value lower than any other azole. Like other azoles, it also acts by impairing the synthesis of ergosterol, an architectural component in fungal cell membrane.

Penetration of ITR into CSF is poor. It is preferred over ketoconazole for systemic mycosis not associated with meningitis. The drug does not inhibit human steroidogenesis, as no anti-androgenic and other hormonal effects have been observed with its use.

ITR is equally effective in continuous and pulse therapies. The pulse therapy is also effective and safe in the elderly patients.

ITR after its introduction in 1984 by Janssen Research Foundation of Belgium, was first approved in 1992 (NDA 20-083) as SPORANOX 100mg capsules to treat blastomycosis and histoplasmosis in AIDS patients. Later on, under the same trade name it was approved for the treatment of onychomycosis of toenails (NDA 20-510 in 1995) and fingernails (NDA 20-694 in 1996). Since 1997, SPORANOX (NDA 20-657) solution has been marketed to treat oropharyngeal candidiasis. The injection solution for systemic mycosis was approved in 1999 (NDA 20-996). Various formulations of ITR are globally sold under the trade names of SPORANOX, CANDITRA, and ITASPOR.

The original IND (69, 847/01-20-2005) for the proposed formulation was submitted by Barrier Therapeutics, Inc. Since August 2008, Stiefel Laboratories, Inc. is the legal owner of Barrier Therapeutics, Inc. The New Drug Application is a 505(b)(1) submission. Cross reference letters for NDA 20-083 (Sporanox capsules) and NDA 20-657 (Sporanox oral solution) were included in the NDA submissions which authorized the Agency to cross-reference the nonclinical toxicology data contained in NDA 20-083 and NDA 20-657 to support this NDA submission.

NDA number: 22-484

Review number: 01

Sequence number/date/type of submission: 1/03-31-2009/original
Pre-clinical Information Amendment
(IND 69, 847; #048/SDN-52/05/27/08)

Information to sponsor: None

Sponsor and/or agent: Stiefel Laboratories, Inc; 20 T.W. Alexander Drive,
Research Triangle Park, NC 27709

Manufacturer for drug substance: Janssen Pharmaceutica NV

Janssen Pharmaceuticaaan 3
B-2440 Geel, Belgium

Reviewer name: Kumar D. Mainigi

Division name: Dermatology and Dental Products (HFD-540)

Drug:

Trade name: Hyphanox™ (itraconazole) Film-coated Tablets

Generic name: Itraconazole

Code name: R051211

Chemical names:

±)-1-[(R*)-sec-butyl]-4-[p-[4-[p[[[(2R*, 4S*)-2-(2, 4-dichlorophenyl)-2-(1H-1, 2, 4-triazol-1-ylmethyl)-1, 3-dioxolan-4-yl] methoxy] phenyl]-1-piperazinyl] phenyl]-Δ²-1, 2, 4-triazolin-5-one *mixture with*

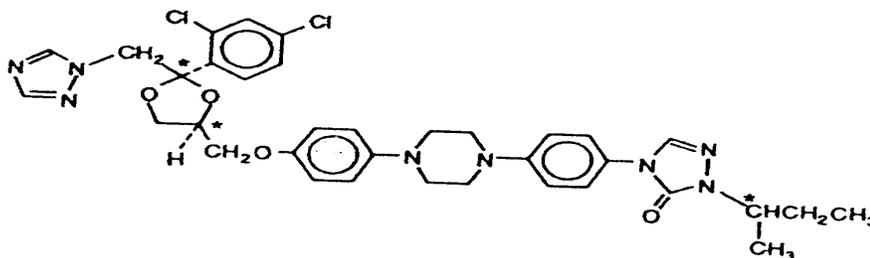
±)-1-[(R*)-sec-butyl]-4-[p-[4-[p[[[(2S*, 4R*)-2-(2, 4-dichlorophenyl)-2-(1H-1, 2, 4-triazol-1-ylmethyl)-1, 3-dioxolan-4-yl] methoxy] phenyl]-1-piperazinyl] phenyl]-A'-1, 2, 4-triazolin-5-one OR

±)-1-[(R*)-sec-butyl]-4-[p-[4-[p[[[(2R, 4S)-2-(2, 4-dichlorophenyl)-2-(1H-1, 2, 4-triazol-1-ylmethyl)-1, 3-dioxolan-4-yl] methoxy] phenyl]-1-piperazinyl] phenyl]-Δ²-1, 2, 4-triazolin-5-one

CAS registry number: 84625-61-6

Molecular formula/molecular weight: C₃₅H₃₈Cl₂N₈O₄/705.64

Structure:



Relevant INDs/NDAs/DMFs:

INDs: 24, 313...itraconazole capsules, 100mg

33, 324...itraconazole capsules 100mg

37, 562...itraconazole oral solution, 10mg/mL

48, 217...itraconazole injection, 10mg/mL

(b) (4)

69, 847...Hyphanox (itraconazole) Film-coated Tablets

NDAs: 20-083...systemic mycosis (approved 09-11-1992)

20-510...onychomycosis of toenails (approved 09-28-1995)

(b) (4)

20-657...oral solution for fungal infection (approved 02-21-1997)

20-694...onychomycosis of fingernails (approved 12-06-1996)

(b) (4)
20-966...injection for the treatment of fungal infection (03-30-1999)

Drug class: Antifungal

Indication: Treatment of onychomycosis of the toenail (b) (4)

Intended clinical population: Subjects (b) (4) years or older

Clinical formulation:

<u>Ingredient</u>	<u>Mg/tablet¹</u>	<u>Function</u>
(b) (4)		
Itraconazole	200.00	Active ingredient
Hypromellose (b) (4) USP	(b) (4)	(b) (4)
(b) (4) lactose		
(b) (4) and		
microcrystalline cellulose (b) (4)		
Crospovidone, NF		
Talc, USP		
Hydrogenated vegetable oil (b) (4) NF		
Colloidal silicon dioxide, NF		
Magnesium stearate, NF (b) (4)		
(b) (4)		
Propylene glycol, NF (b) (4)		
Hypromellose (b) (4) USP		
Propylene glycol, NF		
Titanium dioxide (b) (4), USP		
Talc, USP		
Total film-coated tablet		
(b) (4)		

Route of administration: Oral

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

Studies reviewed within this submission:

1. Two-week oral toxicity study with impurity (b) (4)
2. Ames assay with (b) (4)
3. Mouse Micronucleus assay with (b) (4)

Studies not reviewed within this submission: N/A

2.6.2 PHARMACOLOGY: No new studies were reported.

2.6.2.1 Brief summary: Dermatophytes are responsible for almost 90% cases of onychomycosis of toenails and at least 50% cases of fingernail infections. *T. rubrum* and *T. mentagrophytes* are the most common dermatophytes implicated in these infections. Nondermatophytes such as *Candida albicans* and *Scopulariopsis* species have been also identified as etiologic agents of onychomycosis.

On the whole, ITR (SPORANOX capsules) is approved for the treatment of fungal infections such as blastomycosis, histoplasmosis, aspergillosis, and onychomycosis.

2.6.2.2 Primary pharmacodynamics: The primary target of ITR is the cell membrane in the fungus. The triazole moiety of ITR binds to the heme iron of the fungal cytochrome P-450 enzymes blocking the oxidation caused by these enzymes. The drug specifically inhibits 14 α -demethylase, which is responsible for the oxidative removal of C-14 methyl group from lanosterol or 24-methylene-dihydrolanosterol. It results in accumulation of lanosterol and some other 14-methylated sterols. These sterols are precursors of ergosterol, an architectural membrane component. Lanosterol and its analog methyl sterols due to their protruding methyl groups do not properly fit in the mosaic of membrane causing it to become permeable to protons and eventual burst. The cumulative damaging ITR effects include changes at the ultra-structural level and inhibition of hyphal outgrowth in *C. albicans*.

