CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

PHARMACOLOGY REVIEWS

TOXICOLOGY PAGES 26-48

2. TOXICOLOGY

2.1. Acute Toxicology

2.1.1. Single dose oral toxicity studies in mice and rats (Study Nos. 90155 and 90156; Vol. 1.14 pp. 197-224):

Dose Levels: 300, 500 and 1000 mg/kg body weight.

The aim of these studies was to investigate the acute oral toxicity of UK-92,480 in mice and rats following administration of a single dose.

Groups of male and female (5/sex/dose) Swiss-CD1 mice and Sprague-Dawley rats received a single oral (gavage) administration of UK-92,480. Dose levels chosen were 500 and 1000 mg/kg in mice, and 300, 500 and 1000 mg/kg in rats. The compound was given as an aqueous solution in a volume of 20 ml/kg in mice and 10 ml/kg in rats. The animals were observed for 14 days for clinical signs and mortality. All dead animals and survivors were necropsied.

Results:

Sponsor reports that at 1000 mg/kg 1 male mouse died within 24 hours after drug administration.

In rats, mortality occurred in 3 females at 1000 mg/kg and in 1 female at 500 mg/kg.

The dose of 1000 mg/kg induced clinical signs in both species, generally within 24 hours following the administration, and which persisted less than 24-48 hours. Some of these signs were similar in mice and rats and consisted of partially-closed eyes, hunched posture, tremors, depression, coldness to the touch (with pallor of ears and paws in rats) and prostration.

Female rats were more affected than male rats. Dyspnea was limited to one mouse, and chromodacryorrhea to four female rats. Clinical signs at 500 mg/kg included partially closed eyes in 1 mouse and depression in 1 female rat which died.

No clinical signs were observed in rats at 300 mg/kg.

In both species, the doses administered induced no changes in body weight gain and there were no treatment-related macroscopic changes at gross necropsy.

In these studies, data reported show that the minimal lethal dose (MLDL) level is 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, suggest a sex-linked difference in the sensitivity to acute effects of UK-92,480.

2.1.2. Single dose intravenous toxicity in mice and rats (Study Nos. 91045 and 91046; Vol. 1.14 pp. 225-245):

Male and female Swiss-CD1 mice (5/sex) and male and female Sprague-Dawley rats (5/sex) were given a single i.v. injection of the citrate salt form of UK-92-480-10 (lot #953-27). Mice received a dose of 20 mg/kg and rats received 10 mg/kg. The dose was limited to the solubility of the citrate salt of the compound. The animals were observed for mortality, clinical signs, and body weight changes. After 14 days, all surviving animals were subjected to a gross necropsy.

Results showed that there were no deaths or altered clinical signs. Body weights were unaffected. No abnormalities were noted on necropsy.

It was concluded that i.v. 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.

2.2. Subchronic/Chronic Toxicology

2.2.1. Rats

2.2.1.1. Oral

2.2.1.1.1. 10 day oral range-finding toxicity in rats (Study No. 90080; Vol. 1.14 pp. 246-351):

Testing Facility: Laboratoires Pfizer; Centre de Recherche; Ambroise Cedex; France
Study Number: 90080
Study Date(s): 5/22/90 to 5/31/90
GLP Compliance: Yes

Male and female albino Crl:COBS-VAF-CD(SD)BR rats (5/sex/group; 257 and 191 gms, respectively) were given UK-92,480 (batch no. 1150/262/B) orally by gavage at 50, 150, or 500 mg/kg/day for 10 days. Controls received vehicle (0.5% methylcellulose and 0.1% Tween 80). Additional groups of rats (5/sex/group) were used for plasma drug level determinations (PK rats). Rats were observed for clinical signs, body weights were recorded twice a week, and food consumption was measured. Twenty-four hours after the last dose, blood was taken from for hematology and clinical chemistry. The rats were then sacrificed for a histological examination of lung, heart, liver, and kidneys. The PK rats were bled 1, 3, 5, and 24 hours after dosing on Days 1 and 9 for plasma drug concentrations.

Several deaths were reported in drug-treated rats in both the toxicology and PK groups: one male and two females in the high dose (500 mg/kg) group, and one female in the mid dose (150 mg/kg) group. The causes of death were not determined.

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.

In male rats, a dose-related decrease in triglyceride concentrations was found (44, 56, and 71% for the 50, 150, and 500 mg/kg groups, respectively). The significance of these changes are not clear.

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 (Figure 6). Microscopically, this correlated with an increased incidence of hepatic centrilobular hypertrophy (Table 8). This change was considered to be an adaptive process since it has been found in other cases of liver enzyme induction.

Figure 6

Percent Increase (Compared to Controls) in Relative Liver Weights
in Male and Female Rats Treated with UK-92,480

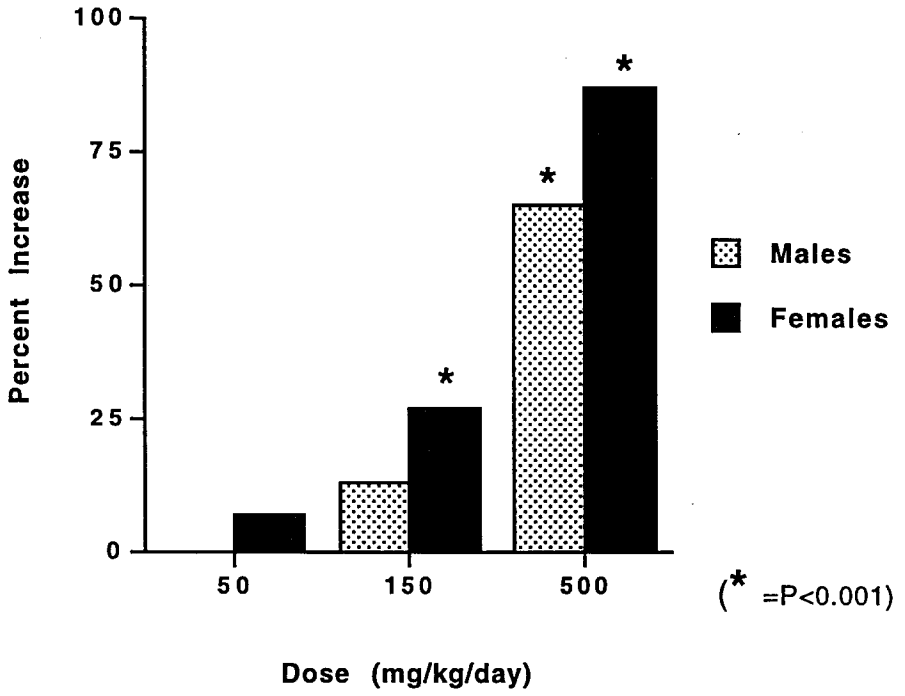


Table 8

Incidence of Hepatic Centrilobular Hypertrophy
in Male and Female Rats Treated with UK-92,480

Dose (mg/kg/day)	Males	Females
150	0/5	3/5
500	5/5	4/5

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 (Figure 7A), while females were exposed mostly to the unchanged drug (Figure 7B). In males, ratios of metabolite to drug, particularly on Day 9, were lower with increasing dose, indicating that metabolism was saturable.

