KETOCONAZOLE

shampoo 2%

2.6.6 Toxicology written summary

Toxicology written summary

Topically applied ketoconazole appears to have a low order of toxicity and is generally well tolerated. Effects in non-clinical studies were observed only at exposures considered sufficiently in excess of the maximum human exposure indicating little relevance to clinical use.
Searches were performed on PubChem and Micromedex database focusing on non-clinical trials for ketoconazole toxicity.

Single dose toxicity

Rats and Mice: Research on rats and mice has shown that acute oral doses of ketoconazole can cause toxicity, including symptoms such as lethargy, decreased activity, and gastrointestinal distress. The severity of toxicity typically depends on the dose administered.

Ketoconazole has been administered by the oral (gavage) and intravenous routes to mice, rats, guinea pigs and dogs. After oral administration toxicity was manifested in mice, rats and guinea pigs by sedation, catalepsy, ataxia, tremors, convulsions and pre-lethal loss of the righting reflex at doses >320 mg/kg. In dogs, toxicity was manifested by diarrhea and vomiting at doses >80 mg/kg. Toxicity after intravenous administration was manifested by spasms, convulsions and dyspnoea in rats, mice and guinea pigs; pre-lethal loss of the righting reflex occurred in mice and guinea pigs, and dogs. Toxicity in dogs was also manifested by licking and convulsions (PubChem_Ketoconazole; Product Monograph Ketoconazole, Teva).

Dogs: Dogs are more sensitive to ketoconazole compared to some other species. Studies have demonstrated that single doses of ketoconazole in dogs can result in symptoms such as vomiting, diarrhea, and neurological effects. Nonclinical study was performed in order to evaluate the type and frequency of adverse effects associated with ketoconazole therapy in dogs treated for skin diseases and any possible influence of dosage, duration of therapy, signalment or concurrent medication. The medical records of 632 dogs treated with ketoconazole (2.6-33.4 mg/kg) were reviewed. Adverse effects occurred in 14.6% (92 dogs) and included vomiting (7.1%), anorexia (4.9%), lethargy (1.9%), diarrhoea (1.1%), pruritus (0.6%), erythema (0.3%) and other adverse effects (2.5%). Of the dogs with other adverse effects, four of 16 (25%) were ataxic and three of these received concurrent ivermectin. Adverse effects were significantly more often recorded in dogs concurrently treated with ciclosporin ($P = 0.034$) or ivermectin ($P = 0.007$). Increased liver enzyme levels were reported rarely, and icterus was not seen in any of the dogs. However, monitoring liver enzymes during therapy is recommended, although this might not necessarily prevent severe idiosyncratic hepatotoxicity (Mayer, et al. 2008).
According to toxicity studies of ketoconazole in various animals' species, after oral, and iv administration, the following results were obtained (PubChem_Ketoconazole):

Organism	**Route**	**LD50**
Rats	oral	166 mg/kg
Rats	iv	86 mg/kg
Mice	oral	618 mg/kg
Mice	iv	41,500 ug/kg
Dog	oral	178 mg/kg

Repeated dose toxicity

Ketoconazole is a major breakthrough although hepatic side-effects as well as interactions with mammalian steroids might rarely occur during prolonged treatment. The prediction of these side-effects is difficult but the potential to interact with mammalian cytochrome P-450 enzymes is considered to be important (Van Cauteren, et al. 1989).

Rats: Slight pathological changes in the kidney, adrenals and ovaries were noted in an 18-month repeated dose rat study. In addition, female rats showed an increase in bone fragility. The No Observed Adverse Effect Level (NOAEL) in both these studies was 10 mg/kg/day. Ketoconazole is inhibitory in vitro at low concentrations to most fungi. Blood levels after oral administration to animals and man greatly exceed these inhibitory concentrations for several hours. The efficacy of this drug has been demonstrated in animal models. Initial clinical evaluation has produced responses to therapy with 200-400 mg/day in 13 of 16 evaluable patients with systemic and superficial fungal infections, involving 10 fungal pathogens. No toxicity has been noted to date in these human studies (Borelli, et al. 1979).

