multiple small vacuoles within the cytoplasm of these cells. As the dose of HPBCD was increased, so was the severity of the damage, with more and more tubules being affected. In the most severe cases, the high dose of HPBCD caused vacuolation of more than 40% of the renal tubular cells.

Injection site lesions and lung granuloma also seemed to be unrelated to the drug since they were also found in control animals.

Conclusion

Liver and lung remain the primary target organs of voriconazole during a 28 day administration. The presence of two impurities ——————————— did not affect the toxicolgy profile of the drug.

16 Study title: Six-month intravenous toxicity in Sprague Dawley rats.
Key study findings: Decreased triglyceride levels, increased relative liver weights, centrilobular hypertrophy. Scattered pale, slightly enlarged hepatocytes, renal tubular vacuolation, vacuolation of the bladder, vacuolation of the transitional epithelium of the renal pelvis, scattered foamy macrophages in the testes were ascribed to SBECD.
Study # 95-96-22
Volume 24
Conducting laboratory and location: Drug Safety Evaluation Central Research, Pfizer Pharmaceuticals Inc. 5-2 Taketoyo-cho, Chita-gun, Aichi, Japan.
Date of study initiation: June 19,1995
GLP compliance: Yes
QA report: Yes
Drug, lot # R12
Formulation/vehicle: Voriconazole was dissolved in an aqueous solution of SBECD

Rats (20 rats/sex/dose) were treated with 2, 5, and 10 mg/kg of voriconazole, intravenously for 6 months. A vehicle control group received SBECD only, an additional control group received saline. Records were kept of mortality, clinical signs, food and water intake,

body weights, ophthalmology, plasma chemistry, hematology, urinalysis, gross necropsy findings, organ weights and histopathology findings. Plasma drug levels were determined at 6 months on 5 supplementary rats/sex/level at 5 minutes and 2, 5 and 24 hours after dosing.

Mortality

No animals died during the course of the study.

Toxicity

Voriconazole administration was associated with decreased triglyceride levels (22 to 33% reduced) in mid and high dose animals. This change was observed in both sexes on day 120, but was only seen in females by day 176.

Relative liver weights were increased in high dose animals (+ 13 %). Minimal centrilobular hypertrophy (hepatocytes enlarged by a factor less than 1.5) was observed in three high dose females. Scattered pale, slightly enlarged hepatocytes were observed in some animals receiving the highest dose of SBECD (4 vehicle control males and 1 high dose male).

Multifocal renal tubular vacuolation of proximal tubules was found in all animals which received 160 mg/kg SBECD with or without voriconazole and so this was ascribed to the vehicle. Renal tubular vacuolation was also seen in 37/40 rats receiving 80 mg/kg and 22/40 rats receiving 32 mg/kg SBECD. More animals and a greater % of cells were affected as the dose of SBECD increased. Cytoplasmic vacuolation of the bladder epithelium and vacuolation of the transitional epithelium of the renal pelvis were also observed with similar frequency. Scattered foamy macrophages were seen in the testes of all males receiving the mid and high doses of SBECD and 14/20 animals in the low dose (SBECD) group.

Conclusion

Intravenous injection of voriconazole formulated in SBECD results in toxic insults to the liver, kidneys and testes. Renal tubular vacuolation is seen in animals treated with SBECD with or without voriconazole and the insult is increasingly more severe as the dose of SBECD increases. These findings suggest that the kidney damage may result from the vehicle SBECD. No NOAEL could be determined since renal tubules were affected in all dose groups.

17. Study title: 14-day intravenous range-finding toxicity in beagle dogs.
Key study findings: Decreased triglyceride levels, increased relative liver weights, centrilobular hypertrophy. Scattered pale, slightly enlarged hepatocytes, renal tubular vacuolation, vacuolation of the bladder, vacuolation of the transitional epithelium of the renal pelvis, scattered foamy macrophages in the testes were ascribed to SBECD.
Study # 90149
Volume # 21

Conducting laboratory and location: Pfizer Centre de Recherche. 37401 Amboise, Cedex, France.
Date of study initiation: November, 1990
GLP compliance: Yes
QA report: Yes
Drug, lot # R1
Formulation/vehicle: Voriconazole was dissolved in an aqueous solution of HPBCD

Two groups of Beagle dogs (1 male and 2 females/dose group) were treated with 1 or 3 mg/kg of voriconazole intravenously. A third group received 10 mg/kg for 2 days then 6 mg/kg from day 3 onwards because of severe clinical signs. Drug was dissolved in an aqueous solution of hydroxypropyl-beta-cyclodextrin, and control animals (one male and two females) received vehicle over the same period. Records were kept of clinical signs, food intake, body weights, plasma drug levels, blood pressure, heart rates, ECG findings, plasma chemistry, hematology, gross necropsy findings and histopathology findings.

There was no mortality. On the first two days of dosing, high dose (10 mg/kg) females showed salivation, tremors, rigid, extended limbs, fixed stare, titubation, regurgitation-like movements, convulsions and pedaling movements. These signs appeared within seconds of drug administration and disappeared approximately two minutes later. As a result the dose of drug was reduced to 6 mg/kg.

Toxic effects in the liver included minimal centrilobular hypertrophy and endoplasmic reticulum proliferation in one high dose male. Drug administration was also associated with dose-related 2 to 3 fold increases in P450 levels. Heart rate was increased by 15 and 22 % in the mid and high dose dogs, respectively. Although there was one high dose animal with increased heart weight, there were no ECG or histopathological correlates which would explain the increased heart rate. Alkaline phosphatase was increased in all high-dose animals, although the rise in the male (39 %) was less than the rise seen in the females (113 % and 65 %).

Focal mineralization of the kidneys as well as injection site inflammation and granulomas were probably excipient related since they were also seen in control animals. This excipient will not be used in the proposed clinical trial.

Drug levels were similar in both sexes, and were dose dependent. Peak plasma drug levels, (measured at 2 hours after drug administration), were generally comparable on days 1 and 8 after accounting for the reduction of the high dose from 10 mg/kg to 6 mg/kg. AUC values, which were similar on days 1 and 8 for the low dose, were decreased on day 8 compared to day 1 at the mid dose. It was unclear whether the trend of reduced AUC with repeated dosing was true for the high dose because of the change from 10 to 6 mg/kg.

Conclusion

Liver, and heart are target organs of this drug. The drug seems to induce its own metabolism. Females seem to be more sensitive to this drug than males.

18. Study title: 1-month intravenous toxicity in beagle dogs with one-month reversibility study at 6 mg/kg.
Key study findings: Miosis, salivation, partially closed eyes, protruding eyes, convulsions, ptosis, decreased activity. Alkaline phosphatase increase, liver weight increase, centrilobular hypertrophy, kidney and heart changes, osteochondropathy.
Study # 93097
Volume # 23
Conducting laboratory and location: Pfizer Centre de Recherche. 37401 Amboise, Cedex, France.
Date of study initiation: December 1993
GLP compliance: Yes
QA report: Yes
Drug, lot # R1
Formulation/vehicle: Voriconazole was dissolved in an aqueous solution of SBECD

Groups of beagle dogs, (3 dogs/sex/dose group) were treated with vehicle (SBECD) or 1, 3, or 6* mg/kg of UK,109 intravenously for 28 or 29 days. *(Initially, high dose animals were treated with 6 mg/kg/day but the occurrence of convulsions led to the lowering of the dose to 4.5 mg/kg for three days (days 5-7) after which 6 mg/kg was administered with a slower rate of injection). In a parallel study, 2 beagle dogs/sex/group were treated with 0 or 6 mg/kg for 28 days and then retained without dosing for one month to assess reversibility of toxic effects. Records were kept of mortality, clinical signs, body weights, cardiovascular parameters, ophthalmologic examinations, hematology, clinical chemistry, urinalysis, necropsy findings, organ weights and histopathology.

No animals died during the study. Within the first hour after dosing, treatment was associated with a number of transient clinical signs including miosis and increased salivation at all doses and partially closed eyes at the mid and high doses. Other signs observed in the high dose animals included protruding eyes following convulsions, ptosis and decreased activity. Changes in hematology parameters were not robust, lacked dose dependence and were reversible at the end of the recovery period. Means were also greatly affected by outlying values due to the relatively small number of dogs/group. Mean plasma alkaline phosphatase increased by 40 and 54 % respectively on days 15 and 29, but had returned to normal at the end of the reversibility period. Liver weight increased in all female dose groups (18-44%), but only showed a 4 % increase in the high dose male group. Even after a month of recovery, mean relative liver weight was increased 30% over controls in females. Centrilobular hypertrophy, with a ground glass appearance of the cytoplasm was observed in these hepatocytes at the end of the dosing period but not in the recovery animals. Kidney and heart changes were similar when controls were compared with treated. Osteochondropathy was noted in the sternum of one mid and one high-

dose male. It was characterized by a single small focus of chondrolysis/osteolysis within the osteogenic zone of the zone plate with secondary replacement by fibrous tissue.

Pharmacokinetics

Table 16 (below) shows the pharmacokinetic parameters measured on day 15 of dosing. Drug levels were similar for both male and females. Both AUC's and Cmax's were dose dependent.

Table 16. Pharmacokinetics of UK-109,496 on day 15 of dosing

Dose (mg/kg)	Cmax[1] (µg/ml)	AUC[2] (µg.h/ml)
1	1.25	3.58
3	3.59	13.6
6	8.05	32.7

[1] Cmax values were measured from blood collected at 5 minutes post dosing. [2] AUC values were calculated from data collected between 5 minutes and 8 hours.

No NOAEL could be determined from this study because all dose groups showed increased liver weights. The NOAEL was estimated to be (<1 mg/kg), which is equivalent to a human dose of 0.54 mg/kg for 28 days.

19. Study title: Six-month intravenous toxicity in beagle dogs.
Key study findings: Miosis, alkaline phosphatase increase, Liver weight increased, Centrilobular hypertrophy, kidney, vacuolation of the renal tubular epithelial cells ascribed to SBECD.
Study 95-96-21
Volume 25
Conducting laboratory and location: Pfizer Centre de Recherche. 37401 Amboise, Cedex, France.
Date of study initiation: December, 1993
GLP compliance: Yes
QA report: Yes
Drug, lot # R1
Formulation/vehicle: Voriconazole was dissolved in an aqueous solution of SBECD

Groups of beagle dogs, (4 dogs/sex/dose group) were treated with vehicle (SBECD) or 1, 3, or 6 mg/kg of UK,109 intravenously for 6 months. Vehicle control dogs were administered SBECD and saline control animals were administered physiological saline. SBECD doses were

0, 96, 16, 48, and 96 mg/kg for saline control, vehicle control, low-, mid-, and high-dose groups, respectively. Records were kept of mortality, clinical signs, body weights, cardiovascular parameters, ophthalmologic examinations, hematology, clinical chemistry, urinalysis, necropsy findings, organ weights and histopathology.

