CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

PHARMACOLOGY REVIEWS

PHARMACOKINETICS SUMMARY/EVALUATION PAGES 117- 142

Summary Table Provided by Drug Sponsor: Single dose pharmacokinetics of sildenafil

Parameter	Mouse	Rat (male)	Rat (female)	Rabbit	Dog	Man[a]
Intravenous Terminal Elimination half-life (h)	1.3 [d]	0.3	1.9	1.8 [e]	5.2	4.0
AUC[b] (ng.h./ml)	174	350	1280	-[e]	1550	1990
Plasma clearance (ml/min/kg)	91	48	13	-[e]	12	9.8
Vol. of distr. (L/kg)	1.0	1.1	2.0	-[e]	5.2	1.5[c]
Free sildenafil in plasma (%)	6	5	5	9	14	4
Oral C_{max}[b] (total) (ng/ml)	30	11	147	44	117	245
T_{max} (h)	0.5	1.0	3.0	2.0	1.1	1.5
AUC[b] (total) (ng.h./ml)	31	51 [f]	252 [g]	190	842	815
Bioavailability (%)	17	15 [f]	44 [g]	-[e]	54	41
C_{max} ratio sildenafil : UK-103,320	4.8	0.2	5.0	1.9	6.9	2.5

[a] data for man from Study 148-208, assuming 70 kg body weight.
[b] normalized to 1 mg/kg dose.
[c] volume of distribution at steady state (V_{ss})
[d] half-life after oral dosing; value not estimable after i.v. dose due to assay insensitivity.
[e] no intravenous data in rabbit. Half-life value after oral dosing.
[f] values derived from DM-96-148-11.
[g] values derived from DM-96-148-10 since evidence of saturable elimination in female rats at higher doses (DM-96-148-11)

3.3. Repeat Dose Pharmacokinetics

3.3.1. Mouse (CD-1)

In a **3-mo** mouse study (**No. 94049**), 10 mice/sex/group were treated with UK-92,480 citrate with 10, 50 and 200 mg/kg/day by gavage. Plasma levels of the drug and the principal N-demethylated metabolite UK-103,320 were determined in blood samples collected at various time intervals on day 63 of study.

Findings reported indicate that plasma levels of the drug and its metabolite were similar in both sexes, and were somewhat dose-related.

In a separate **3-mo** mouse study (**No. 94101**) with doses of 20, 40 and 100 mg/kg/day UK-92,480 by gavage, plasma levels of the drug/its metabolite were determined on day 63 of the study. No sex difference was detected in the exposure to the drug/metabolite **UK-103,320**, however; these compounds increased superproportionally with dose levels.

In the **24-mo** carcinogenicity study in CD-1 mice (No. 95007), the animals were treated with doses of UK-92,480 citrate ranging from 3 up to 30 mg/kg/day by gavage. Plasma levels of the drug were determined in 5 mice/sex/dose level on day 62 of study. The exposure of the parent compound and the demethylated metabolite UK-103,320 was reported as dose-related.

3.3.2. Rat (Sprague-Dawley)

In a 14-day study in which 5 rats/sex were with gavaged with 60 mg/kg/day UK-92,480 citrate (the HD used in the 24-mo rat carcinogenicity study No. 94092), animals blood samples were taken at various hourly intervals up to 24 hrs after dosing. Mean AUC_{1-24hr} values reported for day 14 showed AUC values higher for M than F rats for the unchanged drug (1.67 µg.h/ml vs. 53.5 µg.h/ml) and the N-demethyl metabolite UK-103,320.

In the 1-mo oral toxicity study (**No. 90143**), 10 rats/sex/group were treated with 10, 45 and 200 mg/kg/day UK-92,480, blood samples were taken at various time intervals ranging from 1 up to 24 hrs after dosing on day 23 of study. Plasma mean drug concentration values reported for the 1 h post-dose samples were 0.3, 1.2 and 4.4 µg/ml for M rats, and 1.6, 6.0 and 15.8 µg/ml for F.

Blood conc. of UK-92,480 were higher in F than in M, while the levels of **UK-103,320** metabolite were higher in M than in F. As a result, F rats were exposed predominantly to the unchanged drug, and M to both the drug and the N-demethylated metabolite.

In a 6-mo rat study (**No. 91098**) in which the animals were treated with 3, 12 or 60 mg/kg/day UK-92,480, plasma levels examined of the unchanged drug and the metabolite UK-103,320 on day 176 again results reported showed that M were exposed mainly to the metabolite while F were predominantly exposed to the parent compound.

In the 24-mo carcinogenicity study in rats (**No. 94092**), the animals were treated with doses of UK-92,480 citrate ranging from 1.5 up to 60 mg/kg/day by gavage. Plasma levels of the drug were determined in 6 rats/sex/dose level on day 366 of study. These rats were reported as being exposed in dose-related fashion to the parent compound and the demethylated metabolite UK-103,320. However, drug sponsor stated that M rats were exposed predominantly to the metabolite UK-103,320 whereas the parent compound was the major circulating form in F.

3.3.3. Beagles

In a dog 1-mo oral toxicity study (**No. 90125**) using 3/sex/group the animals were treated with UK-92,480 at 5, 20 and 80 mg/kg/day. The plasma conc. of UK-92,480 and that of the metabolite UK-103,320 were measured at various time intervals on day 21 of study. The proportion of the metabolite relative to the parent compound varied minimally (< 20%) over the dose range examined suggesting to the drug sponsor **no** detectable saturation of this metabolic pathway.

In a dog study (**No. 91058**) using 1/sex to assess the bioequivalence of UK-92,480 citrate and base given ~ 30 mg/kg, p.o. twice- on day 1 and 8 of study as a suspension, and of the citrate salt in gelatin capsules. Results reported indicate that after the administration of the citrate, the plasma concentrations and AUCs of the drug and that of the two metabolites- **UK-103,320** and UK-95,340 were similar to or higher than those seen after the administration of the base as a suspension. In this dog study, all plasma concentrations of the potential metabolite **UK-95,340**, were below the limit of detection of the assay. Findings in this limited study in 2 dogs suggested that the oral bioavailability of the citrate salt in capsules was at least similar to base in suspension.

In a 6-mo study (**No. 91099**) in which the dogs were treated with UK-92,480 citrate in capsules at 3, 15 and 50 mg/kg/day, the plasma levels of the drug and metabolite UK-103,320 were measured at various time intervals on day 334 of study. Plasma levels for the two compounds showed them to be dose-related. The proportion of the metabolite UK-103,320 relative to UK-92,480 again varied minimally (< 20%) as the dose of UK-92,480 increased suggesting no saturation process in the dog.

In a 12-mo dog study (**No. 95039**) treated with UK-92,480 citrate in capsules at 3, 10 and 50 mg/kg/day, plasma levels of the drug and the principal metabolite were measured at various time intervals on day 168 of the study showed that the exposure to the drug and the N-demethylated metabolite **UK-103,320** was dose-related. In none of the dog studies reported there was indication of a difference in the exposure of the drug or the principal metabolite based on sex.

The table below, prepared by drug sponsor, shows some pharmacokinetics parameters obtained for the repeat-dose studies.

Total and free plasma levels of sildenafil and the metabolite UK-103,320 during toxicology studies

		Sildenafil				UK-103,320			
Species (gender) and study	Dose (mg/kg)	Total C_{max} (ng/ml)	Free C_{max} (ng/ml)	Total AUC_{24H} (ng.h/ml)	Free AUC_{24H} (ng.h/ml)	Total C_{max} (ng/ml)	Free C_{max} (ng/ml)	Total AUC_{24H} (ng.h/ml)	Free AUC_{24H} (ng.h/ml)
Mouse (F+M)	3	BLQ		NC		BLQ		NC	
24M 95007	10	70	4.2	NC		BLQ		NC	
	30	780	46.8	NC		630	37.8	NC	
Rat (M)	3	BLQ		NC		70	7.7	200	22
6M 91098	12	BLQ		NC		540	59.4	2500	275
	60	360	18	600	30	3300	363	18900	2079
Rat (F)	3	330	16.5	800	40	70	7.7	300	33
6M 91098	12	1620	81	8000	400	320	35.2	3900	429
	60	8440	422	54100	2705	930	102	12700	1397
Dog (F+M)	3	220	30.8	1800	252	40	5.6	700	98
6M 91099	15	1240	174	13000	1890	200	28	3300	462
	50	6470	906	72800	10192	990	139	15500	2170
Man Study 148-228	**1.43**	**561**	**22**	**1686**	**67**	**254**	**13**	**801**	**40**

BLQ: Below Level of Quantification (< 30 ng/ml), NC: Not Calculated

The table below, prepared by drug sponsor, compares dose and pharmacokinetic safety margins for UK-92,480 and the principal metabolite UK-103,320 based on the estimated NOAELs levels reported for mouse, rat and dog in toxicology studies compared to those in man after administration of the proposed MHTD of 100 mg UK-92,480.