Experimental study was conducted in order to study the effect of ketoconazole induced liver damage, compare with control group and correlate with previous studies. Forty adult male albino rates were used for this study. Group-A served as control animals, received injection of normal saline in dose of 0.05 ml/100 gm of body weight intraperitoneally daily for 03, 07, 05 and 30 days. Group-B received injection of ketoconazole 40 mg/kg of body weigh intraperitoneally daily for 03, 07, 15 and 30 days of treatment. Animals were sacrificed after completion of treatment under ether anaesthesia. The results show that Ketoconazole treated animals showed distortion of hepatic architecture increase size of hepatocytes, decrease nuclear diameter and necrosis of hepatocytes within hepatic lobule as compared to control group-A animals. It was concluded from this study that ketoconazole induced injury is dose and duration of therapy dependent and due to its cost-effective frequent use needs further research in humans (Shaikh, et al 2010).

Repeated 28-day oral toxicity study of ketoconazole in rats based on the draft protocol for the "Enhanced OECD Test Guideline No. 407" to detect endocrine effects was conducted. Seven-

week-old SD rats were administered with ketoconazole daily by oral gavage at doses of 0, 6.25, 25 or 100 mg kg (-1) day (-1) for at least 28 days. The ketoconazole-treated male rats showed reduction of epididymis and accessory sex organ weights, spermatid retention in the seminiferous tubules, decrease of testosterone and increases of estradiol, luteinizing hormone (LH) and follicular stimulating hormone (FSH). A prolongation of the oestrous cycle and increases of estradiol, LH and FSH were observed in the treated female rats. Thyroxin and triiodothyronine were decreased and thyroid-stimulating hormone was increased in both sexes; however, there were no compound-related microscopic lesions in the thyroid gland or changes in the thyroid weight. The endocrine-related effects of ketoconazole could be detected by the parameters examined in the present study based on the Organization for Economic Cooperation and Development (OECD) protocol, suggesting that the Enhanced TG407 protocol should be a suitable screening test for detection of endocrine-mediated effects of chemicals (Shin, et al. 2006).

Kang et al conducted a study aimed to investigate the effect of ketoconazole on aromatase protein levels and to evaluate endocrine disruption effects of ketoconazole. A 20-day-old, 5-week-old and 7 week-old Sprague-Dawley (SD) male rats were dosed with ketoconazole (25, 50, 100mg/kg/day) daily by gavage for 14 days. Ketoconazole significantly decreased the weights of ventral prostate and Cowper's gland in 20-day-old SD male rats. In 5-week-old SD male rats, the weights of seminal vesicle, ventral prostate, LABC and Cowper's gland were decreased. In 7-week-old rats SD male rats the weights of ventral prostate, Cowper's gland were decreased. Ketoconazole significantly decreased serum estradiol in 20-day-old and 5-week-old SD male rats, and significantly decreased serum 5α-dihydrotestosterone (5α-DHT) and testosterone in 7-week-old SD male rats. In comparison with control group, ketoconazole dose dependently increased the aromatase protein levels in 20-day-old rats but decreased in 7-week-old rats. From these results, authors found that ketoconazole altered enzymes related to degradation of testosterone. This suggests that these effects may be one of the action mechanisms of endocrine disruption (Kang, et al 2003).
In the rats from the 6, 12 and 18 month oral diet toxicity studies, radiometry and direct photon absorptiometry were applied to assess the significance of the observed increase in bone fragility. The results indicate a dose-related decrease of cortical bone area which is present in both sexes at 40 and 80 mg/kg and to a lesser extent in females dosed at 20 mg/kg. Bone mineral content per unit of width however was unaffected, demonstrating the absence of osteoporosis. The mechanism for this condition remains unclear - the induction of menopause in female rats could be a contributing factor (Product Monograph Ketoconazole, Teva).

Chronic administration of ketoconazole to male rats and mice resulted in steroid levels comparable with those of control animals. Epididymal sperm motility was only slightly reduced in male mice 4 hours after administration of the drug. No effect on sperm motility was noted after chronic administration in either species studied. In vitro exposure of epididymal sperm to ketoconazole resulted in a significant reduction of sperm motility. Breeding trials after ketoconazole administration resulted in normal fertility and fecundity even at the highest dosage studied (Heckman, et al 1992).