The very high selectivity and specificity of ITR for fungal cytochrome P-450s as opposed to those present in the mammalian cells is due to the long tail structure attached to the asymmetric carbon in the triazole moiety that is common to all azole antifungals. The lower host toxicity of triazoles compared to imidazoles has correlated with their lower affinity for mammalian CYP-450 enzymes and lesser propensity to inhibit mammalian sterol synthesis.

The fungal mitochondria also contain high concentration of ergosterol, and its deficiency may also lead to a dysfunctional membrane. In addition, depletion of ergosterol may also interfere with the regulation of chitin synthesis, another integral component of the fungal cell wall.

ITR is a racemic mixture of four diastereomers (two enantiomeric pairs). Each isomer has varying activity against different fungi. As a result, the racemic mixture offers the widest range of antifungal activity.

2.6.2.3 Secondary pharmacodynamics: Because triazoles are also active against certain bacteria as well (which do not have ergosterol), some (currently unknown) secondary

mechanisms of action also appear to be involved. The development of resistance to azole antifungals in yeasts is well known. The cross-resistance between ITR and fluconazole is frequent but not constant. *C. krusei* and *T. glabrata* are intrinsically resistant to itraconazole. The proposed mechanisms for resistance include reduced permeability of the cell membrane and a mutation in the target fungal enzyme cytochrome P-450. It is also suggested that the wide use of ITR can increase the colonization and infection rates with resistant species in the compromised patients. Development of fungal resistance to azoles has been noted among *Candida* infecting advanced AIDS patients, but has not so far posed significant clinical problem.

2.6.2.4 Safety pharmacology: No new studies were conducted.

Most of the non-clinical safety data for ITR were evaluated in the early 1990s. At that time, the importance of safety pharmacology in the drug approval process was not much recognized. Therefore, except for a few short-term cardiovascular studies conducted in late 1990s, no additional studies were specifically planned to evaluate the drug effect (s) on other vital organ functions. Furthermore, all studies were conducted with Sporanox, none with formulations reviewed in HFD-540. The information provided below was extracted from 1990-92 reviews of Dr. James G. Farrelly (IND 37, 562 and NDA 20-083) from the Division of Anti-viral Drug Products.

Neurological effects: The effects of peritoneal and oral ITR (40mg/kg) on the behavior were investigated in a few general toxicology studies in mice and rats. No significant differences in neurological and behavioral tests were observed. In addition, oral ITR in rats had no effect on food consumption, fecal and urine excretion, castor oil diarrhea, tail withdrawal reaction time, or *Mycobacterium butyricum* arthritis.

Cardiovascular effects: The potential cardiovascular effects of ITR were investigated in several studies in isolated tissues and animals.

In conscious dogs, except for a slight decrease in the systolic blood pressure, oral dose of 500mg ITR did not exhibit any significant change in any other cardiovascular parameters. A single oral dose of 20mg ITR/kg had no effects except, LV dp/dt max tended to decrease slightly at 165 and 210 minutes post-dose only, and the heart rate tended to increase slightly at 60 and 75 minutes post-dose only, causing a modest reduction of stroke volume at intervals between 60-150 minutes.

A group of anesthetized dogs received infusion of 2.5mg/kg solution for one hour, or 10mg/kg solution over one, two, or three hours period. The end results indicated cardiac depression and decreased left ventricular ejection fraction starting at 10 minutes post-dose with a continuous decrease throughout the infusion period. After the infusion, the changed values of all the tested cardiovascular parameters returned to the pre-dose levels, indicating reversible drug effect(s).

In the second study, non-anesthetized (conscious) dogs received daily one hour intravenous infusion of 5, 7.5, and 10mg ITR/kg for 14 consecutive days. The drug had no marked effect on the arterial (systolic, diastolic or mean) blood pressure throughout

the treatment period. Furthermore, no significant effect was observed on the PR interval, QRS duration or QTc interval. However, the heart rate remained consistently high at the highest dose level; a similar effect was observed at the mid-dose level on day 13.

In an oral study, seven conscious dogs received 18mg/kg of drug in gelatin capsules. A variety of cardiovascular changes were determined up to four hours post-dose. A slight transitional increase in systolic blood pressure was immediately recorded. It was followed by a slight decrease in the same pressure between 90 and 160 minutes post-dose.

Based on the outcome of similar observations in humans, the treatment for onychomycosis with ITR is contraindicated in patients with ventricular dysfunction.

In rabbit Purkinje fibers incubated with ITR, the drug did not exhibit any significant effect on the amplitude of the action potential, duration of the action potential, resting membrane potential, effective refractory period, recovery time and the maximum upstroke velocity.

ITR at micromolar and higher concentrations produced direct negative chronotropic and ionotropic effects in isolated spontaneously beating guinea pig right atria. Drug at micromolar levels also inhibited contractions of isolated rat caudal arteries by Ca^{++} ions suggesting a possible calcium entry blocking action.

Pulmonary effects: No studies were conducted.

Renal effects: Effects were not investigated.

Gastrointestinal effects: *In vitro*, ITR up to 40mg/mL level had no effect on the electric potential difference of gastric mucosa; 100mg/kg drug caused no significant damage to the gastric mucosa.

Abuse liability: Not Known.

Other: N/A

2.6.2.5 Pharmacodynamic drug interactions: Oral absorption of ITR is reduced by antacids, H_2 blockers and proton pump inhibitors. Rifampin, phenobarbitone, phenytoin and carbamazepine induce ITR metabolism and reduce its efficacy. On the other hand, clarithromycin and HIV protease inhibitors reduce the metabolism of ITR and raise its blood level.

2.6.2.6 2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.4 PHARMACOKINETICS/TOXICOKINETICS:

No non-clinical studies were conducted with the new formulation. The pharmacokinetic profile of ITR is mostly based on the studies conducted with Sporanox tablets (NDA 20-083) and Oral Solution for Antifungal Infection (NDA 20-657).

2.6.4.1 Brief summary: The pharmacokinetic behavior of oral ITR was investigated in multiple species (rats, guinea pigs, rabbits, cats and dogs). In addition, intravenous doses were administered to determine the absolute bioavailability of the drug. Irrespective of large inter-individual variations recorded in the pharmacokinetic parameters, after single oral dose, ITR was rapidly absorbed in all the species achieving C_{max} at 1-4 hours post-dose. T_{max} was lowest in the rat and highest in the rabbit. In animals as well as in humans, the absorption of solid formulations of ITR is much influenced by gastric acidity, H₂ blockers and proton pump inhibitors. The oral bioavailability of drug is highest when administered after a full meal. However, the absorption of liquid formulations is not affected by food or gastric acidity, and in fact the absorption of such formulations is highest when administered empty stomach. In humans, the steady-state for drug is achieved in 4 days with a half-life of 30-40 hours.

The pharmacokinetic studies in animals and humans have revealed a great affinity of ITR for tissues. Thus, the drug is found at higher concentration levels in tissues than in the plasma. The levels of drug in the skin, sebum, pus, and many fat rich organs are much greater than the corresponding plasma concentrations. The mucous membrane and skin retained the therapeutic concentrations of drug for 1-4 weeks after discontinuation of treatment.

The placenta in rats is semi-protective, and ITR is transported through it to the fetus. The drug was also found in the milk. The highest levels of drug related radioactivity in fetus were found in the mammary glands, ovaries, fetal membrane, and uterus.

In animals, ITR and its active metabolite hydroxyitraconazole (HyITR) are widely distributed in the body, with highest concentrations found in the adrenals, liver, and peri-portal fat. A minimal amount of drug entered the brain. *In vitro* protein binding of ITR was uniformly high (~99.8%) in rat, dog, and human. *In vivo*, the albumin was the main binding protein.

In animals as well as in humans, drug is extensively metabolized in the liver by CYP3A4 isoenzyme system to a large number of metabolites. The anti-fungal activity of the major metabolite HyITR is similar to ITR. In rats, a distinct difference in the rate of metabolism between the two sexes was observed. Though females were exposed to a greater amount of the parent drug, a greater portion of the drug was metabolized in the males.