Species	Ratios (safety factors) for sildenafil			Ratios (safety factors) for UK-103,320	
NOAEL	Dose	free C_{max}	free AUC_{24h}	free C_{max}	free AUC_{24h}
Mouse (M+F)					
3 mg/kg	2	NC	NC	NC	NC
Rat (M)					
60 mg/kg	42	0.8	0.4	28	52
Rat (F)					
60 mg/kg	42	19	40	8	35
Dog (M+F)					
15 mg/kg	10	8	28	2	12
50 mg/kg	35	41	152	11	54

NC indicates parameter not calculable

Data for male and female animals have been combined for mouse and dog since there is no evidence of a gender difference in pharmacokinetics.

Safety ratios have been calculated using data from Study 148-228; *viz.* dose: 100 mg (1.43 mg/kg); free C_{max} (ng/ml): sildenafil: 22, UK-103,320: 13; free AUC (ng.h./ml): sildenafil: 67, UK-103,320: 40.

3.4. Absorption

In clinical studies in healthy M volunteers, the drug was rapidly absorbed with a reported C_{max} reported between 0.5 to 2 hr post dosing.

Excretion of radioactivity were used to determine the extent of gastrointestinal absorption in animals. Effects gastrointestinal (**gi**) transit time of UK-92,480 was studied in mouse and rats, as well as well as biliary excretion and renal/fecal excretion of the drug/metabolites were reported to give an indication of oral absorption of the drug. In mice, single doses UK-92,480 ranging of 200 or 400 mg/kg **po** produced a dose-related reduction in intestinal transit vs vehicle controls. Single doses of the drug ranging from 10 up to 100 mg/kg slowed intestinal transit in M, and only at the 100 mg/kg dose was it slowed in the F mice. In a separate mouse study, the **NOAEL** on intestinal transit time was 1-3 mg/kg **po**. In a 42-day repeat-dose study, daily doses of 200 mg/kg **po** UK-92,480 slowed intestinal transit by day 7 of study up to ~ 42%, and up to 36% by the end of the study when compared to vehicle control.

In rat, single oral doses of UK-92,480 ranging from 10 up to 300 mg/kg po slowed the intestinal transit in M at 100-200 (by 13-30%), and in F at 200 mg/kg (by 21%) vs controls. In support of the adequacy of oral absorption of the drug, drug sponsor stated that in general, the pattern of excretion of UK-92,480 showed only minor differences between the species, genders (in rat), and routes of administration (in man). Further, that since in all the studies, the predominant route of excretion was the feces (~ 73-88% of the dose), in comparison with 6-15% for urine, and that similar pattern of excretion was noted after both oral and iv dosing, these findings suggest that UK-92,480 is well **absorbed orally**. Based on fecal excretion of radioactivity as a result from secretion into the gi tract, the majority of excreted radioactivity in all species studied were reported a being recovered within the first 48 h after administration of the drug.

The table below was prepared by drug sponsor to show the excretion of radioactivity after single doses of [pyrimidine 2-^{14}C-UK-92,480 to mouse, rat, rabbit, dog, and man.

Species (n)	Dose (mg/kg)	Percentage of dose recovered in:				
		Urine		Faeces		Total excretion
		0-24h	0-120h	0-48h	0-120h	0-120h [1]
Mouse (3m;3f)	10 (oral)	6	6	84	85	93
Rat (3m)	45 (oral)	9	9	83	88	98
(3f)	45 (oral)	12	13	73	82	95
Rabbit (3f)	50 (oral)	14	15	66	75	92
Dog (2m)	20 (oral)	7	14	51	73	87
Man (3m)	50mg (oral)	9	12	54	79	91
(3m)	25mg (i.v.)	10	13	48	76	89

[1] Includes cage washings for animal species, carcasses for rat and mouse and exhaled $^{14}CO_2$ for rat.

3.5. Distribution

3.5.1. Tissues

In rat, the distribution of radioactivity after **iv** administration of [^{14}C]-UK-92,480 (4 mg/kg) was studied only by whole body autoradiography. Within 0.1 h after dosing, radioactivity was present in all tissues of M and F rats. At 1 h and 6 h post dose, conc. of radioactivity were generally low, and by 24 h post dose, residual radioactivity was mainly limited to the retina*, substantia nigra and the pigmented skin, which suggests that UK-92,480 and/or its metabolites may have an affinity for melanin. In the F, the levels of radioactivity were generally higher than those in M, and declined more slowly. These differences may reflect the gender difference in elimination of the unchanged UK-92,480. Fecal radioactivity observed in excretion studies suggest a combination of secretion directly into the stomach and limited intestinal secretion via the bile.

The following tables on the concentrations of radioactivity in the tissues of rats after iv dosing with the drug were prepared by the drug sponsor.

CONCENTRATIONS OF RADIOACTIVITY IN THE TISSUES OF MALE RAT AFTER INTRAVENOUS ADMINISTRATION OF [^{14}C]-UK-92,480 AT A NOMINAL DOSE LEVEL OF 4 MG/KG

TISSUE	TIME (h)			
	0.1 male µg eq/g	1 male µg eq/g	6h male µg eq/g	24 male µg eq/g
Adipose tissue				
Brown fat	4.12	0.62	0.23	0.22
White fat	blq	0.37	0.18	blq
Adrenal gland	9.9	1.45	0.30	0.22
Blood	1.33	0.40	0.20	0.12
Bone marrow	3.83	0.57	0.32	0.25
Brain	0.67	blq	0.18	blq
Cardiac muscle	3.39	0.54	0.21	0.08
Eye (retina)	5.88	3.24	1.45	1.45
Harderian gland	4.85	3.2	0.73	0.33
Liver	8.64	3.88	1.31	0.62
Lung	7.23	1.18	0.45	0.12
Nasal/optic sinus	1.68	0.36	0.23	0.25
Pancreas	4.24	1.02	0.55	0.15
Preputial gland	4.05	1.45	0.50	0.27
Pineal gland	3.88	0.73	0.23	0.18
Pituitary gland	5.21	1.09	0.23	0.18
Prostate gland	2.18	0.56	0.23	0.22
Salivary gland	4.36	0.82	0.36	0.18
Seminal vesicle	blq	0.36	0.18	0.21
Skeletal muscle	2.1	0.36	0.18	0.09
Skin (epidermis)	1.73	0.35	0.18	0.08
Sebaceous gland	1.45	0.33	0.20	0.23
Spleen	2.91	0.87	0.50	0.18
Substantia nigra	nm	1.43	0.32	nm
Testis	0.64	0.32	0.18	0.08
Thymus gland	2.43	0.55	0.20	0.22
Thyroid gland	3.6	0.87	0.36	0.33
Upper limit of quantification	23.3	11.6	5.82	2.9
Lower limit of quantification	0.36	0.18	0.091	0.045

alq = Above the upper limit of quantification
blq = Below the lower limit of quantification
nm = No measurement taken.

CONCENTRATIONS OF RADIOACTIVITY IN THE TISSUES OF FEMALE RAT AFTER INTRAVENOUS ADMINISTRATION OF [^{14}C]-UK-92,480 AT A NOMINAL DOS LEVEL OF 4 MG/KG

TISSUE	TIME (h)			
	0.1 female µg eq/g	1 female µg eq/g	6h female µg eq/g	24 female µg eq/g
Adipose tissue				
Brown fat	2.15	2.42	1.38	0.44
White fat	blq	1.09	0.36	0.08
Adrenal gland	3.44	2.11	0.87	0.26
Blood	0.32	0.36	0.36	0.22
Bone marrow	0.94	1.35	0.64	0.36
Brain	blq	blq	blq	blq
Cardiac muscle	1.49	1.41	0.45	0.22
Eye (retina)	2.08	2.45	2.01	2.01
Harderian gland	0.99	5.43	1.45	0.85
Liver	2.48	4.03	2.06	0.70
Lung	2.69	3.37	1.64	0.20
Nasal/optic sinus	0.18	0.34	0.55	0.18
Pancreas	1.57	2.12	1.21	0.32
Pineal gland	2.20	2.38	1.31	0.34
Pituitary gland	2.66	3.11	1.09	0.34
Salivary gland	0.89	1.98	1.11	0.29
Skeletal muscle	0.53	0.63	0.36	0.15
Skin (epidermis)	0.36	0.65	blq	blq
Sebaceous gland	0.19	0.44	blq	blq
Spleen	0.93	2.31	0.73	0.35
Substantia nigra	0.32	alq	0.45	0.53
Thymus gland	0.69	1.13	0.73	0.40
Thyroid gland	2.30	1.59	1.38	0.16
Upper limit of quantification	11.63	11.6	11.6	5.8
Lower limit of quantification	0.091	0.18	0.18	0.045

In pregnant rabbits, at the high doses of UK-92,480 (200 mg/kg **po**), mean drug conc. of the UK-103,320 metabolite were detected in the amniotic fluid (0.11 µg/ml) and in the fetuses (0.96 µg/g). However, at lower doses of the drug (50 and 100 mg/kg **po**), the metabolite level was generally below the detection limits (0.10 µg/g) in these areas examined. No accumulation data were reported for these animals.

