

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**APPLICATION NUMBER 020895**

**CARCINOGENICITY ASSESSMENT COMMITTEE**  
**REPORT**

**AND**

**FDA-CDER RODENT CARCINOGENICITY DATABASE**  
**FACTSHEET**

**CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT  
AND  
FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET**

Thomas Papoian, Ph.D.  
11/12/97

NDA: #20-895  
DRUG CODE#: UK-92,480-10  
CAS#: 171,599-83-0  
DIVISION(s): Cardio-Renal Drug Products (HFD-110)  
DRUG NAME(s): Viagra™ (sildenafil citrate)

SPONSOR: Pfizer  
LABORATORY: Pfizer, Centre de Recherche, 37401 Ambroise Cedex, France  
CARCINOGENICITY STUDY REPORT DATE: 7/11/97 (rat); 7/10/97 (mouse)

THERAPEUTIC CATEGORY: Male erectile dysfunction  
PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: Cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) enzyme inhibitor.

MUTAGENIC/GENOTOXIC: Negative in Ames test, CHO/HGPRT gene mutation test, human lymphocyte clastogenicity, and mouse micronucleus.

**RAT CARCINOGENICITY STUDY:** Single study (#94092)

RAT STUDY DURATION: 104 weeks  
STUDY STARTING DATE: 10/11/94  
STUDY ENDING DATE: 10/10/96  
RAT STRAIN: Sprague-Dawley albino rats, Crl:COBS-VAF-CD(SD)BR  
ROUTE: Orally by esophageal intubation (gavage)  
DOSING COMMENTS: Drug administered at 5 ml/kg body weight

**NUMBER OF RATS:**

- Main study:
  - Control 1 (C1): 60 males and 60 females
  - Control 2 (C2): 60 males and 60 females
  - Low Dose (LD): 60 males and 60 females
  - Middle Dose (MD): 60 males and 60 females
  - High Dose (HD): 60 males and 60 females
  
- Groups for plasma drug level determinations:
  - Low Dose (LD): 7 males and 7 females
  - Middle Dose (MD): 7 males and 7 females
  - High Dose (HD): 7 males and 7 females

**RAT DOSE LEVELS\* (mg/kg/day):**

- Rat Low Dose: 1.5
- Rat Middle Dose: 5.0
- Rat High Dose: 60.0
- \*Dose adjusted during study

**NDA #20-895**

**BASIS FOR DOSES SELECTED:**

- MTD: Selection of high dose (60 mg/kg/day) was based on a rat 6 month repeated dose study in which a decrease in body weight gains of -9% for males and -7% for females was observed. Other effects observed in treated rats, but not used as the basis for the MTD, included: chromodacryorrhea (bloody tears), metabolic changes in the liver (decreased plasma bilirubin and triglycerides, and increased plasma urea, total proteins, and cholesterol), and hypertrophy of the thyroid and adrenal glands.

**PRIOR FDA DOSE CONCURRENCE:** No

**RAT CARCINOGENICITY:** Negative (males and females)

**RAT TUMOR FINDINGS:**

Tumors were analyzed using the Peto's death rate method for fatal tumors and prevalence analysis for incidental tumors (Peto *et al.*, 1980). According to the sponsor, the only statistically significant finding was an increased proliferation in thyroid follicular cells in male rats treated at the high dose of 60 mg/kg/day (combined incidence of hyperplasia, adenoma, and carcinoma;  $P = 0.0056$  for positive trend using the Peto analysis; Table 1). A combined statistical analysis was performed as recommended for a multistage model of carcinogenesis in which thyroid follicular hyperplasia, adenoma, and carcinoma represent a morphological progression from hyperplasia to neoplasia (McConnell *et al.*, 1986). Other proliferative and neoplastic changes in males and females were observed with similar frequencies in the treated and untreated groups.

Table 1

Percent Incidence of Proliferative Changes  
in Thyroid Follicular Cells of Male Rats  
(n = 60)

	Dose (mg/kg/day)				
	C1	C2	1.5	5.0	60.0
Hyperplasia	0	1.7	5.0	1.7	8.3
Adenoma	6.7	0	0	3.3	8.3
Carcinoma	1.7	1.7	0	3.3	0
<b>Combined</b>	<b>8.4</b>	<b>3.4</b>	<b>5.0</b>	<b>8.3</b>	<b>16.6</b>

In a separate study to assess the relationship between liver enzyme induction and thyroxin clearance, female rats were given either vehicle or UK-92,480 orally at 200 mg/kg for 29 days. Results showed that treatment produced an increase in liver and thyroid weights, thyroid follicular cell hypertrophy, increased hepatic UDP-glucuronyl transferase (UDPGT) activity, increased TSH, decreased T3 and T4 hormones, and an increased clearance of exogenous thyroxin. These results were thought to be consistent with the view that the thyroid hypertrophy found in treated rats 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 was not presented, however. Additional experiments assessing induction of genes coding for specific hepatic enzymes, such as UDPGT-specific mRNA levels, would have been able to detect gene induction at the 60 mg/kg dose if such a mechanism were responsible for the thyroid hypertrophy observed in treated rats.

RAT STUDY COMMENTS:

*Mortality:* No drug-related increase in mortality was found (Table 2). Survival in the treated male groups appeared to be higher when compared to the untreated male controls and to all female groups.

Table 2  
Percent Mortality and Percent Survival

	Males			
	Found Dead	Sacrificed as Moribund	Total Unscheduled Deaths	Survival at the End of Study
Control 1+2	56.7	25.0	81.7	18.3
1.5 mg/kg	43.3	15.0	58.3	41.7
5 mg/kg	30.0	35.0	65.0	35.0
60 mg/kg	48.3	21.7	70.0	30.0
	Females			
Control 1+2	34.2	45.0	79.2	20.8
1.5 mg/kg	33.3	41.7	75.0	25.0
5 mg/kg	30.0	48.3	78.3	21.7
60 mg/kg	55.0	30.0	85.0	15.0

*Body Weights:* Mean body weights are shown in Figure 1A (males) and Figure 1B (females). Percent changes in mean body weight gains in male and female rats are shown in Table 3 (Day 1 and Day 723). Results 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.