In rats, more than 90% of the metabolites are excreted in the feces within 2-4 days. In first two days, the excretion in females was low. At 48 hours post-dose, in males about 53% of the radioactivity was found in the feces; this amount was twice that found in females. The urinary excretion in males and females was 7 and 4.5% of the administered radioactive dose, respectively. In dogs, within a week, about 17% and 65% of the radioactivity was excreted in the urine and feces, respectively.

In humans, the pharmacokinetic behavior of ITR was investigated in a 14-day study using a single 200mg film-coated tablet per day. Peak plasma concentrations at steady state for ITR and HyITR were 658 and 974ng/mL, respectively; the corresponding values for AUC₀₋₂₄ were 9047 and 19055ng*h/mL, respectively. At the clinical dose level, the

exposure levels in humans were several folds greater than recorded at NOAEL in rodents, but lower in dogs.

In humans, 3-18% of the dose is excreted in the feces; the urine elimination is less than 0.03 percent. No single metabolite contained more than 5% of the administered radioactive dose.

2.6.4.3 Absorption: In most test species, oral absorption of ITR is rapid, reaching C_{max} within 1-4 hours. In general, the plasma drug concentration was related to the nature of the formulation. For instance, at the same dose level of 10mg/kg, the peak plasma drug concentration was approximately 0.3µg/mL for a PEG formulation and 1µg/mL for a solution formulation. Especially, the absorption in dogs was highly formulation dependent. Thus, the absolute bioavailability in dogs from the oral formulations (10mg ITR/kg/day) ranged from 9-28% with PEG as carrier to 48% with β-cyclodextrin as a vehicle for the intravenous solution form.

A lowest T_{max} was recorded in rats and greatest in rabbits.

In a 10-day oral study (10mg/kg/day) in female rats, the steady-state both for ITR and HyITR were achieved by 6 days of dosing. The average steady-state trough plasma level, determined at 24 hours after the last dose were 0.56µg/mL for ITR and 0.75µg/mL for HYITR. The C_{max} of 1.02µg/mL was achieved for ITR at 6 hours post-dose after repeated drug administration.

In the second 10-day oral rat study at 20mg/kg/day dose level, the steady-state level was achieved in two days in males (2.69 µL/mL) and four days in females (0.55 µL/mL). In a 30-day gavage study (0, 10, 40, 160mg/kg/day) in rats, the plasma drug levels were greater in females at each dose level, and this difference increased with the dose.

In three-month oral study (0, 5, 10, and 20mg/kg/day) in dogs, the combined (4 males+4 females) pharmacokinetic data indicated a dose-related increase in C_{max} and AUC₀₋₂₄ between days 1 and 86 (Table1).

Table1. A summary of combined (male+female) pharmacokinetic data in dogs

Parameter	Low-dose		Mid-dose		High-dose	
	Day1	Day86	Day1	Day86	Day1	Day86
C _{max} (µg/mL)	0.5	1.0	1.0	2.9	1.7	3.6
T _{max} (hr.)	1.8	1.8	2.5	3.0	2.9	2.8
AUC ₀₋₂₄ (µg.h/mL)	4.9	14.8	12.7	44.9	19.9	61.4

A similar pattern was observed in one-year oral dog study (0, 5, 10, 20, and 80mg/kg/day).

The pharmacokinetic behavior was investigated in pregnant rats following a single oral dose (10mg/kg) on gestation day 18. At T_{max} of 4 hours, the C_{max} was only 60% that observed in the non-pregnant controls. The ratio of parent drug to metabolites was 1:2.

Using a single oral and intravenous dose of ^3H -itraconazole (10mg/kg), the placental transfer (gestation day 18) was investigated in rats by whole-body autoradiography. The amount of radioactivity in the placenta was much higher than in the fetus; the amount in fetus hardly exceeded the background counts.

In another rat study, following an oral dose of ^3H -ITR (10mg/kg) on gestation day 18, the levels of the parent drug and metabolites in the plasma and tissues were determined for 4 days. The maximum levels of radioactivity in the fetuses were achieved at 8 hours post-dose; however, all the fetuses together contained only about 1% of the maternal dose. The levels in fetuses were 2-3 times lower than the maternal blood and 6 times lower than in the placenta. However, on day 4, the levels in the fetus and placenta were similar. The elimination of ITR and metabolites was much less efficient from the fetal tissues than from the maternal tissues. By 8 hours, most (62-99%) of the fetal radioactivity was due to parent drug; in contrast most of the radioactivity in the placenta was related to the metabolites. High amounts of radioactivity in the fetus were found in the mammary gland, ovaries, fetal membrane, and uterus.

Due to its poor water solubility ($<1\mu\text{g/mL}$) and low bioavailability ($\sim 50\%$), ITR is categorized as a class II drug according to the Biopharmaceutics Classification System. This property leads to a highly variable and erratic oral absorption. In humans, the oral bioavailability of drug is highest when administered right after the full meal.

2.6.4.4 Distribution: The distribution of ITR and HyITR has revealed that the drug and its metabolites can be found in most tissues at a greater concentration than in plasma.

The distribution of ITR and HyITR was investigated in a 10-day gavage study in female rats (10 groups of 4 rats each) receiving 10mg/kg/day of ITR solution containing 400mg/kg/day of hydroxypropyl- β -cyclodextrin. Blood samples were collected from some groups at 2, 4, and 8 hours after the first dose, pre-dose and at 6 hours after the 4th and 7th dose, and at 2, 4, and 8 hours after the last dose. Tissue and blood samples were collected from other groups at 6, 24, 48, and 96 hours after the first and last dose. The distribution profile is given in table 2.

Table2. Tissue distribution¹ in female rats in a 10-day oral solution study at 10mg/kg/day dose level.

Tissue	-Time (hours) of sample collection-					
	6		24		48	
	ITR	HyITR	ITR	HyITR	ITR	HyITR
Plasma	1.02	1.23	0.66	0.86	0.12	0.23
Brain	0.45	0.06	0.32	ND	0.08	ND
Heart	2.40	1.23	1.90	1.03	0.36	0.34
Lung	1.85	1.47	1.99	1.39	0.29	0.36
Liver	14.60	12.20	7.12	8.18	1.73	3.51
Kidney	5.24	3.64	3.39	2.53	0.62	0.86
Adrenal	26.70	43.40	17.40	31.80	3.40	13.10
Pancreas	4.88	3.16	3.92	2.90	0.73	0.99
Stomach	11.10	0.87	1.61	0.81	0.26	0.22
Small intestine	5.54	3.01	2.10	2.43	0.34	0.69

Large intestine	2.51	1.42	2.12	1.60	0.20	0.27
Ovaries	4.43	0.67	3.21	0.79	0.45	ND
Skin	1.06	0.38	0.59	0.27	ND	ND
Fat (peri-portal)	13.80	ND	14.80	ND	3.55	ND

ND= not detected. The detection limit for HyITR varied between the tissues

¹ µg/mL or µg/g wet tissue

ITR and HyITR were widely distributed with the highest concentrations found in the adrenals, liver, and peri-portal fat. A distinct inter-tissue variation in metabolism was also apparent. However, because the detection limit among the tissues was not uniform, the values in the table 2 should be considered as semi-quantitative. The ratio of ITR to HyITR varied from 0.078 (6 hours post-dose in stomach) to 3.85 (in adrenals at 48 hour), providing an approximate difference of 49-fold.

In the rat dietary study (9 rats /sex), animals received 20mg/kg/day of ITR for ten days. Blood and tissue samples were collected after the last dose. A distinct difference in ITR metabolism in two sexes was observed (Table3). Though females were exposed to a greater amount of the parent drug, a much greater portion of it was metabolized in the male organs. In both sexes, the concentration of HyITR was in the following decreasing order: adrenals, liver, kidneys, and lungs. A minimal amount of drug entered the brain.

Table 3. Tissue distribution in rats fed 20mg ITR/kg/day for 10 days.