3.5.2. Plasma Protein Binding

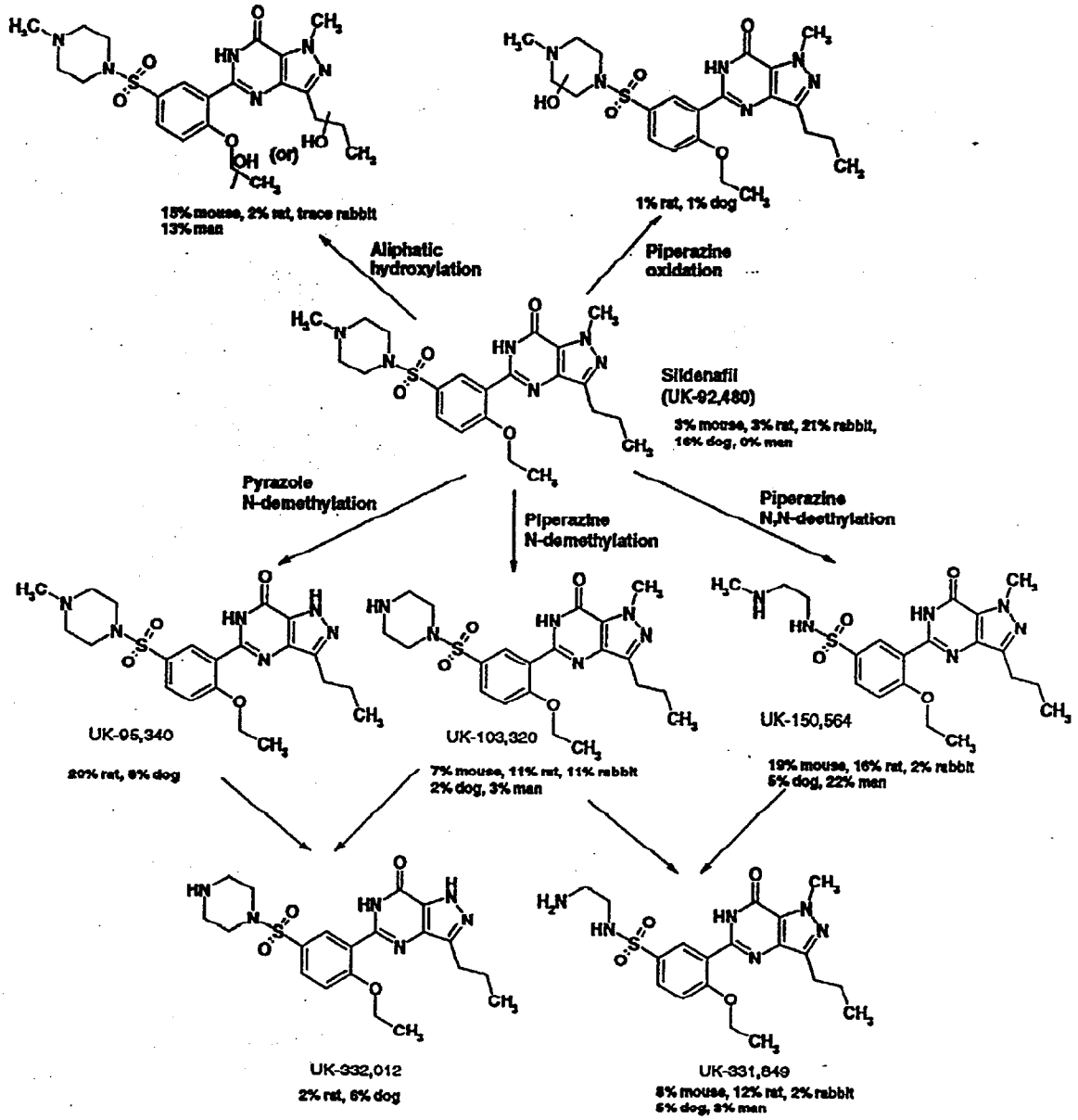
The binding of 14C-UK-92,480 in plasma of mouse, rat, rabbit, dog and man has been measured in vitro using equilibrium dialysis. Plasma protein binding of UK-92,480 was independent of total drug concentration over the range 0.01-10 µg/ml. The extent of binding to plasma proteins of mouse ~ 94%, ~ rat 95%, rabbit ~ 91%, ~ dog 86%, and man ~ 96% From this values it is expected that the unbound fraction of drug in man may be similar to rat and mouse, but lower than dog and rabbit.

BINDING OF [^{14}C]-UK-92,480 IN THE PLASMA OF MOUSE, RAT, RABBIT, DOG AND MAN

Species	Mean percentage bound in plasma for the following initial concentrations.			
	0.01µg/ml	0.1 µg/ml	1µg/ml	10 µg/ml
Mouse	93.4	94.0	94.0	92.9
Rat	94.4	94.9	95.1	94.3
Rabbit	90.5	91.8	91.7	89.9
Dog	82.5	87.0	87.3	86.5
Man	96.2	96.2	96.2	96.5

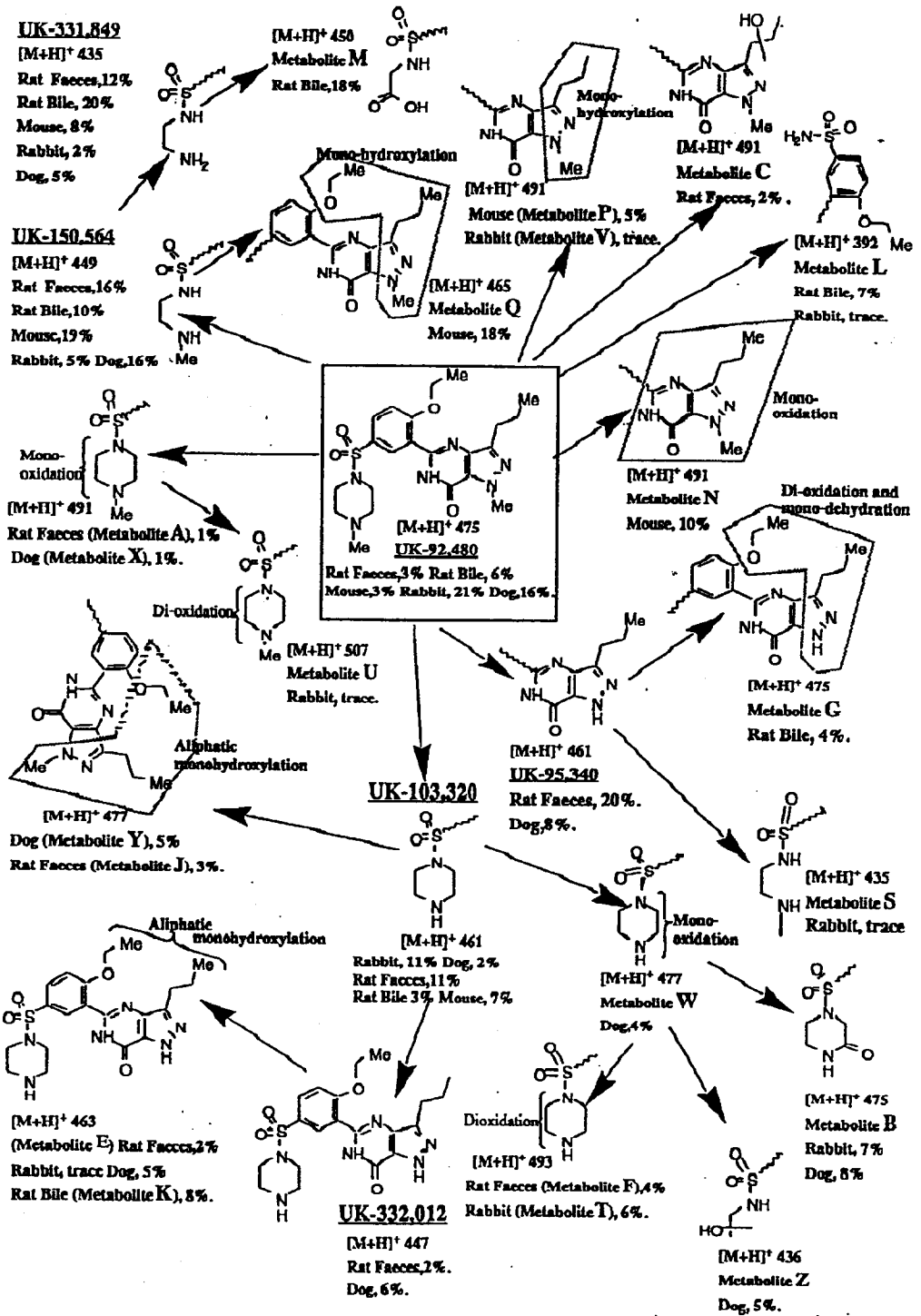
3.6. Metabolism

The following is the proposed metabolic pathway of UK-92,480 submitted by the drug sponsor. The percentages (%) represent the amounts of the administered drug identified in the excreta.



