NONCLINICAL EXPERT REPORT May 1995

Company: JANSSEN PHARMACEUTICA NV **Finished Product:** SPORANOX™ oral solution **Active ingredient:** itraconazole	Expert report referring to part III of the dossier: pharmacodynamics, pharmacokinetics and toxicology.	

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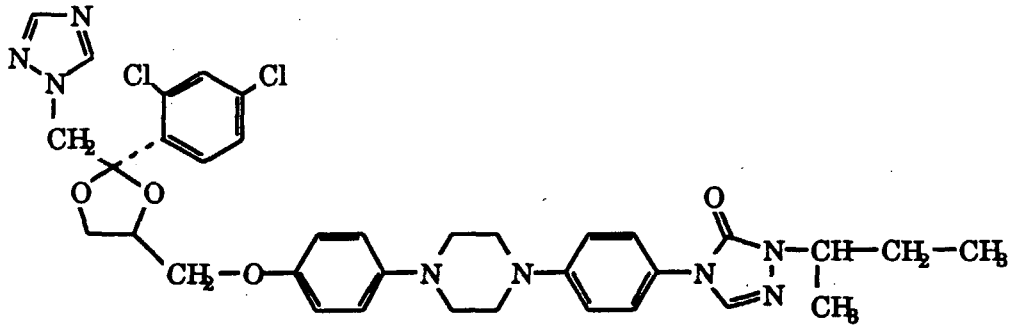
1. INTRODUCTION

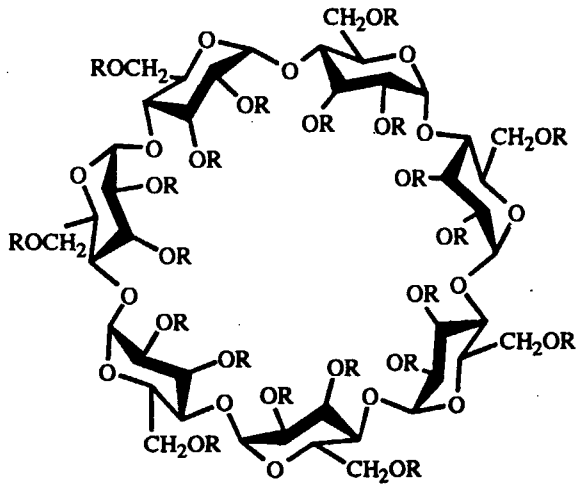
In this expert report, the toxicological and pharmacological properties of itraconazole solubilized in an aqueous hydroxypropyl-β-cyclodextrin solution are critically reviewed. The most important data are concisely summarized in the text and in a few tables. More detailed results are described in a summary report for toxicology and pharmacokinetics (S1, S2) and in the tabulated study reports (S3).

Itraconazole is a proven systemically active antimycotic with an acceptable safety profile and a broad spectrum of activity. Itraconazole capsules have been registered in the late 1980's.



Chemical structure of itraconazole

Hydroxypropyl-β-cyclodextrin (HP-β-CD) is a new pharmaceutical excipient designed to enhance oral absorption and exposure to drugs. It is a cyclic oligosaccharide built up from 7 glucopyranose units with 4.06 to 5.11 hydroxypropyl-groups per molecule of cyclodextrin (as determined by FTIR, Fourier Transform Infra Red Spectroscopy). According to the molar substitution (0.58-0.73), the average molecular weight is between 1350 and 1450. The product is a white powder, soluble in water.
The molecule is able to form inclusion complexes with different compounds ("guest" molecules) into its central cavity and therefore can be used as a "host" molecule for parenteral, oral and local delivery of sparingly soluble and/or unstable drugs.



$R = [(CH_2CH(CH_3)O)]_nH$

$n = 0, 1, 2, 3 \ldots$

Chemical structure of HP-β-CD

The present report mainly focuses upon the use of itraconazole oral solution, i.e. itraconazole solubilized in water using HP-β-CD.

Itraconazole oral solution is indicated for the treatment of oral and/or esophageal candidosis in immunocompromised patients. The proposed dosing regimen is 100 mg (or 2 mg/kg body weight) solubilized in 4 g of HP-β-CD (or 80 mg/kg body weight) twice daily for one or two weeks. In fluconazole-resistant oral and/or esophageal candidosis, the proposed dosing regimen is 100/(4000) to 200/(8000) mg itraconazole/(HP-β-CD) b.i.d. for maximally 4 weeks.

2. PHARMACODYNAMICS

Itraconazole has a broad-spectrum antimycotic activity. With respect to *Candida species*, its activity includes *C. albicans*, *C. krusei* and *C. glabrata*. The high activity of itraconazole is ascribed to the prominent affinity of itraconazole to fungal cytochrome P-450, which is involved in the biosynthesis of ergosterol from lanosterol. Inhibition of the synthesis of ergosterol, which is a vital cell membrane component of fungi, ultimately results in the antifungal effect of itraconazole. At a dose which is 40 times the antimycotic dose, itraconazole is devoid of common central or peripheral drug actions, that would affect vital functions or would limit its specific therapeutic activity. With itraconazole, no relevant pharmacodynamic drug interactions are demonstrated.

In vivo data have shown that enzyme-inducing drugs such as rifampicin and phenytoin significantly reduce the oral bioavailability of itraconazole. Co-medication of itraconazole with H_2-antagonists may also reduce the oral bioavailability of itraconazole. In patients treated with cyclosporin, warfarin and digoxin, monitoring is advised and reduction of the dose might be required on itraconazole co-treatment. Terfenadine, astemizole, cisapride, oral midazolam and triazolam which are mediated through cytochrome P450-3A4 should not be used during treatment with itraconazole at it may inhibit their metabolism (S4).

More detailed information on the primary and general pharmacodynamics of itraconazole is given in the expert report on itraconazole (S5).