<u>Organ</u>	<u>Males</u>		<u>Females</u>	
	<u>ITR</u>	<u>HyITR</u>	<u>ITR</u>	<u>HyITR</u>
Plasma	0.55	1.31	2.69	2.10
	(70) ^a		(43)	
Brain	0.14	ND	1.47	0.04
			(3)	
Lung	0.86	2.12	11.20	8.05
	(71)		(41)	
Liver	4.71	18.8	34.5	31.8
	(80)		(48)	
Kidney	2.37	7.59	17.2	14.4
	(76)		(46)	
Adrenal	7.25	32.8	72.8	73.4
	(82)		(50)	
Muscle	0.57	0.62	5.67	1.74
	(52)		(23)	

^a= Percent of parent drug metabolized into active metabolite

In the six-month multi-dose (0, 10, 40, 160mg/kg/day) rat study, a dose-related non-linear increase in the plasma and tissue levels of ITR and HyITR was observed in the terminal samples (Table 4).

Table 4. Tissue concentration ($\mu\text{g/mL}$ or g of wet tissue) of ITR and HyITR in a six-month dietary study (10, 40, and 160mg/kg/day) in rats.

<u>Tissue</u>	<u>Males</u>		<u>Females</u>	
	<u>ITR</u>	<u>HyITR</u>	<u>ITR</u>	<u>HyITR</u>
<u>Low-dose</u>				
Plasma	0.05 (76) ^a	0.16	0.63 (52)	0.68
Lung	0.18 (69)	0.41	3.09 (25)	1.02
Liver	0.71 (76)	2.31	8.81 (31)	3.99
<u>Mid-dose</u>				
Plasma	0.68 (58)	0.95	4.32 (43)	3.26
Lung	3.15 (76)	10.1	32.6 (23)	9.83
Liver	10.1 (50)	10.3	49.9 (29)	20.4
<u>High-dose</u>				
Plasma	4.37 (42)	3.27	5.12 (52)	5.47
Lung	48.1 (22)	13.6	73.9 (25)	25.2
Liver	69.5 (33)	34.8	94.3 (33)	46.4

^a= Percent of ITR metabolized to HyITR

As in the short-term studies, females contained more ITR, but the concentration of HyITR was much greater in males. In general, in males the tissue concentration of HyITR significantly decreased with the dose, indicating saturation of enzyme (s) involved in its formation. No such pattern was observed in females.

In a 90-day oral dog study (0, 5, 20, or 80mg ITR/kg/day), the tissue levels of the parent drug were determined at 24 hours after the last dose and after one month recovery. The highest drug concentrations were found in the peri-renal fat, adrenal glands and liver at 30, 23, and 17 times the plasma level, respectively. The corresponding values for kidney, heart, and lung were 4-8 times higher. The concentration in the brain was 2 times greater than plasma. At each dose level, the tissue plasma ratios remained constant, indicating no significant drug accumulation in the tissues. At the end of recovery period, the tissue levels of ITR in the high-dose groups were below the detection limit.

A whole body autoradiography study was conducted in pregnant rats (on gestational day 18) after 24 hours of 10mg/kg of oral or intravenous dose of ³H-ITR. Following the oral dose, the highest amounts of radioactivity at 4 hour were found in the liver, the adrenal

cortex, the uterine wall, the vaginal fluid and GI-tract. Medium levels of radioactivity were found in the glandular tissues, uterus, placenta, kidneys, lungs, heart muscle and tongue. The lowest amounts were found in the urine, blood, brain, and fetal tissues. A somewhat comparable distribution pattern was recorded after the intravenous dose.

The *in vitro* plasma protein binding of ITR is very high (99.8%) in rat, dog and human. Because of greater binding to plasma proteins than the blood cells, the blood/plasma ratios were low (rat 0.68, dog 0.61, and in human 0.58). Albumin was the main binding protein.

In humans, ITR has large volume of distribution (10L/kg) with pronounced accumulation in vaginal mucosa, skin, and nails; however, its penetration in CSF is poor. Almost 100% of ITR and HyITR are bound to proteins.

2.6.4.5 Metabolism: In rats and dogs, ITR is extensively metabolized in the liver by CYP3A4 isoenzyme system, and because of its complex structure and existence as a racemic mixture it is metabolized into more than 30 compounds. Most of the metabolites account for less than 1% of the administered dose. ITR also inhibits metabolism of other drugs by occupying CYP3A4 system. This enzyme complex found both in the intestine and liver, exhibits a wide subject variation, leading to a large variation in drug bioavailability.

The prominent metabolic pathways are oxidative scission of the dioxolane ring, aliphatic oxidation at the 1-methylpropyl substituent, N-dealkylation at the substituent, oxidative degradation of the piperazine ring, and triazolone scission. Most of the compounds have lost triazole and dichlorophenyl moieties.

The metabolic profiles are similar in rats and dogs, but some quantitative differences were observed. The formation of excretory metabolites results from the dioxolane scission. More than 20 urinary metabolites have been detected in rat and dog; no individual metabolite accounted for more than 1% of the administered ITR dose. Whereas the low molecular weight (<400 daltons) metabolites were excreted in the urine, the rest (<500 daltons) were found in the feces. Hardly any conjugates were present.

The major urinary metabolite an omega-acid of ITR (R61465) in male rats and male dogs was also the major biliary metabolite in male rats, accounting for 9% of the dose; it was also the major metabolite in the enterohepatic circulation. Furthermore, the limited enterohepatic circulation in female rats was due to a relatively smaller amount of R61465 formed in the female rats. No ITR was detected in the urine.

In feces, the parent drug accounted for 22% of the dose in male rats, 29% in the female rats, and 26% in male dogs. None of the fecal metabolites accounted for more than 10% of the dose in rats, and for more than 5% in dogs.

The antifungal activity of the excretory metabolites was insignificant. The antifungal activity of HyITR was similar to ITR.

In humans, the pharmacokinetic behavior of ITR was investigated in a 14-day study using a single 200mg film-coated tablet per day. Peak plasma concentrations at steady state for

ITR and HyITR were 658 and 974ng/mL, respectively; the corresponding values for AUC₀₋₂₄ were 9047 and 19055ng*h/mL, respectively. Reportedly, at the clinical dose level, the exposure levels in humans were several folds greater than recorded at NOAEL in rodents, but lower in dogs.

In human plasma, ITR and HyITR are usually present in almost equal amounts.

2.6.4.6 Excretion: The excretory profile of drug was investigated following a single oral dose of ³H-ITR to rats of both sexes (10mg/kg) and male beagle dogs (2.5mg/kg). In addition, using the bile-cannulated rats, the first pass metabolism was also investigated.

In rats, more than 90% of the metabolites were excreted in the feces within 2-4 days. In the first two days, the excretion in females was low. The urinary excretion in males and females was 7 and 4.5% of the administered dose, respectively. In males at 48 hours, approximately 53% of the administered radioactivity was found in the bile, it was almost twice the amount found in females.

In dogs, within one week, approximately 17% of the radioactivity was excreted in the urine, and 64.5% was found in the feces.

The terminal t_{1/2} for elimination ranged between 6-10 hours in male rats; t_{1/2} in female rats is twice longer. The apparent t_{1/2} of HyITR was significantly lower than ITR in rats and dogs, while they were similar in rabbits.

In humans, 3-18% of the administered dose is excreted in the feces; the urinary excretion is less than 0.03 percent. Most of the urinary metabolites lack antifungal activity. No single excretory metabolite accounts for more than 5% of the dose.