Figure 1A (Sponsor's Figure 8)

Effect of UK-92,480 on Group Mean Body Weight in Male Rats

Mean Body Weight of Male Groups

Study Number: 94092

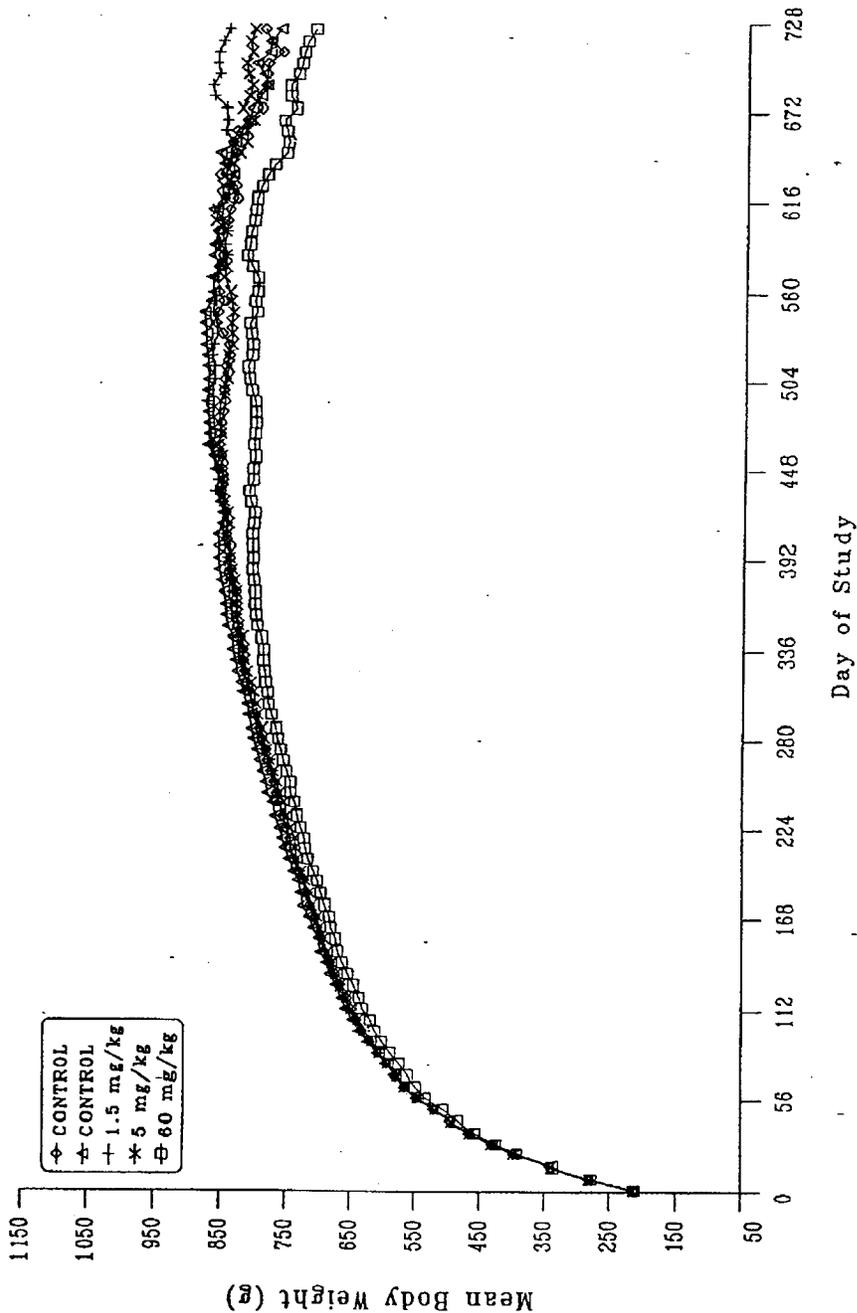


Figure 1B (Sponsor's Figure 9)