No animals died during the study. Within the first hour after dosing, treatment was associated with a number of transient clinical signs including miosis. Miosis was seen in all drug treated animals was more prevalent at the higher doses.

Mean plasma alkaline phosphatase increased by up to 200 %. Increases were seen at all doses in males (for example, + 34 % at 1 mg/kg on day 65) and at the mid and high doses in females.

Pharmacokinetics

Table 17(below) shows the pharmacokinetic parameters measured on days 15 and 172 of dosing. Drug levels were similar for both male and females. AUC's was dose dependent and increased slightly between days 15 and 172.

Table 17 Mean AUC $_{5min-24h}$ (µg.h/ml) values for dogs on days 15 and 172 of dosing.

Dose	Day 15	Day 172
1	3	4
3	15	22
6	44	64

Liver weight increased in mid (+ 5 %, males, + 13 % females) and high (+ 12 % males, +20 % females) dose animals. Centrilobular hypertrophy, was observed in high dose animals. Kidney changes consisted of vacuolation of the renal tubular epithelial cells, which was present in SBECD treated animals and drug treated animals

No NOAEL could be determined for voriconazole since alkaline phosphatase was increased in all dose groups. The NOAEL was estimated to be (<1 mg/kg), which is equivalent to a human AUC of less than 4 µg.h/ml.

20. Study title: Microbial Reverse Mutation Assays (Ames Test)
Key study findings: No evidence of mutagenicity
Study 91-844-01
Volume # 26
Conducting laboratory and location: Department of Safety Evaluation. Pfizer Central Research, Groton Connecticut.
GLP compliance: Yes

QA report: Yes
Drug, lot # R1 and R-2
Formulation/vehicle: Voriconazole was dissolved in DMSO

Salmonella typhimurium strains TA·1535, TA 1537, TA 98 and TA 100 were exposed to UK-109,496 to test the drug's ability to induce reverse mutations. Compound was dissolved in DMSO and cells were exposed to drug at levels of between 0.02 and 10 mg/plate in the presence and absence of S9. Negative and positive controls were run concurrently with each assay and triplicate plates were prepared at each level. S9 fractions were prepared from livers of male rats [Crl:COB CD (SD) BR] which had been dosed five days previously with a single intraperitoneal injection of 500 mg/kg of Aroclor 1254 and were fasted on the day before sacrifice. The number of revertants was determined after at least 60 hours of incubation at 37 degrees C. In the preparation without S9, a dose related reproducible three-fold increase over control value is considered a positive response. In the presence of S9, a positive response is characterized by a dose-dependent, reproducible three-fold increase in the average number of revertant colonies compared to the respective activation control plates. There was no evidence of compound-related increases in the number of revertant colonies per plate at any level of UK-109 that would suggest mutagenic activity with or without S9.

A two-fold increase is usually considered by the FDA to indicate a positive response. The sponsor considered a positive response to be a 3-fold response. In none of the experiments was a 2-fold increase observed. Voriconazole did not display the potential to induce reverse mutations.

21. Study title: Mammalian cell gene mutation assays CHO/HGPRT
Key study findings: Voriconazole does not induce forward mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary eggs
Study #91-844-01
Volume # 26
Conducting laboratory and location: Department of Safety Evaluation. Pfizer Central Research, Groton Connecticut.
GLP compliance: Yes
QA report: Yes
Drug, lot # R1
Formulation/vehicle: Voriconazole was dissolved in DMSO

UK-109,496 was tested for its ability to produce forward mutations at the X-linked hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in the Chinese Hamster ovary cells. Cells were plated in T-75 flasks at a density of 1.25×10^6 cells per flask and exposed to drug in the presence or absence of S9. In the direct mutagenicity experiments (without metabolic activation), drug concentrations were between 965 and 3333 µg/ml. In the metabolic activation

experiments, drug concentrations were between 804 and 2000 µg/ml and drug was dissolved in DMSO. Precipitation occurred above 1667 µg/ml.

The first direct experiment was repeated due to an elevation in the number of spontaneous mutations. In the second direct experiment, drug produced 0-8 mutants per 10^6 survivors. The average number of spontaneous mutants per 10^6 survivors was 1 (acceptance limit <40). There were 41 and 349 mutants per 10^6 survivors respectively for the positive control ethyl methanesulfonate at 50 and 400 µg/ml.

In the metabolic activation experiment, treated cultures produced between 0 and 28 mutants per 10^6 survivors. The average number of spontaneous mutations per 10^6 survivors for negative controls was 26. The positive control group (3-MCA at 5 and 10 µg/ml) produced 124 and 126 mutants per 10^6 survivors, respectively. Based on these test results, UK-109,496 does not induce forward mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary eggs.

22. Study title: In vivo mouse micronucleus assays
Key study findings: Voriconazole does not induce micronuclei.
Study #91-844-01
Volume # 26
Conducting laboratory and location: Department of Safety Evaluation. Pfizer Central Research, Groton Connecticut.
GLP compliance: Yes
QA report: Yes
Drug, lot # R1
Formulation/vehicle: Voriconazole was dissolved in water

The ability of the drug to induce micronuclei in mice was examine by dosing mice once per day for three days at 37.5, 75, and 150 mg/kg/day. Twenty-four hours after the final treatment, the mice were sacrificed, and smears made from the bone marrow of the femora from each mouse. The preparation was stained and 1000 polychromatic erythrocytes were scored for the presence of micronuclei. A positive response was defined as a substantial, dose related and reproducible elevation in the number of micro nucleated polychromatic erythrocytes in the treated animals. Although there was a reduction in the percent of polychromatic erythrocytes in

both sexes in all dose groups, confirming that the dose used were near the maximum tolerated doses, the frequency of multinucleated polychromatic erythrocytes was not increased compared to controls. Positive and negative controls produced the expected responses. Thus, UK-109,496 does not induce micronuclei in polychromatic erythrocytes in male or female mouse.

23. Study title: In vitro Cytogenetics Studies
Key study findings: Voriconazole produces chromosomal abnormalities.
Study #91-844-01
Volume # 26
Conducting laboratory and location: Department of Safety Evaluation. Pfizer Central Research, Groton Connecticut.
GLP compliance: Yes
QA report: Yes
Drug, lot # R1
Formulation/vehicle: Voriconazole was dissolved in DMSO

Human peripheral lymphocytes were collected by venipuncture from healthy donors and cultured in Williams medium E with the [redacted] for 48 hours. Cultures were then pooled, recovered and resuspended in fresh medium, after which they were aliquoted into 5-ml cultures. [redacted] Negative controls were treated with DMSO and culture medium to reach a final concentration of [redacted] Positive controls were exposed to [redacted] After addition of compound, incubation was continued at [redacted] degrees for an additional [redacted] hours with [redacted] present during the final [redacted] hours.

[redacted]

Conclusion

Voriconazole produces a slight increase in chromosomal abnormalities, mostly chromatid breaks, with a few chromosome breaks and, least frequently, rearrangements.
The sponsor argues that the slight magnitude of the increase combined with the lack of clear dose response, render these results non-definitive. However, while the increase in the number of abnormal cells at 1000 may µg/ml may not have been higher than that seen at 750 µg/ml, it was still higher than the control value (2.5 % vs 0.5 %).

Carcinogenicity Studies

24. Twenty-four month in-diet toxicity and carcinogenicity study in rats
Key study findings: Voriconazole produces hepatocellular adenomas and hepatocellular carcinomas when administered orally to rats at 50 mg/kg daily for 24 months.
Study # 96-96-31
Volume # 31
Conducting laboratory and location: Safety Evaluation Laboratory. New Product Development Center. Pfizer Pharmaceuticals Inc. 5-2 Taketoyo-cho, Chita-gun, Aichi Japan
Date of study initiation: October 24, 1996
GLP compliance: Yes
QA report: Yes
Drug, lot #, % purity: Drug batch number R17. Purity, 100%.
Formulation/vehicle: Powdered commercial lab chow

Groups of male and female Sprague Dawley rats (50/sex/dose group) received voriconazole in the diet at 6, 18 or 50 mg/kg for 24 months. The compound was admixed with commercial lab chow. Two identical control groups received unsupplemented diet. Animals were observed at least twice daily for mortality and at least once weekly for clinical signs. Records were kept of body weights, food consumption, hematology, serum chemistry, urinalysis,

necropsy findings, organs weights and histology findings. Plasma concentrations were measured on days 90 and 181. Mortality is shown on Table 18.

Table 18. Mortality after 24 months of voriconazole in rats.

	Control 1+2		6 mg/kg		18 mg/kg		50 mg/kg	
	M	F	M	F	M	F	M	F
Number of animals	100	100	50	50	50	50	50	50
Sacrificed moribund	36	29	11	13	21	16	18	18
Found dead	38	26	28	13	17	9	17	5
Total unscheduled deaths	74	55	39	26	38	25	35	23
Survivors at final sacrifice (%)	26	45	22	48	24	50	30	54

There were significantly more deaths in males than in females, even in control groups.

Voriconazole produced significantly reduced body weights at all doses when administered for 24 months. The decrease was 10% or higher for mid-dose females and high-dose males and females. (see Table 19)

Table 19. Decreases in mean bodyweight on day 715

Dose (mg/kg)	Male	Female
6	9.7	2.4
18	4.3	10
50	13	11

Mean plasma levels were generally (up to about 4-fold) higher for females than for males. Males were far more sensitive to voriconazole toxicity than females, since a four-fold lower exposure of voriconazole produced about twice the mortality in males when compared to females.

Table 20. Mean plasma concentrations of voriconazole µg/ml in rats.