HP-β-CD is a carrier molecule. In view of the limited systemic absorption of HP-β-CD after oral administration and the absence of intrinsic pharmacological activity even at high intravenous doses in animals, HP-β-CD is concluded to be devoid of intrinsic pharmacological activity. The only exceptions are the stimulant effects on intestinal secretion and motility after high oral doses from 1500 mg/kg onwards in rats. In this context, it is suggested that oral overdosing with itraconazole oral solution might lead to gastrointestinal side-effects (diarrhea). Furthermore, as also discussed in the section on repeated dose toxicity (section 4.A.2), HP-β-CD induces an increased CCK-release resulting from bile salt encapsulation by HP-β-CD in the gut lumen. An *in vitro* experiment has given direct evidence of the bile salt encapsulation (S6). CCK-mediated effects are also described for other bile salt complexating agents, such as cholestyramine and with trypsin inhibitors naturally present in human food, e.g. soy protein. In rats, but not in humans, CCK is known to be mitogenic for the exocrine pancreas (S6).

Based on the present data, HP-β-CD seems to have no intrinsic *in vivo* pharmacological activities that might negatively interact with those of itraconazole in the clinical formulation of itraconazole oral solution.

3. PHARMACOKINETICS AND TOXICOKINETICS

In this section, the pharmacokinetics and toxicokinetics of itraconazole, its active metabolite hydroxy-itraconazole and of the encapsulating agent HP-ß-CD after administration of itraconazole in a HP-ß-CD solution to experimental animals are discussed. For an elaborate review of the pharmacokinetics and toxicokinetics of itraconazole and hydroxy-itraconazole in experimental animals after administration of itraconazole in other oral solutions, it is referred to the summary document S2. Additional information on the animal pharmacokinetics and toxicokinetics of HP-ß-CD is also provided in a separate summary (S6).

3.A. Absorption and plasma kinetics

After single oral dosing with itraconazole in a HP-ß-CD solution to female rats, plasma concentrations of hydroxy-itraconazole were higher than those of itraconazole at initial time points (G11). Nevertheless, peak plasma concentrations of the active metabolite were reached at 8 h only. Peak plasma concentrations of the parent drug were reached after 4 h. After peak time, itraconazole was eliminated from plasma with a half-life of about 17 h, and hydroxy-itraconazole with a half-life of about 20 h. The AUC ratio of hydroxy-itraconazole to itraconazole amounted to 1.6.

3.B. Repeated dose pharmacokinetics and toxicokinetics

Upon repeated oral administration of itraconazole for 10 days to female rats, steady-state plasma levels of both unchanged itraconazole and its active metabolite hydroxy-itraconazole were reached within 6 days (G11). However, upon daily gavage of itraconazole for 6 months in the rat, plasma concentrations of both compounds slowly increased during the whole course of the study (G14). In the dog, just as in man, steady-state was reached within 14 days (G19, S4). Plasma concentrations of itraconazole and

hydroxy-itraconazole after repeated oral dosing were 2 to 5 (rats), 3 to 10 (dogs) and 5 to 7 (man) times higher than after a single oral dose.

Itraconazole showed non-linear plasma kinetics in male mice and in rats of both genders (G10, G40). In dogs and in female mice, fairly dose-proportional kinetics were observed (G10, G18, G19). For hydroxy-itraconazole, only in the dog non-linear plasma kinetics were found (G18, G19). Ratios of AUC values of hydroxy-itraconazole to itraconazole decreased with increasing doses in mice, rats and dogs. In mice and rats, plasma concentrations of itraconazole and hydroxy-itraconazole were higher in females than in males, while in dogs, no consistent gender differences were observed.

In general, the plasma kinetics of itraconazole and hydroxy-itraconazole after administration of itraconazole in a HP-β-CD-solution compared fairly well with those after administration of itraconazole in a PEG-formulation.

Upon repeated oral administration of HP-ß-CD in combination with itraconazole, the systemic exposure to intact HP-ß-CD in the rat was very limited (G14). The systemic exposure to intact HP-ß-CD was also very low in the dog (G19). In the latter species, plasma levels amounted to 20 µg/ml at the utmost and they rapidly declined. They were comparable between single and repeated administration (G19). The absolute oral bioavailability of HP-ß-CD when given alone amounted to < 1.0% in the rat and to 3.3% in the dog (S6). In man, the absolute oral bioavailability of HP-ß-CD was calculated at 0.8% at the utmost (S4).

3.C. Plasma protein binding

The plasma protein binding of itraconazole and hydroxy-itraconazole is very extensive (> 99 %), but the free fraction of the metabolite is two to three times higher than that of the parent drug (S2). The free fraction of both compounds is highest in rats (1.5 to 2.3 times higher than in man), followed by dogs (1.2 to 1.6 times higher than in man) and man.

3.D. Tissue distribution and accumulation

Itraconazole as well as hydroxy-itraconazole were extensively distributed to the tissues. Highest concentrations of either compound in rat tissues were reached in the adrenal gland and the liver, lowest in brain. Tissue to plasma (T/P) concentration ratios for itraconazole in these tissues amounted to 27, 13 and less than 1, respectively. Tissue to plasma concentration ratios in most tissues were comparable between parent itraconazole and the active metabolite hydroxy-itraconazole, and for both compounds also between male and female rats (G11, G14). In dogs, highest concentrations of itraconazole were measured in fat, adrenals, skin and liver (T/P ratios of 10 to 27) and for hydroxy-itraconazole in liver, adrenals and kidney (T/P ratios of 2 to 8) (G18, G19). Tissue to plasma concentration ratios of hydroxy-itraconazole in the dog were lower than those of itraconazole. In man, highest itraconazole concentrations were reached in omentum and fat (S4).

After repeated oral administration of HP-ß-CD to dogs, highest tissue levels were found in kidney and bladder, while levels in lung, liver and adrenal were clearly lower (G19). In the rat, only kidney levels of HP-ß-CD were measurable after oral dosing (G14).

In all tissues, concentrations of HP-ß-CD were comparable after administration of HP-ß-CD alone or in combination with itraconazole.

3.E. Comparison of exposure in the animals of toxicology studies and in man

The pharmacokinetics of HP-β-CD after oral administration are similar between experimental animals and man (S6). The systemic absorption of intact HP-β-CD is low. The bulk of an orally administered dose of HP-β-CD is metabolized by the intestinal microflora, and the absorption and tissue distribution of the biodegradation products is limited too. After oral dosing, the gastrointestinal tissues show the highest exposure to intact HP-β-CD as well as to its biodegradation products.