Effect of UK-92,480 on Group Mean Body Weight in Female Rats

Mean Body Weight of Female Groups

Study Number: 94092

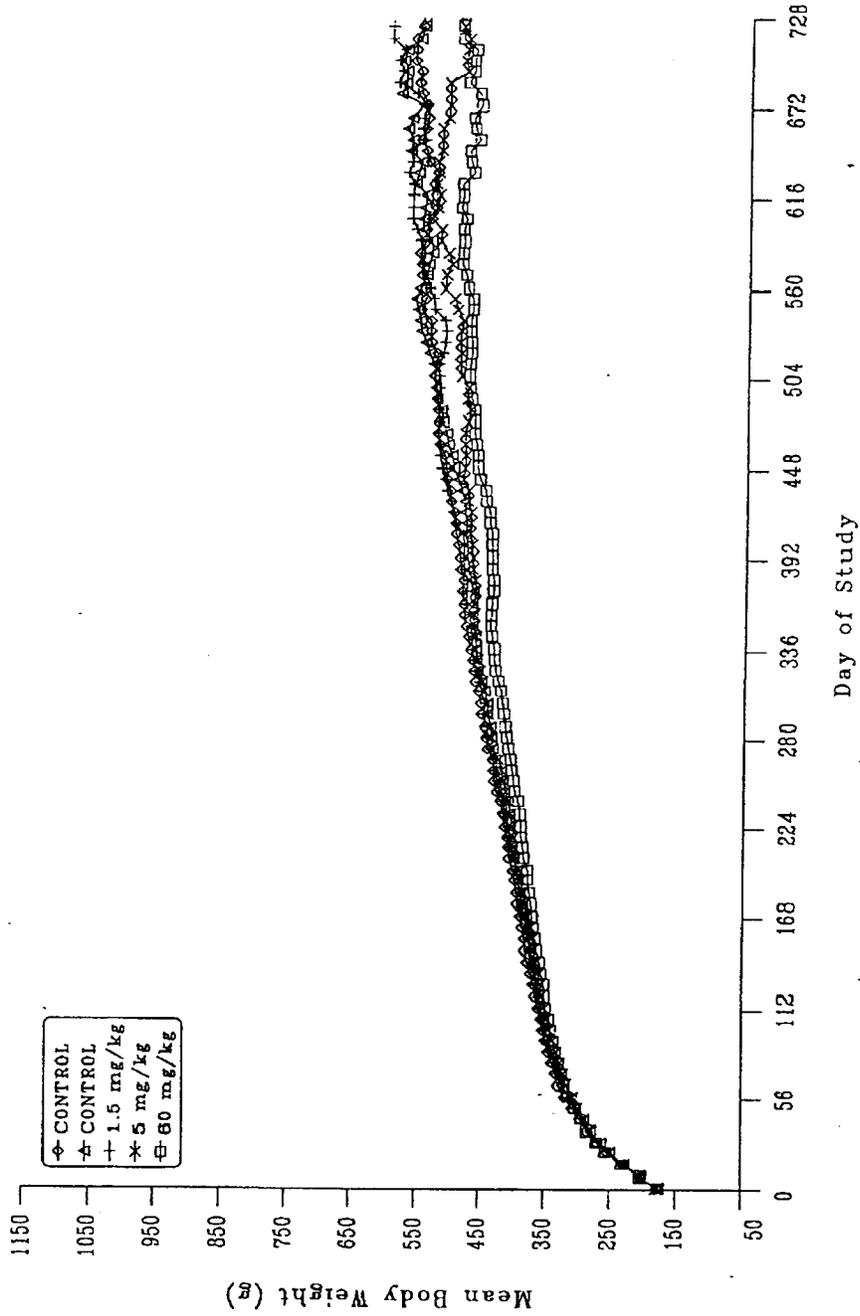


Table 3

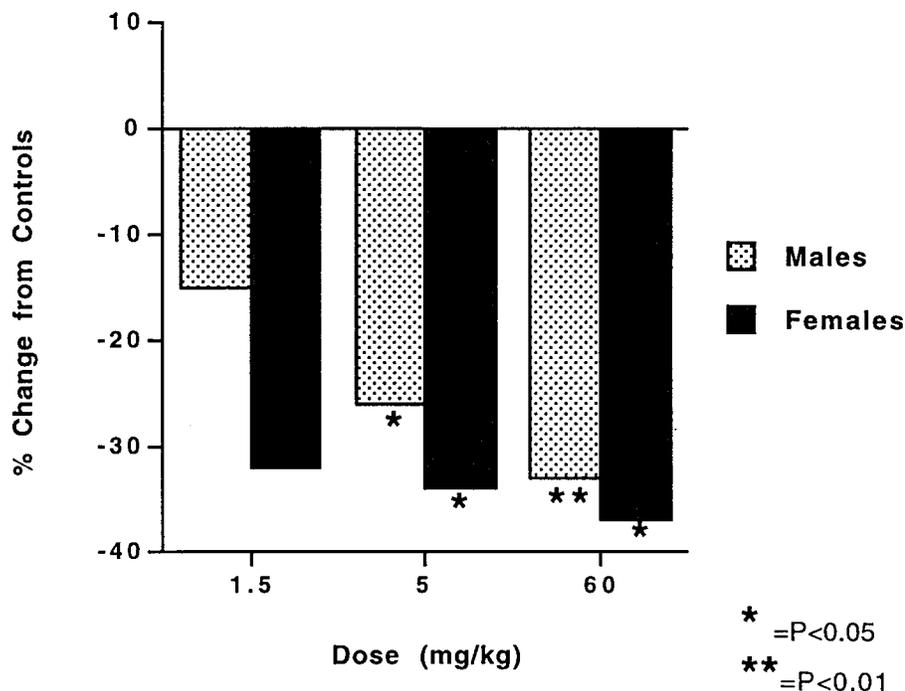
Effect of UK-92,480 on Mean Body Weight Gain in Rats

Sex	Dose (mg/kg/day)	Weight Day 1 (gms)	Weight Day 723 (gms)	Weight Gain (gms)	% Change in Wt. Gain from Controls
M	0	214.6	777.4	562.8	--
	1.5	216.1	845.4	629.3	+11.8
	5	214.7	807.5	592.8	+5.3
	60	213.0	713.8	500.8	-11.0
F	0	176.8	552.2	375.4	--
	1.5	177.4	598.8	421.4	+12.3
	5	178.0	489.5	311.5	-17.0
	60	174.9	491.3	316.4	-15.7

*Non-Neoplastic Pathology:* The only consistent change that was reported was a dose-related decrease in plasma bilirubin in both sexes which was statistically significant ( $P < 0.01$  and  $0.05$ ) at the mid and high doses (Figure 2).

Figure 2

Percent Decrease in Plasma Bilirubin in UK-92,480-Treated Rats



## NDA #20-895

This effect on decreasing plasma bilirubin was thought to be due to the ability of UK-92,480 to increase hepatic uptake and conjugation of bilirubin through increased liver enzyme induction, although there was no evidence of liver enzyme induction, hepatic hypertrophy, or increased liver weight. It was postulated that the mechanism may operate chronically at a low level where liver changes would be undetectable.

*Pharmacokinetics:* UK-92,480 forms two pharmacologically active metabolites, one major and one minor. UK-103,320 is the major pharmacologically active metabolite and has about 50% of the potency of the parent drug. It represents 11% and 3% of the administered dose in rat and man, respectively. A minor pharmacologically active metabolite, UK-150,564, has only about 10% of the potency of the parent drug, and represents 16% and 22% of the administered dose in rat and man, respectively. The terminal elimination half-life was 0.3, 1.9, and 4.0 hours for male rat, female rat, and man, respectively.

Plasma drug levels (AUCs) for UK-92,480 (parent drug) and UK-103,320 (major metabolite) were determined from supplementary rats on Day 366. Mean systemic exposures ( $AUC_{1-8 \text{ hr}}$ ) to UK-92,480 and UK-103,320 are shown in Figure 3A (males) and Figure 3B (females). As can be seen, exposure to UK-92,480 and UK-103,320 was dose-proportional in both sexes. However, males were exposed mostly to the metabolite UK-103,320, whereas females were exposed mostly to the parent drug UK-92,480.

