Efinaconazole: Developmental and reproductive toxicity potential of a novel antifungal azole

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ABSTRACT

Efinaconazole is a new triazole antifungal for topical treatment of onychomycosis. The reproductive and developmental toxicity of efinaconazole was characterized in fertility and early embryonic development (rat), embryo-fetal development (rat and rabbit), and peri/post-natal development (rat) studies in accordance with current ICH guidances. In the fertility study, maternal reproductive toxicity was noted as estrous cycle prolongation (NOAEL = 5 mg/kg/day) but there were no male reproductive effects even in the presence of paternal toxicity (NOAEL = 25 mg/kg/day). Rat embryo-fetal and perinatal pup lethality was the most sensitive (NOAEL = 5 mg/kg/day) efinaconazole developmental toxicity and was noted at maternally toxic doses. Efinaconazole did not affect rabbit embryo-fetal development at maternally toxic doses (NOAEL = 10 mg/kg/day). No malformations were induced by efinaconazole in rats or rabbits. When compared with systemic exposures observed in onychomycosis patients, embryo-fetal toxicity in rats was noted at high (>100-fold) multiples of systemic exposure.

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1. Introduction

Efinaconazole is a novel triazole marketed in the US, Canada and Japan as a topical treatment for onychomycosis, a fungal infection of the nails. Like other antifungal triazoles, efinaconazole inhibits the fungal cytochrome P450 enzyme lanosterol 14α demethylase (CYP51), thereby disrupting ergosterol synthesis and, consequently, membrane integrity and growth in fungi [1]. CYP51 is evolutionarily conserved and, in mammals, mediates conversion of lanosterol to meiosis-activating sterols (MAS); MAS are intermediates in the biosynthesis of cholesterol and may have a signaling role in initiating meiosis and oocyte maturation [2,3]. Azoles have higher affinity for fungal CYP51 compared to the mammalian enzyme and such selectivity contributes to the safety of this therapeutic class [4].

Azoles have been reported to produce reproductive and developmental toxicity in both humans and laboratory animals. The mechanism is unknown but inhibition of mammalian CYP51 as well as other CYPs, e.g. CYP17, CYP19 and CYP26, have been postulated to play a role [5–7]. In rats, fluconazole, voriconazole, and itraconazole prolonged gestation and reduced implantation, embryo-fetal survival, and/or early postnatal survival [8] (2014 package inserts for Diflucan®, Vfend®, Sporanox®). These azoles were teratogenic producing fetal defects such as cleft palate and hydronephrosis/hydroureter. There are case reports in humans of high maternal exposure to fluconazole during early pregnancy associated with craniofacial and skeletal abnormalities [8–10].

The present work characterizes the reproductive and developmental toxicity potential of efinaconazole. Studies were conducted to evaluate the effects of efinaconazole on: (i) fertility and early embryonic development (rats), from premating to conception (males) or implantation (females); (ii) embryo-fetal development (rats and rabbits), from implantation to closure of the hard palate; and (iii) peri/post-natal development (rats), from implantation

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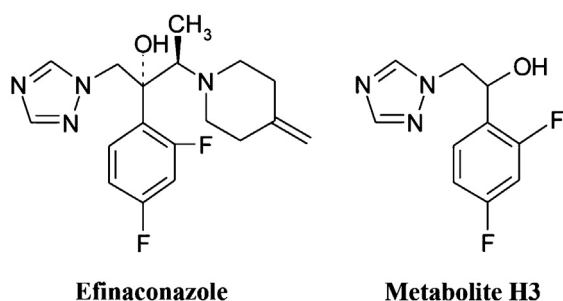[1] Present address: ToxClarity, PO Box 6948, Napa, CA 94581, USA.

Fig. 1. Efinaconazole and H3 metabolite structures.

to weaning and sexual maturity of offspring. Further, the toxicokinetics (TK) of efinaconazole and its major human circulating metabolite, H3, were characterized in plasma (rats and rabbits) and in milk (rats). The margins of safety based on systemic exposure at the NOAELs relative to onychomycosis patients are defined.

2. Materials and methods

2.1. Test chemical

Efinaconazole (Kaken Pharmaceutical Co., Ltd., Shizuoka, Japan), molecular weight 348.40, is lipophilic with a logP of 3.7. Efinaconazole has two chiral carbons and is the R,R stereoisomer, Fig. 1.

Efinaconazole has two chiral carbons and is the R,R stereoisomer configuration. H3 is 2-hydroxyl 2-difluorinated phenyl triazole.

2.2. Animals, dosing regime and evaluations

Studies were conducted in rats and rabbits per 2005 ICH S5(R2) (Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility) and current GLP guidelines; procedures were approved by animal care and use committees, and animal care was in accordance with the United States Animal Welfare Act regulations (9 CFR, Parts 1, 2, and 3) or "Law Concerning the Protection and Control of Animals" (Law No. 105, Japan). All study animals were housed in suspended stainless steel wire bottom cages in climate-controlled rooms. In the perinatal/postnatal study, dams and litters were housed in nesting boxes with corn cob nesting material from GD20 – LD20. Studies were conducted in facilities compliant with the 1996 NRC Guide for the Care and Use of Laboratory Animals.