Figure 7A

Ratios of Mean $AUC_{1\text{-}5hr}$ Values (µg.hr/ml) (Metabolite to Drug)
on Days 1 and 9 in Male Rats

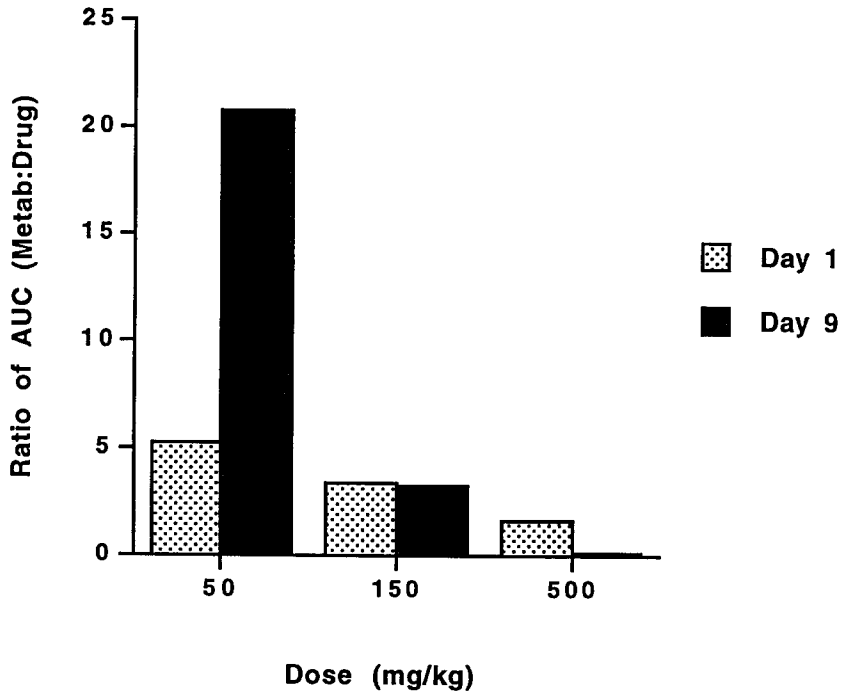
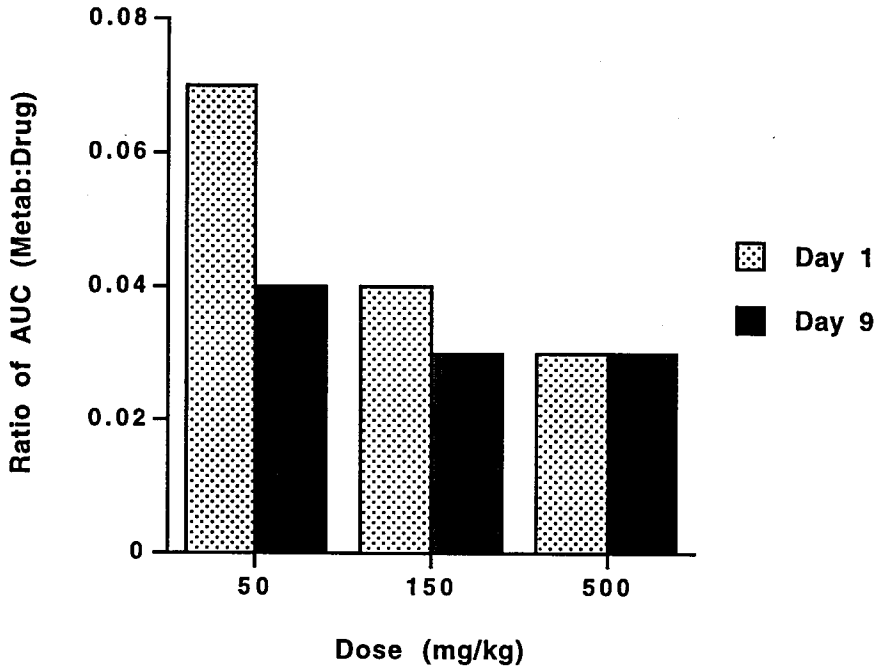


Figure 7B

Ratios of Mean $AUC_{1\text{-}5hr}$ Values (μg.hr/ml) (Metabolite to Drug) on Days 1 and 9 in Female Rats



In summary, doses of 150 and 500 mg/kg/day for 10 days resulted in death, changes in clinical signs, and increases in absolute and relative liver weights. Microscopically, increased liver weights correlated with hepatic centrilobular hypertrophy which may have been due to liver enzyme induction. Triglycerde levels were decreased in all dose groups. Plasma drug concentrations of parent drug and major metabolite showed that males were exposed mostly to the metabolite, while females were exposed mostly to the unchanged drug. In males, metabolism was saturable with increasing dose.

2.2.1.1.2. One month oral toxicity in Sprague-Dawley rats (Study No. 90143; Vol. 1.15 pp. 597-763):

Dose levels: LD-10, MD-45 and HD-200 mg/kg, p.o.

Groups of 10 male and 10 female Sprague-Dawley rats received UK-92,480 by gavage at 0 (vehicle) and at doses of LD-10, MD-45 and HD-200 mg/kg for 29 or 30 consecutive days.

The animals were regularly observed for clinical signs and weighed once a week. Food and water consumption was measured. About 24 hours after the last dose, blood was sampled for clinical chemistry and hematology and urine was collected for clinical chemistry. The animals were then sacrificed, and the organs were weighed and submitted to histopathological examination. Additional groups of 5 males and 5 females were treated with the same doses and used to measure plasma levels of drug and metabolites.

Results:

One HD female used for plasma drug level determination died. Mean values of absolute liver weight when compared to controls showed that males treated with HD had an increase of ~ 22% in mean weight (P= <0.001); also in females, doses of MD and HD increases (> 15 and 43%, respectively; P= <0.01) were also noted. Centrilobular hypertrophy was reported in both sexes. Hypertrophy of the zona glomerulosa of the adrenal glands was seen in HD males and in MD and HD females. Thyroid follicular hypertrophy occurred at the HD in both sexes.

A mild dose-related decrease in circulating red blood cells in MD and HD females; this effect was considered by sponsor as evidence of a regenerative response and also reported, to a smaller extent, in HD males. A moderate neutrophilia was seen in HD males, while a moderate lymphocytosis occurred in MD and HD females. In addition, mesenteric arteritis was diagnosed in 2 MD and 1 HD males and was considered by sponsor to be drug related.

A number of clinical signs and histopathological changes as well as variations in food and water consumption, biochemical parameters, platelets and heart and kidney weights, were seen in treated animals, in most instances at MD and HD doses. Although these observations were not considered to be of toxicological importance by sponsor, a remarkable finding reported consisted of testicular atrophy in some male rats in all groups. The lesion involved less than 10% of the seminiferous tubules but in 1 HD rat where most of the tubules were affected. No remarkable changes in mean absolute testicular was reported. 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 is sex-dependent, females being exposed predominantly to the unchanged drug and males to an almost equal balance of drug and metabolite.

PLASMA DRUG CONCENTRATION:

Since UK-92,480 may undergo demethylation to UK-103,430 and UK-95,340, all plasma samples were assessed for the presence of the three compounds.

UK-92,480:

Plasma UK-92,480 concentrations were much higher in females than in males, with maximal individual values, at 1 or 3 hours, in the following range:

Dose levels (mg/kg)	Range of maximal UK-92,480 concentrations (µg/ml)	
	Males	Females
LD-10		
MD-45		
HD-200		

Comparing drug and metabolite exposure show that UK-103,320 pharmacokinetics are dose- and sex-dependent. In males, as doses increase mainly from 10 to 45 mg/kg, more metabolite is formed, relative to unchanged drug. In contrast, in females, less metabolite is formed as doses increase.

Dose (mg/kg)	Ratio of mean AUC's (metabolite:drug) Males	Females
LD-10	0.5	0.3
MD-45	1.6	0.2
HD-200	1.7	0.1

UK-92,340:

Plasma UK-92,340 concentrations were below the detection limit of the assay (0.03 µg/ml) in most groups except for high-dose females where maximal individual values between 0.07 and 0.12 µg/ml were recorded.