Figure 3A

Mean Exposure ( $AUC_{1-8hr}$ ) to UK-92,480 and UK-103,320 in Male Rats

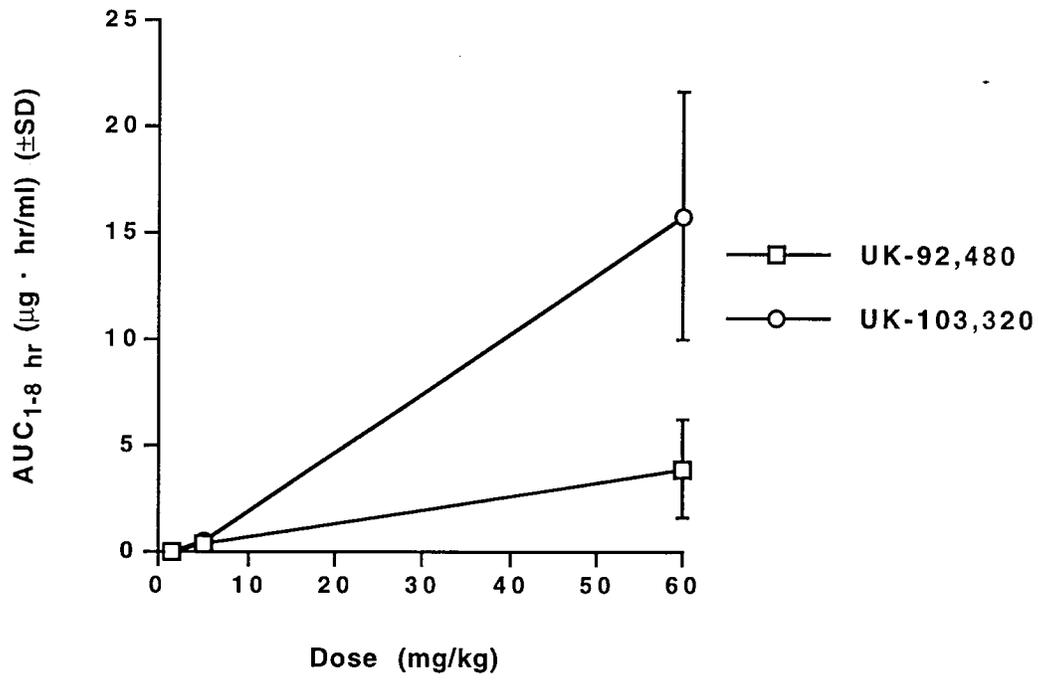


Figure 3B

Mean Exposure ( $AUC_{1-8hr}$ ) to UK-92,480 and UK-103,320 in Female Rats



Comparative AUCs for UK-92,480 and UK-103,320 between normal male human volunteers given the maximum recommended human dose (MRHD) of 100 mg (=1.43 mg/kg based on a 70 kg man) and male and female rats given 60 mg/kg/day are shown in Table 4 (values represent total drug, bound and unbound).

Table 4

Comparative Total AUCs (Total Bound and Unbound) for UK-92,480 and UK-103,320 Between Male Humans and Male and Female Rats

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
Rat (male)	60 mg/kg/day	3.902	15.767
Rat (female)	60 mg/kg/day	32.689	5.554

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 rat is shown in Table 5.

Table 5

## Human and Rat Plasma Protein Binding

Species	UK-92,480		UK-103,320	
	% Bound	Fraction Unbound	% Bound	Fraction Unbound
Man	96	0.04	95	0.05
Rat	95	0.05	89	0.11

Comparison of the male and female rat 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 6. The unbound AUCs were calculated by multiplying the total bound and unbound AUC (Table 4) by the fraction unbound (Table 5). As shown, the total of unbound AUCs in male and female rats given 100 mg/kg/day was 18.0X and 21.0X, respectively the AUC of men given a single dose of 100 mg.

Table 6

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

Species	Unbound UK-92,480 AUC ( $\mu\text{g}\cdot\text{hr}/\text{ml}$ )	Unbound UK-103,320 AUC ( $\mu\text{g}\cdot\text{hr}/\text{ml}$ )	Total of Unbound AUCs ( $\mu\text{g}\cdot\text{hr}/\text{ml}$ )	Multiple of MRHD
Man	0.067	0.040	0.107	--
Rat (male)	0.195	1.734	1.929	18.0X
Rat (female)	1.634	0.611	2.245	21.0X

**Conclusions:** The only statistically significant finding 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 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 18X and 21X the maximum recommended human dose of 100 mg in male and female rats, respectively. Although these values are less than the 25-fold ratio of rodent to human AUC required to qualify as an appropriate endpoint for high dose selection, they do 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.

NDA #20-895

**MOUSE CARCINOGENICITY STUDY:** Single study (#95007)

MOUSE STUDY DURATION: 104 weeks

STUDY STARTING DATE: 1/18/95

STUDY ENDING DATE: 10/28/96

MOUSE STRAIN: CrI: COBS-VAF-CD1 (ICR)BR

ROUTE: Orally by esophageal intubation (gavage)

DOSING COMMENTS: Drug administered at 10 ml/kg body weight

**NUMBER OF MICE:**

- Main study:
  - Control 1 (C1): 55 males and 55 females
  - Control 2 (C2): 55 males and 55 females
  - Low Dose (LD): 55 males and 55 females
  - Middle Dose (MD): 55 males and 55 females
  - High Dose (HD): 55 males and 55 females
  
- Groups for plasma drug level determinations:
  - Low Dose (LD): 5 males and 5 females
  - Middle Dose (MD): 5 males and 5 females
  - High Dose (HD): 5 males and 5 females

**MOUSE DOSE LEVELS\* (mg/kg/day)**

Mouse Low Dose: 3

Mouse Middle Dose: 10

Mouse High Dose: 30

- \*Dose adjusted during study

**BASIS FOR DOSES SELECTED:**

- MTD: 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 (difficulty in breathing) and swollen abdomen. No adverse effects were noted in the 20 mg/kg group after 3 months of treatment.

**PRIOR FDA DOSE CONCURRENCE:** No

**MOUSE CARCINOGENICITY:** Negative (males and females)

**MOUSE TUMOR FINDINGS:**

Tumors were analyzed using the Peto's death rate method for fatal tumors and prevalence analysis for incidental tumors (Peto *et al.*, 1980). Results showed that there were no treatment-related increases in neoplastic lesions.

MOUSE STUDY COMMENTS:

*Mortality:* In contrast to the rat study, treatment in mice produced an increase in mortality in the high-dose males (Figure 4A) and in the mid and high dose females (Figure 4B).

Figure 4A (Sponsor's Figure 1)