Doses were selected based on dose range finding and/or repeat dose toxicity studies. Efinaconazole was administered as subcutaneous (SC) injections once per day at dose volumes of 2 mL/kg in the rat and 1 mL/kg in the rabbit. Dose solution analysis confirmed the accuracy of the dose solutions for each study. The SC administration route achieved a high ratio of systemic exposure to dose [11] and a plasma drug metabolic profile similar to that observed in humans following topical nail efinaconazole administration [12]. Propylene glycol was used as the vehicle based on drug solubility and acceptable tolerability [11]. Topical nail administration is the clinical route in onychomycosis treatment but was not practical for nonclinical studies.

Toxicity endpoints of viability, clinical observations, body weights and food consumption were examined in F0 animals and, in the postnatal development study, F1 pups. Table 1 summarizes study designs and study-specific evaluations.

2.2.1. Fertility and early embryonic developmental toxicity (ICH 4.1.1)

Male Crj:CD(SPF) rats (approx. 10 weeks old at the start of dosing) were administered efinaconazole or propylene glycol vehicle

from 28 days prior to mating, through the mating period, until the day before necropsy (49–53 consecutive days). Females were dosed from 14 days prior to mating, through the mating period, until gestation day (GD) 7 (23–37 consecutive days). Laparohysterectomies were performed on GD 14. Dose levels were 0 (vehicle), 1, 5 and 25 mg/kg/day. The high dose was selected based on results of a 2-week dose range finder (DRF) study where daily SC efinaconazole injection of 50 mg/kg/day produced skin thickening and estrous prolongation (5–7 day cycle) in all rats while 10 mg/kg/day was better tolerated with only 1 of 6 rats with prolonged (6 day) estrous cycle.

2.2.2. Embryo-fetal developmental toxicity in rats (ICH 4.1.3)

Crj:CD(SPF) pregnant female rats (approx. 12 weeks old) were dosed with efinaconazole or vehicle from GD 7 to 17. Dose levels were 0 (vehicle), 2, 10 and 50 mg/kg/day. Laparohysterectomies were performed and all animals were euthanized on GD 20. Doses were selected based on a DRF study in pregnant rats. DRF rats were injected daily with efinaconazole from GD 7 to 17 and the high dose, 50 mg/kg/day, was the maximal tolerated dose based on maternal and placental toxicity.

2.2.3. Embryo-fetal developmental toxicity in rabbits (ICH 4.1.3)

Pregnant New Zealand White [Hra:(NZW)SPF] female rabbits (approx. 6 months old) were administered efinaconazole or propylene glycol vehicle from GD 7 through 20. Dose levels were 0 (vehicle), 1, 5 and 10 mg/kg/day. Animals were evaluated through GD 29. Laparohysterectomies were performed and all animals were euthanized on GD 29. Doses were selected based on a DRF study in pregnant rabbits in which animals were injected SC daily with efinaconazole from GD 7 to 20. The DRF high dose, 50 mg/kg/day, exceeded the maximal tolerated dose and all animals were euthanized on GD 8 due to severe local lesions. The maximal tolerated DRF dose, 10 mg/kg/day, based on lower body weight gain was selected as the high dose for the main study. A 3-day pilot study was conducted in nonpregnant rabbits to determine the tolerability of lower dose volumes and the lowest volume examined, 1 mL/kg/day, was selected for the main study.

Blood samples were collected from satellite animals (3 rabbits/group) prior to dosing and 1, 4, 6, 8 and 24 h after dose administration on GDs 7 and 20. Plasma was analyzed for efinaconazole and metabolite H3 concentrations (Section 2.3).

2.2.4. Developmental and perinatal/postnatal reproductive toxicity (ICH 4.1.2)

Crl:CD(SD) F0 female rats (approx. 10 weeks old) were administered efinaconazole or propylene glycol from day 7 of presumed gestation through day 20 postpartum or day 24 of presumed gestation (for rats that did not deliver a litter). Dose levels were 0 (vehicle), 1, 5 and 25 mg/kg/day. The high dose (25 mg/kg/day) was selected based on the maternal toxicity noted in repeated SC administration of 50 mg/kg/day in pregnant rats (Section 2.2.2) and severe local toxicity in nonpregnant rats injected with 40 mg/kg/day (Jo 2014). Fetal death noted at 50 mg/kg/day in the embryo-fetal developmental toxicity study would preclude F1 evaluations in the perinatal/postnatal study. Therefore, the high dose was set below 50 mg/kg and 25 mg/kg was selected as the high dose. F1 generation pups were not directly administered efinaconazole, but may have been exposed to efinaconazole or its metabolites during maternal gestation (in utero exposure) or via maternal milk during the lactation period.