Mean ± SD UK-92,480 concentrations on day 23:

Mean AUC values (1-5 h data) increased with dose level though not in a linear fashion in both sexes. The greatest increases in drug exposure were seen between the doses of 10 and 45 mg/kg in females and between 45 and 200 mg/kg in males. Overall, drug exposure was 3- to 5-fold higher in females than in males:

Dose (mg/kg)	Mean AUC values (µg.h/ml) Males	Females
LD-10	1.11	3.46
MD-45	2.97	14.68
HD-200	12.78	37.31

UK-103,320:

Plasma UK-103,320 concentrations at the MD and HD levels were much lower in females than in males. Peak concentrations were observed at 1 to 3 hours in males; in females, plasma concentrations changed very little between 1 and 5 hours. UK-103,320 was undetectable 24 hours after dose.

In males, UK-103,320 concentrations were slightly lower than those of the unchanged drug at the low dose, and slightly higher at MD and HD.

In females, UK-103,320 concentrations were much lower than those of the unchanged drug at all dose levels, and increased very little with dose level, particularly between 45 and 200 mg/kg.

Dose Levels (mg/kg)	Mean ± SD UK-103,320 concentrations on day 23 Range of maximal UK-103,320 concentrations (µg/ml) Males	Females
LD-10		
MD-45		
HD-200		

As the dose increased, mean AUC values of male animals increased superproportionally up to 45 mg/kg, but proportionally above 45 mg/kg. The steep increase in AUC values of the metabolite between 10 and 45 mg/kg probably accounts for the small increase in parent compound over this dose range. The exposure to UK-103,320 in males, relative to females, increased with dose levels (from 0.6- to 5.5-fold) as only small, subproportional, elevations of AUC's were registered in females:

Dose (mg/kg)	Mean AUC Values (µg.h/ml)		
	Males	Females	Ratio M:F
LD-10	0.54	0.95	0.6
MD-45	4.87	2.91	1.7
HD-200	21.22	3.89	5.5

2.2.1.1.3. Six month oral toxicity in rats (Study No. 91098; Vol. 1.17 pp. 1322-1708):

Study Dates: 10/17/91 to 4/16/92

Dose levels: LD-3, MD-12 and HD-60 mg/kg p.o.

Groups of 20 male and 20 female Sprague-Dawley rats received UK-92,480-10 by gavage at doses of 0 (vehicle), LD-3, MD-12 or HD-60 mg/kg for 6 months.

The animals were regularly observed for clinical signs and weighed once a week. The methods used were essentially the same as described above in the 1-mo study.

Results:

No treatment-related deaths were recorded. Chromodacryorrhoea was seen in the drug treated groups. Body weight gain and food consumption were increased at the LD and, to a lesser extent, at the MD.

A trend towards a reduced body weight gain was seen at the HD. In the MD and HD groups, there were statistically significant mild to moderate, dose-related increases in absolute and/or relative liver weight, 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 HD (See under Plasma Chemistry below, table prepared by sponsor on page 38.) These changes were suggested to sponsor as drug-induced metabolic changes in the liver.

Increased liver weight was associated with mild centrilobular hypertrophy as seen in the 1-mo study. Thyroid hypertrophy occurred at HD in both sexes and at a lower incidence in MD males. 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 a dose-related incidence at MD and HD.

Some tumors were reported at histopathology. (See table below prepared by sponsor.

Tumors seen in this study were as follows:
- Adrenal cortical adenoma in MD male
- Pituitary adenoma in HD male
- Mammary fibroadenoma in HD male
- Mammary adenocarcinoma in control female and HD female

Drug and metabolite plasma level determinations showed that females were exposed predominantly to unchanged drug while males were exposed almost exclusively to the metabolite.

Table 1 - Plasma Chemistry

Increases in urea were observed at each sampling period in the HD males and females when compared to controls. Plasma triglycerides were reduced vs. control values on days 57, 119 and 182 of study. Decreases in bilirubin were significantly reduced at the 3 recording periods in HD females only. These changes are summarized below by sponsor.

Changes in urea triglycerides and bilirubin in 60 mg/kg group
(percent from controls)

	Day 57		Day 119		Day 182	
	Males	Females	Males	Females	Males	Females
Urea	+19%**	+18%**	+23%***	+24%***	+18%***	+16%**
Triglycerides	-31%**	-11%	-33%**	-22%*	-14%	-33%**
Bilirubin	-15%	-23%**	-6%	-25%**	-5%	-34%***

*, **, ***: statistically significant at p=0.05, 0.01 and 0.001 respectively.

At the end of the study, additional changes were observed in females. There were increases in cholesterol (+23%) or phosphates (+11%) at HD, and an increase in total protein at all dose levels (from +5 to +8%).

Other minor changes, i.e., the decrease in triglycerides in MD females on day 57, the decreased aspartate amino-transferase in HD females on day 119, the increased alanine and aspartate amino-transferase in LD males on day 182, were not considered to be treatment-related by sponsor.

PLASMA DRUG CONCENTRATION:

As UK-92,480 is known to be demethylated to UK-103,320 which is pharmacologically equipotent in vitro, plasma samples were assessed for the presence of the two compounds.

UK-92,480:

UK-92,480 is rapidly metabolized in male rats. In males, drug concentrations were only detected (>0.04 µg/ml) at the HD with individual values between 0.15 and 0.51 µg/ml at 1 hour after dosing. In females, drug concentrations were dose-related. Mean concentrations declined in a similar fashion at all dose levels and became undetectable at 24 hours. Mean AUC values (1-8 hour data) in females increased superproportionally with dose level. At the high dose, drug exposure was about 80-fold higher in females than in males. These data are summarized in the following graphs and tables:

Range of maximal concentrations (UK-92,480) and mean AUC values on day 176

Dose (UK-92,480) (mg/kg)	Concentrations (µg/ml)		AUC 1-8 h (µg.h/ml)	
	Males	Females	Males	Females
LD-3	<0.04		-	0.7
MD-12	<0.04		-	5.0
HD-60			0.4*	31

*This value should be regarded as indicative only. It is shown to allow comparison with female data.

UK-103,320:

Maximal plasma concentrations were similar in both sexes at LD and became higher in males than in females at the higher doses, as shown in the table below. Mean concentrations declined after 1 hour in males and they remained sustained during the first 5 or 8 hours after treatment in females. At 24 hours, plasma concentrations were below the limit of determination of the assay (0.04 µg/ml) (except in two HD animals).

Mean AUC values (1-8 hour data) increased superproportionally to dose level in males but were approximately dose-related in females, leading to a 2-fold greater exposure in males than in females at HD.

Range of maximal concentrations (UK-103,320) and mean AUC values on day 176

Dose (UK-92,480) (mg/kg)	Concentrations (µg/ml)		AUC 1-8 h (µg.h/ml)	
	Males	Females	Males	Females
LD-3			0.1	0.3
MD-12			1.7	2.0
HD-60			12	6.0

Comparing drug and metabolite exposure suggests that UK-103,320 formation is sex-dependent. It may also be dose-dependent as judged by the reduction of the ratio of metabolite to drug at HD in females.

Ratio of mean AUC's (metabolite:drug)

Dose (UK-92,480) (mg/kg)	Males	Females
LD-3	-	0.4
MD-12	-	0.4
HD-60	29	0.2

-: Not calculated

2.2.1.1.4. Investigation of the relationship between liver enzyme induction and thyroxin clearance in rats (Study No. 96010; Vol. 1.19 pp. 2552-2619):

This purpose of this study was to determine if the thyroid hypertrophy observed in rats treated with UK-92,480 could be attributed to induction of thyroid hormone-catabolizing enzymes (UDPGT) in the liver. Reduced levels of circulating thyroid hormone would result in a compensatory increase in TSH levels from the pituitary resulting in thyroid hypertrophy.

Two groups of female Sprague Dawley rats (10/group) were given UK-92,480-10 (lot no. R202) orally at 200 mg/kg/day for 29 days. Two groups received vehicle (aqueous solution of 0.5% methylcellulose plus 0.1% Tween 80). One group of treated and one group of control rats were used for assessment of exogenous thyroxin clearance. The other group of treated and group of control rats were used for measurement of plasma TSH and thyroid hormones, for histopathological examination of liver and thyroid, and for determination of UDPGT (UDP-glucuronyl transferase) activity in the liver.