Dogs:

Ketoconazole toxicosis was first recognized in 1982 in a nonclinical experimental study involving Beagles. This study investigated the usefulness and hazards of ketoconazole treatment for mycotic infections in dogs with escalated supratherapeutic dosages. Hyporexia, weight loss, variable emesis, and increases in serum ALT and ALP activities occurred when administered at 40 and 60 mg/kg/d (18 and 27 mg/lb/d), PO, for 1 year and 20 weeks, respectively. In addition to these signs, jaundice, gastritis, and lethal toxicosis were noted after 2 to 4 weeks of ketoconazole administration at 80 mg/ kg/d (36 mg/lb/d), PO. Adverse clinical reactions to ketoconazole (2.6 to 33.6 mg/kg/d [1.2 to 15.3 mg/lb/d], PO) have been retrospectively characterized in 15% (92/632) of dogs treated at 3 specialty dermatology practices (Mayer, et al 2008). In that study, (Mayer, et al 2008) suspected adverse drug reactions were identified by use of the Naranjo adverse reaction probability scale. An adverse drug reaction was considered to have occurred if clinical signs, clinicopathologic markers, or hepatic histologic abnormalities developed after drug exposure and subsequently improved with drug discontinuation or dose reduction, in the absence of alternative plausible causes (Arimone , et al 1992). Observed adverse effects included vomiting (7%), anorexia (5%), lethargy (2%), and diarrhea (1%) and, less frequently, cutaneous erythema, trembling, and weakness; some dogs had > 1 complication (Mayer, et al 2008).

In Product Monograph Ketoconazole, Teva, the following repeat-dose toxicity studies performed on Beagle dogs for a period of 12 and 6 months and were included:

- Four groups of 3 male and 3 female Beagle dogs received ketoconazole for 12 months at 0, 2.5,10 and 40 mg/kg/day administered in gelatin capsules. Behaviour was normal in the 0, 2.5 and 10mg/kg/day groups; however, at 40 mg/kg decreased appetite and sporadic emesis were noted in all animals during the entire study. No drug-related mortality was observed; however, body weight gain was significantly lower in the high-dose group during the entire experimental period. Heart rate, blood pressure and ECG were normal throughout the experiment. Haematology parameters were unchanged except in the high-dose group, where a marginally low haemoglobin value was noted during the entire dosing period. Urinalyses and serum analyses were normal except in the high-dose group, where a marginal decrease of albumin from week 24 onwards and persistently high alkaline phosphatase and SGPT values during the entire dosing period were noted. Gross pathology was normal in all groups except the high-dose group, where all dogs exhibited dark coloured livers, brownish discoloured pancreas, thymus, adrenals, thyroid, testes, ovaries, lymph nodes and fatty tissue and gray coloured ovaries. In the high-dosed group, the absolute weight of several organs decreased with an increase of the relative organ weight. Absolute and relative liver weight increased in the high-dose group. Histology revealed deposition and marked accumulation of a granular yellowish pigment in macrophages in various tissues (in the ovaries, in the hepatocytes, in the fasciculata and glomerulosa zones of the adrenals, in the biliary epithelium and in the interstitial tissue and Leydig cells of the testis) in a dose-related fashion at 10 and 40 mg/kg/day. The livers of the 40 mg/kg dosed males were devoid of glycogen. Large vacuolated cells were seen in the

fasciculata zone of the adrenals of the high dosed animals of both sexes. Desquamation and spermatid giant cells were conspicuous in some 40 mg/kg dosed dogs and one dog showed reduced spermatogenic activity. Many macrophages with yellowish pigment were seen in the 40 mg/kg dosed dogs and in 2/3 female dogs dosed at 10 mg/kg (Product Monograph Ketoconazole, Teva).