The following is a summary of the proposed metabolic routes of [pyrimidine-2-^{14}C-UK-92,480 in mouse, rat, rabbit and dog.

SUMMARY OF METABOLIC ROUTES OF [PYRIMIDINE-2-^{14}C]-UK-92,480 IN MOUSE, RAT, RABBIT AND DOG



Trace indicates one component from a multicomponent mixture (total amount of mixture < 5%).

Five primary routes of metabolism were described for UK-92,480: N-demethylation of the piperazine ring, loss of a 2 carbon fragment from the piperazine ring, oxidation of the piperazine ring, aliphatic hydroxylation and pyrazole N-demethylation.

The metabolic fate of [pyrimidine-2-^{14}C]-UK-92,480 was studied following single oral doses in mouse, rat, rabbit and dog. Metabolites were isolated and identified in plasma, urine, feces and rat bile.

3.6.1 Circulating Metabolites

In animals and humans treated with UK-92,480, the metabolite UK-103,320 was detected in their plasma, and the metabolite **UK-95,340** and **UK-150,564** (results from the loss of a two carbon fragment from the piperazine ring) were detected in human urine.

3.6.2. Biologic activity of UK-103,320

In *vitro* studies, UK-103,320 was ~ 0.4 X as potent than the parent compound against PDE5 from the human corpora cavernosa (the metabolite also inhibited PDE5 from rabbit's corpora cavernosa), and showed selectivity over human PDE1, PDE2, PDE3, PDE4 and PDE6.

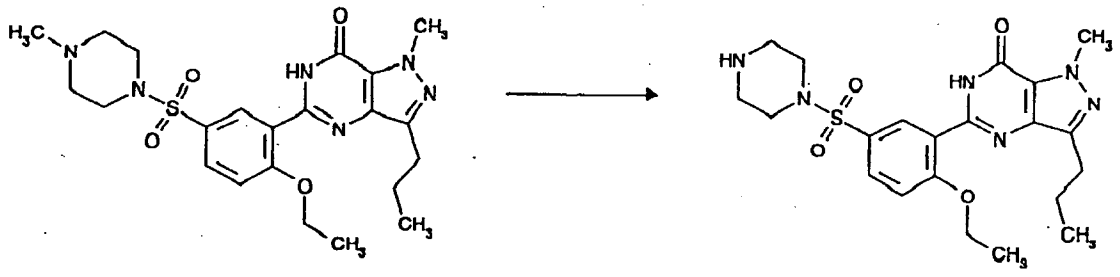
In pharmacology studies using anesthetized rats and dogs, UK-103.320 (0.03 to 3 mg/kg **iv**) was reported as producing dose-related transient falls in MABP; the hypotensive responses were accompanied by tachycardia. (The duration of these effects were reported ~5 min and effects were seen only in some anesthetized dogs.)

In the **1-mo** rat repeat dose study with UK-92,480, a metabolite **UK-95,340** was identified in plasma but at low levels. Although drug sponsor reported under clinical studies that the metabolite **UK-150,564** has ~10% of the potency of UK-92,480 as a PDE inhibitor, reviewer did not readily identify such nonclinical studies, or the biologic activity of UK-95,340, if any.

3.6.3. In vitro Metabolism:

In all animal species studied, disappearance of the drug is reported accompanied by the formation of the N-demethyl metabolite UK-103,320. In vitro metabolism of UK-92,480 has been studied with hepatic microsomes from rat, dog, rabbit and man. **UK-103,320** was also formed when UK-92,480 was incubated in vitro in microsomal fractions prepared from the livers of various animal species and man.

The formation of UK-103,320 by the cytochrome P-450 system:



UK-92,480 UK-103,320

The human and rat liver microsomes assays were described in the NDA. Briefly, liver samples were pooled/homogenated/differentially centrifuged/incubated with cofactors required for drug metabolizing enzymes.

In vitro assays were conducted to determine the potential of UK-92,480 to inhibit 6 cytochrome P450 isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) which are considered to be important in drug metabolism. A high affinity isoform has been identified as **CYP2C9** (reported as being a minor route of metabolism in clinical reports) and a low affinity isoform as **CYP3A4** (reported as the major route of metabolism in clinical reports) based on studies with known inhibitors of these isoenzymes (sulphaphenazole and ketoconazole, respectively). UK-92,480 is only a weak inhibitor of human drug metabolizing cytochrome P450 isoenzymes. Apart from **CYP2C9**, for which the $IC_{50>}$ 160 µM, all other isoforms investigated (CYP1A2, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) were reported as showing IC_{50} values greater than 300 µM. In addition analogous studies with the CYP3A4 substrates terfenadine and testosterone gave IC_{50} values of ~ 100µM and >300µM, respectively.

With F rat liver microsomes, the rate of metabolism was slower than with M, this difference in rate of metabolism was considered consistent by the drug sponsor with the gender difference in rat pharmacokinetics; metabolism (T 1/2) was more rapid in M (~2 min) than in F (129 min) while values for dog (38 min) and humans (45 min) were similar.

In vitro metabolism studies reported indicate that the metabolite UK-103,320 is itself a substrate for cytochrome P450, consistent with its undergoing further metabolism before elimination.

UK-103,320 is only a weak inhibitor of the major human cytochrome P450 isozymes. Apart from CYP2D6 with an IC_{50} of 71 µM, all other isoforms investigated showed IC_{50} values greater than 300 µM. Drug sponsor asserted that at the expected peak plasma concentration of 500 ng/ml (1µM) of the drug for the likely clinical dose range of 25 -100 mg UK-92,480, it is unlikely that the drug will be associated with any drug-drug interactions due to P450 inhibition.

3.7. Excretion

3.7.1. General

In rat and dog, the excretion of [^{14}C]-UK-92,480 has been investigated at the dose levels used for toxicology studies. Briefly, UK-92,480 is well absorbed after oral administration by rat and dog. The drug is rapidly metabolized. Excretion pattern showed only minor differences between the species, genders and routes of administration. The predominant route of excretion was the feces (66-75% of the dose) vs 7-16% for urine. Fecal radioactivity results from secretion into the gastrointestinal tract. Urinary and fecal excretion was relatively rapid, being essentially complete in the first 48 h.

In M rats, 4% of orally dosed radioactivity was excreted in the expired air. This finding suggests that one of the metabolic pathways for UK-92,480 is cleavage of the radiolabelled piperazine ring, resulting in production of [^{14}C]-carbon dioxide. Further evidence for this pathway is identification of the metabolite **UK-150,564** in human feces. Radioactivity (6% of the dose) remaining in rat carcass after 120 h may result from incorporation of one carbon or two carbon fragments from the piperazine ring into endogenous tissues.

In dog and human, urinary elimination of unchanged UK-92,480 was very low (~ or < 2%) in the first 24 h after oral dosing. In man, the metabolite UK-103,320 is eliminated with a similar T 1/2 to that of the parent drug. Little or no UK-103,320 was recovered in human or dog urine after doses of UK-92,480. Newly submitted data indicate that in man this metabolite is further biotransformed to UK-331,849 and other fractions and excreted in the feces. The plasma

concentration of UK-103,320 in man is reported as ~ 30% of that of peak concentration of UK-92,480 itself.

In F rats, plasma levels of UK-103,320 were reported as generally lower than in males when detected after repeated administration of the parent compound.