2.6.4.7 Pharmacokinetic drug interactions: ITR significantly inhibits P450 cytochrome enzymes 3A4 and 2B6, P-glycoprotein function, and active tubular flux of several drugs in kidneys. Thus, the number of drugs interacting with ITR is continuously expanding. Like other azoles, ITR also inhibits P-450 hepatic isoenzymes, causing increase in the plasma levels of other drugs by blocking their catabolism and elimination. The drugs in this category include anticoagulants, oral hypoglycemic agents, and phenytoin. The specific inhibition of liver CYP3A4 by ITR has caused ventricular arrhythmias when concomitantly given with antihistaminic drugs such as terfenadine and astemizole. On the other hand, rifampin, phenytoin and carbamazepine induce ITR metabolism reducing its efficacy.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

Acute Toxicity Studies: In the acute oral (20-320mg/kg) studies, no significant deaths were recorded in the test species (mouse, rat, guinea pig, and dog). The test preparations contained either hydroxypropyl β-cyclodextrin (HPBCD) or propylene glycol (PEG) as a vehicle. The calculated LD₅₀ values in mouse and rat were greater than 320mg/kg; for

guinea pig value ranged between 160 and 320mg/kg, and in dog it was greater than 200mg/kg. The major clinical signs were related to the central nervous system, followed by disturbance in the gastrointestinal system. The effect on the GI-tract was related to HPBCD; the neurological changes also observed with the intravenous doses were considered to be drug related. However, necropsies did not reveal any associated macroscopic lesions.

Repeat Dose Toxicity Studies: Three 90-day oral studies conducted in mice, rats and dogs used PEG 400 as a vehicle.

Protocols and critical findings:

<u>Species</u>	<u>Dose</u> (mg/kg/day)	<u>Administered</u>	<u>Duration</u>
<u>Study 1</u>			
Mice (n=?)	0, 5, 20, 80	gavage	3-month

Critical findings: An increase in alanine and aspartate aminotransferases (ALT and AST) at the high-dose level was associated with relevant histopathologic changes in the liver and adrenals. An increase in ALT and AST activities also observed at the mid-dose level was not associated with any relevant microscopic changes in the liver. The low-dose was established as a NOAEL.

Study 2

Rats 20/sex	0, 10, 40, 160	gavage	3-month
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Critical findings: Adverse effects were much more severe at the high-dose level, especially in females; deaths of 4 males and 8 females were drug related. Changes in the mid- and high-dose animals included decreased erythrocytes, neutrophils, eosinophils, hematocrit, and increased lymphocytes; and increased liver, kidney, heart, and thymus weights, and increased cholesterol level in females. High-dose males had reduced bone growth, fat deposits in zona glomerulosa and zona reticularis of adrenals, and foamy macrophages in thymus. In low-dose females, circling disease, and increased cholesterol level were recorded. No clear cut NOEL was established.

Study 3

Rats (20 and 10 for one-month recovery)	0, 5, 20, 80	gavage	3-month
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Critical findings: No drug related deaths occurred. Cholesterol and phospholipids levels were significantly increased in both the sexes at all dose levels, however, the values returned to normal at recovery. An increase in the liver and adrenal weights in the mid- and high-dose males and the entire drug treated females was recorded. On the other hand, an increase in kidney weights was observed the in mid- and high-dose females and all the drug treated males. An increase in foamy cells in the lungs of mid- and high-dose females

was apparent. At the end of recovery period, the liver and adrenals in females were still enlarged, and foamy cells were still present in the lungs. No clear cut NOEL was established.

Study 4

Rats

(20) 0, 10, 40, 160 dietary admix 6- month

Critical findings: One dead high-dose male had swollen lungs, spleen and lymph nodes. During the last two months of the study, 15/20 high-dose males died; pronounced lesions were found in the lungs (foci with a swollen or irregular surface), tongue (foci and stippling), and swollen adrenals, lymph nodes, and spleen. In the high-dose rats, erythrocytes, hematocrit, Hb, and thrombocytes decreased, more drastically in males. Cholesterol level was increased in all the drug treated animals. A dose-related decrease in urine creatinine and occult blood in females, and a dose-related increase in granular casts were observed in both the sexes. The weights of lungs, spleen, liver and adrenals were increased in both the sexes.

Gross lesions at the mid- and high-dose levels in both the sexes included swollen adrenals, and bone fractures in low-dose males. At the mid- and high-dose levels, reduced growth plate (rib, tibia), and the hypocellular pulp in the teeth were observed. Microscopic lesions in the high-dose males and females included fibrosis, vacuoles, and pigmentation in adrenals, bone fractures, pigmentation, and swollen or vacuolated tubules in kidneys, and foamy cells in lungs. Lesions were more pronounced in females. No clear cut NOEL was established.

Study 5

Rats

(20) 0, 5, 10, 80 dietary admix 12-month

Critical findings: The drug-related deaths occurred at all the dose levels, more so in females. All drug related changes were similar to that observed in the 6-month study. Once again, no NOEL was established.

Study 6

Dogs

(3) 0, 2.5, 10, 40 gelatin capsules 3-month

Critical findings: No deaths occurred. Absolute and relative weights of adrenals were significantly ($p < 0.01$) increased and thymus weights were markedly decreased in the mid- and high-dose dogs. The associated microscopic lesions included discernible hypertrophy of the adrenal cortex and marginal lymphatic hyperplasia in the thymus. The foamy macrophages in the lungs of the mid- and high-dose animals were observed. The NOEL was 2.5mg/kg.

Study 7

Dogs

Carcinogenicity: ITR-mixed diets were fed to mice (2.5, 10, and 40mg/100 g food) for 23 months and to rats (5, 20, and 80/40mg/100g food) for 24 months. In the rat study, the highest dose due to severe toxicity was reduced to 40mg in week 15. Based on the body weight and food consumption data, the approximate concentrations of drug (mg/kg bw) administered were as follows: Mice: 5, 20, and 80. Male rats: 3.2, 13.4, and 25.5; Female rats: 4.7, 22.5, and 52.4.

In inter-group comparison in mice, the high-dose females had a higher mortality rate. A dose-related increase in pigmentation of adrenal glands was also observed. However the tumor burden and rate of tumor formation were similar in the drug treated and control mice.

In high-dose male rats, a slightly higher incidence of soft tissue sarcoma was probably due to hypercholesterolemia resulted from chronic ITR treatment; however, such treatment did not produce sarcomas in dogs and humans. In addition, high-dose in males was also associated with decreased gain in body weight, slightly increased mortality and a few hematological changes. Fibroxanthomatosis-like changes more prominent in the mid- and high-dose rats caused inflammatory reactions, secondary to high cholesterol levels produced by systemic disturbances in lipid and cholesterol metabolism. These changes were also considered to be species-specific.

No statistically significant oncogenic changes were observed in ITR treated female rats.

Reproductive toxicology: One segment I, nine segment II, and one segment III studies were conducted.

In rat segment I gavage study (0, 10, 40, and 160mg/kg/day), no adverse effects on fertility were observed at the mid- and high-dose levels. The severe systemic toxicity in the high-dose rats caused deaths in males (3/24) and females (16/24). In the surviving females, the decreased pregnancy rate was associated with an increased rate of resorption. A statistically significant incidence of wavy ribs was observed in the mid- ($p<0.01$) and high-dose ($p<0.001$) fetuses. The low-dose (10mg/kg) was considered to be the NOAEL for this study.

In two teratogenicity gavage studies (0, 10, 40, 160, and 0, 40, 80, and 160mg/kg/day) in mice, the maternal toxicity at the mid- and high-dose levels was associated with embryotoxicity (reduced litter size and pup weights), and malformations (fused ribs, encephalocele, macroglossia, and incomplete fusion of the skull bones). No teratogenic effects were observed at 40mg/kg/day dose level.

In five segment II rat studies (10, 40, 160mg/kg/day), the drug was administered either via gavage or with the food.

In the dietary study, maternal and embryonic toxicities occurred at the mid- and high-dose levels. Because of 100% resorption at high-dose, the fetuses were not available for evaluation. At the mid-dose level, the teratogenic changes observed in two-third of the fetuses included acrania, ethmocephaly, protrusion of the tongue, and abnormal thymus.

On the other hand in the gavage study, same teratogenic effects were observed only at the high-dose level; no such changes occurred at the mid-dose level. Based on these studies,

the lowest tested dose of 10mg/kg/day was considered to be a NOAEL for teratogenicity in rats. The corresponding value in mice was 40mg/kg. The other three segment II rat studies were mechanistic in nature, and therefore, had no regulatory significance.