- In another experiment, 3 male and 3 female Beagle dogs were orally dosed with gelatin capsules containing increasing doses of ketoconazole (20, 40, 60 mg/kg/day) for a period of 6 months. One animal (60 mg/kg) died of gastroenteritis and nephritis during week 12. A dose-related body weight loss was seen above 20 mg/kg/day coincident with reduced appetite. There was no effect on the ECG at any dose and serum analyses and haematology were normal during the 20 and 40 mg/kg dosage periods. At 60 mg/kg/day an increase in SGOT and haptoglobin, a slight decrease of total protein and albumin and a pronounced increase of alkaline phosphatase and SGPT were seen. Decreases of haematocrit, haemoglobin and RBC were seen in most animals during the 60 mg/kg dosage period. Both serum analyses and haematology normalized during the withdrawal period. Gross pathology revealed a small sized thymus (decreased absolute and relative weight) and swollen liver (increased relative and absolute weight) at 60 mg/kg; however, these effects were reversible since they normalized during the withdrawal period. Histology indicated the presence of macrophages loaded with lipofuscin in the gallbladder, liver, ileum, spleen, lymph nodes, testes, ovaries, adrenals and prostate gland. In the liver, lipo-pigment also accumulated in the hepatocytes. Interstitial parotitis with reduced zymogen storage was seen in one dog. No spermatogenic activity was noted in 2/3 dogs - one having an immature aspect and the other a degenerated germinal epithelium. Lipofuscin loaded Leydig cells were noted in 2/3 dogs. An increase of clear replacement cells and reduced amount of secretion of the prostate was noted in the dogs with no spermatogenic activity (Product Monograph Ketoconazole, Teva).

- Three male and 3 female Beagle dogs were dosed orally with gelatin capsules at a dose of 80 mg/kg/day. Four animals died during the second week of the study, one died at 3 weeks and one during the fourth week. Anorexia with progressive weight loss and exhaustion was seen in all animals. All animals had an increased heart rate, severe gastroenteritis and clinical icterus haematology demonstrated an increase of segmented heterophils and decrease of lymphocytes and platelets. Alkaline phosphatase, SGPT and haptoglobin showed marked increases with increases of SGOT and bilirubin less pronounced. Total protein, albumin and glucose were decreased. Absolute and relative weight increases of the liver and adrenals and a decreased absolute and relative weight of the thymus were observed at necropsy (Product Monograph Ketoconazole, Teva).

Genotoxicity

No evidence of mutagenicity was found with ketoconazole in *in vitro* and *in vivo* studies (Gupta, et al 1994a; Gupta, et al 1994b; Product Monograph_Ketoconazole, Teva; PubChem_Ketoconazole).
In vitro studies: No mutagenic activity was observed in the Ames test with S. typhimurium in the presence of metabolic activating enzymes at doses of ketoconazole up to 50 mcg/plate (Product Monograph_Ketoconazole, Teva).
In vivo Studies: In the dominant lethal test in male and female mice, no increased incidence of mutations was observed in the offspring of male or female animals after doses of ketoconazole up to 160 mg/kg. In the micronucleus test in male mice, no effect was observed at doses up to 80 mg/kg (Product Monograph_Ketoconazole, Teva).
There was no evidence of mutagenicity with the Ames salmonella microsomal activator assay. In addition, ketoconazole single oral doses as high as 80 mg/kg were not mutagenic to germ cells (dominant lethal test in male and female mice). Ketoconazole was also not genotoxic based on the in vivo sister chromatid exchange assay (humans) and dominant lethal and micronucleus tests (mice) (PubChem_Ketoconazole).

Carcinogenicity

No evidence of carcinogenicity was found with ketoconazole in studies of rats and mouse (Contrera, et al 1996; Gupta, et al 1994a). Four groups of 50 male and 50 female albino Swiss mice received doses of 0, 5, 20 and 80 mg/kg/day of ketoconazole administered via the diet for 18 months. There were no significant differences between groups on overall mortality or on the time of death. There were no statistically significant differences between groups in the incidence or type of tumours (Product Monograph_Ketoconazole, Teva).
In a small number of dosed male mice (4-6%) decreased size of testes and mammary stimulation were seen at necropsy. An increased incidence of brownish aspect of the salivary glands was also noted in animals of both sexes dosed with 80 mg/kg/day. The most significant finding was a dose- related increase in pathology of the pancreas (brownish aspect) which was not seen in control animals but occurred in approximately 50% of high-dosed males and females. In the Wistar rat (200 males, 200 females), oral administration of 0,5, 20 and 80 mg/kg/day of ketoconazole in the diet for 24 months did not affect the mortality rate as compared to controls. At necropsy the following dose-related effects were seen: a brownish aspect of the salivary glands and the abdominal fat in medium and high-dosed animals and broken legs in one high-dosed male, 2 medium-dosed females and 10 high-dosed females. These findings have been identified in the 18-month rat toxicity study. The overall incidence of and type of tumours was not significantly different between treated and control groups, except for high-dosed female rats who had a decrease of the overall tumour rate (Product Monograph_Ketoconazole, Teva).