Dose	Males		Females	
	Day 90	Day 181	Day 90	Day 181
6 mg/kg	0.14	0.15	0.40	0.58
18 mg/kg	0.46	0.48	0.89	0.69

50 mg/kg	0.89	0.72	2.4	1.3

Voriconazole administration was associated with a number of hepatic changes including increased liver weights, centrilobular hypertrophy, vacuolation, granuloma, eosinophilic foci, hepatocellular cystic change, adenomas and carcinomas (see Tables 21 to 24 below).

Table 21. Increases in Relative liver weights (%) in voriconazole treated animals compared to controls.

Dose (mg/kg)	Male	Female
6	+16	+6
18	+36	+24
50	+47	+41

Table 22. Hepatic findings in Males

	C1	C2	6	18	50
Centrilobular hypertrophy	0	0	0	0	6
Vacuolation	12	3	12	18	15
Granuloma	4	2	0	1	8
Hepatocellular cystic change	10	9	5	7	20
Eosinophilic foci	6	8	5	8	5

Table 23. Hepatic findings in Females

	C1	C2	6	18	50
Centrilobular hypertrophy	0	0	0	0	9
Vacuolation	24	26	18	24	36
Granulomas	13	15	16	20	26
Hepatocellular cystic change	1	1	1	2	3

Eosinophilic foci	3	7	12	9	12

Table 24. Neoplastic hepatic findings in Rats

	C1	C2	6	18	50
Adenomas					
Males	5	3	1	3	6
Females	5	2	4	5	14
Carcinomas					
Males	1	1	4	1	3
Females	1	0	1	1	1

Voriconazole produced an increase in hepatocellular adenomas in high dose females when compared to control animals. This dose is equivalent to a human dose of 8 mg/kg based on body surface area comparisons. Hepatocellular carcinomas were also increased in males at 6 and 50 mg/kg. These doses were equivalent to 1 and 8 mg/kg based on body surface area comparisons. The recommended human maintenance dose is 400 mg per day or 6.7 mg/kg. Thus adenomas and carcinomas are produced at doses less than or similar to clinical doses.

Conclusion: Voriconazole produces hepatocellular adenomas and hepatocellular carcinomas when administered orally to rats for 24 months at doses comparable to the recommended human maintenance dose.

25. Twenty-four month in-diet toxicity and carcinogenicity study in mice
Key study findings: Voriconazole produces hepatocellular adenomas and hepatocellular carcinomas when administered orally to mice at 100 mg/kg daily for 24 months.
Study no: 96018
Volume # 27
Conducting laboratory and location: Pfizer Centre de Recherche. 37401 Amboise, Cedex, France.
Date of study initiation: September 6, 1996
GLP compliance: Yes
QA report: Yes
Drug, lot #, % purity: Drug lot numbers R14 and R17. Purity, Lot R14, 100%. Purity, Lot R17, 99.7%
Formulation/vehicle: Ground diet ———— was used as the vehicle.

Groups of male and female CD1 mice (50 animals/sex/ dose group) received voriconazole in the diet at 10, 30 or 100 mg/kg for 24 months. The compound was admixed with Ground diet. Two identical control groups received unsupplemented diet. Animals were observed at least twice daily for mortality and at least once weekly for clinical signs. Records

were kept of mortality, clinical signs, body weights, food consumption, hematology, serum chemistry, urinalysis, necropsy findings, organs weights and histology findings. Plasma concentrations were measured on days 86 and 184.

Table 25. Mortality after 24 months of voriconazole in mice and rats.

	Control		10 mg/kg		30 mg/kg		100 mg/kg	
	M	F	M	F	M	F	M	F
Number of animals	100	100	50	50	50	50	50	50
Sacrificed moribund	6	6	3	5	3	2	2	5
Found dead	24	30	12	17	13	22	14	12
Accidental deaths			1	1				
Total unscheduled deaths	30	36	16	23	16	24	16	17
Survivors at final sacrifice (%)	70	64	68	54	68	52	68	66

Mortality is shown on Table 25. Deaths were comparable between the sexes.

Voriconazole had no effect on body weights at doses up to 100 mg/kg when administered for 24 months. Mean plasma levels in mice were generally low (see Table 26). At 10 mg/kg, plasma levels were mostly below the limit of detection (BLD, generally 0.05 or 0.1 µg/ml).

Table 26. Mean plasma concentrations of voriconazole µg/ml in rats.

Dose	Means	
	Day 86	Day 184
10 mg/kg	BLD	BLD
30 mg/kg	0.04	0.09
100 mg/kg	0.78	1.22

Voriconazole administration was associated with a number of hepatic changes including increased liver weights, centrilobular hypertrophy, fatty change, single cell necrosis, pigmentation, eosinophilic foci, basophilic foci hepatocellular cystic change, clear cell foci adenomas and carcinomas (see Tables 27 to 30 below).

Table 27. Increases in Relative liver weights (%) in voriconazole treated animals.

Dose (mg/kg)	Male	Female
10	+20	
30	+41	+16

100	+124	+58

Table 28. Non-neoplastic hepatic findings in Males

	C1	C2	10	30	100
Centrilobular hypertrophy			7	25	45
Fatty change	2	8	8	23	35
Single cell necrosis	6	17	14	15	41
Pigmentation	7	12	10	9	36
Hepatocellular cystic change					7
Basophilic foci	1	4	3	1	18
Eosinophilic foci			1		8
Clear cell foci			2		14

Table 29. Non-neoplastic hepatic findings in Females

	C1	C2	10	30	100
Centrilobular hypertrophy			5	11	35
Fatty change	4	10	12	12	32
Single cell necrosis	26	25	19	23	36
Pigmentation	22	25	20	19	26
Hepatocellular cystic change	3	1	5	1	4
Basophilic foci					4
Eosinophilic foci			1		2
Clear cell foci					

Table 30 Neoplastic hepatic findings in Rats

	C1	C2	6	18	50
Adenomas					
Males	4	2	1	3	27
Females	2			1	11

Carcinomas					
Males	3	2	3	4	9
Females			1		2

Voriconazole produced an increase in hepatocellular adenomas and carcinomas in high dose mice when compared to control animals. This dose is equivalent to a human dose of 8 mg/kg based on body surface area comparisons.

Conclusion:

Voriconazole produces hepatocellular adenomas and hepatocellular carcinomas when administered orally to mice for 24 months at doses comparable to the recommended human maintenance dose (6.7 mg/kg).

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

26. Study title: UK-109,496 fertility study (Japanese Segment I) in Sprague Dawley rats by the oral route.
Key study findings: Voriconazole was associated with reduced ossification, decreased implantation rate and increased incidence of supernumerary ribs. One high dose fetus had major external and visceral anomalies (ablepharia, exencephaly, spina bifida, interventricular septal defect)
Study # 91129
Volume # 36
Conducting laboratory and location: Centre de Recherche. 37401 Amboise Cedex, France.
Date of study initiation: January, 1992
GLP compliance: yes
QA reports: yes
Drug lot R5. Purity: 100%
Formulation/vehicle: voriconazole was formulated in an aqueous solution of methylcellulose containing and — hydrochloric acid

UK109,496 was suspended in an aqueous solution of methyl cellulose containing hydrochloric acid and administered to three groups of 40 female Sprague Dawley rats. Rats were treated for 14 days before mating, throughout the mating period (14 days) and during early gestation (until day 7 post implantation). Dose levels were 3, 10 and 50 mg/kg/day. The males with which the females were mated were treated at the same dose levels for 133 days before mating. Control animals (40 females and 20 males) received the vehicle alone over the same time period and were handled identically..

Date of study initiation: January, 1992
GLP compliance: yes
QA reports: yes
Drug lot #
Formulation/vehicle: voriconazole was formulated in an aqueous solution of methylcellulose containing [redacted] and [redacted] hydrochloric acid

Groups of 7 inseminated female Sprague Dawley rats were treated orally with voriconazole at 10, 40, 80 and 120 mg/kg for 10 days during organogenesis (days 6 to 15 post insemination). The drug was formulated in methyl cellulose (as a 0.5 % aqueous solution) containing [redacted] and HCL. A control group of seven females received vehicle over the same time period. Records were kept of clinical signs, food consumption, body weights, clinical chemistry and mortality. Hysterectomies were performed on day 20 post insemination. Fetuses were sexed, weighed and examined for external and buccal abnormalities. Liver and adrenal tissues were also removed from the dam and subjected to histopathological examination.

Drug produced piloerection and reduced plasma triglycerides and estradiol (by 46-62 %) at all doses. At the high dose, drug also slightly reduced maternal body weight (-6 % on day 9 postimplantation). At 40, 80 and 120 mg/kg, the drug induced increased liver size and weight along with centrilobular hypertrophy and subcapsular hepatic necrosis.

While there was no embryo mortality or adverse effects on fetal body weights, the drug induced cleft palates at 80 and 120 mg/kg (11 % and 19 % respectively). None of the other groups presented with this finding. According to the historical control data, this finding is typically found at a rate of 0.02 %.

Conclusion

This drug is teratogenic and induces cleft palates at a dose equivalent to a human dose of 12.7 mg/kg/day and reduces estradiol levels at doses equivalent to human doses of 1.6 mg/kg/day and higher.

28. Study title: Teratology (Segment II) study in Sprague Dawley rats by the oral route
Key study findings:
Decreased estradiol levels, decereased triglycerides, increased liver size and weight, hepatic centrilobular hypertrophy, cleft palate, supernumerary ribs, minor anomalies of the sternebrae, dilation of ureter and/or renal pelvis, hydroureter and hydronephrosis.
Study # 91111/12
Volume # 37
Conducting laboratory and location: Centre de Recherche. 37401 Amboise Cedex, France.
Date of study initiation: October 1991
GLP compliance: yes
QA reports: yes

Drug lot R4

Formulation/vehicle: voriconazole was formulated in an aqueous solution of methylcellulose containing [redacted] hydrochloric acid

The purpose of the main part of this study was to evaluate the potential maternal toxicity, fetotoxicity and teratogenicity of voriconazole, when administered orally to pregnant rats during organogenesis. A pharmacokinetic study was run in parallel to determine the drug levels in maternal plasma, amniotic fluid and fetal tissues.