Table 3-1 shows the exposure of the animals in various toxicology studies to itraconazole and to the 'active moiety' (= sum of itraconazole and hydroxy-itraconazole) after administration of itraconazole in oral solution (40 % HP-ß-CD) as compared to the exposure after administration of itraconazole via the food (rats) or in PEG-capsules (dogs). The no-toxic-effect dose levels (NOEL) in the rat amounted to 5 mg/kg/day for itraconazole in oral solution and to 10 mg/kg/day for itraconazole administered via the food. In dogs, the NOEL amounted to 5 mg/kg/day for itraconazole in oral solution as well as in PEG-capsules. The exposure to the unbound active moiety at the NOEL is 1.8 times (male rats) and 1.1 times (female rats) higher after administration of itraconazole in a HP-ß-CD solution than after administration as a PEG-premix via the food. In dogs, the exposure to itraconazole at the NOEL is 2.0 times higher for the HP-ß-CD solution than for PEG-capsules. Safety factors for the administration of itraconazole in HP-ß-CD solution are similar or slightly higher than for the administration of itraconazole as a solid dosage form. Itraconazole in solid dosage forms is on the market in many countries for several years.

Figure 3-1 shows plasma levels of the free (= unbound) active moiety upon repeated oral dosing with itraconazole in a HP-ß-CD solution in humans in comparison with those at the various dose levels of toxicology studies in rats and dogs. The animals in the toxicology studies have been subjected (sub)chronically to plasma levels up to 2.2 (dogs), 4.3 (male rats), or 8.5 (female rats) times higher than those obtained in man during repeated oral administration of itraconazole at 200 mg o.d. (S4).

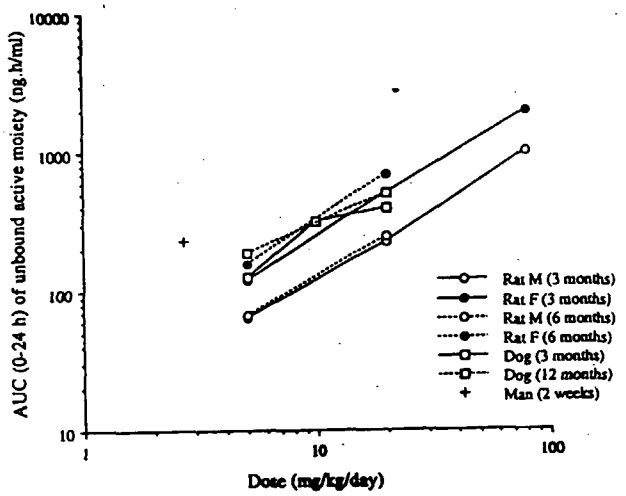


Figure 3-1: Exposure of humans to the unbound active moiety (itraconazole + hydroxy-itraconazole) upon repeated oral administration of itraconazole in HP-ß-CD solution at 200 mg o.d., in comparison with that in male (M) and female (F) rats and dogs upon (sub)chronic oral administration of itraconazole, also in HP-ß-CD solution, in (sub)chronic toxicity studies.

Table 3-1: **Pharmacokinetic parameters related to the exposure to itraconazole either with or without its active metabolite hydroxy-itraconazole in the animals of the toxicology studies after multiple oral dosing of itraconazole in a HP-ß-CD solution in comparison with the exposure after administration of itraconazole via the food (rats) or in capsules (dogs). No-toxic-effect levels (NOEL) are indicated in bold italics.**

	Rat M		Rat F		Rat M			Rat F			Dog M + F				
ITRACONAZOLE															
Duration (ref.)	6 months (G14)				6 months (G41)						12 months (G19)		12 months (G42)		
Dose (mg/kg/day)	*5*	20	*5*	20	***10***	40	160	***10***	40	160	*5*	20	*5*	20	80
	(40 % HP-ß-CD solution)				(PEG-premix administered via the food)						(40 % HP-ß-CD solution)		(PEG-capsules)		
C_{max} (µg/ml)	0.317	1.69	0.790	4.54	0.046[a]	0.681[a]	4.37[a]	0.628[a]	4.32[a]	5.12[a]	1.60	4.19	0.625	2.71	4.35
$AUC_{0\text{-}24\ h,\ total}$[d] (µg.h/ml)	(2.02)[c]	17.7	12.4	72.8	1.10[b]	16.3[b]	105[b]	15.1[b]	104[b]	123[b]	23.6	78.0	10.2	47.4	93.1
$AUC_{0\text{-}24\ h,\ free}$[e] (ng.h/ml)	5.46	47.8	33.5	197	2.96	44.1	283	40.7	280	332	49.6	164	21.4	99.6	196
ACTIVE MOIETY (= sum of itraconazole and hydroxy-itraconazole)															
C_{max} (µg/ml)	0.902	3.14	1.56	7.40	0.203[a]	1.63[a]	7.64[a]	1.08[a]	7.58[a]	10.6[a]	-	-	0.986	4.58	6.73
$AUC_{0\text{-}24\ h,\ total}$[d] (µg.h/ml)	(8.74)[c]	39.2	25.7	125	4.87[b]	39.0[b]	184[b]	25.8[b]	182[b]	254[b]	44.7	131	21.1	98.6	158
$AUC_{0\text{-}24\ h,\ free}$[e] (ng.h/ml)	66.6	243	155	695	37.2	251	994	138	991	1526	185	504	91.1	427	611

[a] Serum concentration at autopsy [b] Calculated from serum concentration at autopsy times 24 h ($C^{ss,av}$ x 24 h) [c] Calculated from $AUC_{0\text{-}24\ h}$ on day 36, using the ratio of $AUC_{0\text{-}24\ h}$ on day 183 to that on day 36 in female rats [d] $AUC_{0\text{-}24h}$, total = AUC for the sum of free (= not bound to plasma proteins) and bound drug [e] $AUC_{0\text{-}24h}$, free = AUC for free (= not bound to plasma proteins) drug, calculated from $AUC_{0\text{-}24h}$, total x free fraction (fu). The fu amounted to 0.27% in male rats and to 0.21% in dogs. For female rats, the fu was taken equal to the value in male rats (0.27%). For the calculation of $AUC_{0\text{-}24h}$, free for the active moiety, the free fraction of itraconazole and hydroxy-itraconazole were taken into consideration.