Two rats in the treated groups died on days 2-3. Weights of liver and thyroid relative to body weight were increased 51% and 20%, respectively, when compared to untreated (control) rats. Microscopically, liver showed minimal hypertrophy, while thyroid follicular cell hypertrophy was diagnosed in 7/10 treated rats. Liver UDPGT activity (µmol pNP/min/mg protein) was significantly increased from 0.27 in controls to 5.35 in treated rats. Plasma TSH increased 47%, but plasma thyroid hormones levels remained unchanged. Thyroxin clearance (ml/min/kg)

increased about 50% in treated rats. Also, the thyroxin elimination half-life (t1/2 β) decreased from 13.7 hours to 9.9 hours.

These experiments showed that treatment of rats with UK-92,480 at 200 mg/kg/day for 29 days resulted in induction of the thyroid hormone-catabolizing enzyme UDPGT, increased liver weights (although hypertrophy was minimal), increased clearance and elimination of thyroxin from the plasma, increased pituitary TSH (although plasma thyroid hormone levels were unchanged, possibly because of increased production by the thyroid), and increased thyroid weight and hypertrophy. Whether similar effects occurred at the lower doses (≤ 60 mg/kg) used in the carcinogenicity studies below was not determined.

2.2.1.2. Intravenous

2.2.1.2.1. 13 day intravenous range-finding in Sprague-Dawley rats (Study No. 90139; Vol. 1.15 pp. 433-525):

Testing Facility: Laboratoires Pfizer; Centre de Recherche; Ambroise Cedex; France
Study Number: 90139
Study Date(s): 10/10/90 to 10/22/90
GLP Compliance: Yes

Previous symptomatology studies in rats (up to 60 mg/kg i.v.) used an acidic (pH <2) formulation of UK-92,480 to increase solubility. This resulted in difficulty (local intolerance) in repeating i.v. administration. The present study used a high dose of 10 mg/kg, the limit of solubility at pH 4, which was considered compatible with repeated i.v. dosing.

Male and female Sprague-Dawley rats (5/sex/group; 241 and 181 gms, respectively) were given UK-92,480 (batch no. R1) i.v. at 2.5, 5, or 10 mg/kg/day for 13 days. Controls received vehicle (dextrose solution pH 4). Rats were observed for clinical signs and irritation at the injection site. Measurements were taken for body weights and food and water consumption. After the last dose, blood was taken for hematology and clinical chemistry. Rats were then sacrificed and subjected to necropsy which included gross examination, weights, and microscopic examination of heart, kidney, liver, and lung.

No deaths were reported. A transient redness of the ears was observed which was probably due to the vasodilatory properties of the drug. No other abnormalities were reported. It was concluded that UK-92,480 at up to 10 mg/kg/day for 13 days i.v. in rats was well tolerated and produced no evidence of toxicity.

2.2.1.2.2. One month intravenous toxicity in rats (Study No. 91044; Vol. 1.16 pp. 965-1110):

Testing Facility: Laboratoires Pfizer; Centre de Recherche; Ambroise Cedex; France
Study Number: 91044
Study Date(s): 3/29/91 to 4/25/91
GLP Compliance: Yes

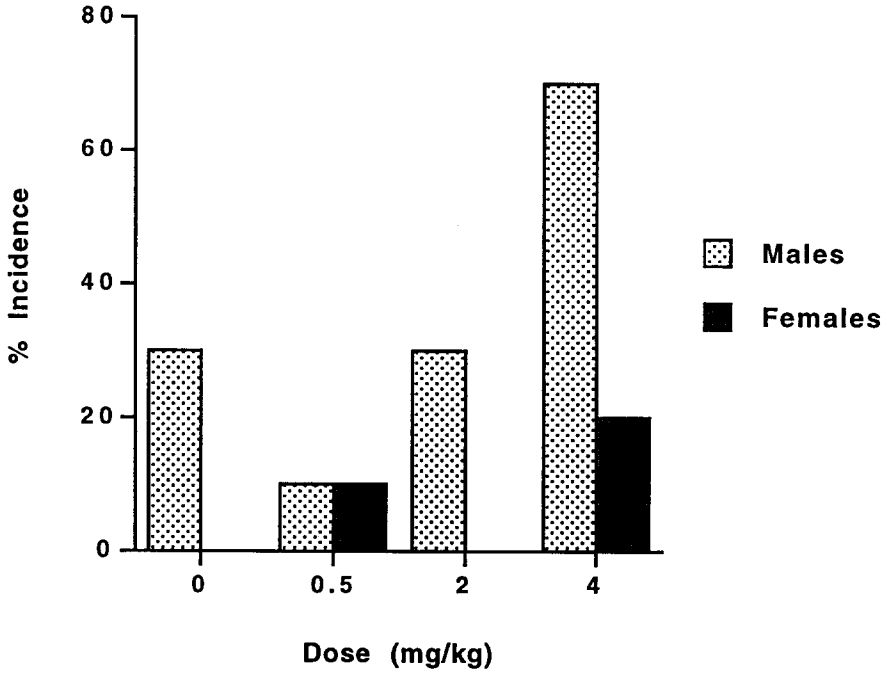
Male and female Sprague-Dawley rats (10/sex/group; 267 gms for males and 187 gms for females) were injected with UK-92,480 (lot no. 953-27) i.v. at 0.5, 2, or 4 mg/kg/day for 28 days. Controls received vehicle (5% mannitol solution). Rats were observed for clinical signs and weighed once per week. An ophthalmological exam was performed before and at the end of treatment. One day after the last dose, blood was taken for hematology and clinical chemistry. Urine was collected for urinalysis. Rats were then sacrificed and a necropsy performed which included a gross exam. Weights of several organs were taken. Microscopic exam of 34 tissues was performed.

No deaths were reported. The only noticeable finding was a chronic inflammation in the myocardium (left and right ventricles). Although this lesion was found in controls, the incidence

in the high-dose males was about twice as high as that found in controls (Figure 8). The significance of these findings were not clear to the sponsor, and cannot be explained by the known pharmacological properties of the drug.

Figure 8

Percent Incidence of Myocardial Chronic Inflammation in Rats Treated with UK-92,480



2.2.2. Dogs

2.2.2.1. Oral

2.2.2.1.1. Ten day oral range-finding toxicity in dogs (Study No. 90081; Vol. 1.14 pp. 352-432):

Testing Facility: Laboratoires Pfizer; Centre de Recherche; Ambroise Cedex; France
Study Number: 90081
Study Date(s): 5/29/90 to 6/7/90
GLP Compliance: Yes

Male and female Beagle dogs (1 male and 2 females/group; 8.1 and 8.5 kg, respectively) were given UK-92,480 (batch no. 1150/262/B) orally by gavage at 10, 30, or 100 mg/kg/day for 10 days. Controls received vehicle (0.5% methylcellulose and 0.1% Tween 80). Dogs were observed for clinical signs, and body weights were recorded twice a week. ECGs and blood pressure were recorded before treatment and before and 2 hours after treatment of Days 3, 8, or 10. Heart rate was calculated from the ECG data. Twenty-four hours after the last dose, blood

was taken from for hematology and clinical chemistry. Blood was also taken 1, 3, 6, and 24 hours after administration on Days 1 and 9 for plasma drug concentration of unchanged drug and two metabolites. The dogs were sacrificed after the last treatment for a histological examination of lung, heart, liver, and kidneys.

No deaths were reported. Altered clinical signs were observed and consisted of 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.

Systolic blood pressure was decreased in the mid and high dose groups 2 hours after dosing on Days 3 and 8 when compared to baseline values (values before daily treatment) (Figure 9). Heart rates in the mid (30 mg/kg) and high (100 mg/kg) dose groups showed an increase 2 hours after treatment on Days 3 and 8 when compared to baseline values (Figure 10).