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Reproductive and developmental toxicity

Imidazoles inhibit testicular and male reproductive function by inhibiting testosterone secretion, testicular interstitial fluid production and luteinising hormone (LH) secretion regulatory systems (Adams et al. 1998). Ketoconazole may have effects on the endocrine system and liver (Gupta, et al 1994b). Animal tests show that this substance possibly causes toxicity to human reproduction or development (PubChem_Ketoconazole). At high doses (0, 6.25, 25 or 100 mg/kg/day), ketoconazole blocks testicular and adrenal biosynthesis. A repeated 28-day oral toxicity study of ketoconazole in rats showed a decrease in testosterone and an increase in oestradiol, luteinising hormone, and follicle stimulating hormone (Shin et al. 2006). The specific sites of ketoconazole inhibition in testicular steroidogenesis confirm the observation that ketoconazole is a potent inhibitor of androgen biosynthesis in several species. A relatively potent inhibitory effect of ketoconazole on CYP17A1 activity in Leydig cells from human testes, stallions, and pigs was observed. Ketoconazole (30 or 300 µg/l) in fish consistently suppressed ex vivo testosterone gonadal synthesis in both sexes and 17β-oestradiol (E2) in females during the exposures, and ketoconazole exposure also reduced plasma T concentrations in men and E2 in women (Maretta, et al 2023). Ketoconazole has been shown to cause gynaecomastia in humans (Thompson and Carter,1993).

To determine the effects of ketoconazole on androgen production and androgen dependent tissues in the rat, adult male rats were administered varying doses of ketoconazole every 12 hours. Serum testosterone declined to unmeasurable levels at ketoconazole doses of greater than 4 mg. This dose was sufficient to completely inhibit the testosterone surge induced by the administration of a potent luteinizing releasing hormone agonist. The ventral prostate weight of normal rats and the volume of the Dunning R3327H androgen dependent prostatic cancer declined at the same rate in animals treated with ketoconazole or castration. Ketoconazole may be an effective agent to treat androgen dependent diseases (Trachtenberg, 1984).

Recent evidence that ketoconazole depresses testosterone synthesis in humans prompted us to investigate the effects in rats of its administration alone or in combination with the gonadotropin releasing hormone super agonist analogue leuprolide. Plasma luteinizing hormone, testosterone, and ketoconazole levels as well as ventral prostate weight and tumour growth in rats bearing the androgen-dependent Dunning R3327H model of prostate adenocarcinoma were measured. The results indicate that combining ketoconazole with leuprolide achieves greater suppression of testosterone than either agent alone. When such protocols are applied to humans with prostate

cancer, more extensive effects may be expected because of the greater sensitivity of patients than of the rodent species to these agents (English, et al. 1986).

In animal fertility studies, dose and duration-dependent fertility impairment was observed in male and female rats. Although oral ketoconazole doses up to 2.4 times the MRHD in female rats had no effects on fertility, doses of 4.5 times the MRHD or greater resulted in decreased pregnancy rates and number of implantation sites. In male rats, oral ketoconazole doses 12 times the MRHD administered for 3 days decreased fertility; however, doses 24 times the MRHD for 3 days resulted in complete loss of fertility. Decreased fertility also occurred in male rats at doses 1.4 times the MRHD administered up to 3 months in duration. In a separate study, decreased sperm motility, decreased sperm count, increased abnormal sperm, and atrophy of the testes were observed in male beagle dogs administered oral ketoconazole at doses 5.2 times the MRHD for up to 4 weeks. The effects were reversible following cessation of treatment (Micromedex_Ketoconazole, 2024).