In general, safety factors calculated using tissue concentrations are more indicative for the safety than safety factors calculated using plasma concentrations. Such 'tissue concentration-based' factors can often not be calculated, since measurement of tissue concentrations in humans is only rarely performed. However, for itraconazole, limited data on tissue concentrations in humans are available and the data are sufficient to allow such comparison (Table 3-2). From the table, it follows that the tissue distribution of itraconazole differs between the animal species and man. T/P ratios for fat, skin, muscle and lung compare fairly well between animals and man. Hence, for these tissues, exposure ratios based on plasma concentrations are similar to those based on tissue concentrations and they are indicative of the safety. In contrast, T/P ratios for liver (a target tissue) and kidney are a factor of 2 to 5 times higher in rats and dogs as compared to man, which means that exposure ratios based on plasma concentrations appear to be underestimated as compared to those based on tissue concentrations. This might also be the case for other (target) tissues.

Table 3-2: Tissue-to-plasma or -to-serum concentration ratios of itraconazole in rat, dog and man after administration of itraconazole in a HP-ß-CD solution.

Reference	Duration Formulation	Dose (mg/kg/day)	Tissue-to-plasma or -serum (T/P) ratios[a] (for concentrations or AUC values)		
Rat					
				Male	Female
G14 A9	6 months (40 % HP-ß-CD solution)	5 20	Liver Kidney	17 5.0	9.5 2.2
G11	10 days (7.6 % HP-ß-CD solution)	10	Adrenal gland Perirenal fat Liver Pancreas Ovaria Kidney Lung Heart Skin Muscle Brain		28 26 13 6.3 6.2 5.3 3.0 2.8 1.5 1.3 0.49
Dog					
				Male + Female	
G19 A11	12 months (40 % HP-ß-CD solution)	5 20	Adrenal gland Liver Kidney Bladder Pancreas Lung	14 10 4.8 3.3 2.9 2.3	
G18 A10	3 months (30-50 % HP-ß-CD solution)	5 10 20	Fat Adrenal gland Skin Liver Kidney Pancreas Lung Muscle Brain	30 16 10 9.9 4.3 3.0 2.1 1.4 0.93	

a) Mean value for the different dose levels.

Table 3-2: **Tissue-to-plasma or -to-serum concentration ratios of itraconazole in rat, dog and man after administration of itraconazole in a HP-ß-CD solution.**

Reference	Duration Formulation	Dose (mg/kg/day)	Tissue-to-plasma or -serum (T/P) ratios[a] (for concentrations or AUC values)	
Man				
S4	various	200 mg o.d. or b.i.d.	Fat	23
			Skin	3.1 - 11
			Bone	4.7
			Stomach	3.8
			Liver	3.5
			Spleen	3.1
			Muscle	2.4
			Lung	0.9 - 2.4
			Kidney	1.5

a) Mean value for the different dose levels.

4. TOXICOLOGY

Itraconazole is used worldwide for the oral treatment of superficial and systemic mycoses, with a good clinical record and a relatively low incidence of side-effects (S7). For the approval of this use, the systemic toxicological properties of itraconazole have been determined in a comprehensive set of toxicity studies, i.e. single and repeated dose toxicity; reproduction including fertility, embryotoxicity, teratogenicity and peri- and postnatal toxicity; mutagenicity *in vivo* and *in vitro*, and carcinogenicity.
These studies have been summarized and extensively discussed in an expert report for the registration file of itraconazole capsules, with a latest update made in 1993 (S5).

Itraconazole is now developed as an oral solution with HP-β-CD as an excipient. Since HP-β-CD is a new excipient, it has also been tested in an extensive set of toxicity studies, which are summarized and discussed in S6.

In the present expert report, emphasis will be given to the safety evaluation of itraconazole oral solution.

In order to assess the safety of itraconazole formulated as an oral solution in HP-β-CD, a limited package of toxicity studies, confined to single and repeated dose toxicity and immunotoxicity, has been conducted. For a more extended review of the findings in these studies it is referred to S1.
The specific protocols for each study were designed to satisfy the regulations of the major countries worldwide. The animal species used were mainly rats, mice and dogs.

These species are considered appropriate

- because they were the main species used in the toxicity studies performed with the two compounds separately
- because of their reliability for toxicity studies
- because of the possibility to compare the results with historical control data obtained in other, similar studies conducted with the same strains in the same testing facilities.

The single dose toxicity was studied by administration via the oral and the intravenous route in mice, rats and dogs. The studies were performed before the first International Conference on Harmonization (ICH1), which explains the conduct of the dog studies.

For the evaluation of repeated oral use of itraconazole/(HP-β-CD), rats were daily dosed for 3 and 6 months, while dogs were orally dosed for 3 and 12 months. Good Laboratory Practices have been applied for these studies. Additionally, the results of a three-month pilot toxicity study in mice are also included in this expert report.

Other toxicity studies evaluating effects on reproduction or mutagenicity have not been performed with the oral solution. These effects were extensively studied with itraconazole and HP-β-CD alone (S5, S6), and exposure levels of itraconazole in laboratory animals, using various formulations up to a toxic dose range are sufficiently documented. The same applies to HP-β-CD.

Carcinogenicity studies with itraconazole oral solution were not considered necessary, from a regulatory point of view. Neither itraconazole nor HP-β-CD are considered to be primary carcinogens. Both itraconazole and HP-β-CD in separately conducted carcinogenicity studies have positive tumor findings mediated through compound-specific epigenetic mechanisms, and no adverse interaction for carcinogenicity between both compounds is envisaged. Moreover, itraconazole oral solution is indicated for a treatment period of maximally four weeks.

Immunotoxicity studies in mice, performed as a phase IV commitment for the US-FDA approval of itraconazole capsules, were also performed with the oral solution and therefore are also included in this expert report.

4.A. Single and repeated dose toxicity

4.A.1. SINGLE DOSE TOXICITY

Itraconazole/(HP-β-CD) was administered orally and intravenously to mice, rats and dogs from both genders (A1-A6). The results are summarized in Table 4-1.