Drug sponsor reported clinical studies that concentrations of UK-92,480 in the ejaculate at 1.5 and 4 h post-dose, were 18% and for the metabolite UK-103,30 17% of the concentrations of these compounds in the plasma (of 5% and 15%, respectively) at the same time points.
Overall, of the total radioactivity excreted in the feces, the parent drug accounted for ~ 3% in mouse and rat, ~ 21% in rabbit, and ~ 16% in dog.

3.7.2. Biliary and transintestinal secretion (DM-97-148-15)

In 2 anesthetized M rat with cannulated bile ducts were treated with 4 mg/kg iv [pyrimidine 2-^{14}C]-sildenafil. The mean excretion of radioactivity into bile and urine up to 6h post-dose was reported to be ~ 45.5% and 6.1% of the dose, respectively, with ~ 21.5% detected in the g.i tract and 4.3% in the liver. The drug sponsor asserted that these results are consistent with those from excretion studies, and those of the distribution in the whole body autoradiography, that radioactivity in feces of M rats results from both biliary excretion and direct secretion into the g.i. tract.

In conclusion, the pharmacokinetics and bioavailability of UK-92,480 have been investigated in the mouse, rat and dog using a specific HPLC method for assaying the drug/metabolites in biological fluids. The drug is absorbed orally by rat and dog and distributed into the tissues in the rat. In man, UK-92,480 absorption is rapid with C_{max} being attained within 1 hour in the fasted state. In man, the elimination is bi-phasic with a terminal T 1/2 of ~ 4 h. The N-demethylating the drug (at the piperazine ring) results in the metabolite **UK-103,320** and loss of two carbon fragment from the same ring results in the metabolite **UK-150,564**. In all species studied, **UK-103,320** was detected in their plasma, and the drug and the metabolite **UK-150,564** the excreta. In *vitro* studies, UK-103,320 was ~ 0.4 X as potent than the parent compound against PDE5 from the human corpora cavernosa (the metabolite also inhibited PDE5 from rabbit's corpora cavernosa). In anesthetized rats and dogs, this metabolite given **iv** induced dose-related transient falls in MABP and tachycardia. The extent of plasma protein binding for mouse, rat, rabbit, dog and man was 94%, 95%, 91%, 86% and 96%, respectively.

UK-92,480 is only a weak inhibitor of human drug metabolizing cytochrome P450 isoenzymes, and the metabolite UK-103,320 is only a weak inhibitor of the major human cytochrome P450 isozymes. Apart from CYP2D6 (which is involved in biotransformation of numerous drugs) with an IC_{50} of 71 μM, all other isoforms investigated showed IC_{50} values greater than 300 μM.

A high affinity isoform has been identified as CYP2C9 (reported as being a minor route of metabolism in clinical reports) and a low affinity isoform as **CYP3A4** (reported as the major route of metabolism in clinical reports) based on studies with known inhibitors of these isoenzymes

4. LABELING (Package Insert)

The following changes to the package insert are recommended:

Carcinogenesis, Mutagenesis, Impairment of Fertility

No evidence of drug related carcinogenicity was revealed in a 24-month study in rats at doses up to 42 times the Maximum Recommended Human Dose (MRHD) on a mg/kg basis (approximately 9 times the MRHD on a mg/m^2 basis) and in an 18-21 month study in mice at doses up to 7 times the MRHD on a mg/kg basis (approximately 0.6 times the MRHD on a mg/m^2 basis).

No teratogenic effects, impairment of fertility or adverse effects on peri/postnatal development were found in reproduction studies in rats and rabbits following oral administration of sildenafil. In vitro bacterial mutagenicity, in vitro mammalian cell mutagenicity and clastogenicity, and in vivo clastogenicity tests were negative.

There was no effect on sperm motility or morphology after single 100 mg oral doses of VIAGRA in health volunteers.

Pregnancy, Nursing Mothers and Pediatric Use

VIAGRA is not indicated for use in newborns, children, or women.

Pregnancy Category B. Reproduction studies have been performed in rats at doses up to 140 times the MRHD on a mg/kg basis (approximately 24 times the MRHD on a mg/m^2 basis) and in rabbits at doses up to 140 times the MRHD on a mg/kg basis (approximately 56 times the MRHD on a mg/m^2 basis) and have revealed no evidence of impaired fertility or harm to the fetus due to sildenafil. There are, however, no adequate and well-controlled studies in pregnant women.

5. OVERALL SUMMARY AND EVALUATION

In previous clinical studies with UK-92,480 as a vasodilator for the treatment of angina, a remarkable drug effect noted in some subjects was penile erection. Vasorelaxation noted with the drug is considered due to the increased levels of cyclic guanosine monophosphate (cGMP) resulting from the inhibition of the phosphodiesterase (PDE) that hydrolyzes the nucleotide. Based on findings that the predominant PDE of the smooth muscle corpus cavernosum is the type V (other PDEs are also present, e.g., a cGMP-stimulated cAMP PDE_{II} and cGMP-inhibited cAMP PDE_{III}) and that several members of the $CGMP_V$-specific isoenzyme family have been identified in vascular smooth muscle and platelets, sponsor undertook further studies to the determine the mechanism of action of UK-92,480.

The physiological mechanism responsible for erection of the penis involves the release of nitric oxide (NO) from nerve endings and endothelial cells in the corpus cavernosum during sexual stimulation. Nitric oxide then activates the enzyme guanylate cyclase, which results in increased levels of cyclic guanosine monophosphate (cGMP). cGMP produces vascular smooth muscle relaxation in the corpus cavernosum and causes an increase in penile blood flow and an erection. This sinusoidal engorgement works to maintain an erection by inhibiting venous return from the penis by compressing the veins responsible for draining the corpus cavernosum.

Sildenafil is a potent and selective inhibitor of cGMP-specific phosphodiesterase type 5 (PDE5). PDE5 is responsible for degradation of cGMP in the corpus cavernosum. When the NO/cGMP pathway is activated during sexual stimulation, inhibition of PDE5 by sildenafil results in increased levels of cGMP in the corpus cavernosum and increased relaxation of corpus cavernosal smooth muscle cells in response to sexual stimulation. This causes an increase in penile blood flow and an erection. Thus, sexual stimulation is required for an erection while sildenafil helps maintain one.

Sildenafil has between an 80- and nearly 20,000-fold selectivity for PDE5 found in human corpus cavernosum compared with human PDE2, PDE3, and PDE4. Sildenafil has a 10-fold selectivity for human PDE5 over human retinal PDE6.

5.1. Pharmacology

Sponsor identified the dose-response for sildenafil's relatively specific cavernosal effects *in vitro* (3-300nM) and *in vivo* (10-300 µg/Kg iv). Since PDE5 also occurs elsewhere (platelets and skeletal, vascular, and visceral muscle) - and the other PDE isoenzymes are widely distributed - sponsor also examined general (safety) pharmacology at dosages 10 to approx. 30 X those affording PDE5 selectivity.

Mechanism of action. Six PDE subtypes have been characterized, and the relative potency of sildenafil for inhibiting each was identified. Sildenafil selectively and potently inhibited PDE5 which specifically degrades cyclic guanosine monophosphate (cGMP): for human enzymes, sildenafil had a >1000 fold selectivity for PDE5 over PDE2, PDE3, PDE4; an 80 fold selectivity over PDE1 (found in human cardiac ventricle); and about 10 fold selectivity over PDE6 (found in human retina). Accordingly, it is expected to inhibit the degradation of cGMP without affecting that of cyclic adenosine monophosphate (cAMP) in vivo. The selectivity (4,629-fold) of sildenafil for human PDE5 (IC50 = 3.5 nM) over human PDE3 (IC50 = 16.2 µM) is important given the known cardiovascular activity of PDE3 inhibitors, including intracellular cAMP- dependent proarrhythmogenicity.

Safety pharmacology: Sildenafil dose-relatedly changed the kinetics of the light response of the dog retina in situ, including slowing of the rate of hyperpolarization, at a threshold plasma level approx. 4x greater than that maximally effective on the corp. cavernosum. Such activity is consistent with the effect of sildenafil on PDE6, presence of PDE6 in the retina, and the role of cGMP in phototransduction.

In conscious dogs, no remarkable hemodynamic changes were seen at up to at least 10X blood levels achieving targeted cavernosal effects; at 30 X "therapeutic" dosages, modest changes within ± 20% occurred in cardiac output , total vascular resistance , and heart rate, with no cardiotonic activity. Lack of cardiovascular activity reflects relative absence of PDE3- blocking activity. Consistent with radioligand receptor binding studies *in vitro*, sildenafil had neither adrenergic, cholinergic, serotonergic or histaminergic blocking activity nor sympathomimetic or ganglion stimulating or blocking activity in cats, at up to 3 mg/Kg iv, i.e. at least 30X dosage effective on dog cavernosum. It did not facilitate induction of, or interfere with electroconversion of, PES-induced ventricular fibrillation in dogs at 30-100 X therapeutic iv dosage. It prolonged bleeding time in rats (+60%) and rabbits (+30%) at 0.3 -1.0 mg/Kg iv., i.e., 30-100X the iv doses active on the dog cavernosum.