Survival Plot in Male Mice

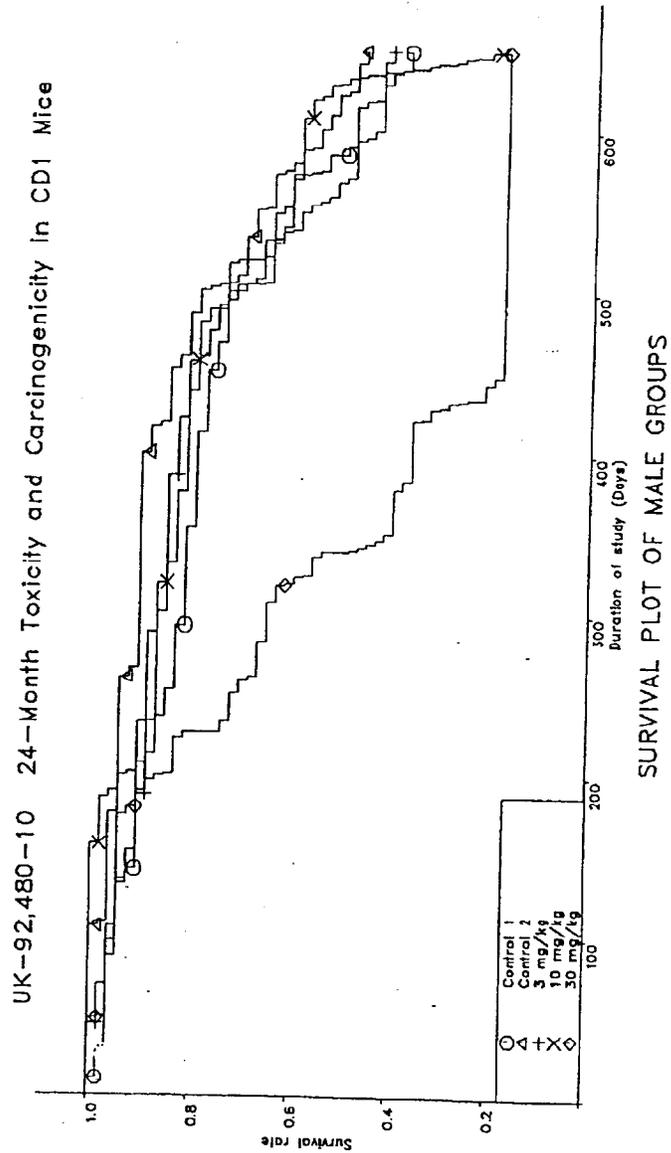
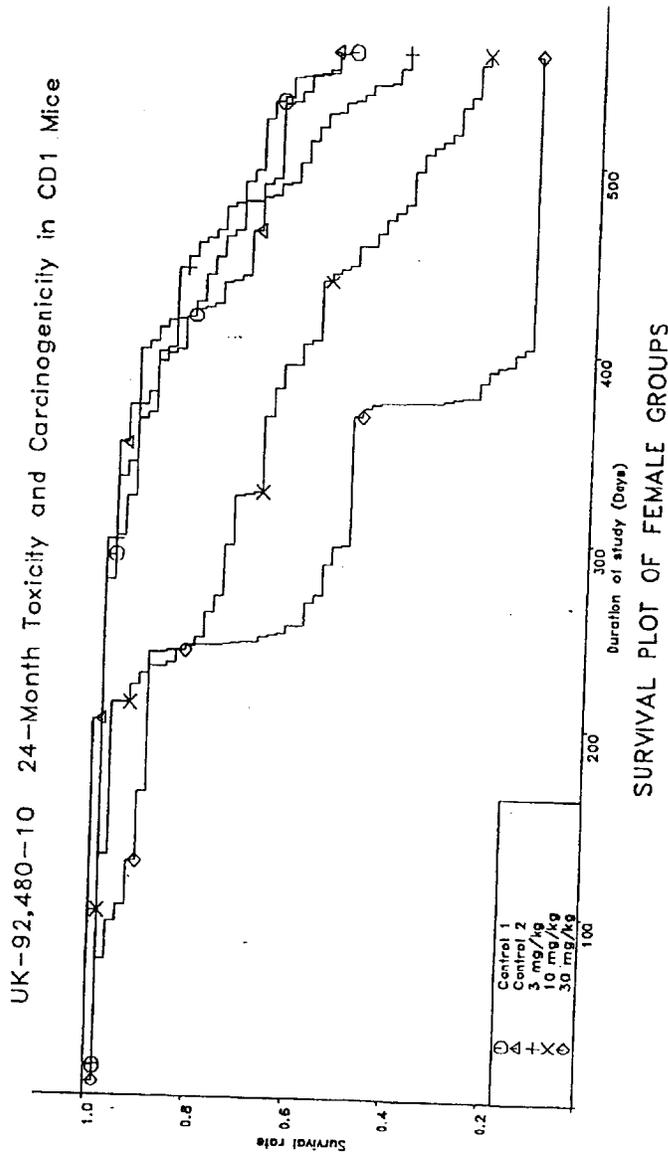


Figure 4B (Sponsor's Figure 2)

Survival Plot in Female Mice



When survival dropped to near 20%, groups of mice were sacrificed. This occurred after 15.1 months (Day 454) in high dose males (18% survival) with the remaining groups (control, low, and mid doses) being sacrificed after 21.7 months (Day 650). The high dose females were sacrificed after 13.5 months (Day 405; 13% survival). When survival in the mid dose females reached 24% after 18.6 months (Day 559), control, low and mid dose female groups were sacrificed. Times of sacrifice and percent survival at sacrifice are summarized in Table 7.

Table 7

Times of Sacrifice and Percent Survival at Sacrifice in Mice

Sex	Dose (mg/kg)	Time of Sacrifice		% Survival at Sac
		Days	Months	
Male	Control 1+2	650	21.7	43
	3	650	21.7	42
	10	650	21.7	22
	30	454	15.1	18
Female	Control 1+2	559	18.6	55
	3	559	18.6	40
	10	559	18.6	24
	30	405	13.5	13

Body Weights: Mean body weights are shown in Figure 5A (males) and Figure 5B (females).

Figure 5A (Sponsor's Figure 3)