For toxicokinetics evaluation, blood samples were collected from satellite animals (3 rats/time point/group; 9 animals/group total) prior to dosing and 0.5, 1, 4, 8, and 24 h after dose administration on GD 7 and 17 and on lactation day (LD) 20. In addition,

Table 1
Study design overview.

Study	Species (number animals/group)	Dose levels (mg/kg/day)	F0 evaluations	F1 evaluations
Fertility and early embryonic development (ICH 4.1.1)	Rat [Crj:CD(SPF)] (16 males and 16 females)	0, 1, 5 and 25	*Males*: Macroscopic pathology Organ weights (liver, spleen, kidneys, adrenal glands, prostate, testis, seminal vesicle, and epididymis) Histopathology (liver, spleen, skin, and any tissues with macroscopic findings) Sperm counts, morphology and motility *Females*: Vaginal smears (days until copulation) Macroscopic pathology Number of corpora lutea, implantations, dead/live embryos, the indices of insemination, pre-implantation loss and post-implantation loss Histopathology (ovaries, uteri, and any tissues with macroscopic findings)	Not applicable
Embryo-fetal development (ICH 4.1.3)	Rat [Crj:CD(SPF)] (20 females)	0, 2, 10 and 50	Macroscopic pathology Number of corpora lutea, implantations, dead/live embryos Indices of implantation and embryo-fetal mortality Histopathology of any tissues with macroscopic findings	External abnormalities Fetal weight and sex Placental weight and diameter About 50% of fetuses/dam examined for visceral abnormalities (head, abdomen, and thoracic organs) and the remaining fetuses examined for skeletal abnormalities and ossification progress
Embryo-fetal development (ICH 4.1.3)[a]	Rabbit [Hra:(NZW)SPF] (23 females)	0, 1, 5 and 10	Macroscopic pathology (uteri, placentae and ovaries) Placental and gravid uterine weights Number of fetuses, early and late resorptions, total implantations and corpora lutea.	Fetal weight and sex, observation of all fetuses for external, visceral and skeletal malformations and developmental variations
Peri-/post-natal development (ICH 4.1.2)[a]	Rat [Crl:CD(SD)] (25 females)	0, 1, 5 and 25	Maternal behavior, litter observations, natural delivery, pup body weights, viability index (live pups on LD 4/liveborn pups on LD 1), lactation index (live pups on LD 21/live pups on LD 4) and dam and pup necropsy observations	Sexual maturation (vaginal patency and preputial separation) Learning and memory (passive avoidance and M-type watermaze tests) Mating performance, Cesarean-section-enabled counts and descriptions (implantation sites, corpora lutea, resorptions, and fetal survival; placental size, color, and shape; fetal sex and gross external observations), testes and epididymides weights, and necropsy observations

Viability, clinical observations, body weights and food consumption examined in F0 and F1 (postnatal development study) animals.

[a] Toxicokinetics were assessed in these studies.

milk samples were collected from 5 rats/dose group following IV injection of oxytocin approximately 4 h after dose administration on LD 14. Plasma and milk were analyzed for efinaconazole and metabolite H3 concentrations (Section 2.3).

2.3. Toxicokinetics

Plasma concentrations of efinaconazole and H3 (Fig. 1) were determined in samples collected in the rat peri/postnatal and rabbit embryo-fetal development studies using validated (FDA's Guidance on Bioanalytical Method Validation, 2001) LC-MS/MS methods. The lower limit of quantitation for both analytes was 0.10 ng/mL in rats and 1.0 ng/mL in rabbits.

Concentrations of efinaconazole and H3 were determined in rat milk using a non-validated, non-GLP LC-MS/MS method with a lower limit of quantitation of 0.10 ng/mL for both analytes.

Noncompartmental pharmacokinetic parameters were calculated using WinNonLin 5.0.1 (Pharsight Corp., Sunnyvale, CA). For rats, the mean values for the samples at each sampling time were used for the toxicokinetic analysis.

2.4. Statistical analysis

Data were analyzed for statistically significant ($p \leq 0.05$) differences from control values using either parametric or nonparametric tests based on a statistical decision tree. In rat studies, homogeneity of variance was evaluated with Bartlett's test. Homogeneous data were analyzed with a post hoc Dunnett's test if $p \leq 0.05$ in a parametric ANOVA. Nonparametric data (i.e. unequal variance in Bartlett's test, $p \leq 0.001$) were tested with Kruskal–Wallis ANOVA followed by Dunnett's (Seg I and Seg II) or Dunn's (Seg III) test. Clinical or other proportion Seg III data were analyzed using the

Table 2
Efinaconazole effects on rat estrous cycle length (ICH 4.1.1).