In developmental studies in rats the incidence of stillborn foetuses increased from a control value of 0.5% to 32.7% in rats dosed with 40 mg/kg and cannibalization of young occurred in two litters. In mice a significant decline in sperm motility and density in cauda epididymis was noted. A sharp decline in fertility (50% negative) in ketoconazole treated mice was observed. A significant reduction in the total protein and sialic acid contents of testes, epididymis, seminal vesicle and ventral prostate were noticed. The cholesterol contents of testes were raised while fructose contents of seminal vesicle were reduced significantly. The ketoconazole treatment altered the biochemical milieu of the reproductive tract. In the rabbit, ketoconazole produces evidence of maternal toxicity, embryotoxicity and teratogenicity at a high dose of 40 mg/kg/day (PubChem_Ketoconazole).

Ketoconazole adversely affects spermatogenesis in rodents, but knowledge on adverse efects of prolonged administration of ketoconazole on the fertility of male dogs is lacking. A case of reversible infertility with azoospermia in a male American Stafordshire terrier treated with ketoconazole is reported here. A seven-year old male American Stafordshire terrier treated for 3 months with ketoconazole for a persistent Malassezia dermatitis displayed reduced libido and mating of 3 bitches had been unsuccessful. The dog was presented at the clinic 40 days after the treatment had been stopped. At frst presentation, low libido and complete absence of sperm in the ejaculate (azoospermia) associated with low testosterone level were found. Repeated examinations revealed that sperm quality and testosterone level had restored 100 days after ketoconazole had been withdrawn. Thereafter, the dog successfully mated 2 bitches. It was concluded that treatment with ketoconazole for 3 months may have led to reversible infertility characterized by azoospermia. Therefore, owners of study dogs should be informed of this risk prior to initiating such treatment and in case of infertility, previous treatment with ketoconazole should be considered as a possible cause. (Domosławska and Zduńczyk, 2021).

A single oral dose (300 mg kg^{-1}) of ketoconazole induced reversible immobilization of rat epididymal spermatozoa at 8-24 h after dosing. This occurred when the drug concentrations in cauda epididymal fluid and seminal plasma were at their peak (18.0 $\pm$ 7.3 and 13.5 $\pm$ 3.0 µg ml^{-1} respectively), and which was preceded by a peak plasma concentration (C_{max}) of 64.82 $\pm$ 2.47 µg ml^{-1} at 5.15 $\pm$ 0.68 h (Tmax). In contrast, rete testis fluid collected from the same animals

contained only minute amounts of ketoconazole (0.47 $\pm$ 0.34 μg ml^{-1}). Plasma testosterone concentration showed a sharp decline within 4 h of dosing, followed by a recovery from suppression, even after administration of a low dose (100 mg kg^{-1}) which did not affect sperm motility. These findings suggest that ketoconazole gains access to the post-testicular sex organs and affects the mature spermatozoa therein much more readily than it affects testicular spermatogenesis. Synthesis and screening of compounds with a related molecular structure but which exhibit more pronounced spermicidal and less pronounced anti-androgenic effects are thus suggested in the hope that rapidly acting and reversible male contraceptives might be identified and developed (Wang, et al 1992).

Ketoconazole (KTC) is a model pharmaceutical representing imidazole and triazole pesticides, which inhibit fungal growth through blocking a cytochrome P450 (CYP)-mediated step in ergosterol biosynthesis. Several of these fungicides have been shown to be reversible inhibitors of CYPs in vertebrates (primarily mammals), including CYP isoforms involved in the pathway that converts cholesterol to active sex steroids. In these studies, the investigators assessed the effects of KTC on aspects of steroidogenesis and reproductive function in the fathead minnow (Pimephales promelas). Exposure of spawning adults to the fungicide for 21 d significantly decreased egg production at a water concentration as low as 25 microg/l. Despite evidence of reduced ex vivo testosterone production by gonads from KTC-exposed fathead minnows, circulating plasma concentrations of sex steroids (testosterone, 17beta-estradiol) were not affected. Exposure to KTC caused an increase in the gonadosomatic index in both sexes and, in males, the fungicide caused a marked proliferation of interstitial (Leydig) cells. In addition, mRNA transcripts for two key steroidogenic enzymes, cytochrome P450 side-chain cleavage (CYP11A) and cytochrome P450 c17alpha hydroxylase/17,20 lyase (CYP17), were elevated by exposure to KTC. Both the changes in transcript levels and proliferation of gonad tissue represent potential adaptive or compensatory responses to impaired steroidogenic capacity. Overall our data indicate that, although KTC does adversely affect steroidogenesis and reproduction in the fathead minnow, the fish can compensate to some degree to mitigate effects of the fungicide. This has important implications for the interpretation of data from tests with endocrine-active chemicals (Ankley, et al. 2007).