Drug was suspended in an aqueous solution of methyl cellulose containing [redacted] and [redacted] hydrochloric acid. In the main study, drug was administered by oral gavage to 20 inseminated females at 10, 30 and 60 mg/kg/day during organogenesis (days 6 to 17 post insemination). A control group received vehicle only over the same period. Records were kept of clinical signs, body weights and on day 17 postconception, blood was withdrawn for biochemical evaluations. Hysterectomies took place on day 20 and reproductive parameters recorded. Livers were weighed and examined histologically. All fetuses were sexed, weighed and examined for external and buccal anomalies, skeletal anomalies/degree of ossification and visceral malformations. In the parallel pharmacokinetics study, groups of 5 inseminated female rats were treated at 10, 30 and 60 mg/kg to determine voriconazole concentrations in maternal plasma, amniotic fluid and fetal tissues.

Mortality

There were no drug-related deaths. In the control group, one animal was sacrificed, nonpregnant, while at 10 mg/kg, one animal died due to a gavage error. At 30 mg/kg one animal was sacrificed pregnant but with only traces of implantation, while at 60 mg/kg one animal was sacrificed nonpregnant.

Pharmacokinetics

Maternal plasma voriconazole levels were sustained during the first 5 hours of dosing and maximal values were between 3.3 and 3.9 µg/ml at 10 mg/kg, between 8.3 and 11.5 µg/ml at 30 mg/kg and between 13 and 19 µg/ml at 60 mg/kg. $AUC_{(1-5h)}$ values were approximately dose related and ranged from 13.7 after 10 mg/kg to 57 µg.h/ml after 60 mg/kg. Drug concentration in fetal homogenates were similar to those found in maternal plasma at 5 hours (3.7, 10, and 17 µg/g after 10, 30 and 60 mg/kg, respectively). In amniotic fluid, drug levels were about half of those noted in maternal plasma and ranged between 1.92 µg/ml after 10 mg/kg and 9.1 µg/ml after 60 mg/kg.

Toxicity

Changes observed in the dams included decreased estradiol levels, which were 75% or more decreased at all doses, with levels being below the limit of detection (6.6 pg/ml) in 0/19,

14/19, 18/19 and 18/19 animals in the control, 10, 30 and 60 mg/kg treatment groups, respectively. At the high dose, drug was associated with decreased triglycerides (-31%) and liver changes, including increased size and weight and centrilobular hypertrophy.

In the fetuses, cleft palate was observed in 1/300, 1/288 and 8/301 animals at 10, 30 and 60 mg/kg doses respectively. At all dose levels, there was an increased incidence of variations/minor anomalies and major visceral anomalies: supernumerary ribs, minor anomalies of the sternebrae (at 60 mg/kg only), dilation of ureter and/or renal pelvis, hydroureter and hydronephrosis. When considered as a class of toxicities, kidney problems showed a dose-related increase (16, 23, 51 and 55 % incidence in the control, 10, 30 and 60 mg/kg groups respectively). Mean fetal body weights were slightly increased in males at all doses and mid dose females.

Conclusion

UK-109,496 is teratogenic. Drug administration in pregnant rats is associated with decreased estradiol levels, decreased triglycerides and liver hypertrophy in dams. Pups show an increase in cleft palates and variations/minor anomalies and major visceral anomalies: supernumerary ribs, minor anomalies of the sternebrae (at 60 mg/kg only), dilation of ureter and/or renal pelvis, hydroureter and hydronephrosis. Effects were seen at the lowest dose tested which is equivalent to a human dose of 1.6 mg/kg/day (based on body surface area considerations). Women included in the trial must be made aware of the potential risks involved in taking this drug and should be advised to use effective contraceptive methods.

29. Study title: Preliminary reproduction study in rats by the oral route
Key study findings: Pregnancy rate reduced, gestational length was increased, labor was prolonged. Eight of the 15 animals treated at 50 mg/kg never delivered, either dying or being sacrificed moribund. The mean litter size at birth, after 24 hours and after 4 days were significantly decreased in all treated groups when compared to controls. Among the dead pups examined, three presented with cleft palate.
Study # 91113
Volume # 36
Conducting laboratory and location: Centre de Recherche. 37401 Amboise Cedex, France.
Date of study initiation: October 1991
GLP compliance: yes
QA reports: yes
Drug lot R4
Formulation/vehicle: voriconazole was formulated in an aqueous solution of methylcellulose containing hydrochloric acid

This study was designed to determine the effects of voriconazole on the fertility of the male and female rat as well as the viability and perinatal development of their offspring. Drug (suspended in an aqueous solution of methyl cellulose containing [redacted] hydrochloric acid) was administered by gavage to groups of 10 males and 20 females before mating, throughout the mating period and during gestation and lactation at daily dose levels of 3, 10 and 50 mg/kg. Control animals received vehicle over the same period. Records were kept of clinical signs, body weights, fertility of the F_O generation and the viability and perinatal development of the pups.

In this study, 20/20 control, 20/20 low dose, 15/20 mid dose, and 19/20 high dose females had been inseminated by rats, as indicated by the presence of spermatozoa in vaginal smears. Copulation rate was significantly reduced in the mid-dose group compared to controls. Pregnancy rate was 100 %, 85 %, 93 % and 79 % respectively in the control, low- mid and high dose groups. Pregnancy rate was significantly reduced in the high dose group...

Gestational length was increased in treated groups compared to control. Labor was prolonged and, in the high-dose group (in most cases), delivery did not occur. In the littering females, the average duration of parturition in the control, low, medium and high-dose groups were 21.3, 21.7, 21.8, and 22.3, days respectively. Eight of the 15 animals treated at 50 mg/kg never delivered, either dying or being sacrificed moribund. In these females, clinical signs included ataxia, prostration, piloerection and cold to the touch.

The mean litter size at birth, after 24 hours and after 4 days were significantly decreased in all treated groups when compared to controls.

Table 31. Effects of UK-109,496 on pup development in rat

UK-109 dose (mg/kg)	Number of pups		
	Total at birth	Deaths by day 1	Deaths by day 4
0 (Control)	338	3	7
3	265	12	14
10	193	20	29
50	76	48	58

Among the dead pups examined, three presented with cleft palate: One at 10 mg/kg and two at 50 mg/kg.

Summary

The oral administration of drug to male and female rats at 3, 10 and 50 mg/kg from mating to lactation produced lower pregnancy rates and difficulties in delivering leading to death. Litter sizes were also reduced, as was pup survival. Cleft palate was found in the pups from dams receiving 10 and 50 mg/kg of drug.

30. Study of effects on Pre- and Post-natal development, including maternal function, in Sprague-Dawley rats by the oral route.
Key study findings: Increased gestational length, duration of parturition, number of dead pups at birth. Decrease in the number of viable pups at birth. Decrease in implantation sites at 3 and 10 mg/kg.
Study # 98032/033
Volume # 37
Conducting laboratory and location: Pfizer Centre de Recherche. 37401 Amboise, Cedex, France.
Date of study initiation: September 10, 1998
GLP compliance: Yes
QA report: Yes
Drug, lot #, % purity: Drug batch number R12. Purity, 100 %.
Formulation/vehicle: Voriconazole was formulated in 0.5% methylcellulose containing —

The purpose of the study was to evaluate the potential adverse effects of voriconazole on the pregnant/lactating female and on the development of the conceptus and the offspring following exposure of inseminated females from implantation to weaning.

Drug was suspended in an aqueous solution of methyl cellulose containing — and — hydrochloric acid. In the main study, drug was administered by esophageal intubation to 32 inseminated females at 1, 3 and 10 mg/kg/day for 36 days (day 6 post insemination to day 20 post partum). A control group received vehicle only over the same period. Records were kept of clinical signs, mortality, body weights. The day on which pups were born was designated day 0. The number of pups and any external abnormaliteis were recorded. After randomisation on day 4 pp, 4 pups/sex/litter were retained for further study. The remainder were culled and 1 culled pup/sex/litter was examined at necropsy and any visceral anomalies were recorded. Before

weaning the pups were assessed for surface righting and air righting reflexes, appearance of superior incisors and eyelid opening. After weaning, the pups were assessed for vaginal opening and the presence of prepucial fissure. Ophthalmoscopic examinations, spontaneous activity tets, water Maize learning tasks and passive avoidance tests were also conducted. Two rats/sex/litter were then mated and F2 pups examined.

Mortality

At the highest dose, one animal was found dead on day 24 p.i. Two females were sacrificed as moribund on day 23 p.i. One female was sacrificed without littering on day 24 p.i. Three of 4 of these females showed piloerection, pale skin, and were cool to touch. Dead pups were found in the uteri of these females indicating dystocia. Four females were also sacrificed on day 1 or 2 p.p. since they had no surviving pups. Two of these were cannibalising their pups. At 3 mg/kg one female (who showed piloerection and pale skin) was sacrificed because it bore no viable pups.

Table 32. Reproduction parameters

	Control	1 mg/kg	3mg/kg	10 mg/kg
Pregnant females (of 32)	30	31	30	32
Pups at birth	14.1	14.5	13.1	13.1**
Viable pups at birth	13.5	14.2	12.7	10.8**
Dead pups at birth	0.6	0.3	0.5	2.4**
Duration of parturition	13h32m	13h31m	14h06m	15h38m
Length of gestation	21.6	21.4	21.9	22.1**

Voriconazole was associated with increased gestational length, duration of parturition, number of dead pups at birth and a decrease in the number of viable pups at birth. At 10 mg/kg, these findings were clearcut but the same trends were obvious at 3 mg/kg. The 4-day survival index was also decreased for 10 mg/kg rats (92.8 % compared to 95.7 % for controls). There was also a decrease in implantation sites at 3 and 10 mg/kg. There were no differences between the control and low dose animals. At 10 mg/kg, all developmental landmarks were obtained earlier than in control groups. In addition, at 3 mg/kg eyelid opening of male pups occurred earlier than in controls.

Conclusions

Voriconazole administration to rats at 3 or 10 mg/kg produced parturition disorders, which led to maternal mortality and decreased perinatal survival. There were no apparent adverse effects seen in the F2 animals. The NOAEL for reproductive effects was 1 mg/kg.