Efinaconazole (mg/kg/day)	Estrous cycle length[a]		Continuous diestrous[a]
	4 days[b]	5 days	
0	16/16 (100)	0/16 (0)	0/16 (0)
1	16/16 (100)	0/16 (0)	0/16 (0)
5	15/16 (94)	1/16 (6)	0/16 (0)
25	10/16 (63)	2/16 (13)	4/16 (25)

[a] Number animals/total (%).
[b] Normal estrous cycle length is 4 days.

Variance Test for Homogeneity of the Binomial Distribution. Rabbit data were processed tested as parametric for selected data (i.e. maternal and fetal body weight, gravid uterine weight and placental weight) or nonparametric (all other endpoints). Rabbit parametric data were tested with ANOVA and Dunnett's while nonparametric data were tested with Kruskal–Wallis ANOVA and post hoc Dunn's.

3. Results

3.1. Fertility and early embryonic developmental toxicity (ICH 4.1.1)

No animals died in any group throughout the experiment, and no efinaconazole related changes were observed in clinical signs, body weight changes and food consumption (data not shown). Local toxicity, i.e. microscopic subcutaneous irritation and inflammation, was noted in all study groups and thickened skin at the injection site was observed at $\geq$5 mg/kg/day.

In males, absolute and relative spleen weights were increased (25% and 22%, respectively) and slight extramedullary hematopoiesis was observed at efinaconazole doses of 25 mg/kg/day. Dose-related incidence of microvacuolation of peripheral hepatocytes in the liver was observed at 5 and 25 mg/kg/day. No other test article effects were noted in study evaluations of males.

In females during the pre-mating phase, the normal 4-day estrous cycle was prolonged by one day in 1/16 (6%) and 2/16 (13%) animals in the 5 and 25 mg/kg/day groups, respectively, and 4 high dose rats had continuous diestrous, Table 2. All estrous cycles recovered to normal during the pre-mating period and there was no efinaconazole effect on copulation. Three of the 4 animals noted in constant diestrous during the pre-mating period were successfully mated and became pregnant. There were no other test article effects on study evaluations of females.

The paternal and maternal local toxicity NOAELs were 1 mg/kg/kg based on injection site inflammation. The fertility NOAELs were 25 mg/kg/day based on an absence of fertility effects at the high dose. The NOAEL for estrous prolongation was 5 mg/kg/day and early embryonic development NOAEL was 25 mg/kg/day based on absence of toxicity findings at the high dose.

3.2. Embryo-fetal developmental toxicity in rats (ICH 4.1.3)

In dams, local toxicity was observed as injection site reactions which included thickening of the skin and subcutaneous nodules at 10 and 50 mg/kg/day. Efinaconazole dependent systemic effects included reduction in food consumption (GD15–GD20; 5% decrease; $p < 0.05$), enlarged spleen, and splenic extramedullary hematopoiesis and proliferation of megakaryocytes at 50 mg/kg/day. No changes in the number of corpora lutea or implantations were observed.

Signs of efinaconazole embryo-fetal toxicity at the high dose (50 mg/kg/day) included embryo-fetal deaths, decreased live fetuses, increased placental weight and, diameter, and microscopic placental lesions, Table 3.

An increase in the number of unilateral and bilateral rudimentary lumbar ribs was also observed at the high dose (27.2% at 50 mg/kg/day vs. 3.7% at 0 mg/kg/day, $p < 0.01$) but considered a skeletal variation and not a fetal malformation [13]. Furthermore, Marr et al. reported that supernumerary ribs noted prenatally and on PND 21 were not permanent and disappeared by PND 63 [14]. No efinaconazole-related changes in any other study evaluations were observed.

The maternal toxicity NOAEL of efinaconazole was 10 mg/kg/day based on reduced body weight gain and food consumption, extramedullary hematopoiesis, and local toxicity. The embryo-fetal toxicity NOAEL was 10 mg/kg/day. The fetal malformation NOAEL was 50 mg/kg/day based on an absence of malformations at the high dose.

3.3. Embryo-fetal developmental toxicity in rabbits (ICH 4.1.3)

Three and 2 females in the control and 10 mg/kg/day groups, respectively, were euthanized after 8–15 days of dosing (between GD 15 and 22) due to severe lesions at the injection sites. Because these local effects were observed in control animals, they were likely due to the irritant properties of propylene glycol. At necropsy, no efinaconazole-related macroscopic findings were noted for these animals.

Maternal systemic effects included increased incidence of soft stool and lower (35%) mean body weight gains with a trend

Table 3
Efinaconazole embryo-fetal toxicity in rats (ICH 4.1.3).