Teratogenicity

There are no adequate and well-controlled studies of ketoconazole use during pregnancy. Animal studies have shown the teratogenic and embryotoxic potential of this drug when administered through gavage at higher doses in pregnant rats (Amaral, et al 2023; Mineshima, et al. 2012). High doses of ketoconazole are embryotoxic and teratogenic in rats (Gupta, et al 1994a).

Syndactyly and oligodactyly in fetuses have been reported after the administration of 80 mg/kg (10 times the maximum recommended human dose) of ketoconazole in pregnant rats. High incidence of fetal resorption as well as a significant number of stillbirths showing embryotoxicity and fetotoxicity has been revealed by another study using different doses (10, 25 or 50 mg/kg) of ketoconazole in pregnant rats on gestational days 6 through 21. There have been few data describing the use of this drug in human gestation (Amado et al., 1990; Amaral, et al 2023).

Dystocia was reported at ketoconazole doses higher than 10 mg/kg (higher than 1.25 times the maximum human dose). It is postulated that maternal toxicity and resulting teratogenicity may be due to the particular sensitivity of the female rat to ketoconazole, as evidenced by an oral LD (50) of ketoconazole given by gavage to the female rat of 166 mg/kg which compared to 287 mg/kg in the male rat. In the rabbit, ketoconazole produces evidence of maternal toxicity, embryotoxicity and teratogenicity at a high dose of 40 mg/kg/day (PubChem_Ketoconazole).
Azole derivatives have teratogenic effects in rodents. In one study, malformations and their sensitive windows induced by high-dose ketoconazole (KCZ), an azole derivative, without maternal toxicity were investigated. In addition, the malformation spectrum determined was compared with that induced by vitamin A palmitate (VAP). Pregnant rats were administered a single dose of KCZ by oral gavage on specific individual days from gestational days 8 to 15 (GDs 8-15). Maternal animals were subjected to necropsy on GD 20, and the obtained fetuses were examined for external, visceral and skeletal malformations. The malformation spectrum of VAP was identified from available published data (Noda, Sato, and Udaka, 1982) and a complementary study (single administration of VAP at 1 200 000 IU kg(-1)). Embryonic lethality was observed in dams given KCZ on GDs 9-12 with peak incidence on GDs 10 and 11 with complete resorption. KCZ induced major malformations included cleft palate, digital anomalies, misshapen limbs and unique discontinuous ribs, and the sensitive window for each was identified. Compared with the malformations induced by VAP, unique malformations (e.g. discontinuous ribs by KCZ, neural tube defects by VAP), similar malformations with similar sensitive windows (e.g. digital and limb malformations) and similar malformations with different sensitive windows (e.g. embryonic lethality and cleft palate) were distinguished, suggesting that the mechanisms of several of the types of KCZ-induced malformation are related to excessive vitamin A (Mineshima, et al. 2012).
In animal studies, embryotoxicity (ie, increased resorptions, increased number of stillbirths, and delayed parturition) and delays in maturation were observed in mice orally administered ketoconazole during organogenesis at doses 0.8 times the maximum recommended human dose (MRHD) based on body surface area (BSA). However, maternal toxicity or malformations were not observed at doses up to 1.5 times the MRHD based on BSA, and there were no treatment-related developmental effects at dose 0.3 times the MRHD. In rats, embryotoxicity (ie, resorbed fetuses and stillbirths), and teratogenicity (ie, syndactylia, oligodactylia, waved ribs, and cleft palate), possibly as a result of maternal toxicity, were noted in rats orally administered ketoconazole at doses up to 4.8 times the MRHD. Similarly, an oral ketoconazole dose of 6 times the MRHD administered to rats on a single day during gestation days 9 to 15, resulted in increased resorptions and external malformations, including cleft palate, micromelia, and digital anomalies (brachydactyly, ectrodactyly, syndactyly). In a separate study, increased resorptions and skeletal malformations were observed following oral administration of ketoconazole to pregnant rabbits at doses up to 4.8 times the MRHD (Micromedex_Ketoconazole, 2024).