31. Study title: Oral maternal toxicity study in Japanese White rabbits

Key study findings: Increased gestational length, duration of parturition, number of dead pups at birth. Decrease in the number of viable pups at birth. Decrease in implantation sites at 3 and 10 mg/kg.
Study # 919671
Volume # 39
Conducting laboratory and location: Safety Evaluation Laboratory. New Product Development Center. Pfizer Pharmaceuticals Inc. 5-2 Taketoyo-cho, Chita-gun, Aichi, Japan
Date of study initiation: September 10, 1998
GLP compliance: No
QA report: Yes
Drug, lot #, % purity: Drug batch number R12. Purity, 100 %.
Formulation/vehicle: Voriconazole was formulated in 0.5% methylcellulose containing ——

Groups of 4 or 5 pregnant Japanese White rabbits were treated with voriconazole at 10, 40 and 100 mg/kg/day for 12 days (days 6-18 of gestation, during organogenesis). The drug was formulated in methyl cellulose (as a 0.5 % aqueous solution) containing ——— and HCL, and control rabbits received vehicle only. Blood samples were taken from the 100 mg/kg dams on day 18 of gestation at 0, 0.5, 1 and 4 hours post dose. On day 29 of gestation, all dams were sacrificed and fetuses examined.

There were no drug-related deaths among the dams during the study. Maternal plasma levels were 11, 11 and 6 µg/ml at 0.5, 1 and 4 hours postdose respectively. At 24 hours postdose, drug levels were below the limit of detection.

Rabbits treated with 100 mg/kg/day voriconazole showed a slight decrease in food consumption, but there was no corresponding reduction in bodyweight gain. One of the four dams given 100 mg/kg/day aborted on day 28 of gestation. Although an increase in embryo mortality (24 %) and a decrease in the number of live fetuses (6) were observed at 10 mg/kg/day, compared to controls (3 % and 10 respectively) these differences were not considered to be drug-related because they did not occur at the higher doses.

Conclusion

Voriconazole, at 100 mg/kg/day in rabbits, produces a slight decrease in food consumption, but this is not associated with a reduction in body weight gain.

32. Study for effects on Pre- and Post-natal development, including maternal function, in Japanese White rabbits by the oral route.
Key study findings: Voriconazole administration is associated with increased embryomortality including early and late resorptions. Cervical rib and extra sternebral ossification site are also increased with voriconazole treatment.
Study # 98-96-72

Volume # 37
Conducting laboratory and location: Pfizer Drug Safety Evaluation Central Research. Pfizer Pharmaceuticals Inc. 5-2 Taketoyo-cho, Chita-gun, Aichi Japan.
Date of study initiation: November 10, 1998
GLP compliance: No
QA report: Yes
Drug, lot #, % purity: Drug batch number R17. Purity, 100 %.

The purpose of the main part of this study was to evaluate the potential maternal toxicity, fetotoxicity and teratogenicity of voriconazole, when administered orally to pregnant Japanese White rabbits during organogenesis. A pharmacokinetic study was run in parallel to determine the drug levels in maternal and fetal plasma.

Drug was suspended in an aqueous solution of methyl cellulose containing [redacted] and [redacted] hydrochloric acid. In the main study, drug was administered by oral gavage to 20 inseminated females at 10, 40 and 100 mg/kg/day during organogenesis (days 7 to 19 post insemination). A control group received vehicle only over the same period. Records were kept of clinical signs and body weights. Hysterectomies took place on day 29 and reproductive parameters recorded. All fetuses were sexed, weighed and examined for external and buccal anomalies, skeletal anomalies/degree of ossification and visceral malformations. In the parallel pharmacokinetics study, groups of 4 inseminated female rats were treated at 10, 40 and 100 mg/kg (days 7-21) to determine voriconazole concentrations in maternal and fetal plasma.

Mortality

There were no drug-related deaths. One intermediate dose animal died on gestation day 15 due to gavage error. Another was found with hind limb paralysis due to a handling accident and was sacrificed. In the control group, one animal aborted on gestation day 22. A number of animals were sacrificed non-gravid.

Table 33 Pharmacokinetics of voriconazole

Dose	$AUC_{(0\text{-}24h)}$ (µg*h/ml)	Cmax (µg/ml)	Tmax (h)
10 mg/kg	0.8	0.3	1
40 mg/kg	13	4.6	0.8
100 mg/kg	67	15	0.5

Table 34 Pharmacokinetics of voriconazole

Dose	[Maternal] at 1h (µg/ml)	[Fetal] at 1h (µg/ml)	Fetal/Maternal
10 mg/kg	1	0.8	0.8
40 mg/kg	4.5	3.7	0.8

100 mg/kg	15	11	0.7

Maternal plasma voriconazole levels increased with increasing dose and time to peak plasma levels decreased with dose from 1 hour at 10 mg/kg to 0.5 h at 100 mg/kg. Mean fetal concentrations also increased with dose and the ratio of fetal to maternal levels was between 70 and 80 %.

Toxicity

Does dosed at 100 mg/kg experienced a reduction in food consumption. Although this was not associated with a reduction in bodyweight, there was a reduction in bodyweight gain. Embryomortality and early resorptions were increased in mid and high dose animals and there was an increase in dead fetuses and late resorptions at the high dose. Fetal bodyweights were also decreased at the high dose males (see Table 35 below)

Table 35: Embryotoxic effects of voriconazole

	Control	10 mg/kg	40 mg/kg	100 mg/kg
Embryomortality %	4	4	9	11
Early resorptions	4	5	8	13
Late resorptions	2	0	1	4
Dead fetuses	0	1	0	2
Fetal bodyweights (males,g)	47	45	47	43

Fetal abnormalities consisted of extra sternebral ossification site and cervical rib. The numbers of fetuses and litters affected increased with increasing dose. (see Table 36 below).

Table 36 : Fetal abnormalities in Study 98-96-72

	Control	10mg/kg	40 mg/kg	100 mg/kg
Extra sternebral ossification site	0 [0]	2 [2]	3 [3]	6 [13]
Cervical rib	2 [3]	2 [2]	3 [5]	15 [43]

Litters[fetuses]

Summary and Conclusions

Voriconazole administration is associated with increased embryomortality including early and late resorptions. Cervical rib and extra sternebral ossification site are also increased with

voriconazole. Effects were clearly evident at 100 mg/kg ($AUC_{(0-24h)}$ of 67 µg*h/ml), but effects were ocassionally seen at the 40 mg/kg dose ($AUC_{(0-24h)}$ of 13 µg*h/ml).

Overall Summary and Conclusions

Toxicology studies were conducted with oral and intravenous voriconazole. Studies were up to 24 months duration for the oral formulation and up to six months duration for the intravenous formulation. In preclinical toxicology testing, voriconazole adversely effected the eyes, liver, heart, kidney and the unborn fetus. Voriconazole also produced hepatocellular adenomas and carcinomas in experimental animals.

These changes are reflected in the label and there are no findings that would preclude the approval of this drug.

Owen G. McMaster, Ph.D.
Pharmacology/Toxicology Reviewer, DSPIDP

Concurrences:
HFD-590/OfficeDirector/GoldbergerM
HFD-590/Pharm/ToxTL/HastingsK

Disk:
HFD-590/HastingsK
cc:
HFD-590 Original.
HFD-590 Original 1
HFD-590/Biopharm/MeyerJ
HFD-590/Biopharm/ColangeloP
HFD-590/BiopharmTL/AjayiF
HFD-590/PM/SalibaJ
HFD-590/Chem/HolbertG
HFD-590/ChemTL/SchmuffN
HFD-590 Division File
HFD-590/Micro/GoseyL
HFD-590/MicroTL/BalaS
HFD-590/MO/Alivisatos
HFD-590/MO/CoxE

HFD-590/MO/JohannLiangR
HFD-590/MO/PowersJ
HFD-590/MO/TiernanR
HFD-590/MOTL/CavailleCollM
HFD-590/Pharm/McMasterO
HFD-590/PharmTL/HastingsK
HFD-590/Stat/DixonC
HFD-590/StatTL/HigginsKar
HFD-340

Appendices

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

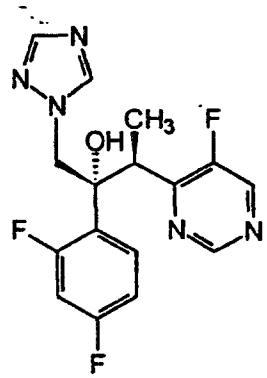
/s/

David Hussong
7/12/01 11:13:42 AM
MICROBIOLOGIST

Peter Cooney
7/12/01 01:02:17 PM
MICROBIOLOGIST

Date Submitted: August 29, 1995
Date Assigned: September 1, 1995
Date Completed: July 31, 1996

DiV



Sponsor: Pfizer, Inc.
235 East 42nd St.
New York, N.Y. 10017

Drug: UK-109,496, voriconazole
Molecular formula: $C_{16}H_{14}N_5F_3O$
Molecular weight: 349.3

Chemical name: (2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoro-4-pyrimidinyl-1-(1H-1,2,4-triazol-1-yl)-2-butanol.

Formulation: UK-109,496 will be administered orally, as a tablet for this IND. Tablets contain voriconazole in addition to lactose, pre-gelatinized starch, croscarmellose sodium, povidone, magnesium stearate and white Opadry. It may also be administered as an intravenous infusion in sulphobutyl ether-β-cyclodextrin sodium salt (CP-217,861-02 or SBECD). Most phase II efficacy studies to date have been performed using an oral capsule and an intravenous infusion in the excipient hydroxypropyl-β-cyclodextrin (HPBCD).

Related DMF's: ———————

Indications: 1. Invasive aspergillosis and invasive candidiasis
2. Empiric therapy of systemic mycoses

Introduction

UK-109,496 was discovered at Pfizer Central Research Laboratories in Sandwich England and is a potent triazole antifungal agent. UK-109,496 is active *in vivo* in a range of animal models of fungal infection, including systemic infections with *Aspergillus, Candida* spp. (including *C. krusei* and *C. glabrata*, which are less susceptible to other azoles), and *Cryptococcus neoformans* in either normal or immunocompromised mice.

The clinical efficacy, safety and tolerability of UK-109,496 has been tested in approximately 260 patients in three phase II studies in Europe; oropharyngeal candidiasis in HIV positive patients, acute aspergillosis in immune compromised patients and chronic fungal infections in non-neutropenic patients. In protocol 150-302, a seven-day, oral, double-blind, dose ranging study in 165 HIV+ patients with oropharyngeal candidiasis, the drug was well tolerated and the only significant drug-related event reported by the sponsor was visual disturbance. These effects were dose-dependent and resolved fully in all patients at follow-up.