Parameter	Efinaconazole (mg/kg/day)			
	0 (vehicle)	2	10	50
Embryo-fetal deaths (dams with resorptions)	0/19 (0)[a]	0/20 (0)	0/20 (0)	4/20[*] (20)
Live fetuses[b]	14.47 ± 2.2 (96)	13.7 ± 3.5 (93)	13.7 ± 1.7 (95)	10.7 ± 5.2[*] (70)
Placental weight[b] (g)	0.44 ± 0.05	0.46 ± 0.10	0.47 ± 0.04	0.80 ± 0.09[*]
Placental diameter[b] (mm)	13.47 ± 0.68	13.46 ± 1.04	13.65 ± 0.78	15.84 ± 0.64[*]
Placental histopathology[c]				
Decidual cell vacuolar degeneration	0/5	0/5	v. slight-3/5, slight-1/5	slight-4/5
Decidua basalis fibrinoid necrosis	0/5	0/5	v. slight-1/5	v. slight 1/5, slight-3/5, moderate-1/5
Dilation	0/5	0/5	0/5	slight-3/5, moderate-2/5
Fibrinoid deposit	0/5	0/5	0/5	v. slight-3/5, slight-2/5
Intervillous hemorrhage	0/5	0/8	0/5	v. slight-1/5, moderate-1/5

[a] Number animals/total (%).
[b] Mean ± SD.
[c] Examined in the first 5 dams/group.
[*] $p \leq 0.05$.

Table 4
Efinaconazole toxicity in pregnant rabbits (ICH 4.1.3).

Efinaconazole (mg/kg/day)	Body weight gain (g)		Food consumption GD 7–21 (g/animal/day)
	GD 7–21	GD 21–29	
0	353 ± 174[a]	11 ± 142	185 ± 41
1	377 ± 132	2 ± 134	181 ± 34
5	316 ± 129	28 ± 148	169 ± 31
10	229 ± 178[*]	85 ± 110	163 ± 33

[a] Mean ± SD.
[*] $p \leq 0.05$.

Table 6
Efinaconazole effects on lactating rats (ICH 4.1.2).

Efinaconazole (mg/kg/day)	Body weight gain GD7–20 (g)	Body weight LD 1 (g)	Body weight gain LD 1–4 (g)
0	109 ± 17[a]	292 ± 19	6 ± 9
1	112 ± 13	290 ± 22	7 ± 9
5	106 ± 11	287 ± 14	8 ± 11
25	109 ± 20	278 ± 17[*]	16 ± 8[**]

[a] Mean ± SD.
[*] $p \leq 0.05$.
[**] $p \leq 0.01$.

for lower food consumption at 10 mg/kg/day relative to controls, Table 4.

During the post-dosing period between GD 22 and GD 29, body weight gain became comparable to the control group. No other efinaconazole-related maternal changes were noted at necropsy, and no efinaconazole-related changes in fetal parameters were observed.

Exposure (C_{max} and AUC) to efinaconazole and H3 increased with increasing dose on both GD7 and GD20, Table 5.

The increases in plasma AUC_{0-24} were approximately linear from 1 to 10 mg/kg/day. Efinaconazole exposure increased from GD7 to GD20, indicating drug accumulation with repeated dosing. Exposure to H3 was substantially lower than efinaconazole.

The maternal toxicity NOAEL for efinaconazole was 5 mg/kg/day (GD20 AUC_{0-24}: efinaconazole = 2311 ng h/mL, H3 = 30 ng h/mL) based on adverse lower mean body weight gains and decreased food consumption at 10 mg/kg/day. The embryo-fetal development NOAEL was 10 mg/kg/day (GD20 AUC_{0-24}: efinaconazole = 3881 ng h/mL, H3 = 59 ng h/mL) based on the lack of effects on embryo-fetal development at the highest dosage level evaluated.

3.4. Developmental and perinatal/postnatal reproductive toxicity (ICH 4.1.2)

No test article related deaths and no dose-dependent increases in adverse clinical findings were observed in the F0 generation during the gestation period. At necropsy, adhesions of abdominal organs or tissues occurred in all dosage groups (in ≥12% of animals per dose group), with slightly greater incidence at 5 (20% of animals) and 25 (24% of animals) mg/kg/day. These findings were consistent with other repeat-dose SC rat toxicity studies with this test article and vehicle [11]. These adhesions were likely related to the spread of inflammation related to SC administration of the propylene glycol vehicle and exacerbated with apparent dose-dependence by efinaconazole.

Efinaconazole did not affect body weight and body weight gain during the gestation period. However, LD 1 body weight was significantly reduced (6.6%, $p \leq 0.05$) in the 25 mg/kg/day dosage group, Table 6.

This weight loss was not correlated with litter size. Maternal body weight gain during LDs 1 through 4 was significantly

increased (46%, $p \leq 0.01$) at 25 mg/kg/day; this weight gain was not correlated with the number of pups found dead on lactation day 1, suggesting the difference from control was not related to lower nutritional demand. Body weight gains were then generally comparable among the four groups for the remainder of the lactation period. No test-article related changes in absolute and relative feed consumption were observed during either the gestation or lactation periods.