Figure 9

Effect of UK-92,480 on Systolic Blood Pressure in Dogs
(Two Hours after Dosing)

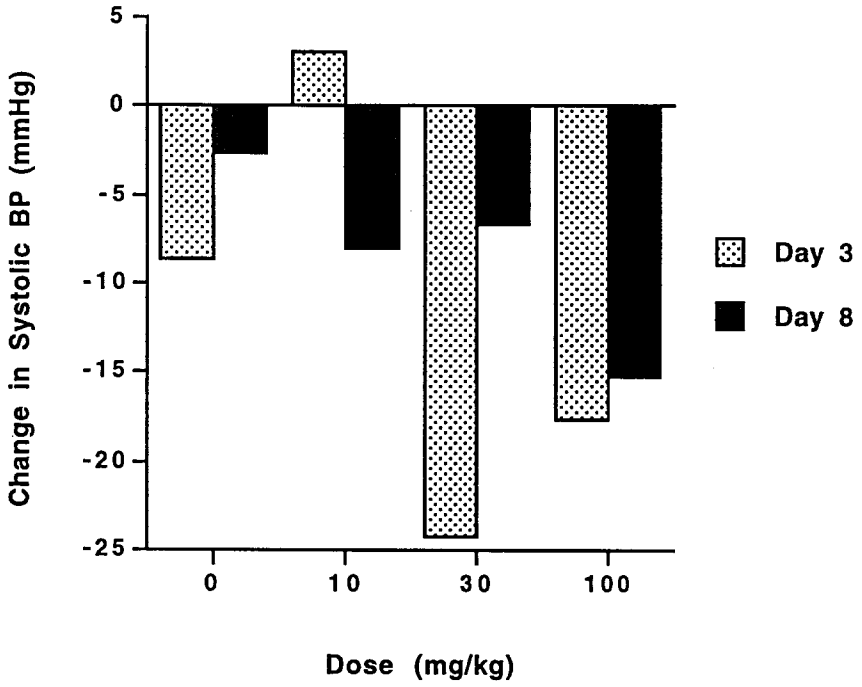
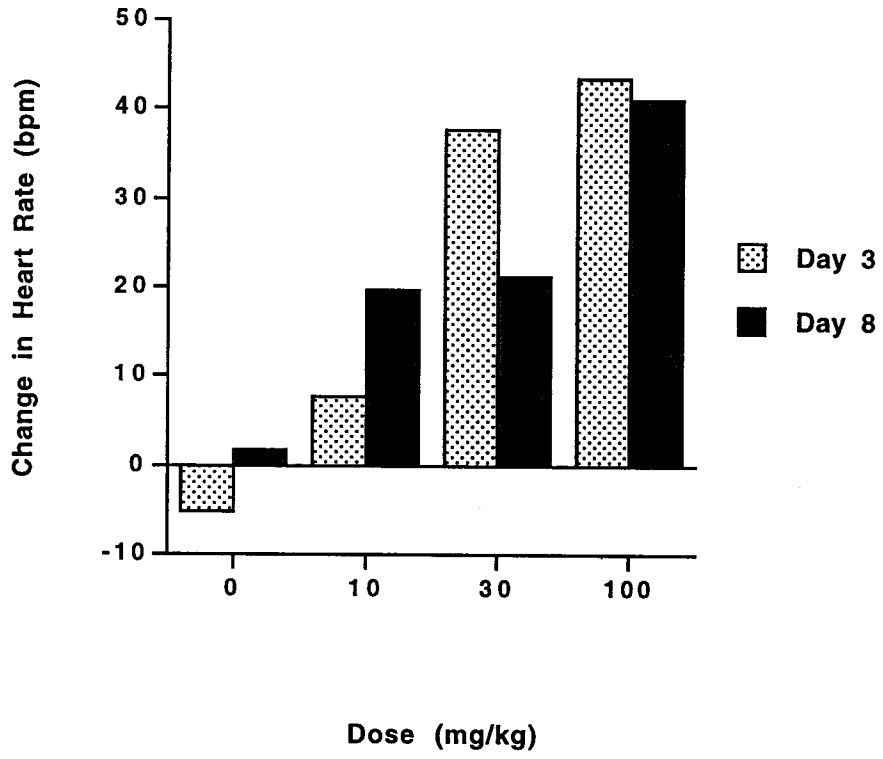


Figure 10

Effect of UK-92,480 on Heart Rate in Dogs
(Two Hours after Dosing)



ECG data two hours after treatment of Days 3 and 8 showed a trend to decreased PQ and QT intervals at the high dose (100 mg/kg) when compared to pre-dose values on Day -1 (Table 8). These effects were may have been related to the increased heart rates observed which, in turn, may have been in response to the decrease in systolic blood pressure.

Table 8

Effect of UK-92,480 on Increasing PQ and QT Intervals (msec) in Dogs

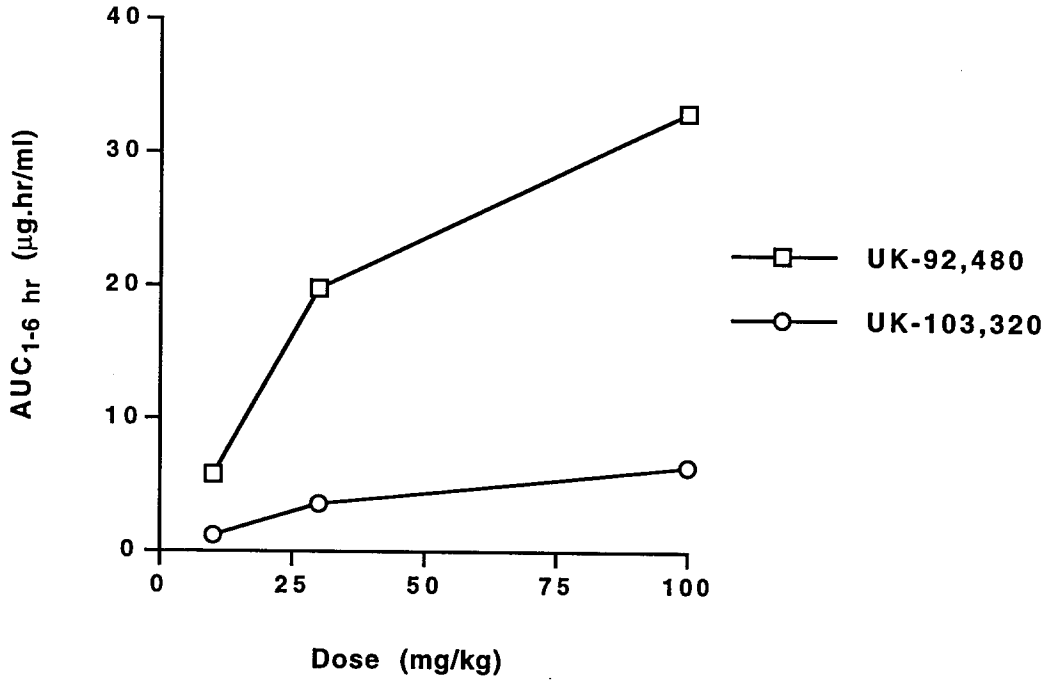
	PQ Interval (msec)			QT Interval (msec)		
Dose (mg/kg)	Day -1	Day 3	Day 8	Day -1	Day 3	Day 8
0	93	93	89	186	205	190
10	104	108	105	202	193	197
30	102	107	103	195	190	195
100	110	**96**	**87**	197	**177**	**179**

Two dogs in the high dose group showed a 45% and 65% increase in plasma cholesterol when compared to pre-dose values. Relative liver weights were slightly increased 30, 28, and 27% in the 10, 30, and 100 mg/kg groups, respectively when compared to the single control dog, and may reflect individual variation. 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.