Table 4-1: Single dose toxicity studies with itraconazole/(HP-β-CD)

Species	Route	Sex	LD_{50}-values (mg itraconazole/ (HP-β-CD/kg bwt)	Ref.
mouse	oral	M&F	> 100/(2500)	A1
mouse	intravenous	M&F	> 44.1/(1764)	A2
rat	oral	M&F	> 100/(2500)	A3
rat	intravenous	M&F	> 17.6/(704)	A4
dog	oral	M&F	> 100/(2500)	A5
dog	intravenous	M&F	> 17.6/(704)	A6

Upon oral administration, all animals survived the maximum dose of 100/(2500) mg/kg body weight (bwt). Clinical responses mainly consisting of soft feces or diarrhea were seen in all three species. As they were also present in the mice and rats dosed with HP-β-CD alone, these phenomena were considered to be due to the osmotic effects of HP-β-CD (S6).
A single intravenous dose of 44.1/(1764) mg/kg bwt in mice and 17.6/(704) mg/kg bwt in rats and dogs did not result in mortality. Clinical effects observed were transient

hyperpnea in rats and mice, and dyspnea and loss of righting reflex in dogs. These effects were only seen during the first hours after injection.
These results indicate that there is no interaction between itraconazole and HP-β-CD after a single-dose administration.
At the highest dose tested in animals, i.e. 100/(2500) mg/kg, no mortality occurred, indicating that the risk of fatalities after accidental overdosing in man is low. When calculated for a person of 50 kg, this would correspond to an intake of 500 ml of the solution containing 10 mg itraconazole/ml.

4.A.2. REPEATED DOSE TOXICITY

For the evaluation of repeated oral use of itraconazole/(HP-β-CD), mice, rats and dogs were daily dosed for 3 months (A7, A8, A10). In addition, chronic oral toxicity studies were performed in rats (6 months, A9) and dogs (12 months, A11). The duration of these studies was done in conformity with the requirements of the major regulatory authorities worldwide.

Table 4-2: Repeated dose toxicity studies with itraconazole/(HP-β-CD)

study	itraconazole/(HP-β-CD) doses in mg/kg bwt	Ref.
3 month pilot toxicity in mice	0/(0), 0/(800), 5/(200), 20/(800), 80/(3200)	A7
3 month toxicity in rats	0/(0), 0/(4000), 5/(1000), 20/(2000), 80/(4000)	A8
6 month toxicity in rats	0/(0), 0/(800), 5/(200), 20/(800), 30/(0)	A9
3 (+1)* month toxicity in dogs	0/(500), 5/(300), 10/(400), 20/(500)	A10
12 month toxicity in dogs	0/(0), 0/(800), 5/(200), 20/(800), 20/(0)	A11

*: + 1 month of recovery

Summary of findings

In the studies performed with itraconazole/(HP-β-CD) in mice, rats and dogs, the itraconazole dose of 5 mg/kg body weight was virtually not toxic. In rats, the only effects observed were, as expected from the toxicological profile of itraconazole, slightly increased serum cholesterol and phospholipid-levels, and minimal histological changes of the adrenal cortex (swelling), but without any cytopathological changes. In dogs, slight histological changes of the mononuclear phagocytosing system (MPS) mainly consisting of an increase in foamy macrophages and adaptive changes in the urinary tract (swelling and vacuolation of epithelial cells in the renal pelvis and the urinary bladder) were the only effects observed, and they were mainly attributable to HP-β-CD.

In mice (A7), dosing at 20/(800) and 80/(3200) mg/kg for 3 months resulted in slight to more pronounced toxicity. Serum aspartate and alanine aminotransferase levels were elevated, and at 80/(3200) mg/kg, associated with histological liver changes (eosinophilic aspect of hepatocytic cytoplasm and increase in individual cell necrosis). Histological examination further revealed modifications of the adrenal cortex (swelling) at 80/(3200) mg/kg bwt. In addition, HP-β-CD-related adaptive changes of the urinary tract were noted at 20/(800) and 80/(3200) mg/kg bwt.

In **rats** dosed for **3 months** (A8), 20/(2000) and 80/(4000) mg/kg body weight were moderately toxic doses. At these doses, target organs or tissues were similar to those observed with itraconazole alone (S5) i.e. the adrenal cortex, the liver, the MPS, and the ovaries. Increased serum cholesterol and phospholipid-levels were also observed. Changes related to the HP-β-CD-vehicle were slightly decreased hematocrit and hemoglobin, a slightly lowered number of red blood cells, and adaptive changes of urinary bladder and renal pelvis.

Dosing in rats for **6 months** (A9) at 20/(800) mg/kg and at 30/(0) mg/kg, a dose of itraconazole in PEG resulting is a similar systemic exposure (section 3.B), revealed toxicity mainly characterized by some altered blood and serum variables, and histological changes in MPS, liver and adrenal cortex. HP-β-CD-related effects in the 20/(800) and the 0/(800) mg/kg dosed groups were limited to histological changes in urinary tract and to an increased pancreas weight, but without apparent histological changes.

In **dogs** dosed for **3 months** (A10), itraconazole-related toxicity at 10/(400) and 20/(500) mg/kg body weight was confined to a slightly decreased body weight gain, some altered blood and serum variables, a slightly increased adrenal weight and foci of foamy cells in the lungs. The latter finding was also seen in the 0/(500)-vehicle dosed group, indicating that it is at least partially related to HP-β-CD.
Oral administration of HP-β-CD further led to slightly decreased hematocrit and hemoglobin and a slightly lowered number of red blood cells. After one month of recovery, the changed parameters in the 0/(500) and 20/(500) mg/kg dosed groups showed good reversibility.

Repeated dosing at 20/(800) or 20/(0) mg/kg for **12 months** (A11) mainly produced reduced body weight gain and some changed blood and serum variables. At these doses, histological examination revealed itraconazole-related effects on the MPS and the adrenal cortex. Apart from transient softening of the stools and adaptive urinary tract changes, no other HP-β-CD-related effects were present.

Discussion

The results of these studies indicate that, at the tested doses, the (sub)chronic toxicity profile of itraconazole in HP-β-CD was similar to that of itraconazole alone. This conclusion is further substantiated by the fact that peak plasma concentrations and AUC-values were comparable in solid (PEG) and liquid (HP-β-CD) formulations (section 3.B).