In the rabbit segment II study (0, 25, 50, 100mg/kg/day), no teratogenic effects were observed.

In the rat segment III study (0, 5, 20, and 80mg/kg/day), maternal toxicity was observed only at the high-dose level. However, no embryotoxic, external anomalies, or behavioral changes were observed at any dose level.

Based on the outcome of multiple reproductive and developmental toxicity studies, ITR was placed in Pregnancy Category C.

Special toxicology: The sponsor has provided outline summaries of a few published reports concerning hepatic- and immuno-toxicities of ITR.

Hepatotoxicity was investigated in male rats receiving a single intraperitoneal dose of 10, 100, or 200mg ITR/kg, or a daily dose of 10, 50, and 100mg ITR/kg for 14 days.

(Somchit et al. 2004) A single dose of 100 or 200mg ITR caused mild hepatocellular degeneration but had no effect on the ALP (alkaline phosphatase) or ALT (alanine aminotransferase) activities. The NOAEL was established at 10mg/kg. Repeated dosing produced a statistically significant dose-dependent increase in ALP and AST (aspartate aminotransferase), hepatocellular necrosis, degeneration of periacinar and midzonal hepatocytes, bile duct hyperplasia, biliary cirrhosis, and giant cell granulomas.

Pretreatment with phenobarbital, an inducer of CYP decreased the ITR associated hepatotoxicity. On the other hand, pretreatment with CYP inhibitor SKF 525A increased the ITR hepatotoxicity (Somchit et al 2006). The inhibition of CYP3A activity by ITR was dose-dependent. The drug had no effect on the activity of CYP1A or 2E.

In vitro assays indicated that ITR may inhibit human T-lymphocyte proliferation, suppress neutrophil chemotaxis, deoxyglucose uptake, and hexose-monophosphate activity at 10µg/mL concentration level. Pawelec et al, 1991a and 1991b; Vuddhakul et al, 1990).

Data from a mouse oral study conducted by the sponsor indicated that at 10mg ITR/kg concentration, drug had no effect on the antibody response to either a T cell-dependent or independent antigen as measured by the number of splenic IgM plaque forming cells. However, the oral 40mg/kg dose slightly suppressed the IgM response to both antigen types (Cools et al, 1992). In the 21-day mice oral study (0, 40, and 80mg/kg/day), a significant reduction was observed in the spleen and thymus weights, the PFC response to T cell-dependent antigen, and WBC counts. It was also observed that at 40 and 80mg/kg/day, ITR also significantly decreased the phagocytic activity of WBC (Kim and Ahn, 1994).

References:

Somchit N, Norshahida AR, Hasiah AH., et al. (2004) Hepatotoxicity induced by antifungal drugs itraconazole and fluconazole in rats: a comparative *in vivo* study. *Human & Experimental Toxicology* 23:519-525.

Somchit N, Wong CW, Zuraini A., et al. (2006) Involvement of phenobarbital and SKF 525A in the hepatotoxicity of antifungal drugs itraconazole and fluconazole in rats. *Drug and Chemical Toxicology* 29:237-253.

Pawelec G, Ehninger G, Rehbein, K., et al. (1991a) Comparison of immunosuppressive activities of the antimycotic agents itraconazole, fluconazole, ketoconazole and miconazole on human T-cells. *Int. J. Immunopharm* 13(2/3): 299-304.

Pawelec G, Toschonek K and Ehninger G (1991b) The antifungal agent itraconazole exerts immunosuppressive effects on alloreactivity but not on natural immunity *in vitro*. *Int. J. Immunopharm* 13(7): 875-879.

Vuddhakul V, Mai GT, McCormack JG et al. (1990) Suppression of neutrophil and lymphoproliferative responses *in vitro* by itraconazole but not fluconazole. *Rmac* 12(6): 639-645.

Cools M, Aerts F, and Van Wauwe J (1992) Lack of immunosuppression by ketoconazole and itraconazole. *Int. J. Immunopharm* 14(6): 1011-1017.

Kim JH and Ahn YK (1994) The effects of itraconazole on the immune responses in ICR mice. *The Journal of Toxicological Sciences* 19:7-15.

2.6.6.2 Single-dose toxicity: N/A

2.6.6.3 Repeat-dose toxicity: The sponsor in a Pre-clinical Information Amendment (IND 69, 847, serial number 048 dated May 27, 2008) informed the Division that during processing of ITR 200mg film-coated tablets, an organic impurity (b) (4) was found to be present in the drug product at concentration which require qualification based on ICH Q3B (R2) [Impurities in New Drug Products, July 2006]. The highest detected amount of this thermal stress impurity accounted for (b) (4) of the drug product, therefore, requiring qualification (b) (4) according to ICH guideline. The sponsor has conducted one animal and two genotoxicity studies to support the safe qualification of (b) (4)

Study title: Two-week oral toxicity qualification study of itraconazole spiked with impurity (b) (4) in rat.

Key study findings: Oral doses of ITR with or without 0.6% (b) (4) were well tolerated by rats of both sexes. The NOAEL with or without impurity in both sexes was 40mg/kg/day, the highest dose tested.

Study no.: TOX 8572

Volume #, and page #: N/A

Conducting laboratory and location: Toxicology/Pathology
Johnson & Johnson Pharmaceutical
Research & Development, L.L.C.
Raritan, NJ

Date of study initiation: 10-12-2007

GLP compliance: Yes

QA report: yes

Drug, lot #, and % purity: ITR, batch number ZR05121PUC921
Impurity (b) (4), batch number WVLA-0058-042-3

Methods

Doses: Groups:

1. 0.0mg ITR/kg/day (vehicle control)
2. 10mg/kg ITR/day with 0.6% (b) (4)
3. 10mg/kg ITR/day
4. 40mg/kg ITR/day with 0.6% (b) (4)
5. 40mg/kg ITR/day

Species/strain: Rats/Crl:CD(SD)

Number/sex/group or time point (main study): 5

Route, formulation, volume, and infusion rate: Oral (gavage) in 10mL/kg in
0.5% hypromellose

Satellite groups used for toxicokinetics or recovery: None

Age: 8 weeks

Weight: 181-303g

Sampling times: Blood samples (after overnight fasting) for clinical pathology and urine samples were collected during the second week of treatment.

Mortality: Animals were checked daily for morbidity and mortality and twice weekly for clinical signs of toxicity.

No deaths occurred during the study period.

Clinical signs: No drug or impurity related clinical signs of toxicity were observed.

Body weights: Body weights were determined prior to study initiation, and on days 1, 6, and 13.

The terminal body weights in all male groups with/without (b) (4) were slightly decreased (2-6%); however, no dose related trend was recorded. In females, the increase (3%) in the mean body weight at the highest dose level was the same with/without the impurity.

Food consumption: Food consumption was recorded three days prior to the initiation of drug treatment, and weekly thereafter.

No significant inter-group differences in food consumption were observed.

Ophthalmology: No examinations were conducted

EKG: Not recorded

Hematology: Blood samples from all animals were used to determine 10 hematologic parameters, and coagulation parameters such as prothrombin time, activated partial thromboplastin time and fibrinogen.

No intergroup differences in any hematologic or coagulation parameters in groups with/without the impurity were observed.

Clinical chemistry: A total of 19 parameters were determined.

Values in both dose levels of ITR for all parameters were similar with or without the impurity. All values were comparable with the mean control values.

Urinalysis: Twelve major parameters were determined.

No differences in values between the drug groups with or without the impurity were recorded. All values were comparable to values in the control group.

Gross pathology: A total of 46 tissues/organs were subjected to necropsy examination.

No drug, impurity, or drug plus impurity related gross lesions were observed.

Organ weights: After the gross pathology examination, the following organs were weighed: adrenal glands (both), brain, heart, kidney (both), liver, lung, ovary (both), spleen, testis (both), and thymus.

No inter-group differences in organ weights were recorded.

Histopathology: Adequate Battery: yes

Peer review: yes

All tissues/organs subjected to necropsy were also processed for the histopathological examination.