Efinaconazole increased perinatal pup mortality at 25 mg/kg/day, Table 7.

Mean liveborn litter size was slightly reduced (1.6%) while the numbers of pups found dead on LD 1 and on LDs 2 through 4 were significantly increased and viability index decreased (10%).

No test article-related deaths, clinical findings, and necropsy findings were observed in the F1 generation after weaning. No test-article related changes were observed during the post-weaning, pre-cohabitation and/or gestation periods.

In F1 pups, there were no effects on neurological development as evaluated in passive avoidance and water maze tests. There were no effects on sexual maturation in F1 pups. The average day that preputial separation for male rats occurred earlier at 25 mg/kg/day, 44.2 ± 2.2, relative to vehicle controls, 46.2 ± 2.9, but was within the observed historical ranges at the Testing Facility and not considered toxicologically important.

No test article-related effects on reproductive development were observed in the F1 generation. There were no test article-related fetal gross external alterations.

Maternal systemic exposure (C_{max} and AUC_{0-24}) to efinaconazole and H3 increased with increasing dose on GD7, GD17 and LD 20, Table 8. C_{max} and AUC increased from GD 7 to GD 17 but generally decreased from GD17 to LD 20.

Efinaconazole and H3 were detected in milk collected 4 h after dosing on LD 14 Table 9.

In rats dosed at 1, 5 and 25 mg/kg, milk concentrations for efinaconazole increased in a less than dose proportional relationship while H3 metabolite levels were roughly dose proportional. In contrast to plasma, the concentrations of efinaconazole were substantially higher than H3 in milk, which may be explained by the higher lipophilicity of efinaconazole compared to H3. Excretion of efinaconazole and H3 into milk correlated with

Table 5
Efinaconazole and H3 plasma exposure in pregnant rabbits (ICH 4.1.3).

Dose (mg/kg/day)	Day of dosing	Efinaconazole		H3	
		C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)	C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)
1	GD 7	18.6 ± 5.4[a]	181 ± 38	0	0
	GD20	51 ± 4.0	384 ± 63	0	0
5	GD 7	164 ± 106	1242 ± 346	2.3 ± 1.1	20.5 ± 11.9
	GD20	284 ± 109	2311 ± 461	3.3 ± 0.6	37.6 ± 15.6
10	GD 7	193 ± 80	2355 ± 521	3.1 ± 0.8	48.2 ± 22.8
	GD20	334 ± 89	3881 ± 804	4.0 ± 0.6	59.4 ± 14.5

[a] Mean ± SD.

Table 7
Efinaconazole effects on rat peri and postnatal survival (ICH 4.1.2).

Efinaconazole (mg/kg/day)	Liveborn pups	Litter size[b]	Perinatal pup mortality[a]		Viability index[c]	Lactation index[d]
			LD 1	LD 2–4		
0	317	13.8 ± 2.4	0/317 (0)	6/317 (1.9)	311/317 (98.1%)	305/311 (98.1)
1	332	13.3 ± 1.4	1/332 (0.3)	5/331 (1.5)	326/332 (98.2%)	323/326 (99.1)
5	311	13.5 ± 1.9	5/311 (1.6)	3/306 (1.0)	303/311 (97.4%)	285/303[**] (94.0)
25	266	11.6 ± 3.3	13/266 (4.9)[**]	20/253 (7.9)[**]	233/266 (87.6%)[**]	232/233 (99.6)

[a] Number dead/total number pups in dose group (%).
[b] Mean ± SD (%).
[c] Number of live pups on LD4/number of liveborn pups on LD1.
[d] Number of live pups on LD21/number of live pups on LD4.
[**] $p \leq 0.01$.

Table 8
Toxicokinetics of efinaconazole and H3 in plasma of pregnant and lactating rats (ICH 4.1.2).

Efinaconazole (mg/kg/day)	Day of dosing	Efinaconazole		H3	
		C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)	C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)
1	GD 7	7.11	54.9	15.1	219
	GD17	14.5	120	17.8	290
	LD20	9.15	85.8	19.1	241
5	GD 7	38.1	364	56.7	913
	GD17	46.4	552	113	1602
	LD20	29.1	422	84.5	1131
25	GD 7	57.7	1033	145	2567
	GD17	189	3363	373	8186
	LD20	128	2253	276	5810

Table 9
Concentrations of efinaconazole and H3 in milk of lactating rats (Seg III).

Efinaconazole (mg/kg/day)	Concentration on lactation day 14, 4 h post-dosing (ng/mL)[a]	
	Efinaconazole	H3
1	54.1 ± 24.9	11.0 ± 3.0
5	1536 ± 1232	52.4 ± 20.8
25	2157 ± 662	256 ± 59

[a] Mean ± SD.

decreases in the plasma exposures (AUC) between GD 17, when dams are not producing milk, and LD 20.