Neither basal gastric acid secretion nor gastrointestinal motility were affected in the rat at up to 10 mg/Kg p.o.

A circulating metabolite (UK-103,320) identified in dog, rabbit, rat, mouse and man also showed PDE5 selectivity and, where tested, biological activity - including altered retinal response to light - similar to that of parent.

5.2. Toxicology

5.2.1. Acute Toxicology

Single oral dose studies in rats and mice found the minimal lethal dose to be between 500-1000 mg/kg in mice and between 300-500 mg/kg in rats. In rats the severity of clinical signs in females and the mortality which occurred in females only suggested a sex-linked difference in the sensitivity to acute effects of UK-92,480.

Single dose i.v. studies showed that administration of UK-92,480-10 to mice at 20 mg/kg and to rats at 10 mg/kg produced no evidence of acute toxicity.

5.2.2. Subchronic/Chronic Toxicology

5.2.2.1. Rats

5.2.2.1.1. Oral

Ten day oral toxicity studies in rats found deaths in the 150 and 500 mg/kg/day groups. Palpebral (eyelid) closure and chromodacryorrhea (bloody tears) were observed in the 150 and 500 mg/kg groups. Dyspnea and salivation occurred in the 500 mg/kg groups. There were significant increases in absolute and relative liver weights in the high dose (500 mg/kg) males and in the mid (150 mg/kg) and high (500 mg/kg) dose females. Microscopically, this correlated with an increased incidence of hepatic centrilobular hypertrophy. This change was considered to be an adaptive process since it has been found in other cases of liver enzyme induction. Plasma drug concentration ratios of UK-92,480 and the major pharmacologically active metabolite, UK-103,320, showed that males were exposed mostly to the metabolite, while females were exposed mostly to the unchanged drug.

One month oral toxicity studies in rats found increased absolute liver weights at the 45 and 200 mg/kg/day doses. Centrilobular hypertrophy was reported in both sexes. Hypertrophy of the zona glomerulosa of the adrenal glands was seen in 200 mg/kg dose males and in 45 and 200 mg/kg dose females. Thyroid follicular hypertrophy occurred at the 200 mg/kg dose in both sexes. The dose of 10 mg/kg appeared to be the no-adverse effect level (NOAEL). N-demethylation of UK-92,480 to UK-103,320 was found to be an important route of UK-92,480 biotransformation

in male rats. The transformation rate was sex-dependent; females being exposed predominantly to the unchanged drug and males to an almost equal balance of drug and metabolite.

Six month oral toxicity studies in rats found increased liver weights, the increases being more prominent in the females. Decreases of plasma bilirubin and triglycerides, and increases in plasma urea, total proteins and cholesterol were seen at 60 mg/kg/day. These changes were suggested to sponsor as drug-induced metabolic changes in the liver. Thyroid hypertrophy occurred at 60 mg/kg/day in both sexes and at a lower incidence in males given 12 mg/kg/day. This change was considered by sponsor to be a secondary phenomenon related to increased hepatic clearance of thyroid hormone. Hypertrophy and increase in weight of the zona glomerulosa of the adrenal gland was seen at 12 and 60 mg/kg/day.

5.2.2.1.2. Intravenous

Thirteen day i.v. toxicity studies in rats found no toxicity at doses up to 10 mg/kg/day. One month i.v. toxicity studies found about a two-fold increased incidence of a chronic inflammation in the myocardium when compared to controls. This effect could not be explained by the known pharmacological properties of the drug.

5.2.2.2. Dogs

5.2.2.2.1. Oral

Ten day oral toxicity studies in dogs found emesis and salivation in the high dose (100 mg/kg) group, conjuctival redness in the mid (30 mg/kg) and high (100 mg/kg) dose groups, and lacrimation in all dose groups (10-100 mg/kg/day). Heart rates were slightly increased at the 30 and 100 mg/kg doses. Decreased PQ and QT intervals at the high dose (100 mg/kg) may have been related to the increased heart rates observed. Plasma cholesterol was increased 45-65% at the 100 mg/kg dose. Microscopic analysis found a focal arteritis in the right coronary artery of one high dose female. Although such lesions may occur spontaneously in Beagle dogs, it has been associated in dogs with PDE3 inhibitors.

One month oral toxicity studies found a mild coronary arteriopathy in one high-dose (80 mg/kg/day) animal. Six month oral toxicity studies found a moderate increase in heart rate and subsequent decrease in PQ and QT intervals at the high dose of 50 mg/kg/day. These effects were considered related to the vasodilatory properties of the drug. A high dose male showed a number of clinical signs and changes in hematological parameters and plasma chemistry associated with a disseminated necrotizing panarteritis. Two high dose males showed qualitatively similar arteritis in the thymus which drug sponsor considered to be an expression of a latent spontaneous arteritis "precipitated by the treatment but not caused by it."

Twelve month toxicity studies in dogs found no treatment-related effects of body weight or blood pressure. There were no noteworthy drug-related changes in hematology, clinical chemistry, or urinalysis. Heart rates were increased 2 hours after treatment as measured on several days. The increased heart rates may have been a compensatory response to the vasodilatory effects of the drug. ECG results showed that there were increases in P amplitude (atrial contraction) and decreases in PQ (time between atrial and ventricular contraction) and QT (time between the beginning of ventricular contraction and repolarization) intervals in the high-dose dogs. These changes correlated with the increases in heart rates observed. The changes were within the sponsor's historical range for dogs, and were not considered toxicologically significant. On microscopic examination, a periarteritis was observed in 3/4 high-dose males, 1/4 high-dose females. and 1/4 low-dose females. It was characterized by a mononuclear infiltrate in the adventitia and media accompanied by intimal proliferation and fragmentation of the internal elastic lamina. In females, the periarteritis was focal and restricted to a coronary vessel, while in affected males it involved the heart and other organs.

The major toxicological finding of the 12-month study was the occurrence of a periarteritis in 3/4 high-dose (50 mg/kg) males. Periarteritis was also found in a previous 6-month toxicity

study in 2/4 male dogs treated with 50 mg/kg. This condition, also known as idiopathic febrile necrotizing arteritis, occurs spontaneously on a rare occasion in Beagle dogs. Clinical pathology changes in this syndrome include neutrophilia, high fibrinogen levels, anemia, increased alkaline phosphatase, and decreased sodium and chloride. These changes were found in the high-dose male dogs, but in none of the controls indicating that these effects were drug-related. The total of unbound AUCs in dogs given 50 mg/kg/day was 48.9X the AUC of men given a single dose of 100 mg. However, the value of 48.9X the human AUC represents a relatively large safety margin with respect to the possible development of drug-induced arteritis in man. The NOAEL for the 12 month study in dogs was 10 mg/kg/day. Systemic exposure (sum of unbound AUCs) in dogs to 10 mg/kg (NOAEL) was 7.8X the human exposure at 100 mg (1.4 mg/kg).

Even with the relatively large safety margin, the occurrence of periarteritis in high-dose male dogs would be a cause for concern in human patients because of the difficulty associated with finding clinical evidence of a focal vascular infiltration. However, the periarteritis is reminiscent of a vasculitis seen in dogs with a variety of vasodilators given at hypotensive and tachycardic dosages.

5.2.2.2.2. Intravenous

Fourteen day i.v. toxicity studies in dogs found liquid feces, an inhibition of pupillary reflex, increased plasma cholesterol, and an increased heart rate at doses of 5-10 mg/kg/day. The increased heart rate was considered a pharmacological response to the drug. The no-effect level was 2.5 mg/kg/day.

One month i.v. toxicity studies found no drug-related effects on cardiovascular parameters (ECG, BP, HR) at doses up to 4 mg/kg/day. No other drug-related effects were noted. It was concluded that UK-92,480 given to dogs i.v. at up to 4 mg/kg/day for 28 days produced no evidence of toxicity.

5.2.3. Carcinogenicity

5.2.3.1. Rat

The dose of 60 mg/kg/day chosen as the high dose for the two year rat carcinogenicity study was based on data from several toxicity and pharmacokinetic studies which are summarized as follows: (1) doses above 60 mg/kg resulted in mortality and hypertrophy of several organs, (2) a dose of 60 mg/kg for 6 months resulted in similar adaptive responses and a moderate decrease in body weight gain (-9% in males and -7% in females), and (3) the sums of the AUC levels for free parent and metabolite in rats given 60 mg/kg for 14 days were 27X and 40X for male and female rats, respectively, the human exposure at the maximum recommended dose of 100 mg/day.