No drug, impurity, or drug plus impurity related microscopic lesions were observed.

2.6.6.4 Genetic toxicology

Study title: Bacterial reverse mutation assay with a confirmatory assay.

Key findings: Under the assay conditions, ITR spiked with 0.6% of (b) (4) tested non-mutagenic in all the tester strains with or without S9 mix.

Study no.: 7677-106

Conducting laboratory and location: (b) (4)

Date of study initiation: 09-11-2008

GLP compliance: Yes

QA reports: yes

Drug, lot #, and % purity: ITR: ZR051211PUJ561/100%
(b) (4): WVLA-0058-042-3

Methods

Salmonella strains: TA98, TA100, TA1535, TA1537, and WP2uvrA

E. coli strain: WP2uvraA

Doses used in definitive study: 33.3, 100, 333, 1,000, 2,500, and 5,000µg/plate

Basis of dose selection: The test preparation was evaluated in tester strains TA100 and WP2uvraA at concentrations ranging from 6.67 to 5,000µg/plate with or without S9 mix. Cytotoxicity (the growth inhibitory effect) of the test material was determined to select appropriate doses for the main assay. Cytotoxicity in strain TA100 is considered to be representative of that observed in all other *Salmonella* test strains. TA100 comparatively exhibits high spontaneous frequency (revertant colonies/plate).

Negative control: DMSO (vehicle)

Positive controls: TA98: 2-nitrofluorene and benzo[a]pyrene

TA100 and TA 1535: Sodium azide, 2-aminoanthracene

TA1537: ICR-191, 2-aminoanthracene

WP2uvrA: 4-nitroquinoline-N-oxide and 2-aminoanthracene

Incubation and sampling times: The assay plates were incubated for 52±4 hours at 37⁰C±2⁰C. Revertant colonies were counted by automated counter and as well by hand.

Based on the results of dose range-finding assay, ITR spiked with 0.6% (b) (4) was further evaluated in all tester strains at dose levels ranging from 33.3 to 5,000µg/plate with or without S9 mix. The test was validated with findings in positive controls. No increase in the revertant colonies was recorded for any tester strain. However, the mean vehicle control values for TA100 with or without the metabolic activation system were not within the acceptable range for this strain. Therefore, the test material was once again re-tested with the same strain in a repeat mutagenicity assay at concentration levels of 50, 100, 250, 500, 1,000, 2,500, and 5,000µg/plate. All the positive and vehicle control values were within the acceptable ranges.

Results: Under the test conditions, ITR spiked with (b) (4) was negative in the bacterial reverse mutation assay.

Study title: *In vivo* Mouse Bone Marrow Micronucleus Assay

Key findings: Under the experimental conditions, ITR spiked with (b) (4) tested negative in the Mouse Micronucleus Assays.

Study no.: 7677-107

Conducting laboratory and location: (b) (4)

Date of study initiation: 07-17-2008

GLP compliance: Yes

QA reports: Yes

Drug, lot#, and %purity: ITR: 040199S/100%

(b) (4): WVLA-0058-042-3

Methods: The clastogenic activity (i.e. disruption of the mitotic apparatus) of ITR spiked with 0.6% impurity was investigated by detecting micronuclei in polychromatic erythrocytes (PCE) isolated from the bone marrow of treated mice.

Strain/species: 8 weeks old male (34-40g) and female (25-27g) Hsd: ICR (CD-1) mice (3/sex/group). This out bred strain known for its maximum heterogeneity tends to eliminate strain-specific response to test substance.

Basis for dose selection: The dose range-finding study was conducted to essentially find the maximum tolerated dose by examining mice for signs of systemic toxicity, morbidity, and mortality. Animals received 3 daily gavage doses of 2,000mg/kg/day of the test material in an aqueous vehicle containing 0.3% Tween 80 and 2% Methocel E15.

All animals remained healthy during 3 days of dosing and 2 days of observation periods. The validated dose of 2000mg/kg was selected as the highest dose for the micronucleus assay.

Doses used in definitive study: 0, 500, 1,000, and 2,000mg/kg/day

Negative control: Vehicle 0.3% Tween 80+ 2% Methocel E15 in deionized water

Positive control: Cyclophosphamide (160mg/kg in water)

Experimental details: Since no relevant toxicological differences between the sexes were observed in the dose range-finding study, only males were employed in the main micronucleus assay involving the following test groups:

<u>Group (n=5)</u>	<u>Dose</u> (mg/kg/day)
Positive control	160 once
Vehicle control	0.0mg/kg ITR
Low-dose	500 ITR spiked with 0.6% (b) (4)
Mid-dose	1000 ITR spiked with 0.6% (b) (4)
High-dose	2000 ITR spiked with 0.6% (b) (4)

Animals received gavage doses at volume of 20mL/kg.

At the end of the observation period, bone marrow extracted from tibias of each animal was used to prepare slides at designated time points. All slides were scored for micronuclei and PCE to NCE ratio. The percent micronucleated cells (micronucleus frequency) were determined by analyzing at least 2000 PCEs/animal. The PCE:NCE ratio was determined by scoring at least 500 erythrocytes/animal.

Results: Throughout the study period, animals in any treatment group did not exhibit any signs of toxicity. ITR spiked with (b) (4) was not cytotoxic to bone marrow at any dose level, nor did it induce any statistically significant increase in PCEs. In addition, the

PCE:NCE ratios at all dose levels were almost similar (Table1). The assay was well validated with the comparative data obtained from the vehicle and positive controls.

Table1. Summary data of micronucleus assay

<u>Group</u>	<u>% Micronucleated PCEs</u> (Mean value/SD)	<u>PCE:NCE Ratio</u> (Mean value/SD)
Vehicle control	0.08/0.06	0.35/0.10
Positive control	2.10/0.39*	0.39/0.08
Low-dose	0.03/0.03	0.49/0.10
Mid-dose	0.05/0.04	0.44/0.09
High-dose	0.03/0.04	0.48/0.12

*Significantly greater than the vehicle control, $p \leq 0.01$

2.6.6.5 Carcinogenicity: No studies were conducted with the clinical formulation.

2.6.6.6 Reproductive and developmental toxicology: No studies were conducted with the clinical formulation.

2.6.6.7 Local tolerance: No studies were conducted with the clinical formulation.

2.6.6.8 Special toxicology studies: No studies were conducted with the clinical formulation.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: After the approval of 100mg ITR (SPORANOX) capsules in 1992, the drug has been commonly used at a dose level of 2x200mg/day. It is tolerated at or below the 200mg/day level; however, significant gastric intolerance had been reported at or above 400mg/day level. Infrequent adverse effects have included hypertriglyceridemia, edema, urticaria, anaphylaxis, erythema multiforme, neuropathy, hypertension, leucopenia, and nephritic syndrome. A few rare cases of hepatic injury and fulminant hepatotoxicity have also been reported. The use of ITR in patients with a history of heart failure and liver disease is contraindicated. A clear boxed warning to this effect has been included in the label.

The second critical safety issue is of multiple drug interactions. ITR inhibits CYP3A4, a key enzyme in the metabolism several drugs. As expected, a number of drugs interact with ITR reducing its efficacy by enzyme induction, or raising its blood level by inhibiting its metabolism. Because of such induction/inhibition kinetics, the list of drugs that can interact with ITR is continuously growing.

The non-clinical safety profile of the proposed 200mg tablets is essentially based on studies conducted to support the safety of SPORANOX (200mg) tablets (NDA 20-083,

approved in 1992) for blastomycosis and histoplasmosis in AIDS patients, and SPORANOX solution for oropharyngeal candidiasis (NDA 20-657, approved in 1997). Both drugs were reviewed and approved by the Division of Antiviral Drug Products. Most of these studies were conducted prior to ICH guidelines in early 1990s. At that time the importance of Safety Pharmacology in drug development and approval process was not much recognized, therefore, no such studies were specifically planned for itraconazole (ITR) formulations.