In protocol 150-303, an open non-comparative study using 200 mg p.o. b.i.d. for up to 24 weeks, in chronic aspergillosis or candidiasis of non-neutropenic patients, the adverse events recorded included visual disturbance, skin rash and markedly elevated liver function tests. In protocol 150-304, an open, noncomparative study of UK-109,496 (6 mg/kg intravenous every 12 hours for one day then 3 mg/kg every 12 hours for 6-27 days followed by 200 mg b.i.d. p.o. for a total of 24 weeks) in acute aspergillosis of neutropenic and other immunocompromised patients, elevated liver function tests resulted in three withdrawals from the study.

Proposed Clinical Protocol

In the first phase of the proposed study, patients will be randomized to receive either 200 mg bid of oral UK-109,496 or placebo for 14 days. Safety and tolerance of this dose will be assessed by the sponsor. If 200 mg bid (400 mg/day or 8 mg/kg for a 50 kg patient) is judged to be well tolerated, then the next stage of the study will be performed to evaluate the safety of 300 mg UK-109,496 (versus placebo) b.i.d in a new group of patients (12 mg/kg/day) . If that dose is judged to be well tolerated, the final phase of the study will evaluate the dose of 400 mg of UK-109,496, b.i.d. (versus placebo) in a new group of patients (16 mg/kg/day).

Toxicology Studies Summary:Oral acute and repeat-dose toxicity studies with UK-109,496.

1. Single dose oral toxicity in mice and rats. Study # 90157, 90158.
2. UK-109,496: One month oral toxicity in Sprague Dawley rats. Study # 90144.
3. One month oral toxicity/reversibility in Sprague Dawley rats. Study # 91030.
4. UK-109,496: One month oral toxicity in beagle dogs. Study # 90145.
5. UK- 109,496: 3 month dietary prechronic toxicity in CD-1 mice. Study # 92104.
6. Six month oral toxicity study in Sprague Dawley rats-with reversibility at 10 mg/kg.
7. Six month oral toxicity study in beagle dogs, with reversibility at 8 mg/kg.
8. Microbial Reverse Mutation Assays (Ames Test) Study 91-844-01.
9. Mammalian cell gene mutation assays CHO/HGPRT
10. In vivo mouse micronucleus assays
11. In vitro Cytogenetics Studies

Toxicology Studies Review

1. Single dose oral toxicity in mice and rats. Study # 90157, 90158. Pfizer Centre de Recherche. 37401 Amboise, Cedex, France. Drug batch number R1. December 1990. GLP study.

Groups of male and female Swiss CD-1 mice and Sprague Dawley rats (2-5 animals/sex/ dose group) received a single oral (esophageal intubation) administration of UK-109,496 at 100, 300 and 500 mg/kg. The compound was suspended in a 0.5% aqueous solution of methyl cellulose 4000 cps containing 0.1 % [redacted], acidified with HCL at [redacted] and administered at a standard volume of 10 ml/kg bodyweight. Animals were observed for 14 days. Mortality is shown on Table 1.

Table 1. Mortality after single dose of UK-109,496 in mice and rats.

Dose (mg/kg)	Mouse	Rat
100	0/10	0/10
300	10/10	10/10
500	4/4	4/4

Deaths occurred between 3 and 24 hours (500 mg/kg dose) and 5-72 hours (300 mg/kg). Clinical signs common to both species included mydriasis titubation, depression, prostration, partially closed eyes and dyspnoea. Additional signs in mice included convulsions, swollen abdomens and corneal opacification. Additional signs in rats included salivation, rigid, extended hind limb and body weaving.

Conclusion: Minimum lethal oral dose levels were between 100 and 300 mg/kg for both rats and mice, doses equivalent to human doses of between 8 and 48 mg/kg.

2. UK-109,496: One month oral toxicity in Sprague Dawley rats. Study # 90144. Pfizer Centre de Recherche. 37401 Amboise, Cedex, France. Drug batch number R1. November 1990. GLP study.

UK-109,496 was administered orally for one month to groups of Sprague Dawley rats, 10/sex/group at dose levels of 3, 10, 30 and 80 mg/kg. The compound was suspended in a 0.5% aqueous solution of methyl cellulose 4000 cps containing 0.1 % [redacted], acidified with HCL at [redacted] and administered at a standard volume of 10 ml/kg bodyweight. A control

group of animals received vehicle only. Additional groups of 5 rats/sex/dose group were used for plasma drug level determinations. Blood was withdrawn for plasma drug measurements at 1, 3 and 5 hours after dosing on days 1 and 26. Additional samples were taken at 24 hours on day 1.

Mortality There were no deaths during this study.

Toxicokinetics

Plasma drug exposure was approximately dose-related and was higher in females than in males. Drug exposure was also reduced on repeat dosage, presumably due to autoinduction (see Tables 2 and 3).

Table 2. Mean AUC (1-5h)'s in rats treated orally with UK-109,496 Day 1.

Dose (mg/kg)	Mean AUC (1-5h) (μg.h/ml)	
	Males	Females
3	3.5	6.5
10	13	18
30	66	73
80	165	166

Table 3. Mean AUC (1-5h) in rats treated orally with UK-109,496, Day 26.

Dose (mg/kg)	Mean AUC (1-5h) (μg.h/ml)	
	Males	Females
3	2.0	4.7
10	14	23
30	37	52
80	96	112

The main target organ for toxicity was the liver, and changes included, increased liver weights centrilobular hypertrophy, increased cholesterol in females, decreased triglycerides in males as well as induction of hepatic microsomal cytochrome P450 contents and proliferation of smooth endoplasmic reticulum. Mild multifocal hepatic necrosis was seen in two high-dose males. Adverse events also included increased plasma proteins, decreased urinary volume, thyroid follicular hypertrophy and pituitary vacuolation, mild decreases in red blood cells and slightly increased platelet counts. There was also a slight increase in adrenal weights in the females tested at 30 and 80 mg/kg, but these were not accompanied by any histopathological changes.

A NOAEL was calculated at 3 mg/kg, a dose equivalent to a human dose of 0.48 mg/kg/day for one month.

3. One month oral toxicity/reversibility in Sprague Dawley rats. Study # 91030. Pfizer Centre de Recherche. 37401 Amboise, Cedex, France. Drug batch number R1. February, 1991. GLP study.

This study was designed to assess the reversibility of the adrenal and liver changes seen in the previous one month study. Groups of 5 male and 5 female Sprague Dawley rats were treated orally for one month with UK-109,496 at 0, 10 and 30 mg/kg and then were either sacrificed or kept untreated for another month. The compound was suspended in a 0.5% aqueous solution of methyl cellulose 4000 cps containing 0.1 % [redacted] acidified with [redacted] HCl and administered at a standard volume of 10 ml/kg bodyweight. Control rats received vehicle only. Clinical observations were restricted to recording mortality and body weights were recorded weekly. Clinical chemistry and hematology was assessed at the end of the study or after the reversibility period. Animals were then sacrificed and necropsied. Liver and adrenals were weighed and subjected to histopathological examination.

Induction of P450, increased liver weights, centrilobular hypertrophy and proliferation of smooth endoplasmic reticulum, increased plasma proteins and decreased bilirubin concentration and urinary volume were all reversed after the one month recovery period. The increases in adrenal weights, seen in the previous one month study, were not seen in this (smaller) study.

Conclusion

Some of the effects of UK-109,496 on the rat liver, and plasma and urine chemistry are reversible. However, the study was not designed to determine whether or not the liver necrosis, seen in study 91030 at 80 mg/kg, was reversible.

4. UK-109,496: One month oral toxicity in beagle dogs. Study # 90145. Pfizer Centre de Recherche. 37401 Amboise, Cedex, France. Drug batch number R1. November 1990. GLP study.

Groups of 3 male and 3 female beagle dogs received single daily oral doses of 3, 6, and 12 mg/kg UK-109,496 for one month. Groups of 3 dogs/sex received 24 mg/kg for 14 or 16 days before the animals died or were sacrificed. The compound was suspended in an aqueous solution of methyl cellulose 4000 cps containing ——— and administered at a standard volume of 1 ml/kg bodyweight. Control groups received 1 ml/kg of vehicle over the one month period. Records were kept of clinical signs, food intake, body weight, cardiovascular measurements, ophthalmology, hematology, clinical chemistry and plasma drug levels. At the end of the study all animals were sacrificed and subjected to necropsy. A full histopathology panel of organs underwent histopathological examination and the brain, heart kidneys liver ovaries, spleen, testes and adrenals were weighed. Cytochrome P450 levels were measured in the liver, which was also examined by electron microscopy. On days 1 and 16, the dogs were bled by venepuncture at 2, 5, 8, and 24 hours after dosing for plasma drug level determination.

Mortality

At the 24 mg/kg dose, one female dog was found dead on day 15 (before dosing) and another (female) on day 17. Another (male) dog was sacrificed in a moribund condition on day 17.

While dogs treated at 3 mg/kg showed only minor rises in alkaline phosphatase, the next higher dose (6 mg/kg) was also associated with decreased cholesterol levels. At 12 mg/kg, additional toxic effects included a moderate decrease in plasma fibrinogen levels, an occasional QT interval value exceeding the upper limit of the normal range of 240 milliseconds in two dogs. A bilateral lesion, seen on ophthalmoscopic examination, which was situated on the fundus and consisted of depigmented streaks in the non-tapetum, was only seen in one animal at 12 mg/kg and was not attributed to treatment. Also at 12 mg/kg, three of six animals had endocardiosis. Dogs receiving the highest dose, 24 mg/kg, showed mydriasis, salivation, emesis, anorexia, lack of feces, depression, prostration, dryness of the nose, mucopurulent exudates, weight loss, decreased heart rates (30% decreased) , markedly increased hemoglobin, red blood cell count and packed cell volume and moderately increased prothrombin times. In addition, decreased calcium (-20 %), potassium (-30-40 %) and glucose (-30-70 %) and total proteins (-10-50%) as well as increased aspartate and alanine aminotransferase. High-dose animals also had increased liver, spleen, adrenal and renal weights and decreased testicular weights. Other findings included thick black bile, pancreas embedded in a gelatinous material, discolored kidneys, small spleen, testes and prostate, and red zones on the endocardium, fatty liver, tubular vacuolation of the kidney, atrophy of the salivary glands, thymic atrophy, testicular tubular atrophy, focal acute vasculitis (brain) and bone marrow atrophy.