The maternal toxicity NOAEL for efinaconazole is 5 mg/kg/day (LD20 AUC_{0-24}: efinaconazole = 422 ng h/mL, H3 = 1131 ng h/mL) based on injection site reactions and LD 1 body weight loss. The reproductive toxicity NOAEL in the dams is 25 mg/kg/day based on the lack of effects at the high dose. The NOAEL for viability in the offspring is 5 mg/kg/day based on increased perinatal pup mortality, reduced liveborn litter size, and increased pup mortality on lactation days 1 through 4 observed at 25 mg/kg/day.

4. Safety assessment

Topical efinaconazole 10% solution has demonstrated safety and efficacy in the treatment of onychomycosis [15–17]. In onychomycosis patients, the highest individual $AUC_{0-24\,h}$ at the maximum recommended human dose (MRHD) was 25.3 ng h/mL for efinaconazole and 141.5 ng h/mL for metabolite H3 [12]. Safety factors based on comparison of animal NOAEL efinaconazole AUC exposures to human AUC were calculated for developmental and reproductive toxicity, Table 10.

TK exposure was not measured in the rat fertility and embryo-fetal developmental toxicity studies so exposure was estimated based on 2821 ng h/mL female day 90 AUC at 10 mg/kg/day determined in a 6-month SC repeat dose rat toxicity study [11].

5. Discussion

Efinaconazole treatment during gestation produced developmental toxicity in rats but not rabbits; F1 survival and placental effects were the most sensitive endpoints. Effects on F1 survival were consistent in both the embryo-fetal developmental study and the perinatal/postnatal study. Embryo-fetal and early postnatal mortality manifested as decreased liveborn litter size and/or increased neonatal death, while placental effects presented as increased weight and diameter, vacuolar degeneration and fibrinoid necrosis. These rat developmental toxicities are common to both drug and pesticide azole products [5] (Vfend). The perinatal and postnatal study results agree with results from the rat developmental study and there were no efinaconazole effects on F1 sexual maturation, reproductive function or neurological development.

Although many antifungal azoles are teratogenic, efinaconazole did not induce malformations in rats and rabbits. Teratogenic azole antifungals include ketoconazole, itraconazole, fluconazole and voriconazole (2013 package insert for Extina®; Sporanox, Diflucan, Vfend). The differences in teratogenicity findings between efinaconazole and teratogenic azoles may be due to systemic exposure and/or intrinsic activity at the azole teratogenicity target(s). Exposure data at teratogenic doses for most azole drugs is not available but a comparison of voriconazole teratogenic exposure to efinaconazole can be made. Voriconazole rat teratogenic effects occurred at exposures more than 10-fold above the efinaconazole high dose exposure (Vfend). Intrinsic teratogenic activity of efinaconazole is difficult to determine as the azole teratogenic target is not known although there are several proposed targets, including CYPs (e.g. CYP51, CYP19 and CYP26) and Hox gene expression.

Antifungal azoles are known to inhibit mammalian cytochrome P450 enzymes (CYP) in addition to the fungal target enzyme [5]. Efinaconazole inhibition of mammalian CYP51, CYP19 and CYP26 has not been studied. Efinaconazole does inhibit some drug metabolizing CYP enzymes, CYP2C9 and CYP3A4, in vitro at submicromolar concentrations (Jublia 2013 FDA Summary Basis of Approval). Several antifungal azoles have been reported to inhibit
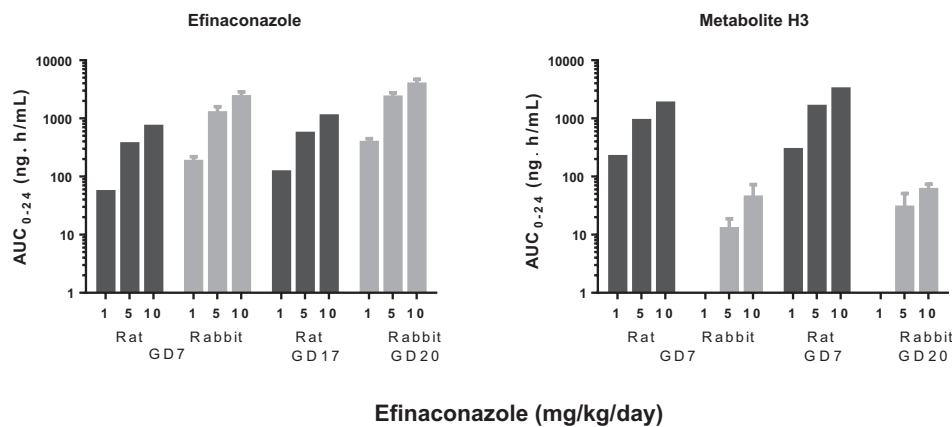
Fig. 2. Species differences in plasma parent drug and metabolite exposure.

Table 10
Efinaconazole developmental and reproductive safety.