HP-β-CD-related changes were slight and limited to transient softening of the stools, slight decreases in hematocrit, hemoglobin and red blood cells, foamy cells in the lungs and urinary tract changes.
Most importantly, there was no increase in toxicity with itraconazole/(HP-β-CD) when compared with both compounds given separately, indicating that no adverse interactions between itraconazole and HP-β-CD were evidenced. A comparison of the toxicity profile of itraconazole oral solution in rats and dogs after 3 months and 6 - 12 months of dosing indicated that there is no time-dependent progression of toxicity.

The main **itraconazole-related** target organs or tissues were:

- the adrenal cortex and the ovaries
- serum cholesterol and phospholipids in rats

- the liver
- the mononuclear phagocytosing system (MPS) i.e. lungs, lymph nodes, liver, and spleen

These effects were discussed in detail in the nonclinical expert report on itraconazole capsules (S5) and are shortly evaluated for their relevance in the present document.

Endocrine system

Itraconazole/(HP-β-CD) dose-dependently affected the adrenal cortex (swelling) in mice, rats and dogs, and in rats also, but to a lesser extent, the ovaries (increase in clear interstitial tissue). In *in* vivo and *in vitro* experiments, itraconazole is shown to interact with cytochrome P450- mediated reactions involved in the steroid synthesis in adrenals and gonads (S5).

The effects on the endocrine system observed with itraconazole/(HP-β-CD) were qualitatively and quantitatively similar to those observed at a bioequivalent dose of itraconazole alone, showing that HP-β-CD did not negatively influence the effect on this target.

With regard to the clinical relevance of these findings, itraconazole is a marketed drug, and experience of clinical human safety studies and postmarketing data have indicated an acceptable safety profile with regard to adrenals and ovaries (S7).

Serum cholesterol and phospholipids

A dose-related increase in serum cholesterol and phospholipids was demonstrated in rats. This rat-specific effect was also noted with itraconazole alone (S5). HP-β-CD did not enhance the effect on this target. Possible hypotheses for the rat-specific increase in cholesterol have been suggested (S9).
With regard to the relevance in man, evaluation of cholesterol levels in patients on long-term itraconazole capsule therapy (S7) indicate no association between itraconazole therapy and increases in serum cholesterol. In patients treated with itraconazole/(HP-β-CD) in the therapeutic dose range of 100 to 200 mg b.i.d., no effects on serum cholesterol and phospholipids were shown either, further sustaining that this rat-specific effect is clinically irrelevant (S10).

Some changes seen with itraconazole/(HP-β-CD) were also seen with **both HP-β-CD and itraconazole** separately, i.e. the liver and the MPS.

Liver

As also described in the previous expert report on itraconazole capsules (S5) and in the nonclinical safety evaluation of HP-β-CD (S6), the liver is a target organ for both itraconazole and HP-β-CD overdosing.

Itraconazole-related effects in the studies conducted with the oral solution were seen in mice, rats and dogs. In mice and rats, serum transaminases were elevated. Histological examination in male mice at 80/(3200) mg/kg bwt and in rats at 80/(4000) mg/kg bwt revealed slightly increased individual cell necrosis. In dogs, increased serum transaminases were not associated with histological liver changes.

HP-β-CD in rats at 0/(4000) and 80/(4000) mg/kg bwt also slightly elevated serum transaminases.

However, in general, no adverse interaction on the liver was seen with itraconazole/(HP-β-CD).

In patients, itraconazole oral solution was dosed at 100 to 200 mg o.d or b.i.d. for one to four weeks. No relevant effect on serum parameters specific for liver function could be demonstrated (S10).
The relevance of the effects on the liver was further assessed.

In an *in vitro* study (Q3), the cytotoxicity of itraconazole, HP-β-CD and itraconazole/(HP-β-CD) towards human and dog hepatocytes was compared. In human hepatocytes, cytotoxicity was observed at itraconazole concentrations of 7 to 10 µg/ml or 1350 times the free plasma concentrations of about 4.5 to 5.2 ng/ml after repeated oral doses of 200 mg b.i.d. in healthy volunteers. For HP-β-CD, plasma concentrations after oral administration of 8 g were less than 1 µg/ml. Cytotoxicity was only observed at a concentration of 400 µg/ml, indicating a safety margin of at least 400.

With itraconazole/(HP-β-CD), no relevant increase in cytotoxicity was evidenced, which further confirms the absence of a negative interaction between the two compounds with regard to the effects on the liver. In dog hepatocytes, cytotoxicity was even slightly lower with itraconazole/(HP-β-CD) than with both compounds tested separately.

Mononuclear phagocytosing system (MPS)

The changes observed were characterized by an increase in foamy macrophages in various tissues, i.e. lungs, spleen, liver and lymph nodes. These effects were also seen with itraconazole alone (S5) and indicate an interference of itraconazole with the MPS. The altered adrenocortical hormone synthesis was considered to be one of the factors to initiate these effects. Macrophages might also contain an increased amount of itraconazole causing slight cytological changes. Electron microscopy of the foamy macrophages revealed an increase in inclusions containing amorphous, dense material and lipid-like droplets.

In addition, an HP-β-CD-related increase in foamy macrophages was also observed (S6). Ultrastructural examination revealed that the foamy cells corresponded with macrophages containing secondary lysosomes filled with heterogeneous inclusions. This finding was not unexpected considering the physiological action of these macrophages. No adverse evolution towards a more specific cytopathology (e.g. phospholipidosis) was observed.

In order to further investigate these findings, two immunotoxicity studies (Q1, Q2) in mice (doses: 0/(0), 2.5/(100), 10/(400) and 40/(1600) mg/kg bwt) were performed where a possible adverse effect on the immune system was measured by the number of splenic IgG and IgM plaque forming cells. Itraconazole/(HP-β-CD) up to 10/(400) mg/kg did not negatively affect the immune response, whereas only at a clearly toxic dose of 40/(1600) mg/kg, a slight tendency to a reduced plaque-forming cell response was observed. These findings allow to conclude that immunosuppressive effects, if at all, are of secondary nature. No primary immunotoxic potential was evidenced from these studies in mice.

The relevance of these effects on the MPS in patients in the therapeutic dose range of 100 to 200 mg b.i.d. is considered low.

Human data from clinical trials and postmarketing experience confirm the safety of itraconazole with regard to the immune system (S7).