Toxicokinetics

Drug exposure was measured after the first and sixteenth doses at 2, 5, 8 and 24 hours after dosing. Drug levels were approximately dose-related and repeated administration led to reduced exposure after 3, 6, and 12 mg/kg. Treatment at the higher dose (24 mg/kg) resulted in drug accumulation. On a biochemical level, treatment with 3, 6, or 12 mg/kg produced an increase of similar magnitude, in the hepatic cytochrome P450 content, while the degree of induction appeared to be less at 24 mg/kg (see Tables 4 and 5)

Table 4. Range of maximal drug concentrations (μg/ml) in beagle dogs on days 1 and 16

Dose (mg/kg)	Day 1. Range of maximal drug concentrations (μg/ml)	Day 16. Range of maximal drug concentrations (μg/ml)
3	[redacted]	[redacted]
6	[redacted]	[redacted]
12	[redacted]	[redacted]
24	[redacted]	[redacted]

Table 5. Mean $AUC_{(2\text{-}24h)}$ (μg.h/ml) after repeated exposure to UK-109,496 on Days 1 and 16.

Dose (mg/kg)	$AUC_{(2\text{-}24h)}$ (μg.h/ml) Day 1	$AUC_{(2\text{-}24h)}$ (μg.h/ml) Day 16
3	24	14
6	64	43
12	170	124
24	332	867

Conclusion: Although there were effects seen in the dogs after 30 days of treatment with UK-109,496 at 3 mg/kg/day , 6 mg/kg seems to be the lowest safe dose. This is equivalent to a human dose of 3.2 mg/kg for one month. At the higher doses toxic effets of this drug include changes in the eyes, liver and heart.

5. UK- 109,496: 3 month dietary prechronic toxicity in CD-1 mice. Study # 92104. Pfizer Centre de Recherche. 37401 Amboise, Cedex, France. Drug batch number R1. November 1990. GLP study.

CD-1 mice, 10/sex/group were fed for three months with a diet supplemented with appropriate amounts of UK-109,496 to result in an average daily intake of intake of 50, 100 or 150 mg/kg. Animals were housed individually and were subjected to a twelve hour light/dark cycle. Records were kept of mortality, clinical signs, body weight, food and water consumption, hematology, clinical chemistries, necropsy findings, select organs weights, histopathology findings (heart, kidney, lungs, liver, pancreas, ovary) cytochrome P450 contents and electron microscopy of liver. Food consumption was measured automatically using an electronic balance connected to a computer. Plasma drug levels were determined in 18 supplementary mice/sex/dose level on day 80 at 0400, 0800, 1200, 1600, 2000 and 2400h.

Mortality

One high dose animal from the main study was found dead on day 42. No cause of death was recorded, but the mouse died without any premonitory sign. One low dose animal from the pharmacokinetics study section died from an anesthesia accident.

Toxicity

The liver was the target organ of UK-109,496 toxicity. Low dose UK-109,496 (50 mg/kg) was associated with decreased neutrophil counts (-44%) and decreased monocytes (-46%), increased aspartate aminotransferase and alanine aminotransferase (females only, see Table 6), increased liver weights (+26 %) , hepatocellular hypertrophy, mild hepatocellular fatty changes, subcapsular necrosis of the liver (2/20 low dose animals) and hepatic microsomal cytochrome P450 increases.

At the higher doses, toxicity also included increased neutrophils (+55 %)and monocytes (+39 %) and increased triglycerides (+73 % in high dose males). Livers were enlarged, pale or marbled with single cell necrosis, proliferation of smooth endoplasmic reticulum and accumulation of cytoplasmic vacuoles. Occasionally, there were differences in the susceptibility between males and females. See table 7 for a summary of the microscopic observations in the liver.

Table 6. Increases in aminotransferase activity in treated females (% control)

Dose (mg/kg)	ALAT increase (% control)	ASAT increase (% control)
50	+40 %	+35 %
100	+65 %	+31 %
150	+106%	+53 %

Table 7 Incidence summary of microscopic observations in liver

Finding	Control	50 mg/kg	100 mg/kg	150 mg/kg
Infiltration, mononuclear cells	0	0	1m	0
Centrilobular hypertrophy	0	8m	17(9m,8f)	17(10m,7f)
Necrosis, single cell	0	0	0	3(m)
Necrosis, subcapsular	0	2(1m,1f)	2(f)	1(f)
Cystic change, hepatocellular	0	0	0	1f
Mitoses	0	0	0	1m
Fatty change	0	4(1m,3f)	17(9m,8f)	17(8m,9f)

Ten males and ten females were examined per group. m-males, f-females

All the necrosis in the male rats were discribed as minimal. At 50 mg/kg, there was minimal subcapsular necrosis and at 150 mg/kg the lesions were described as minimal single cell necrosis. In the females, the 50 mg/kg dose produced moderate subcapsular necrosis, while the lesion at the two higher doses were described as mild.

Table 8 shows the exposure of the animals over 24 hours.

Table 8. UK-109,496 AUC values over 24 hours, males versus females

Dose (mg/kg)	AUC_{0-24h} (μg.h/ml)	
	Male	Female
50	3	4
100	7	14
150	30	32

Drug exposure increases with dose, but is greater in females than in males. The increase in exposure with dose is superproportional at 150 mg/kg/day in males and at 100 and 150 mg/kg/day in females.

Conclusion: The liver was the target organ of UK-109,496 toxicity. Increased aspartate aminotransferase and alanine aminotransferase, increased liver weights, hepatocellular hypertrophy, hepatocellular fatty changes, necrosis of the liver and hepatic microsomal cytochrome P450 increases were among the toxicities associated with UK-109,496 administration for three months in the diet.

6. Six month oral toxicity study in Sprague Dawley rats-with reversibility at 10 mg/kg. Pfizer Centre de Recherche. 37401 Amboise, Cedex, France. Drug batch number R3. September 1991. GLP study.

Groups of Sprague Dawley rats, 20 per sex per dose group, were treated orally (by esophageal intubation) with UK-109,496 at 3, 10 and 50 mg/kg on a daily basis for six months. Drug was formulated in an aqueous solution of methyl cellulose and —— acidified with —— hydrochloric acid and administered in a volume of 10 ml/kg. Control rats (20 rats/sex) received vehicle alone over the same period. Records were kept of body weights, food consumption, ophthalmology, hematology, clinical chemistries, plasma drug levels. Rats surviving to the end of the studies were sacrificed and subjected to necropsy. Adrenals, brain, heart, kidneys, liver, spleen and testes were weighed and a complete panel of organs subjected to histopathological examination. The liver was examined using electron microscopy. In a supplementary experiment, designed to assess the reversibility of any toxic effects seen after six months, 10 rats/sex were treated with 0 or 10 mg/kg/day for 6 months after which they were allowed to recover for two months before sacrifice. In a second supplementary experiment, groups of 5 animals/sex/dose level were treated for six months and used to determine drug levels . On days 1 and 176 of the study, blood was collected for plasma drug level assays at 0200, 0500, 1000 and 2400h.

Mortality

There were no deaths during this study which could be ascribed to the drug substance. No animals from the high dose group died. Deaths in the other dose groups were as follows: Control group: 3/60, low dose group: 1/50, mid dose group: 3/70 animals .

Clinical signs, observed in all dose groups (including control animals) included brown deposits on the muzzle or around the eyes (chromodacryorrhoea), cutaneous lesions (alopecia, crusts, irritation or cutaneous outgrowths). Since these findings were also detected in control animals, they may be related to the excipient.

Other changes included decreases in hemoglobin, red blood cell count, packed cell volumes, red cell distribution width, mean corpuscular volume, platelet volume, lymphocyte count, total white cell count and increases in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, platelet count (although the low dose females had slightly lower platelet counts). Bilirubin levels were decreased up to 50 %. Reductions were observed in alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase although these changes were generally transient and moderate. Cholesterol was increased in high dose females, but it was decreased in some males. The drug was also associated with an increase in albumin, total protein, and calcium. Decreases were seen in chloride levels. Potassium levels decreased in females and increased in males. A slight decrease in cholesterol level was still detectible in males, two months after the end of treatment. Drug was also associated with decreased urine pH, increased urine density and decreased urine volume. Except where otherwise noted, these changes were mild, (generally less than 20%) and were usually reversible.

Post-mortem changes included increased liver weights (males and females), and increased kidney weights (females). Adrenal weights increased (+15-24%) in females and decreased (-16 %) in males, while cardiac weights increased in females (+9%) and decreased in males (-9%). None of these changes were present at the end of the recovery period. Histologically, rats showed dose related increases in incidence and severity of hepatic centrilobular hypertrophy, which, in the high dose animals, extended to midzonal and periportal regions. High dose animals also showed hypertrophy of thyroid follicular cells. There was also a mild dose-related increase in the incidence and severity of chronic progressive nephropathy. Other changes, seen only in males included focal atrophy of the exocrine pancreas (1/20, 5/20 and 5/20 males at the low, mid and high doses) , and vacuolation of the pituitary (1/20 and 3/20 at the low and high doses.

Pharmacokinetics

On day one, plasma UK 109,496 concentrations were maximal 2 or 5 hours after dosing and were generally higher in females than in males. At 24 hours, no drug was detected at the low and mid doses and only very low levels (0.07 to 3.68 μg/ml) were noted at the high dose. Mean AUC's (2-10 hours) were 3.3 and 1.6 fold higher in females than in males at the low and mid doses respectively and increased superproportionately to dose in males but not in females. On day 176, drug concentrations were lower and declined more rapidly than on day one, with no drug detected 24 hours after dosing. AUC's were lower on day 176 compared to day one (25% at the low dose and 60 to 80% at the high dose). Drug exposure was higher in females than in males at all dose levels and approximately proportional to dose.

Mean AUC $_{(2\text{-}10\ h)}$ values μg.h/ml on day 1

Dose (mg/kg)	Males	Females
3	2.8	9.3
10	24	39
50	199	189

Mean AUC $_{(2\text{-}10\ h)}$ values μg.h/ml on day 176

Dose (mg/kg)	Males	Females
3	2.1	6.8
10	9.4	23
50	48	73

Mean AUC values were higher in females than in males at the two lower doses on day 1 and at all doses on day 176. Mean AUC values were also lower on day 176 compared to day 1. Since the drug has been shown in other studies to induce its own metabolism, this result is not entirely unexpected.