Pregnancy
There are no adequate and well-controlled studies in pregnant or lactating women. Data on a limited number of exposed pregnancies indicate no adverse effects of topical ketoconazole on

pregnancy or on the health of the foetus/newborn child. Animal studies have shown reproductive toxicity at doses that are not relevant to the topical administration of ketoconazole. No effects on the breastfed newborn/infant are anticipated. Plasma concentrations of ketoconazole were not detectable after topical administration of ketoconazole 2% shampoo to the scalp of non-pregnant humans. Plasma levels were detected after topical administration of ketoconazole 2% shampoo on the whole body. There are no known risks associated with the use of ketoconazole 2% shampoo in pregnancy or lactation (SmPC Nizoral 2% shampoo, Thornton & Ross Ltd. UK, 23/10/2020).
During an animal study, dystocia (difficult labor) was observed in rats administered oral ketoconazole at doses greater than one-fourth the MRHD during the third trimester of gestation (Micromedex_Ketoconazole, 2024).

Breastfeeding
Available evidence and/or expert consensus is inconclusive or is inadequate for determining infant risk when used during breastfeeding. The physician has to weigh the potential benefits of drug treatment against potential risks before prescribing this drug during breastfeeding. Plasma concentrations of ketoconazole were not detectable after topical administration of ketoconazole 2% shampoo to the scalp of non-pregnant humans. Plasma levels were detected after topical administration of ketoconazole 2% shampoo on the whole body. There are no known risks associated with the use of ketoconazole 2% shampoo in pregnancy or lactation (SmPC Nizoral 2% shampoo, Thornton & Ross Ltd. UK, 23/10/2020).

Local Tolerance
Local tolerance studies for ketoconazole shampoo would assess its safety and potential for irritation or sensitization when applied to the scalp and skin. These studies are important for ensuring that the shampoo is well-tolerated by users and does not cause adverse reactions when used as directed. Sung, et al conducted a case study on female cocker spaniel dog and provide evidence suggesting that ketoconazole shampoo-triggered pemphigus-like reactions most probably represent a unique contact drug-triggered PF, which has never been described in veterinary medicine. The case also highlights the need for clinicians to understand the mechanism of contact drug-triggered PF in order to ensure appropriate diagnosis and treatment. Furthermore, this report emphasizes that a careful topical medication history, including shampoo, is essential in the workup of cases of pemphigus (Sung, et al 2017).
Ketoconazole is a widely used imidazole antifungal agent. True contact allergy to topical ketoconazole is rare, and few cases of patients with contact allergy to ketoconazole have been reported. Allergies to inactive ingredients, especially vehicles and preservatives, are more common than allergies to ketoconazole itself (Warshaw, 2014).
Ketoconazole shampoo has an excellent safety profile, with mild adverse events occurring in only 1% to 7% of patients. The most commonly reported adverse effects for topical KTZ are pruritus, dry skin, and burning sensation; these may be attributed KTZ or vehicle being contact

irritants Imidazole antifungals have the potential to cause allergic contact dermatitis (ACD) and photoallergic contact dermatitis. KTZ is a moderate irritant and could lead to sensitization reactions (Choi, et al 2019; Sinawe and Casadesus, 2022). After topical application, irritation and burning sensation were reported (Kaur and Kakkar, 2010). The most common side effects are dryness of the scalp and hair and application site reaction such as itching, burning, stinging and contact dermatitis (Dias, et al, 2013; Sinawe and Casadesus, 2022). Kubicki, et al presented case of ketoconazole shampoo-induced hair discoloration (Kubicki, et al 2020).

Other toxicity studies

Not applicable.