Plasma drug concentrations of UK-92,480 were slightly higher on Day 9 than on Day 1. There were no differences between males and females. Maximal concentrations were measured after 1-3 hours. Concentrations of the major metabolite, UK-103,320, were lower than unchanged drug, and values on Day 9 were similar to values on Day 1. $AUC_{1\text{-}6\ hr}$ values on Day 9 for UK-92,420 and UK-103,320 are shown in Figure 11. Values showed a dose-dependent increase, but was not linear between the mid and high doses. This may reflect decreased absorption at the high dose.

Figure 11

Mean $AUC_{1\text{-}6\ hr}$ Values (µg.hr/ml)
of UK-92,480 and UK-103,320 in Dogs (Day 9)



2.2.2.1.2. One-month Oral Toxicity in Dogs (Study # 90125; Vol. 1.15 pp. 764-964):

Dose Levels: LD-5, MD-20, and HD-80 mg/kg/day

Groups of 3 male and 3 female Beagles received UK-92,480 by gavage at 0 (vehicle) and doses of 5, 20 and 80 mg/kg for 1 month. The animals were observed daily for clinical signs and weighed regularly. Electrocardiograms, blood pressure and heart rate were recorded before and during the treatment period. Blood was sampled for hematology and clinical chemistry before the start of the study, on day 15 and about 24 hours after the last dose. Plasma drug concentrations of unchanged drug and two metabolites were measured 1, 3, 6 and 24 hours after dosing for 21 days.

Results:

Measurements of plasma concentrations of drug and metabolites showed that N-demethylation of the piperazine groups is a significant route of UK-92,480 biotransformation, and that there was no detectable saturation of this metabolite pathway over the dose range used.

A moderate decrease in blood pressure at the high dose and increases in heart rate at the mid and high doses were noted. A mild coronary arteriopathy was seen in one high-dose animal but was not considered to have any significance to man. At MD and HD, the drug induced a low incidence of emesis and transient salivation. A moderate incidence of soft and liquid feces was noted at all doses. A moderate increase in plasma cholesterol was seen at HD.

2.2.2.1.3. Six-month Oral Toxicity in Dogs (Study # 91099; Vol. 1.17 pp. 1709-1981):

Dose Levels: LD-3, MD-15 and HD-50 mg/kg/day

Groups of 4 male and 4 female Beagle dogs received UK-92,480-10 in capsules at doses of 3 and 15 mg/kg for 6 months. The HD group of 4 animals/sex received 80 mg/kg for the first 5 days; the treatment was interrupted for 2 days, then the animals received 20 mg/kg for 4 days, 40 mg/kg for the 2 following days and 50 mg/kg afterwards. Four animals per sex were kept as control and received placebo capsules. All dogs were observed daily for clinical signs and weighed regularly. Electrocardiograms, blood pressure and heart rate were recorded before and during the treatment period. Blood was sampled for hematology and clinical chemistry before the start of the study after about 2, 4 and 6 months of treatment. Urine was collected for clinical chemistry before and at the end of the treatment period. Plasma drug concentrations of unchanged drug and a metabolite were measured 1, 3, 6 and 24 hours after dosing for 168 days. About 24 hours after the last dose, the animals were sacrificed and submitted to pathological examinations.

Results:

Analyses of plasma drug and metabolite indicate that N-demethylation of the piperazine groups is a significant route of UK-92,480 metabolism in the dog and that no saturation of this process occurs when the dose increases up to 50 mg/kg.

Emesis, resistance to compound administration and salivation were seen when the animals were treated with an initial high dose of 80 mg/kg and were related to gastric intolerance. The incidence and frequency of the signs were much lower after 50, 15 and 3 mg/kg.

A moderate increase in HR and subsequent decrease in PQ and QT intervals were reported at HD; mean QT interval decreased at MD and HD (~-12% on weeks 15 and 23, respectively.); these effects were considered related to the vasodilatory properties of the drug.

Hematologic changes followed no trend; changes noted were not considered by sponsor to be drug related. Plasma chemistry showed variable changes; except for increases in plasma cholesterol and globulin, other changes consisted of increases and decreases in alkaline phosphatase and albumin.

Compared to controls, both HD male/females showed increases (~ 25%) in relative liver weights, and HD females showed a 23% increase in absolute liver weight.

Increases were seen in the absolute/relative weights of adrenal glands of males and females, while the LD females showed a decrease in relative weights.

A HD male showed a number of clinical signs and changes in hematological parameters and plasma chemistry associated with a disseminated arteritis. Sponsor asserts that these changes correspond to a syndrome of polyarteritis which occurs sporadically in Beagle dogs. Microscopic findings reported included disseminated necrotizing panarteritis in thymus, mediastinal lymph nodes, thyroid, epididymides, optic meninges, etc, in one HD male; other changes included unilateral testicular infarction in this dog.

Other changes reported included thymic atrophy in control (2 of 8) and drug treated dogs (at LD-2/8; MD- 4/8 and HD-4/8). Two HD males showed qualitatively similar arteritis in the thymus which drug sponsor considers to be an expression of a latent spontaneous arteritis "precipitated by the treatment but not caused by it."

According to sponsor, intimal proliferation in cardiac blood vessels reported was graded and there were no differences in the severity of this histopathologic change between control and treated dogs.

The results of the current study showed that the doses of 50 and 15 mg/kg induced some changes in biological parameters but no direct toxic effects. No compound-related adverse effects were seen after administration of 3 mg/kg.

PLASMA DRUG CONCENTRATION:

UK-92,480 is demethylated in dogs to UK-103,320 which is pharmacologically equipotent to the parent drug.

Plasma levels UK-92,480 were similar in dogs regardless of sex with maximal individual values observed at 1 or 3 hrs; by 24 hrs mean concentrations had declined by at least 90% at the HD and MD relative to 6-hr values and below detection limits at the LD.

Range of maximal concentrations and mean AUC values on day 168
(males and females)

Dose (mg/kg)	Maximal Concentrations (µg/ml)	AUC 1-6h (µg.h/ml)
3		0.91
15		5.23
50		27.9

Plasma levels of the metabolite UK-103,320 were also similar in dogs regardless of sex and lower than parent compound. Peak concentrations were observed at about 3-6 hrs and were still detected at 24 hrs at MD and HD. Mean AUC values increased with dose level as did the unchanged drug.

Range of maximal UK-103,320 concentrations and mean AUC values

Dose (mg UK-92,480/kg)	Maximal Concentrations (µg/ml)	AUC 1-6h (µg.h/ml)	Ratio of AUC's metabolite:drug
3		0.21	0.23
15		0.90	0.17
50		4.08	0.15

2.2.2.1.4. Twelve-month oral toxicity study in Beagle dogs (Study No. 95039; Vol. 1.18 pp. 1982-2524):

Testing Facility: Pfizer, Centre de Recherche, Amboise Cedex, France
Study Number: 95039
Study Date(s): 5/18/95 to 5/14/96
GLP Compliance: Yes

Male and female Beagle dogs (4/sex/group; 11.2 kg for males and 9.2 kg for females) were given UK-92,480-10 (batch numbers R109, R112, and R202) orally (in capsules) at doses of 3, 10, or 50 mg/kg/day for 363-363 days. Controls received capsules containing placebo.

Dogs were observed for clinical signs and food intake was measured. Motor function was assessed on Days 301 and 362. Body weights were recorded weekly. Blood pressure and ECG recordings were performed before the start and on Days 12-14, 194-197 and 342-345 of the study, before and about 2 hours after treatment. An ophthalmological examination was performed before the start of the study, after 2 weeks of treatment and at the end of the study. Plasma drug concentrations were measured on Day 334, at 1, 3, 6, and 24 hours after dosing. Hematology and plasma clinical chemistry investigations were performed on 3 occasions before the start of the treatment period and on Days 91, 187, 278 and 363 of the study for standard parameters. Plasma myosin and creatine kinase were measured every other week from Day 307 to the end of the study. In addition, blood was sampled for hematology and plasma chemistry from high-dose animals M32 on Day 209 and M33 on Day 117. Urinalysis was performed before the start of the treatment, on Day 183 and at the end of the study.