The liver remains the main site of toxic effects after six months of therapy with UK-109,496. A number of the post-mortem changes (such as increased liver weights and kidney weights) were not present at the end of the recovery period. Other effects noted included thyroid follicular cell hypertrophy, nephropathy, focal atrophy of the exocrine pancreas and vacuolation of the pituitary.

7. 6 month oral toxicity study in beagle dogs, with reversibility at 8 mg/kg. Pfizer Centre de Recherche. 37401 Amboise, Cedex, France. Drug batch number R3. September 1991. GLP study.

Groups of beagle dogs, four/sex/dose group were treated with oral UK109 at doses of 4, 8 and 12 mg/kg/day (capsules) for six months. The high-dose group was initially treated with 15 mg/kg for 8 days but this dose caused unacceptable levels of adverse reactions. The dose was therefore stopped and, after a six day recovery period, animals were treated with 12 mg/kg from day 15 onwards. Control animals were given two placebo capsules per day over the same period. To determine the reversibility of observed changes, supplementary groups of two dogs/sex were treated at 0 and 8 mg/kg for six months after which treatment was withdrawn and animals observed for two months before sacrifice. Records were kept of clinical signs, food intake, body weights, cardiovascular findings, ophthalmology, hematology, clinical chemistry of plasma and urine, necropsy findings, organ weights, histopathology, cytochrome P450 measurements and liver electron microscopy findings.

Mortality

There were no deaths during this study.

Toxicity

Clinical signs were severe in the animals treated with 15 mg/kg/day. Effects included absence of stool (first observed between 3-6 days after dosing began), anorexia (starting on day 5) depression (starting on day 8) and body weight loss (between days 1 and 7). After this, the dose was reduced to 12 mg/kg/day, the growth of these dogs resumed at a rate similar to that of controls, and fully compensated for their initial rate loss by day 50. There were sporadic (although statistically significant) variations in body weights in all dose groups, however these changes were generally in the 10 % range. There were no significant changes in cardiovascular parameters that seemed drug-related. One (mid-dose) animal showed premature ventricular contractions and another (low dose) animal showed a right deviation of the QRS axis.

In females, significant changes were observed in platelet counts (<47% increase), plateletcrit (<29% increase) mean platelet volume (<12 % decrease). These findings, along with a statistically significant increase in activated partial thromboplastin time (APTT) were comparable to values in concurrent controls two months after cessation of treatment. The main treatment-related change in plasma chemistry was an increase in mean plasma alkaline phosphatase in most treatment groups, which was dose-dependent and rose as high as 518 % of control at the high dose. Other changes included moderately elevated alanine aminotransferase activity, decreased mean albumin levels, decreased calcium, decreased total protein and decreased mean cholesterol. Plasma chemistry was normal at the end of the two month

reversibility period.

A dose-related increase in absolute (<83 % increase) and relative (<73 % increase) liver weight was observed in both sexes. Livers occasionally appeared enlarged and/or dark or marbled. One high-dose animal presented with a large white area which was firm. On a microscopic examination, all treated animals except one low dose female presented with hepatic centrilobular hypertrophy. In the mid and high-dose groups, this was accompanied by a dose related increase in the number of multinucleated hepatocytes. Foci of subcapsular hemorrhage and single cell necrosis were observed in a few hepatocytes. Liver changes were reversible after two months. Other histopathological changes included vacuolation of the outer part of the zona fasciculata of the adrenal glands in high dose animals and tubular giant cells within the seminiferous tubules.

Electron microscopy uncovered moderate proliferation of smooth endoplasmic reticulum, with displacement of nucleus and organelles to the periphery of the cell.

Treatment of dogs with 8 mg/kg UK-109,496 caused an increase in the specific content of hepatic microsomal cytochrome P450.

Conclusion:

The administration of UK-109,496 to dogs for 6 months at doses up to 8 mg/kg/day produced changes in the liver which were reversible after a two month recovery period. This dose is equivalent to a human dose of 4.3 mg/kg/day for six months. It is not clear why the sponsor neglected to assess the reversibility of the lesions at the high dose.

8. Microbial Reverse Mutation Assays (Ames Test) Study 91-844-01. Department of Safety Evaluation. Pfizer Central Research, Groton Connecticut.

Salmonella typhimurium strains TA 1535, TA 1537, TA 98 and TA 100 were exposed to UK-109,496 to test the drug's ability to induce reverse mutations. Compound was dissolved in DMSO and cells were exposed to drug at levels of between 0.02 and 10 mg/plate in the presence and absence of S9. Negative and positive controls were run concurrently with each assay and triplicate plates were prepared at each level. S9 fractions were prepared from livers of male rats [Crl:COB CD (SD) BR] which had been dosed five days previously with a single intraperitoneal injection of 500 mg/kg of Aroclor 1254 and were fasted on the day before sacrifice. The number of revertants was determined after at least 60 hours of incubation at 37 degrees C. In the preparation without S9, a dose related reproducible three-fold increase over control value is considered a positive response. In the presence of S9, metabolic activation is characterized by a dose dependent, reproducible three-fold increase in the average number of revertant colonies compared to the respective activation control plates. There was no evidence of compound-related increases in the number of revertant colonies per plate at any level of UK-

109 that would suggest mutagenic activity with or without S9.

Comment: A three-fold increase is considered a positive response by the sponsor although a 2-fold increase is usually considered the standard. In none of the experiments was a 2-fold increase observed.

9. Mammalian cell gene mutation assays CHO/HGPRT. Department of Safety Evaluation. Pfizer Central Research, Groton Connecticut.

UK-109,496 was tested for its ability to produce forward mutations at the X-linked hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in the Chinese Hamster ovary cells. Cells were plated in T-75 flasks at a density of 1.25×10^6 cells per flask and exposed to drug in the presence or absence of S9. In the direct mutagenicity experiments (without metabolic activation), drug concentrations were between 965 and 3333 μg/ml. In the metabolic activation experiments, drug concentrations were between 804 and 2000 μg/ml and drug was dissolved in DMSO. Precipitation occurred above 1667 μg/ml.

The first direct experiment was repeated due to an elevation in the number of spontaneous mutations. In the second direct experiment, drug produced 0-8 mutants per 10^6 survivors. The average number of spontaneous mutants per 10^6 survivors was 1 (acceptance limit <40). There were 41 and 349 mutants per 10^6 survivors respectively for the positive control ethyl methanesulfonate at 50 and 400 μg/ml.

In the metabolic activation experiment, treated cultures produced between 0 and 28 mutants per 10^6 survivors. The average number of spontaneous mutations per 10^6 survivors for negative controls was 26. The positive control group (3-MCA at 5 and 10 μg/ml) produced 124 and 126 mutants per 10^6 survivors, respectively. Based on these test results, UK-109,496 does not induce forward mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary eggs.

10. In vivo mouse micronucleus assays. Department of Safety Evaluation. Pfizer Central Research, Groton Connecticut.

The ability of the drug to induce micronuclei in mice was examine by dosing mice once per day for three days at 37.5, 75, and 150 mg/kg/day. Twenty four hours after the final treatment, the mice were sacrificed, and smears made from the bone marrow of the femora from each mouse. The preparation was stained and 1000 polychromatic erythrocytes were scored for the presence of micronuclei. A positive response was defined as a substantial, dose related and reproducible elevation in the number of micro nucleated polychromatic erythrocytes in the treated animals. Although there was a reduction in the percent of polychromatic erythrocytes in both sexes in all dose groups, confirming that the dose used were near the maximum tolerated doses, the frequency of multinucleated polychromatic

erythrocytes was not increased compared to controls. Positive and negative controls produced the expected responses. Thus, UK-109,496 does not induce micronuclei in polychromatic erythrocytes in male or female mouse.

11. In vitro Cytogenetics Studies. Department of Safety Evaluation. Pfizer Central Research, Groton Connecticut.

Human peripheral lymphocytes were collected by venipuncture from healthy donors and cultured in Williams medium E with the ——— for 48 hours. ——— Negative controls were treated with DMSO and culture medium to reach a final concentration of 1 %. Positive controls were exposed to ——— After addition of compound, incubation was continued at— degrees for an additional 24 hours with ——— present during the final 3 hours.

For the activation assay, 48 hours after the culture initiation, cells were exposed to UK-109,496 and S9 mix for three hours. This was followed by centrifugation and resuspension of the recovered cells in complete Williams medium E. Incubation was continued for an additional 21 hours, with —— present for the final 3 hours. Negative control cultures were exposed to DMSO (1 %), while positive control cultures were exposed to

[redacted]

Conclusion

UK-109,496 produces a slight increase in chromosomal abnormalities, mostly chromatid breaks, with a few chromosome breaks and, least frequently, rearrangements. The sponsor argues that the slight magnitude of the increase combined with the lack of clear dose response, render these results non-definitive. However, while the increase in the number of abnormal cells at 1000 may μg/ml may not have been higher than that seen at 750 μg/ml, it was still higher than the control value (2.5 % vs 0.5 %).

Summary and conclusions

Minimum lethal oral dose levels were between 100 and 300 mg/kg for both rats and mice, doses equivalent to human doses of between 8 and 48 mg/kg.Toxic effects of voriconazole are observed mainly in the liver but other target organs include the heart, adrenals, eyes, thyroids, pituitary and kidneys. Some of these effects are reversible. The NOAEL for one month of therapy was calculated at 3 mg/kg, a dose equivalent to a human dose of 0.48 mg/kg/day. Plasma drug exposure was gender-dependent and nonlinear in all species investigated. In most species, drug exposure was reduced on repeat dosage, presumably due to autoinduction.

The sponsor's first proposed study, will randomize patients to receive either 200 mg bid of oral UK-109,496 (3.3 mg/kg/day) or placebo for 14 days. Safety and tolerance of this dose will be assessed by the sponsor before a higher dose is initiated. The nature of the toxic effects and the reversibility of the significant findings, combined with the escalating design of the study should make this study safe.

/S/

Owen McMaster
Pharmacology Reviewer

Concurrences:
HFD-530/GChikami /S/ 8/2/94
HFD-530/JFarrelly /S/ 8/20/94

cc:
HFD-530 Original IND
HFD-530 Division File
HFD-340
HFD-530/Biopharm/BDavitt
HFD-345
HFD-530/Chem/MJarski
HFD-530/CSO/VKinsey
HFD-530/Micro/SBala
HFD-530/MO/TWu
HFD-530/Pharm/OMcMaster