Effect of UK-92,480 on Group Mean Body Weight in Male Mice

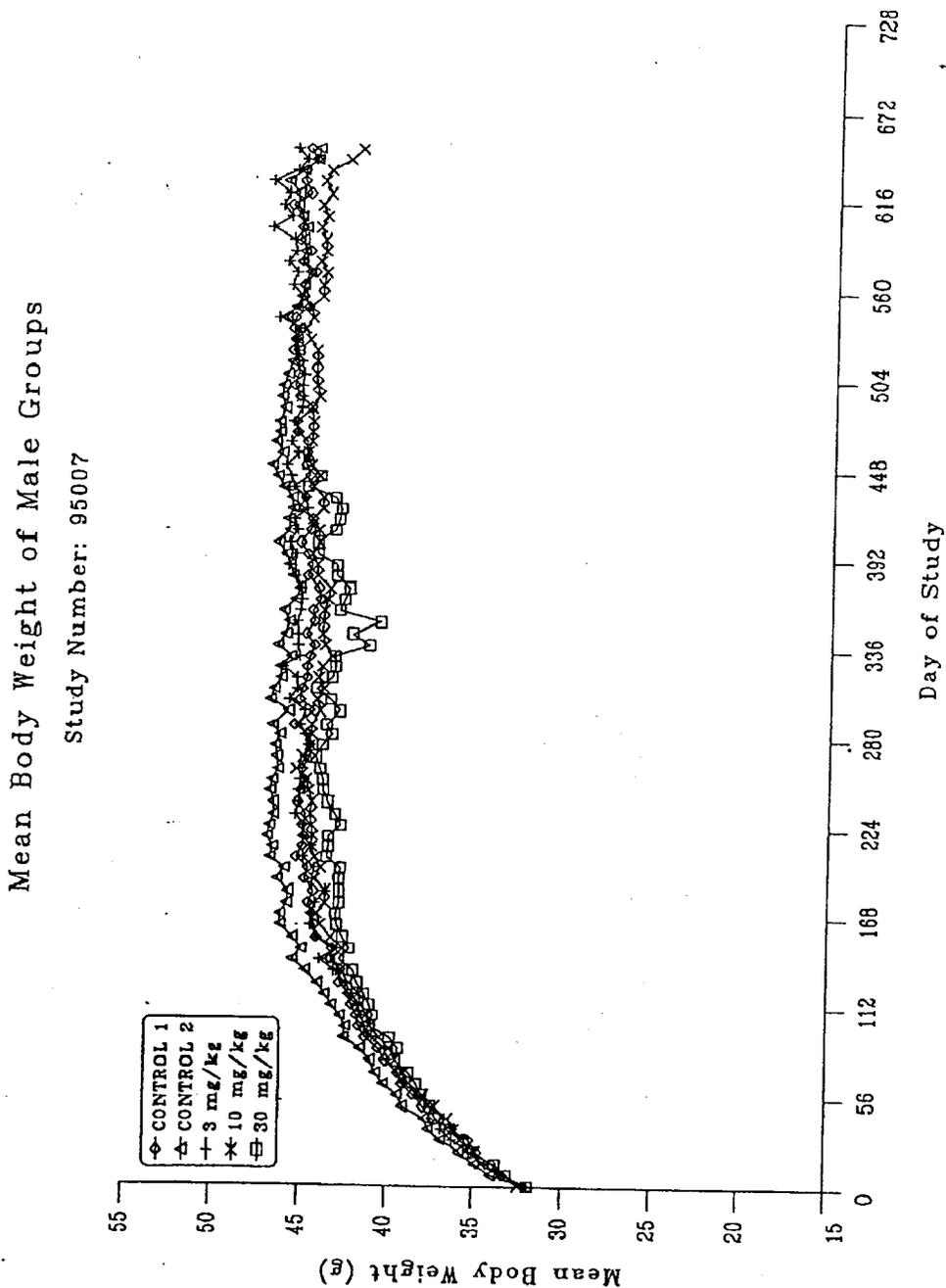
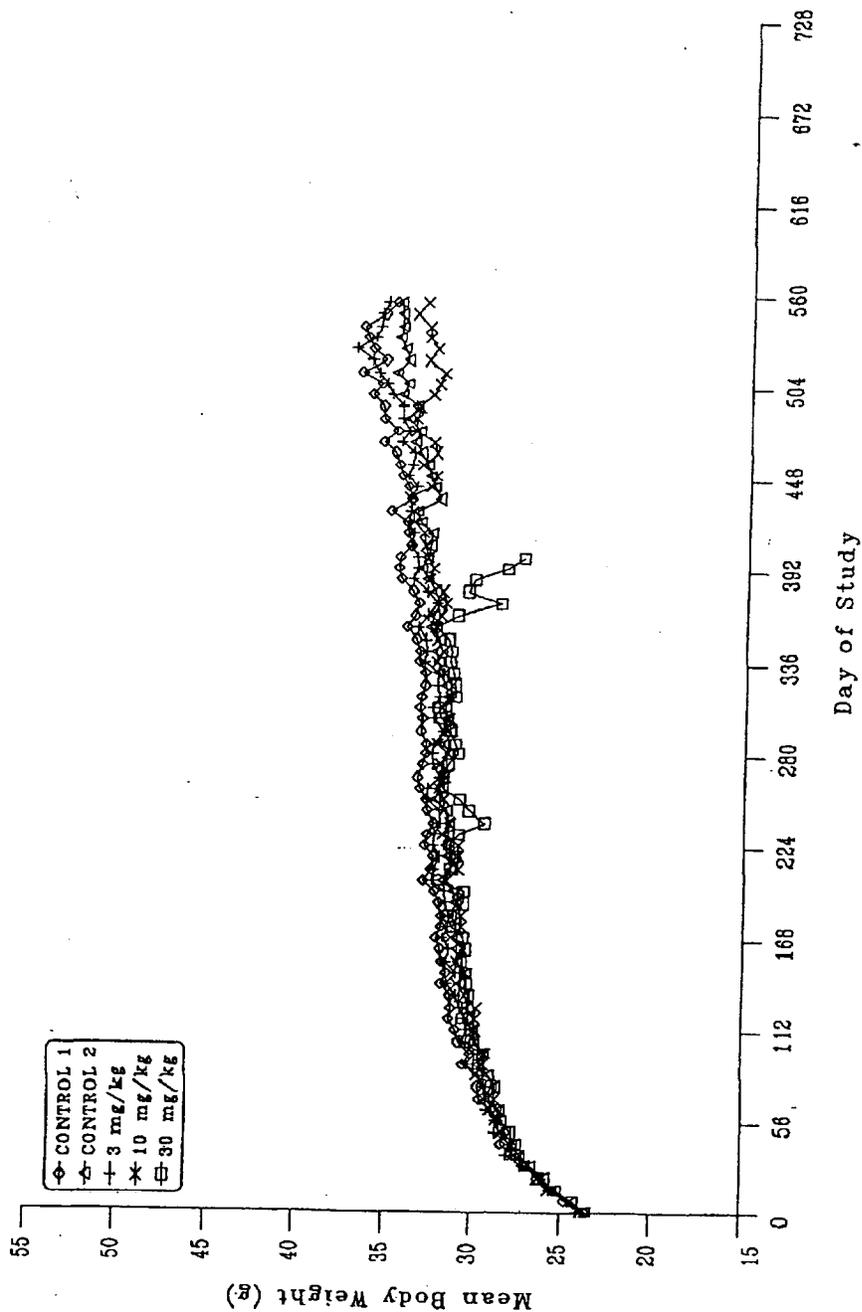


Figure 5B (Sponsor's Figure 4)

Effect of UK-92,480 on Group Mean Body Weight in Female Mice

Mean Body Weight of Female Groups

Study Number: 95007



Percent changes in mean body weight gains in male mice are shown in Table 8A. Weights of treated mice at sacrifice are compared to control mice at the same time point. Results showed that mid dose (10 mg/kg) males gained 24% less weight than controls on sacrifice Day 645, while high dose (30 mg/kg) males gained 6.4% less weight than controls on sacrifice Day 449. Apparently, early death in the high dose males was not associated with significant weight loss as was found in the mid dose males at a later time point.