The only statistically significant finding in the two year oral carcinogenicity studies in rats was an increased proliferation in thyroid follicular cells in male rats treated at the high dose of 60 mg/kg/day. This was expressed as the combined incidence of hyperplasia, adenoma, and carcinoma as recommended for a multistage model of carcinogenesis. Evidence from another study was presented to suggest that the mechanism for this effect was due to induction of hepatic UDPGT which increased the clearance of thyroid hormone and caused a compensatory increase in plasma TSH which, in turn, stimulated the thyroid gland. Evidence for such a mechanism at the 60 mg/kg dose used in the carcinogenicity studies was not presented.

No drug-related increase in mortality was found. Percent changes in mean body weight gains in male and female rats showed that high dose males (60 mg/kg/day) gained 11.0% less weight than controls, while mid and high dose females gained 17.0% and 15.7% less weight, respectively than controls. These values are an acceptable MTD according to ICH-S1C guidelines ("no more than 10% decrease in body weight gain relative to controls").

Systemic exposure to total unbound drug (sum of the parent drug UK-92,480 and the principle pharmacologically active metabolite UK-103,320) was calculated to be 34X and 38X the maximum recommended human dose of 100 mg in male and female rats, respectively. These

results suggest that the lack of a carcinogenic effect in rats was not due to inadequate systemic exposure to sildenafil. A statistical review of tumor incidence in the rat study by the Division of Biometrics is pending.

5.2.3.2. Mouse

Selection of the high dose (30 mg/kg/day) was based on a mouse 3 month repeated dose study in which mortality occurred in 1/20 animals in each group treated with 40 or 100 mg/kg UK-92,480-10, but not in the groups treated with 20 mg/kg. The cause of death, which occurred from the sixth week of treatment, was due to gastrointestinal dilation, and was associated with dyspnea and swollen abdomen. No adverse effects were noted in the 20 mg/kg group after 3 months of treatment.

Tumor analysis in the two year carcinogenicity studies using the Peto's death rate method for fatal tumors and prevalence analysis for incidental tumors showed that there were no treatment-related increases in neoplastic lesions.

In contrast to the rat study, treatment in mice produced an increase in mortality in the high-dose (30 mg/kg/day) males and in the mid (10 mg/kg/day) and high dose (30 mg/kg/day) females. The male and female high dose (30 mg/kg) groups were terminated early after only 13-15 months on treatment. The remaining groups were sacrificed after about 19-22 months of drug administration because of near 20% survival in the mid dose (10 mg/kg) groups. The increased mortality in drug-treated mice was shown to be due to gastro-intestinal dilation. Separate studies demonstrated a drug effect on reducing intestinal transit which was thought to be due to relaxation of gastrointestinal smooth muscle. This effect was postulated to be due to the drug's pharmacological properties of drug-induced PDE-5 inhibition which reduces cGMP breakdown and leads to reduced gastrointestinal motility. The extent of the slowed intestinal transit correlated with the increased incidence of death due to gastro-intestinal dilation in both male and female mice. The fact that mice appeared to be more sensitive than rats may explain the absence of mortality due to gastro-intestinal dilation in the rat studies. Target organ (gastro-intestinal) toxicity and subsequent death should qualify the mid dose as an acceptable MTD in both male and female mice according to ICH-S1C guidelines ("target organ toxicity").

Drug treatment for 19-22 months reduced weight gain in the mid dose groups by 24% and 17% in males and females, respectively, when compared to controls. The reductions in weight gain for the mid dose groups should also be considered as an acceptable MTD according to ICH-S1C guidelines ("no more than 10% decrease in body weight gain relative to controls").

Although AUC values were not calculated, plasma drug levels (C_{max}) of total unbound drug (sum of the parent drug UK-92,480 and the principle pharmacologically active metabolite UK-103,320) in mid dose male and female mice was calculated to be only 0.1X and 0.2X, respectively, that present at the maximum recommended human dose of 100 mg. This value was only 0.6X when the multiple of the maximum recommended human dose (MRHD) was expressed as surface area (mg/m^2).

Although the extent of systemic exposure to UK-92,480 in the mouse studies was lower than the MRHD, the doses used were limited due to excessive toxicity. This was shown by increased mortality due to gastro-intestinal dilation and reduced body weight gains in both the mid (10 mg/kg) and high (30 mg/kg) dose groups. Therefore, although mice in the mid dose (10 mg/kg) groups received doses of drug for >18 months that were essentially toxic, there were no significant increases in neoplastic lesions. A statistical review of tumor incidence in the mouse study by the Division of Biometrics is pending.

5.2.4. Reproduction Toxicity

The toxic potential of oral doses of UK-92,480 citrate* on fertility and reproduction was evaluated in rats (Sprague-Dawley) and rabbits (NZW). These GLP studies were conducted in France.

5.2.4.1. Rat

Rats (20 adult M and F/dose group) were treated with 0, 3, 12 and 60 UK-92,480 citrate mg/kg/day po to evaluate their fertility. The M with which the F were mated, were treated for 9 wks prior to the mating period (2 wks), and until the sacrifice of the F (day 20 pi) for F and day 20 post-insemination for M. From the findings reported, it may be concluded that UK-92,480 citrate given at oral doses given prior to- and during the mating period, and during gestation induced **no** adverse effects on fertility of either sex, and no embryo- or fetotoxicity. The **NOAEL** on fertility for both M and F rat may be considered 60 mg/kg (~ 420 mg/M^2 representing about 7 X the MHTD in M).

Reviewer considers that treatment with UK-92,480 was associated with some maternal toxicity at the HD 60 mg/kg/day (~ 420 mg/M^2 assuming a 250 g rat) because of the reported moderate decrease in triglycerides (~30% vs controls) together with minor decreases in plasma proteins, and statistically significant decreases in some liver enzymes (i.e AP, ALT and AST), phosphate levels. However, at this time, the drug is **only** intended for use by M with penile dysfunction.

Artificially inseminated rats were treated orally with 0, 10, 50, and 200 mg/kg UK-92,480 per day during the period of organogenesis (6-17 of pregnancy) to evaluate maternal toxicity, fetotoxicity and embryotoxicity. The doses tested were based on a preliminary study in pregnant rats with doses at the same doses of UK-92,480.

The pregnant rats in this the definitive study were sacrificed on 20 of pregnancy. At the HD of 200 mg/kg (~ 1700 mg/M^2), hematologic changes were reported in the dams (slight decrease in Hgb, RBC counts), and dose-related changes in clinical chemistry (decreases in mean plasma triglycerides) mild increase in liver weight/centrilobular hypertrophy considered a sign of xenobiotic-metabolizing enzymes. The body weight of M fetuses from HD dams showed a reduced body weight (at ~ 28X the MHRD of 100 mg UK-92,480) when compared to the F fetuses reported as slight fetotoxicity.

No clear evidence of drug-related external, skeletal or visceral abnormalities were reported.

5.2.4.2. Rabbit

Artificially inseminated rabbits were treated orally with 0, 10, 50, and 100 mg/kg UK-92,480 per day during the period of organogenesis (6-18 of pregnancy) and sacrificed on gestation day 28, to detect any adverse effects on the dam or development of the embryo/fetus. One MD and 1 HD rabbit aborted on days 21 and 19 pi. Toxicologically significant signs in the dams included inconsistent changes in body weight (increased at the LD/MD and decreased at HD). Notable fetal findings included external abnormalities (1 omphalocele and 1 gastroschisis in MD fetuses), visceral abnormalities (ventricular septal defect complicated with left ventricular hypertrophy/atrophy of right ventricle/pulmonary artery). Although these visceral abnormalities were marginally above the highest incidence reported in their historical controls, these findings were not considered drug related. The principal metabolite UK-103,320 was reported present in

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* CHEMISTRY review stated that drug sponsor has indicated that lots of the drug , used in genotoxicity and teratogenicity studies contain the impurity evels. Drug sponsor has used these studies to assert that .oes not induce reproduction toxicity.

the amniotic fluid. The NOAEL for the parent compound on the dam and developing embryos/fetuses appears to be ~ 10 mg/kg (~ 100 mg/M^2 assuming a 2.5 kg rabbit).

5.2.5. Genetic Toxicity

UK-92,480 has been evaluated in a range of *in vitro* and *in vivo* tests to detect genotoxic activity. (1 bacterial and 3 mammalian cells assays.) These GLP studies were conducted in the drug sponsor's laboratories in the US.