On Days 363-364, dogs were sacrificed, necropsied, and nine organs were weighed. A histopathological examination was carried out on 33 tissues.

No deaths were reported. Clinical signs in 2/4 high-dose males were noted. These included: pain, arched back, hyperthermia, increased salivation, and tremor. Also, redness of the conjunctiva in these high-dose males was thought to be due to the vasodilatory properties of the drug. There were 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 (Table 9). The increased heart rates may have been a compensatory response to the vasodilatory effects of the drug.

Table 9

Percent Changes in Mean Heart Rates Two Hours after Treatment
Compared to Predose Values

Group	Day 12	Day 194	Day 342
Control	-4	-4	-4
3 mg/kg	+2	-4	0
10 mg/kg	+5	+13*	+10*
50 mg/kg	+27***	+32***	+42***

(* = P<0.05; *** = P<0.001)

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 (Table 10). 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.

Table 10

Percent Changes in Mean PQ and QT Intervals and P Amplitude
Two Hours after Treatment Compared to Predose Values

	Day 12	Day 194	Day 342
PQ Interval	-10**	-9*	-12***
QT Interval	-8**	-7**	-17***
P Amplitude	+8	+20*	+31*

(* = P<0.05; ** = P<0.01; *** = P<0.001)

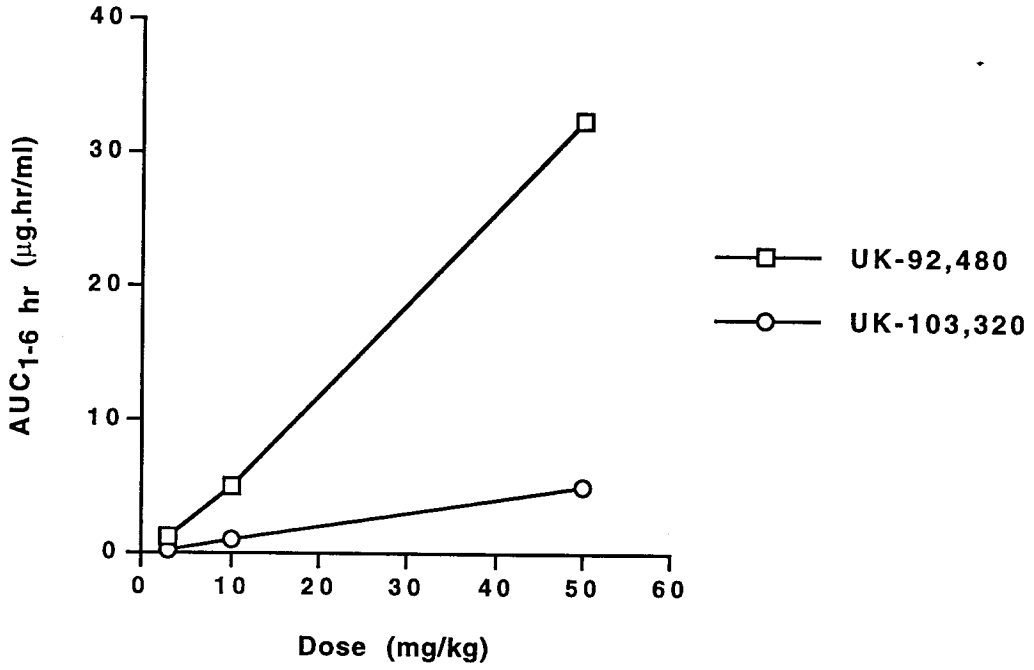
Except for dark kidneys in one high-dose male, there were no significant drug-related changes noted on organ weights or on macroscopic exam (necropsy).

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. Other microscopic findings included accumulation of hemosiderin in the liver of one high-dose male.

Pharmacokinetics of UK-92-480 and the pharmacologically active metabolite UK-103,320 are shown in Figure 12. Since there were no differences between male and female dogs, values are combined. C_{max} was 1-3 hours after dosing. Results showed that AUC values were dose-proportional. Also, the proportion of parent drug to metabolite showed little variation with increasing dose, suggesting that the metabolic pathway was not saturated.

Figure 12

Mean $AUC_{1\text{-}6\ hr}$ (μg.hr/ml) in Dogs
(Males and Females Combined; Day 334)



The major toxicological finding of this 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 (Study No. 91099). 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. It was concluded that the NOAEL for this study in dogs was 10 mg/kg/day.

The occurrence of periarteritis in high-dose male dogs given 50 mg/kg/day may be a cause for concern in human patients because of the difficulty associated with detecting systemic changes due to a focal inflammation. To determine the relative systemic exposures between the maximum recommended daily dose in man (100 mg = 1.4 mg/kg), the NOAEL dose (10 mg/kg/day) in dogs, and the dose that produced arteritis in dogs (50 mg/kg/day), AUCs were determined and are shown in Table 11 (values represent total drug, bound and unbound).

Table 11

Comparative Total AUCs (Total Bound and Unbound) for UK-92,480 and UK-103,320 Between Male Humans and Beagle Dogs (Males and Females Combined)

Species	Dose	UK-92,480 AUC (μg·hr/ml)	UK-103,320 AUC (μg·hr/ml)
Man	100 mg/70 kg	1.686	0.801
Dog	10 mg/kg/day	4.97	1.01
	50 mg/kg/day	32.45	4.96

Since pharmacologic activity for sildenafil (UK-92,480) and its active metabolite (UK-103,320) is represented by the unbound fraction, the percentage of plasma protein binding for both human and dog is shown in Table 12.

Table 12

Human and Dog Plasma Protein Binding

	UK-92,480		UK-103,320	
Species	**% Bound**	**Fraction Unbound**	**% Bound**	**Fraction Unbound**
Man	96	0.04	95	0.05
Dog	86	0.14	86	0.14

Comparison of the dog AUCs for total drug exposure (sum of unbound UK-92,480 and UK-103,320 AUCs) as a multiple of the maximum recommended human dose (MRHD) of 100 mg is shown in Table . The unbound AUCs were calculated by multiplying the total bound and unbound AUC (Table 11) by the fraction unbound (Table 12). As shown in Table 13, 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. This value represents a relatively large safety margin with respect to the possible development of drug-induced arteritis in man. 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).

Table 13

Dog Multiple of MRHD as a Function of Total Drug Exposure (Sum of Unbound AUCs of UK-92,480 and UK-103,320)

Species	Dose (mg/kg)	Unbound UK-92,480 AUC (μg·hr/ml)	Unbound UK-103,320 AUC (μg·hr/ml)	Total of Unbound AUCs (μg·hr/ml)	Multiple of MRHD
Man	1.4	0.067	0.040	0.107	--
Dog	10	0.696	0.141	0.837	**7.8X**
	50	4.543	0.694	5.237	**48.9X**

2.2.2.2. Intravenous

2.2.2.2.1. Fourteen day intravenous range-finding toxicity in Beagle dogs (Study No. 90142; Vol. 1.15 pp. 526-596):

Testing Facility: Laboratoires Pfizer; Centre de Recherche; Ambroise Cedex; France
Study Number: 90142
Study Date(s): 10/24/90 to 11/6/90
GLP Compliance: Yes

Male and female Beagle dogs (2 males and 1 female/group; 9.5 kg for males and 8.7 kg for females) were given UK-92,480 (batch no. R1) i.v. at 2.5, 5, or 10 mg/kg. Controls received vehicle (dextrose solution pH 4). Dogs were observed for clinical signs are weighed twice a week. ECGs, blood pressure, and heart rates were measured before treatment and during the study. Blood samples were taken for hematology and clinical chemistry before the study and 24 hours after the last dose. Animals were then sacrificed and a necropsy performed which consisted of organ (heart, kidneys, liver, and lung) weights, gross exam, and microscopic exam of the same five organs.