Table 8A

Effect of UK-92,480 on Mean Body Weight Gain in Male Mice

Dose (mg/kg)	Weight (gms) Day 1	Weight (gms) Day 449	Weight (gms) Day 645	Weight Gain (gms) (D=Day)	% Change in Wt. Gain from Controls
0	32.1	46.2	45.0*	14.1 (D449) 12.9 (D645)	--
3	32.0	--	46.0*	14.0 (D645)	+8.5
10	32.4	--	42.2*	9.8 (D645)	-24.0
30	31.9	45.1*	--	13.2 (D449)	-6.4

(\* = last weight taken)

Percent changes in mean body weight gains in female mice are shown in Table 8B. Weights of treated mice at sacrifice are compared to control mice at the same time point. Results showed that mid dose (10 mg/kg) females gained 17% less weight than controls on sacrifice Day 554, while high dose (30 mg/kg) females gained 60% less weight than controls on sacrifice Day 400.

Table 8B

Effect of UK-92,480 on Mean Body Weight Gain in Female Mice

Dose (mg/kg)	Weight (gms) Day 1	Weight (gms) Day 400	Weight (gms) Day 554	Weight Gain (gms) (D=Day)	% Change in Wt. Gain from Controls
0	23.6	33.7	34.8*	10.1 (D400) 11.2 (D554)	--
3	24.0	--	35.4*	11.4 (D554)	+1.8
10	23.8	--	33.1*	9.3 (D554)	-17.0
30	23.5	27.5*	--	4.0 (D400)	-60.4

(\* = last weight taken)

If the male and female high dose groups are excluded because of early sacrifice (less than 18 months of treatment), criteria for an MTD may still be met using the mid dose groups which showed 24% and 17% reductions in weight gains for males and females, respectively.

*Non-Neoplastic Pathology:* The major pathological finding was gastro-intestinal dilation in treated mice which was the principle drug-related cause of death, particularly in high-dose males (33% incidence; Table 9). The percent incidence in high dose females was 9%. No deaths due to gastro-intestinal dilation were found in controls, indicating that this was a drug-related effect.

Table 9

Incidence of Death in Drug-Treated Mice  
Due to Gastro-Intestinal Dilation

Sex	Dose (mg/kg)	Incidence (%)
<b>Male</b>	0	0/55 (0)
	3	2/55 (4)
	10	1/55 (2)
	30	18/55 (33)
<b>Female</b>	0	0/55 (0)
	3	0/55 (0)
	10	2/55 (4)
	30	5/55 (9)

Additional studies in mice (Study Nos. 96094 and 97028) have shown that UK-92,480, after a single oral administration, slowed intestinal transit which was thought to be due to relaxation of gastrointestinal smooth muscle. Mice appeared to be more sensitive than rats (Figure 6), and the extent of slowed intestinal transit correlated with the incidence of death due to gastrointestinal dilation in both male and female mice (Figures 7A and 7B).

Figure 6

Effect of UK-92,480 on Mean Intestinal Transit in Mice and Rats  
(% Relative to Controls)

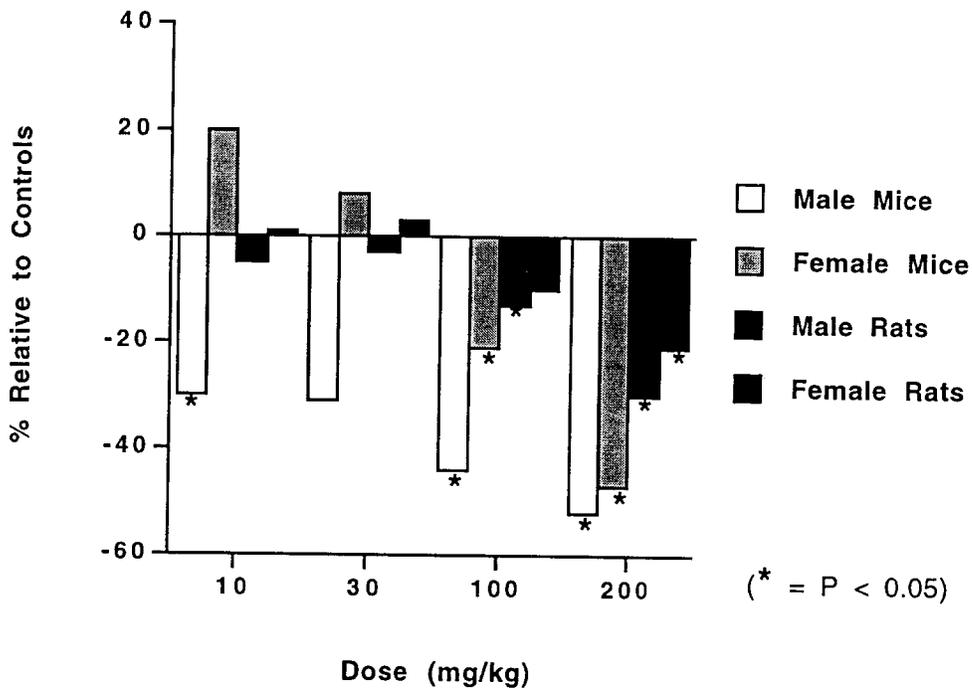


Figure 7A

Correlation Between Reduction in Intestinal Transit  
and Death Due to Gastro-Intestinal Dilation (Male Mice)