In the Ames test, with or without metabolic activation, UK-92,480 (0.002 up to 1 mg/plate) did not display mutagenic activity in the 4 S. typhimurium strains. In the CHO/HGPRT mammalian cell gene mutation assay, concentrations of the drug from 65 up to 240 µg/ml showed no evidence of dose-related increase in the frequency of 6-thioguanine-resistant mutant cells. *In vitro* mammalian cytogenetic assays were used to detect potential clastogenic activity of UK-92,480. The L5178Y mouse lymphoma cells (preliminary test for dose-ranging) and human lymphocyte cultures (definitive test) were exposed to UK-92,480 without and with exogenous metabolic activation (liver S9 fraction from Aroclor-induced rat). Concentrations of the drug tested ranged from 10 to 25µg/ml without metabolic activation (direct method) and 100 up to 250 µg/ml with metabolic activation. Although in the definitive assay an increase in abnormal human lymphocyte cells was noted in cultures treated with the metabolically activated drug (100 up to 250 µg/ml), a repeat assay at the same concentrations of UK-92,480, revealed no statistically significant increase in the number of abnormal cells compared to both the concurrent and historical negative controls. It must be noted that in all assay where the drug was metabolically activated, the plates contained crystals which were identified by the term "compound". The proposed International Conference on Harmonization (ICH) Genotoxicity Guidelines is not absolutely clear in how to treat precipitates. It recognizes that heavy precipitates may interfere with interpretation of findings. Overall, UK-92,480 did not show clear evidence of clastogenic activity in this assay.

For the *in vivo* micronucleus assay in CD-1 mice bone marrow, both sexes were treated with UK-92,480 at 500, 1000 and 2000 mg/kg/day p.o. for 3 days. UK-92,480 did not produce any significant dose-related increase in the frequency (%) of micronucleated polychromatic erythrocytes (MNPCE), suggesting no chromosomal damage by the drug. Compared to solvent controls, there was a dose-related decrease in % PCE suggesting cytotoxicity at doses dosages near the MTD.(Bioavailability of the drug in mouse was reported ~17%) One way to interpret these findings is that since there was no significant increase in the number of MNPCE the drug may be considered nonclastogenic in this study at up to dosages cytotoxic for bone marrow. Although the genotoxic potential of metabolites of the drug or the impurity analog of UK-92,480 was not directly tested), drug sponsor has conducted a battery of tests (Ames test to detect reverse mutation, CHO/HGPRT mammalian cell gene mutation assays, in vitro mitogen-stimulated human lymphocytes assay to detect clastogenic activity, and in vivo mouse bone marrow metaphase assay to detect chromosomal aberrations) in which metabolically activated UK-92,480 was used, and no clear evidence of genotoxicity was reported.

5.3. Pharmacokinetics (ADME)

The pharmacokinetics studies with UK-92,480 were conducted in the UK using the mouse, rat and dog and a specific HPLC method for assaying the drug/metabolites in biological fluids. Data reported indicate that UK-92,480 is absorbed orally by mouse, rat and dog and distributed readily into the tissues in the rat (detected by autoradiographic studies with ^{14}C drug). In rat, autoradiographic studies showed that, after the iv administration of ^{14}C labeled drug radioactivity was detected in organs by 0.1 h post-dose; the liver, adipose tissue, adrenal gland, retina and pigmented tissue showed affinity for the drug. By 24 hr most of the radioactivity remained in the retina; the long term significance of this finding in unknown. However, this finding is in keeping with the reported activity of cGMP in vertebrate retinal rods.

In man, UK-92,480 absorption is rapid with C_{max} being attained within 1 hour in the fasted state, and the elimination is bi-phasic with a terminal T 1/2 of ~ 4 h.

When the drug is N-demethylated at the piperazine moiety this results in principal metabolite **UK-103,320,** and loss of two carbon fragment from the piperazine ring results in the metabolite **UK-150,564**. These 2 metabolites are weak PDE5 inhibitors compared to the parent compound and were formed in all species studied, and excreted in bile and found in feces. The unchanged drug and the metabolite **UK-150,564** were detected in human urine.

In *in vitro* studies, UK-103,320 was less potent than the parent compound against PDE5 from the human and rabbit corpora cavernosa. In vivo studies with anesthetized rats and dogs, showed that the metabolite UK-103,320 given **iv** produced dose-related transient falls in MABP and tachycardia. UK-92,480 is only a weak inhibitor of human drug metabolizing cytochrome P450 isoenzymes. CYP2C9 was reported as being a minor route of metabolism in clinical reports and **CYP3A4** as the major route of metabolism in clinical reports based on studies with known inhibitors of these isoenzymes.

Gender differences in the metabolism in rat was also reported in vivo in that M were more exposed to the unchanged drug than to the principal metabolite UK-150,564 while F rats the reverse was reported.

The drug binds to plasma protein (protein not identified); % binding in man was reported as ~ 96%, rat ~ 95%, mouse ~ 94%, rabbit ~ 91% and dog 86%. In vitro studies indicated that plasma protein binding of the drug was independent of total drug concentration over the range 0.01-10 µg/ml.

In summary, UK-92,48 (**sildenafil**), showed **no** evidence of genotoxic potential in a battery of vivo and in vitro assays. Further, the drug provoked no alarming toxic effects after repeat oral doses to rat (up to 12 mg/kg/day for 6 mo; ~ 84 mg/M^2 representing **~ 1.36 X** the **MHRD** of 100 mg in man), and dog (up to 3 mg/kg/day for 12 mo; ~ 60 mg/M^2 assuming a 9 kg dog representing **~ 0.9 X the MHRD** assuming a 60 kg man). Since sildenafil is specifically indicated for men, it should be noted that at the doses tested (3, 12 and 60 mg/kg UK-92,480) in the M/F rat fertility studies, the **NOAEL** may be considered 60 mg/kg (~ 420 mg/M^2 representing about **7 X** the MHRD); this number was obtained by using a km (conversion factor) of 7 for rat and 37 for man. Drug sponsor has previously reported (Correspondence dated: 05-11-95; Serial No. 009) that histopathologic "examination of the testes was carried out in rat and dog," treated for 6 mo each at dose levels of 60 and 200 mg/kg/day, respectively, showed "... no adverse effect on spermatogenesis..." However, the drug/principal metabolite have been reported in human semen, and females might be exposed by vaginal contact with these compounds. Although F rats were exposed orally to UK-92,480 and, possibly vaginally with the drug/metabolites with unimpaired fertility, we recommend that the possibility of vaginal exposure should appear in the labeling.

5.4. Conclusions

Studies conducted *in vitro* and *in vivo* over relatively large dose ranges project no adverse cardiovascular (including proarrhythmogenic and hemorrhagic), autonomic, cytotoxic, tumorigenic/genotoxic, or reproductive liabilities. The periarteritis occurring in dogs, especially male, at high multiples of the human dosage is reminescent of that provoked by a variety of innocuous vasodilators in animals at hypotensive and tachycardiac dosages. It was not associated with any parenchymal end-organ damage, either acute or chronic. However, sildenafil changes the canine retinal response to light at approximately 4X the dosage producing maximal cavernosal activity in dogs.

6. RECOMMENDATIONS

From a preclinical safety perspective, we recommend that the application NDA #20-895 (sildenafil; Viagra®) be approvable with the recommended changes in labeling (see page 129).

The Executive CAC has recommended that a commitment may be necessary from the sponsor (it could be Phase 4) to carry out a 3 month dose ranging study in rats with a wider range of dosing to better characterize the relationship between dose, body weight, and toxicity. This information may make it possible to determine how close the doses were in the rat two year carcinogenicity study to a Maximum Tolerated Dose (MTD). The recommendation was based on pharmacokinetic data submitted by the sponsor in which a 25-fold ratio of rat to human plasma AUC was not acheived in the study (18X and 21X in male and female rats, respectively). However, the sponsor calculated rat systemic exposure using 1-8 hour AUCs, while the human study used 1-24 hour AUCs. Recalculation with the linear trapezoidal rule of the rat AUCs using 1-24 hours has shown that rats in the two year study were exposed to a greater than 25-fold multiple of the human AUC (34X and 38X in male and female rats, respectively; see page 62). Therefore, the rat two year carcinogenicity study should be considered adequate as performed without the need for additional studies.

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cc:
Orig. NDA
HFD-110
HFD-110/ G. Buehler
HFD-110/ E. Barry
HFD-110/ A. DeFelice
HFD-110/ T. Papoian
HFD-024/J. DeGeorge
HFD-345 (Scientific Invest.)
HFD-400/ J. Contrera