Finally, some changes observed in the repeated dose toxicity studies were related to the HP-β-CD-vehicle, i.e. the transient softening of the feces, slight decreases in hematocrit, hemoglobin and red blood cells, the CCK-mediated effects on the pancreas and the adaptive changes of the urinary tract. These findings are briefly discussed especially for their relevance to man when exposed to itraconazole/(HP-β-CD).

Softening of the feces

In dogs dosed at 20/(800) and 0/(800) mg/kg bwt, transient softening of the feces was observed. This was most likely related to the osmotic water retention in the large intestine. The osmotic effect of HP-β-CD leads to an accelerated intestinal propulsion and enhanced weights of the intestinal contents as measured in a pharmacodynamic model in rats (S6). As a consequence, an increased amount of softened feces could be observed.

In patients, diarrhea was sporadically noticed, but the frequency was not higher than expected in the study patient population and in the control groups (S8).

Hematological changes

At 20/(2000) and 80/(4000) mg/kg bwt in rats and at 20/(500) mg/kg bwt in dogs, slightly decreased hematocrit, hemoglobin and red blood cells were observed. These effects were reversible after one month of recovery. These effects are quite commonly encountered in toxicology studies and are considered to be rather non-specific findings occurring at toxic doses.

The relevance of these findings to man is considered low (S8).

Pancreas

In rats dosed for 6 months with 20/(800) mg/kg bwt of itraconazole/(HP-β-CD), a slightly increased pancreas weight was present, not associated with apparent histological changes. These effects are considered related to the increased cholecystokinin (CCK) release resulting from bile salt encapsulation by HP-β-CD in the gut lumen (S6). This further leads to a decrease in free bile acids in the gut lumen causing disinhibition of the luminal feedback mechanism between bile salts and CCK-release. CCK-mediated effects are also described for other bile salt complexating agents, such as cholestyramine and with trypsin inhibitors naturally present in human food, e.g. soy protein. In rats, CCK is known to be mitogenic for the exocrine pancreas. After six months of exposure, this effect only led to the increase in pancreas weight, without altering the normal structure.

The relevance of these findings with itraconazole/(HP-β-CD) is considered low, since the mitogenic effect of CCK is rat-specific, and since the increased pancreas weight was only seen in rats dosed for six months at the dose of 20/(800) mg/kg bwt, not at lower doses and not after 3 months of dosing. For a more extensive discussion on this target, it is referred to the nonclinical safety evaluation of HP-β-CD (S6).

Urinary tract changes

Dose-dependent HP-β-CD-related effects on the urinary tract were noted at all doses in rats and dogs. They consisted of histological changes such as swelling and vacuolation of cells in the renal pelvis and the urinary bladder. Electron microscopically, these light microscopic findings corresponded with the enlargement of secondary lysosomes filled with heterogeneous inclusions. Highest tissue levels of HP-β-CD were also measured in kidney and urinary bladder (section 3.D).

As also described for mannitol, dextran and other sugars, the lysosomal enlargement is most probably related to the pinocytotic uptake of HP-β-CD and its incorporation in the phagolysosomes followed by osmotic retention of water (S6). In conclusion, these findings are considered to be the result of pharmacokinetic processes and to be of an adaptive nature. This is further substantiated by the facts that the changes in the urinary tract did not worsen in time, that no histological changes indicative of renal or urinary bladder cell cytotoxicity were evidenced, and that renal function was not relevantly impaired in rats and dogs. In humans, administration of itraconazole oral solution did not reveal any changes in urine variables indicative of an adverse effect on renal function (S10).

4.B. REPRODUCTION

The set of reproduction studies performed with itraconazole alone and in combination with HP-β-CD alone covers the regulatory needs worldwide. An extended review and discussion of fertility studies, embryotoxicity and teratogenicity studies, and peri-and postnatal toxicity studies is given in the respective expert reports (S5, S6).

Itraconazole was not primary antifertile, or did not produce primary adverse effects on reproduction in rats and rabbits. However, at higher, maternally toxic doses, it has, as is the case for other systemically active antifungal azoles, embryotoxic and teratogenic properties in rodents, which are, at least partially related to the adrenocortical effects (S5). As described in the section on repeated dose toxicity (section 4.A.2), adrenal effects were seen to the same extent with itraconazole / (HP-β-CD).

HP-β-CD did not produce adverse effects on fertility or embryotoxicity. It was not teratogenic, as discussed in the nonclinical safety evaluation of HP-β-CD (S6).

Pharmacokinetic data have shown that, in rats, pregnancy has little effect on the disposition of itraconazole as seen after single doses of itraconazole in β-cyclodextrin solutions (S2). Therefore, reproduction studies with itraconazole/(HP-β-CD) were not considered necessary and labeling information should be similar to that for itraconazole capsules.

The estimated teratogenic risk in patients is considered low. Nevertheless, extrapolation from one species to another remains difficult. Therefore, itraconazole oral solution should only be given to pregnant women in life-threatening conditions and when in these cases, the potential benefit outweighs the potential harm for the fetus.

4.C. Mutagenicity

No mutagenicity studies were performed with itraconazole/(HP-β-CD).

In previous studies, itraconazole alone was not mutagenic in a DNA repair test, in Ames tests in *Salmonella typhimurium* and *Escherichia coli*, a sex-linked recessive lethal test in *Drosophila melanogaster*, a mouse lymphoma test, a chromosome aberrations test in human lymphocytes, a micronucleus test in mice and dominant lethal tests in male and female mice (S5).

HP-β-CD also was investigated for its effects on DNA-damage in an unscheduled DNA-synthesis test, on gene mutations in Ames tests in *Salmonella typhimurium* and *Escherichia coli* and a mouse lymphoma test, or on chromosome aberrations in human lymphocytes and in two micronucleus tests in mice (S6).

Since both compounds did not show mutagenic properties in any of the test systems, itraconazole/(HP-β-CD) also is not considered to have a mutagenic potential.

4.D. Carcinogenicity

No carcinogenicity studies were conducted with itraconazole/(HP-β-CD).
The results of the carcinogenicity studies with itraconazole indicated no primary carcinogenic effect in mice up to 80 mg/kg bwt, and in rats up to 20 mg/kg bwt. In male rats dosed at the toxic dose of 80 mg/kg bwt, a slight increase in soft tissue sarcomas was demonstrated. As discussed in the expert report on itraconazole (S5), this rat-specific pathogenesis is initiated by the increases in serum cholesterol, resulting in cholesterol-mediated histological changes at toxic doses. The relevance of these findings is low, since in patients, serum cholesterol levels were not elevated (S10).