No non-clinical studies were ever conducted with the proposed clinical formulation or for that matter with any ITR formulation evaluated in HFD-540. In animals as well as in humans, ITR has been proved to be one of the most toxic antifungal imidazoles known.

The oral sub-chronic and chronic studies were conducted in rats (5-160mg/kg/day via gavage or dietary admix) and dogs (5-80mg/kg/day in gelatin capsules). Of the two species, rats were much more sensitive to ITR, and the adverse effects at the mid- and high-dose levels (20-160mg/kg/day) were severe and life threatening. These lesions/adverse effects included significantly decreased erythrocytes, neutrophils, eosinophils, hematocrit, increased lymphocytes, increased weights of liver, kidneys, heart, thymus, lungs, adrenals and spleen, increased levels of cholesterol and phospholipids, bone fractures, reduced growth plate (ribs and tibia), reduced thickness (tibia), and hypocellular pulp in the teeth.

Reevaluation of the original data (by this reviewer) had indicated that in the rat studies, no clear cut NOAEL was established i.e. the drug exhibited some irreversible toxicity at all the tested dose levels. These studies were conducted at dose levels ranging from 0.35-11.5 times (in mg/kg) the proposed single human dose of 200mg/day. It must be mentioned that a clear cut NOAEL of 40mg/kg was established in a short-term (2-week) rat study conducted to investigate the potential toxicity of an organic impurity in the ITR preparation.

In dogs, the adverse effects were restricted to hematologic (decreased erythrocytes and thrombocytes), occult blood in the urine, and the irreversible adrenal hypertrophy at the mid- and high-dose levels (10-80mg/kg/day). The NOAEL of 2.5mg/kg established in the 3-month study was extended to 5mg/kg in the 12-month study. Taking into account a NOAEL of 5mg/kg in dogs, the margin of safety for a 200mg/day clinical dose will be 1.5 times in terms of body weight, and 0.8 times in terms of body surface area (mg/m^2).

During the Phase 3 trials, (b) (4) at a maximum concentration of (b) (4) was found in the clinical formulation. To meet the impurity qualification under ICH Q3B(R2), the sponsor had conducted one short-term animal and two genotoxicity studies. The potential systemic toxicity of (b) (4) was evaluated in a two-week oral rat study (0, 10, and 40mg ITR/kg/day with or without 0.6% (b) (4)). Absolutely, no impurity related systemic toxicity was observed, and the NOAEL for ITR was 40mg/kg with or without the impurity. Assuming 100% absorption, a subject will receive a maximum of (b) (4), providing a safety margin of 927 times of the maximum recommended human dose (MRHD) in terms of body surface area (mg/m^2).

In conclusion, in terms of systemic toxicity, the test species (rat, dog) were much more sensitive to ITR than humans, resulting in a very low margin of safety. However, irrespective of the non-clinical safety limits, based on the extensive long-term clinical experience with 200mg ITR twice a day, twelve weeks of treatment with a single 200mg dose per day is not expected to produce any significant undesirable effects.

ITR tested non-genotoxic in multiple *in vivo* and *in vitro* tests. Also, the drug with or without (b) (4) tested non-mutagenic and non-clastogenic in Ames assay and mouse micronucleus test, respectively.

The drug did not exhibit any carcinogenic potential in mice receiving oral doses up to 80mg/kg/day (2 times of MRHD) for 23 months. In the 24-month rat study, a slightly increased incidence of soft tissue sarcoma was observed in males administered 25mg/kg/day (1.3 times MRHD). These tumors may have been related to hypercholesterolemia caused by chronic treatment with ITR in rats; since hypercholesterolemia was not observed with such treatment in dogs or humans, this adverse effect seems to species-specific. Compared to untreated controls, female rats receiving 50mg/kg/day (2.5 times MRHD) had a statistically insignificant increase in squamous cell carcinoma in lungs (2/50), an uncommon spontaneous tumor in rats.

Toxicity observed in male and female rats at dose levels of up to 40mg ITR/kg/day (2 times MRHD), did not affect the reproductive functions in either of the sexes. The levels of mitochondrial and microsomal enzymes from rat and bovine testes and adrenals were not significantly affected at the highest *in vitro* treatment level of 7.3µg/mL. Studies in animals and humans have also revealed that ITR had no effect on the steroidogenesis in testes and adrenals.

In oral studies, maternal toxicity, embryotoxicity, and teratogenicity were observed in rats at dose levels of 40-160mg/kg/day (2-10 MRHD). Similar observations were made in mice at 80mg/kg/day (2 MRHD) dose level. The teratogenic changes in rats included some major skeletal deformities; in mice, abnormalities included encephaloceles and macroglossia.

Unresolved toxicology issues (if any): None

Recommendations: I have no objection to the approval of this New Drug Application.

3 pages of Draft Labeling have been Withheld in Full immediately following this page as B4 (CCI/TS)

Application
Type/Number

Submission
Type/Number

Submitter Name

Product Name

NDA-22484

ORIG-1

STIEFEL
LABORATORIES
INC

HYPHANOX 200MG FILM-
COATED TABLETS

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

/s/

DAIVENDER K MAINIGI
11/24/2009

BARBARA A HILL
11/24/2009
I concur

Filability of NDA 022484

**Division of Dermatologic and Dental Drug Products (HFD-540)
Pharmacology/Toxicology Checklist for NDA Filing Meeting**

Date: 05-14-2009

Reviewer: Kumar D. Mainigi

NDA Number: 22-484

Drug Name: Hyphanox (itraconazole) 200mg Film-coated Tablets

CAS Number: 84625-61-6

Drug Type: 3S

Drug Class: Antifungal

Indication: Treatment of onychomycosis of toenail

Route of Administration: oral

Date CDER Received: March 31, 2009

User Fee Date: January 30, 2010

Date of Draft Review: September 30, 2009

Sponsor: Stiefel Laboratories, Inc.

Fileability: On initial overview of the NDA application:

- (1) Does the pharmacology/toxicology section of the NDA appear to be organized in a manner to allow a substantive review to be completed? **Yes**
- (2) Is the pharmacology/toxicology section of the NDA indexed and paginated in a manner to enable a timely and substantive review? **Yes**
- (3) Is the pharmacology/toxicology section of the NDA sufficiently legible to permit a substantive review to be completed? **Yes**
- (4) Are all required (*) and requested IND studies completed and submitted in this NDA (carcinogenicity, mutagenicity, teratogenicity*, effects on fertility*, juvenile studies, acute studies*, chronic studies*, maximum tolerated dosage determination, dermal irritancy, ocular irritancy, photocarcinogenicity, animal pharmacokinetic studies etc)? **Yes**
- (5) If the formation to be marketed is different from the formulation used in the toxicology studies, has the sponsor made an appropriate effort to either repeat the studies using the to be marketed product or to explain why such repetition should not be required? **N/A**
- (6) Are the proposed labeling sections relative to pharm/tox appropriate (including human dose multiples expressed in either mg/m² or comparative serum/plasma levels) and in accordance with 201.57? **Yes**

- (7) Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions with the sponsor? **Yes**
- (8) On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted a rationale to justify the alternative route? **Yes**
- (9) Has the sponsor submitted a statement (s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? **Yes**
- (10) Has the sponsor submitted the data from the non-clinical carcinogenicity studies, in the STUDIES electronic format, for the review by Biometrics? **N/A**
- (11) Has the sponsor submitted a statement(s) that the pharm/tox studies have been performed using acceptable, state-of other art protocols which also reflect agency's animal welfare concerns? **Yes**
- (12) From pharmacology perspective, is this NDA fileable? If "no", please state below why it is not. **Yes**
- (13) If the NDA is fileable, are there any issues that need to be conveyed to sponsor? If so specify: **No**
- (14) Issues that should not be conveyed to the sponsor: **N/A**

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

/s/

Kumar Mainigi
5/14/2009 09:01:49 AM
PHARMACOLOGIST

Barbara Hill
5/14/2009 01:40:56 PM
PHARMACOLOGIST