Study	Endpoint(s)	Efinaconazole (mg/kg/day)	AUC (ng h/mL)	AUC ratio animal/MRHD[b]
Fertility and early embryonic developmental toxicity	Fertility NOAEL	25	7052[a]	279
	Prolonged estrous NOAEL	5	1411[a]	56
Rat developmental toxicity	EFT LOAEL and malformation NOAEL	50	14105[a]	559
	EFT NOAEL	10	2821[a]	112
Rabbit developmental toxicity	EFT and malformation NOAEL	10	3881	154
Developmental and perinatal/postnatal toxicity	EFT LOAEL and postnatal NOAEL	25	2253	89
	EFT NOAEL	5	422	17

[a] Estimate based on female day 10 mg/kg/day 90 AUC in 6-month subcutaneous repeat dose rat toxicity study.
[b] Highest individual AUC at the maximum recommended human dose.

CYP19 (aromatase), a key enzyme in the biosynthesis of estrogen [6], at sub-micromolar (e.g. bifonazole $IC_{50} = 0.02\,\mu M$) to high micromolar (e.g. fluconazole $IC_{15} = 140\,\mu M$) concentrations [18]. Inhibition of CYP19 results in lower estrogen levels, and estrogen decreases were noted in epoxiconazole, ketoconazole, fluconazole, and voriconazole rat developmental toxicity studies [19] (Diflucan; Vfend). Lower estrogen during rat gestation is associated with a variety of effects including increased placental weights and prolongation of parturition (Diflucan). Estrogen levels were not measured in the efinaconazole studies, but the finding of increased placental weights in rats is consistent with lower estrogen due to CYP19 inhibition.

Teratogenicity has been associated with ablation or reduction of CYP51 activity; CYP51 knock-out mice develop skeletal and cardiac malformations, consistent with fluconazole-induced teratogenicity, although potency for CYP51 inhibition and teratogenicity do not correlate [7–10,20].

CYP26 inhibition is also a proposed azole teratogenic target [5]. CYP26 catabolizes retinoic acid, a well-established teratogen. In animals, azole antifungals produce a spectrum of malformations similar to patterns of retinoic acid teratogenicity (i.e. craniofacial and axial defects), and in vitro, azoles modulate gene expression in embryos similar to retinoic acid [21]. Retinoic acid is important to axial specification during normal embryonic development via Hox genes expression and alterations of embryonic retinoic acid levels leads to dysmorphogenesis [22]. Efinaconazole was not teratogenic and has not been tested for inhibition of mammalian CYP51 or CYP26 or effects on gene expression.

Efinaconazole was embryotoxic in rats but not rabbits; efinaconazole exposures were roughly equivalent between the rat and rabbit while metabolite H3 exposures in the rat were 100-fold higher than in the rabbit, Fig. 2.

These data may suggest a role for H3 in embryo-fetal mortality but H3 embryotoxicity has not been investigated. Another explanation is that other azoles (e.g. ketoconazole, itraconazole and fluconazole) are teratogenic in rats but not rabbits [23]; thus, species differences in efinaconazole toxicity may be due to species sensitivities and not to the H3 metabolite.

No effects of efinaconazole on rat fertility or early embryonic development were observed at the high dose (25 mg/kg/day) or, based on estimated AUC, 279 times the maximum human exposure in onychomycosis therapy. The lack of efinaconazole effects on rat fertility is consistent which other pharmaceutical azoles (itraconazole, voriconazole and fluconazole) and differs from ketoconazole impairment of rat fertility. The estrous prolongation NOAEL was 5 mg/kg/day, 56-fold higher than the MRHD AUC. No fetal malformations were found at the rat developmental toxicity high dose, 50 mg/kg/day (559-fold MRHD). Embryo-fetal toxicity was noted at the high dose with a 10 mg/kg/day NOAEL or 112-fold MRHD. The rabbit developmental toxicity NOAEL establishes a 154-fold exposure multiple for efinaconazole. The dose levels in the perinatal and postnatal rat developmental study were slightly different compared to the developmental toxicity study and are probably the reason for differences in NOAELs. The perinatal NOAEL establishes a 17-fold exposure multiple for efinaconazole compared to human exposure at the MRHD. These exposure multiples support the safety of topical efinaconazole treatment of onychomycosis.

6. Conclusions

Efinaconazole reproductive and developmental toxicity included F1 mortality, placental effects and estrous cycle prolongation. These toxicities are consistent with marketed azole antifungal drugs. Unlike many marketed azole antifungals efinaconazole was not teratogenic. Efinaconazole did not affect embryo-fetal development in rabbits even in the presence of maternal toxicity.

When compared with maximal systemic exposures observed in onychomycosis patients treated with topical nail administration of efinaconazole 10% solution, developmental and reproductive toxicity in rats was noted only at high (>100-fold) multiples of human systemic exposure.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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