No deaths were reported. Liquid feces occurred in treated dogs only. The amplitude of the pupillary reflex was diminished in the mid and high doses. There were no effects on blood pressure. Heart rates increased 15-65 bpm in the 10 mg/kg group 2 hours after drug administration. QT intervals were decreased in both the high dose males two hours after drug treatment. A mild leukocytosis and neutrophilia were observed in one animal in each of the mid and high dose groups. Plasma cholesterol increased 50% in two high dose dogs.

Liver weights increased 30% in two dogs from the high dose groups. An arteritis of the coronary artery was observed in one female in the low dose group.

It was concluded that UK-92,480 when given to dogs i.v. at doses of 5 and 10 mg/kg for 14 days produced liquid feces, an inhibition of pupillary reflex, increased plasma cholesterol, and an increased heart rate. The increased heart rate was considered a pharmacological response to the drug. The no-effect level was 2.5 mg/kg/day.

2.2.2.2.2. One month intravenous toxicity in dogs (Study No. 91041; Vol. 1.16 pp. 1111-1321):

Testing Facility: Laboratoires Pfizer; Centre de Recherche; Ambroise Cedex; France
Study Number: 91041
Study Date(s): 4/4/91 to 5/2/91
GLP Compliance: Yes

Male and female Beagle dogs (3/sex/group; 9.5 kg for males and 8.5 kg for females) were injected with UK-92,480 (lot no. 953-27) i.v. at 0.5, 4, or 4 mg/kg/day for 28 days. Controls received vehicle (5% mannitol solution). Dogs were observed for clinical signs. Pupil size and pupillary reflex to light was performed before and at the end of treatment. Body weights were recorded weekly. ECGs, systolic blood pressure, and heart rates were recorded periodically before and two hours after dosing. Blood was sampled before treatment and after 13 and 28 days of treatment for clinical chemistry and hematology. Urine was collected before and at the end of treatment for urinalysis. One day after the last dose, dogs were sacrificed and a necropsy performed which consisted of a gross exam, weighing of several organs, and microscopic exam of 35 tissues.

No deaths were reported. Clinical signs consisted of emesis and salivation were unrelated to treatment. No effects were reported on pupil size or pupillary reflex to light. There were no drug-related effects on cardiovascular parameters (ECG, BP, HR). 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.

2.2.2.2.3. Bioequivalence between base and citrate in dogs (Study No. 91058; Vol. 1.19 pp. 2525-2551):

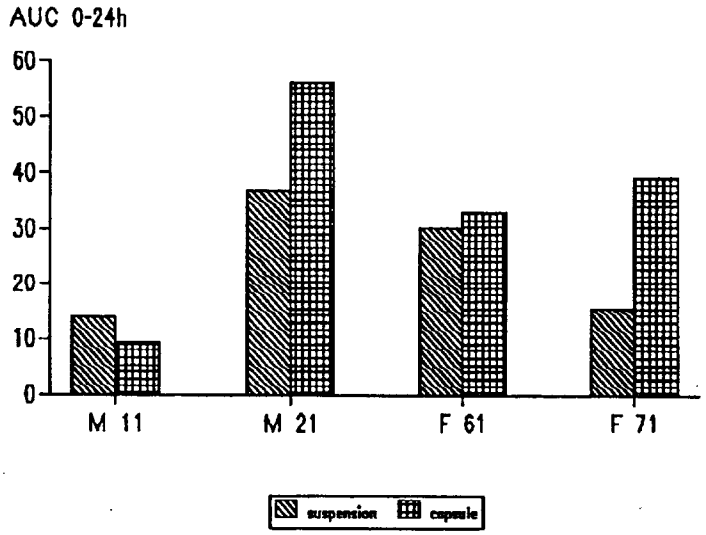
Early toxicity studies were conducted with the free base (UK-92,480) while further toxicity studies and clinical trials were conducted with the citrate salt (UK-92,480-10). This study assessed in dogs the oral bioequivalence of a suspension of the base and of capsules of the citrate salt.

Male and female Beagle dogs (1/sex/group; 10.5 kg for the male and 8.9 kg for the female) were given oral doses of either UK-92,480 (free base as an acidic suspension in a solution of 0.5% methylcellulose with 0.1% Tween 80) or UK-92,480-10 (citrate salt in capsules). Eight days later the treatment groups were reversed so that comparisons could be made within each animal. Each dog received 300 mg (=28.5 to 33.7 mg/kg). Blood was sampled multiples times for up to 24 hours. Plasma levels were determined by HPLC for the parent (UK-92,480) and for two metabolites (UK-95,340 and UK-103,320). UK-103,320 is pharmacologically equipotent to the parent.

One male (M11) vomited shortly after administration on days 1 and 8. Its plasma levels were, therefore, lower. In the other dogs, the AUC levels for the citrate capsule were either the same or higher than the free base suspension (Figure 13). The AUC levels for UK-103,320 were 19-32% of those of the unchanged (parent) drug. The range of AUCs (µg.hr/ml) for the active metabolite UK-103,320 were 5.03-7.71 for the base and 8.72-10.47 for the citrate. AUC levels for the other metabolite UK-95,340 were below the limit of detection (<0.05 µg/ml).

Figure 13

Comparative AUCs (µg.hr/ml) between Base in Suspension and Citrate Capsules in Dogs



It was concluded that the oral bioavailability in dogs of the citrate salt capsules, which was the form used clinically, was equal to or better than that of base in acidic suspension.

2.2.3. Mice

2.3.3.1. Three-month oral (gavage) prechronic toxicity study in CD1 mice (Study No. 94049; Vol. 1.30 pp. 7328-7657):

(CD-1 [Crl:COBS-VAF-CD1(ICR)BR(France)] (GLP Study No. 94049 conducted in Pfizer Centre de Recherche, Amboise, France)

The purpose of this study was to assess the oral toxicity of UK-92,480-10 when given to mice at the doses ranging from 10 up to 200 mg/kg/day, and to select the oral doses for a 24-month toxicity/carcinogenicity study in the same species.
It most be noted that the mouse carcinogenicity study had been started by the time this 3-mo study was submitted for review. Drug sponsor did not submit a protocol or solicit comments from FDA prior to starting the carcinogenicity study.

Material and methods for this 3-mo study are described in the study report. Briefly, the toxicity study consisted of 5 treatment groups of 10 mice/sex/group at 0, 10, 50, 100 and 200 UK-92,480-10 mg/kg/day, and for concurrent toxicokinetics, an additional 3/animals/sex/treatment groups were used.
For both studies, the drug was suspended in a 0.5% sol. of methylcellulose containing 0.1% Tween 80. Controls were treated with the vehicle.

During the study, mice were observed daily for signs of toxicity and clinical signs, body weight, hematology, moribundity, clinical chemistry, drug/metabolite plasma concentrations, and mortality. Plasma drug or metabolite levels were determined in the toxicokinetics groups.

Necropsy was performed on all animals in the main study. A number of organs were weighed and histopathological examinations were carried out on a range of tissues from mice found dead, sacrificed moribund or at scheduled sacrifice in the main study groups.

RESULTS

In this study, a total of 14 mice died or were sacrificed moribund: Control 1 F; LD- 1 F; MD-1 3 M; MD-2 3 M and 1 F, and at HD- 3 M and 2 F. The minimum lethal dose (MLD) of UK-92,480-10 in this study was 50 mg/kg/day; cause of death was gastrointestinal (g.i.) dilatation. Other animals died due to gavage accidents, and 1 HD F died of unknown causes according to drug sponsor.