The results of the studies with HP-β-CD indicated no primary carcinogenic potential in mice (S6). In rats, a dose-dependently increased incidence of exocrine pancreas tumors and an increased incidence of well-circumscribed and well-differentiated polypous neoplasms in the large intestine (at the high dose only) were demonstrated. Overall survival was not affected in any dosage group.

The exocrine pancreas tumors resulted from a CCK-mediated rat-specific mechanism (S6). CCK is a powerful secretagogue and, specifically in rats, also acts as a mitogen and therefore increases cellular hyperplasia of the acinar cells of the exocrine pancreas, leading to neoplasia after longterm treatment.

The polypous neoplasms in the large intestine seen in high dosed rats, are part of a mucosal hypertrophic response representing an adaptation to increased osmotic activity of high doses of HP-β-CD. This hypertrophic response is well known to occur with a wide range of natural and chemically modified ingredients such as starches and other polysaccharides that are recognized as safe for human consumption (S6).

It can be concluded that neither itraconazole nor HP-β-CD are considered to be primary carcinogens. Both itraconazole and HP-β-CD in separately conducted carcinogenicity studies have positive tumor findings mediated through compound-specific epigenetic mechanisms, and no adverse interaction for carcinogenicity between both compounds is envisaged. Furthermore, the subchronic and chronic toxicity studies with itraconazole in combination with HP-β-CD gave no evidence for an adverse interaction between both compounds with regard to the findings, related to each compound, in the carcinogenicity

studies. Moreover, itraconazole oral solution is indicated for the treatment of oral and/or esophageal candidosis for a treatment period of one up to four weeks.

4.E. Other information

In order to evaluate the influence of different storage conditions of the formulation intended for marketing, two limited bioassays were conducted upon request of pharmaceutical development. More specifically, the acute and the repeated dose oral toxicity of samples of the same batch of itraconazole/(HP-β-CD) stored at different temperatures were compared. For more information it is referred to the chemical-pharmaceutical expert report on itraconazole oral solution (S11).

In a pilot **acute** toxicity study (Q4) in female rats, one sample was stored for 25 months at a temperature of 30° C. This batch showed a slight change of color. The second sample was stored for the same time period at 4° C, and showed no change of color. When administered once at the limit dose of 2 ml/kg, corresponding to a dose of 20/(800) mg/kg bwt, no mortality and no adverse clinical effects were observed in either of the samples.

In the **repeated** dose oral toxicity in rats (Q5), a first sample was stored at 4° C for 14 months and the second sample was stored at 40° C for 14 months. Both were administered orally by gavage for one month at doses of 5/(200) and 20/(800) mg/kg bwt. A vehicle group was dosed at 0/(800) mg/kg bwt. No mortality occurred. No toxic effects were noted at 5/(200) mg/kg bwt. Toxic effects at 20/(800) mg/kg bwt were expected from the toxicological profile and were similar in both samples.

It can be concluded that storage of the clinical formulation of itraconazole/(HP-β-CD) at higher temperature does not relevantly alter the safety profile as determined in the existing set of toxicity studies.

5. ENVIRONMENTAL RISK ASSESSMENT

An environmental risk assessment for 10 mg/ml itraconazole oral solution (R1), has been performed in accordance with the Phase I EC draft guidelines III/5504/94 - Draft 6 (dd. 08.12.94).

Results indicated that predicted environmental concentrations (PEC) were as follows:

PEC itraconazole in surface water	0.0023 µg/l	(< 0.01 µg/l)
PEC itraconazole in soil	0.038 µg/kg	(< 10 µg/kg)
PEC itraconazole in sediment	0.0023 µg/kg	(< 10 µg/kg)

After the assessment of potential risks to the environment posed by 10 mg/ml itraconazole oral solution, we can conclude that the product is of no concern to the environment and no further action, i.e. a Phase II assessment, is required.

6. OVERALL CONCLUSION

The available animal toxicity and nonclinical pharmacokinetic data on itraconazole oral solution as well as the separate evaluation with itraconazole and HP-β-CD alone support the safety of itraconazole oral solution.

Itraconazole is already available on the market for several years in a solid formulation in capsules for the treatment of various mycotic infections. It possesses a good record of efficacy as well as an acceptable safety profile (S7).

Itraconazole when tested in a comprehensive set of toxicity studies can be considered as safe. The main targets for oral overdosing were the adrenals, the liver, the ovaries and the MPS. Their clinical relevance has been assessed. Itraconazole was not primary antifertile, or primary adverse for reproduction in rats and rabbits. However, at higher, maternally toxic doses, it has embryotoxic and teratogenic properties in rodents. Itraconazole is not genotoxic or primary carcinogenic.

Separate studies on HP-β-CD have shown that this carrier molecule has a low toxicity potential and is not mutagenic, teratogenic or primary carcinogenic. There was no increase in toxicity with itraconazole/(HP-β-CD) as compared to both compounds given separately, indicating that no adverse interactions between itraconazole and HP-β-CD were evidenced. This also applies to the compound-specific tumor findings in rats, mediated through epigenetic mechanisms that were found not to be applicable to man.

From the exposure data of the active moiety (= sum of itraconazole and hydroxy-itraconazole) in the animals of the toxicity studies and in man, exposure ratios calculated for itraconazole in oral solution were similar to slightly higher than those calculated for itraconazole in solid dosage forms. Therefore, an acceptable safety of itraconazole oral solution is also evident from a pharmacokinetic point of view.

In conclusion, the available information provides evidence that itraconazole oral solution is considered safe for the indication of oral and/or esophageal candidosis in immunocompromised patients at the proposed dosing regimen. From a nonclinical safety point of view, and next to its benefit as discussed in the clinical expert report (S8), itraconazole oral solution does not bear an additional risk for systemic side-effects in patients when compared with the itraconazole oral capsule formulation.

8. INFORMATION ON THE EXPERT

[redacted]

9. AUTHORS AND AREA OF RESPONSIBILITY