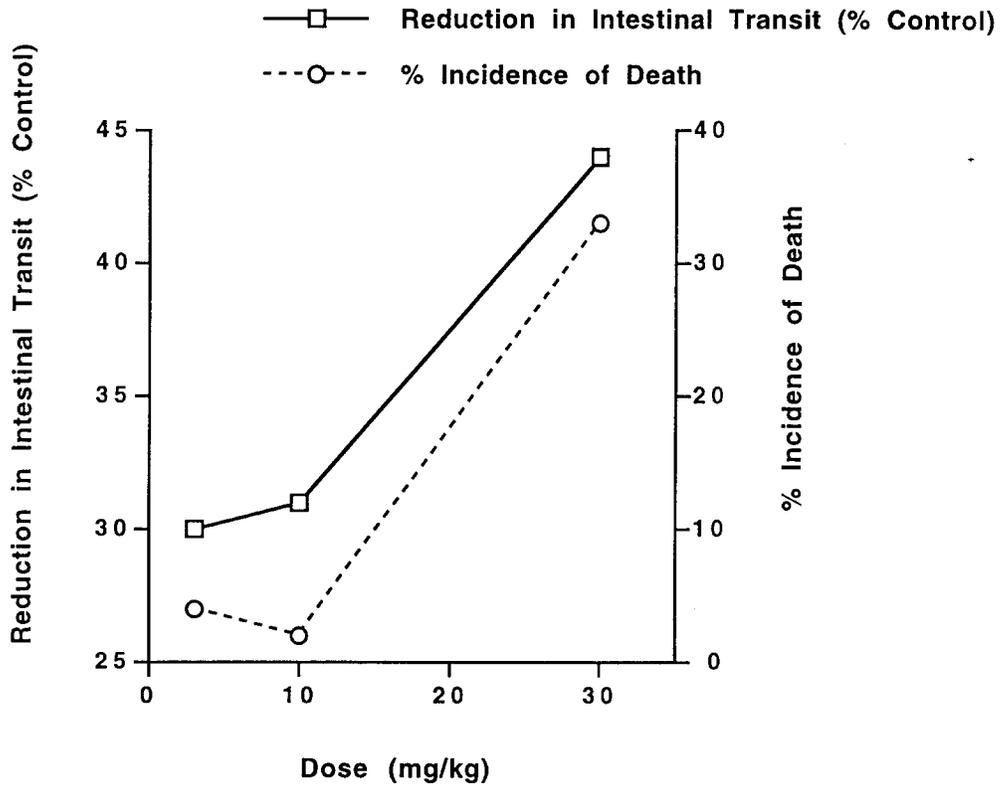
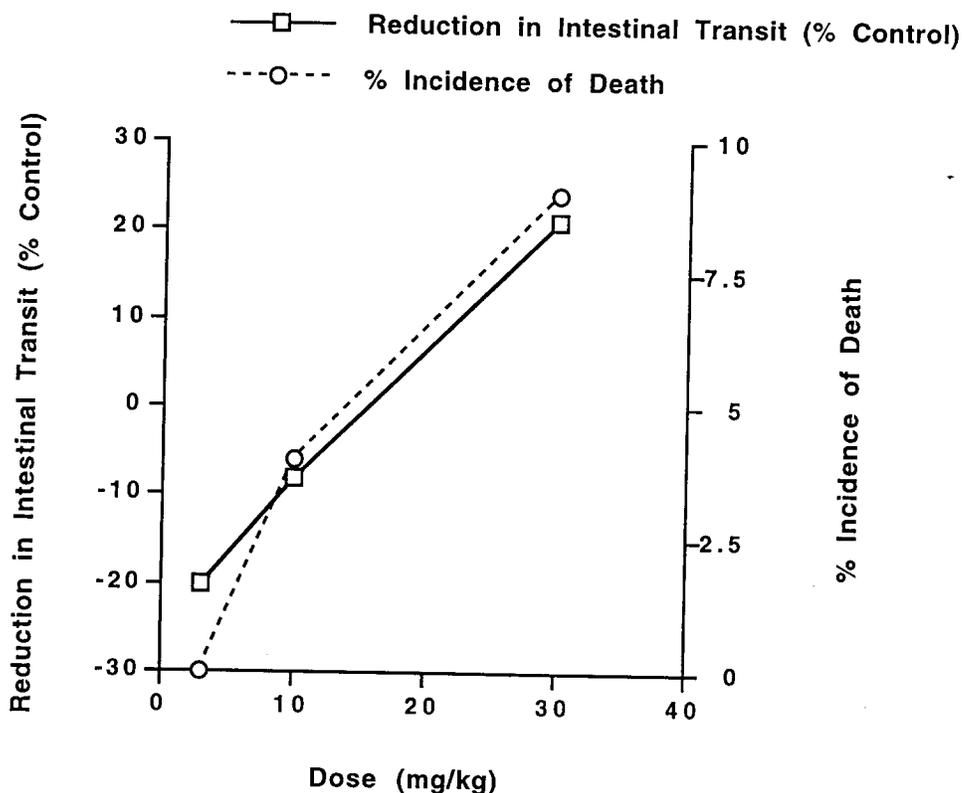


Figure 7B

Correlation Between Reduction in Intestinal Transit  
and Death Due to Gastro-Intestinal Dilatation (Female Mice)



This effect on slowing intestinal transit was considered to be consistent with the drug's pharmacologic properties, since other studies have shown that nitric oxide inhibits gastrointestinal motility by increasing the level of intracellular cGMP in smooth muscle cells (Stark and Szurszewski, 1992). Similarly, selective inhibition of the cGMP-specific phosphodiesterase 5 by UK-92,480 may also lead to reduced gastrointestinal motility by preventing the breakdown of cGMP in gastrointestinal smooth muscle cells.

**Pharmacokinetics:** As discussed for the rat studies, UK-92,480 forms two pharmacologically active metabolites, one major and one minor. UK-103,320 is the major pharmacologically active metabolite and has about 50% of the potency of the parent drug. It represents 7% and 3% of the administered dose in mouse and man, respectively. A minor pharmacologically active metabolite, UK-150,564, has only about 10% of the potency of the parent drug, and represents 19% and 22% of the administered dose in rat and man, respectively. The terminal elimination half-life was 1.3 and 4.0 hours for mouse and man, respectively.

Plasma drug levels ( $C_{max}$ ) for UK-92,480 (parent drug) and UK-103,320 (major metabolite) were determined from supplementary mice on Day 62. AUCs were not calculated. Mean drug levels one hour after dosing ( $C_{max}$ ) to UK-92,480 and UK-103,320 are shown in Figure 8A (males) and Figure 8B (females). As can be seen, exposure to UK-92,480 and UK-103,320 was dose-proportional in both sexes. As was the case in rats, male mice were exposed mostly to the metabolite UK-103,320, whereas female mice were exposed mostly to the parent drug UK-92,480.

Figure 8A

Mean Drug Levels ( $C_{max}$ ) for UK-92,480 and UK-103,320 in Male Mice  
(One hour after dosing on Day 62)

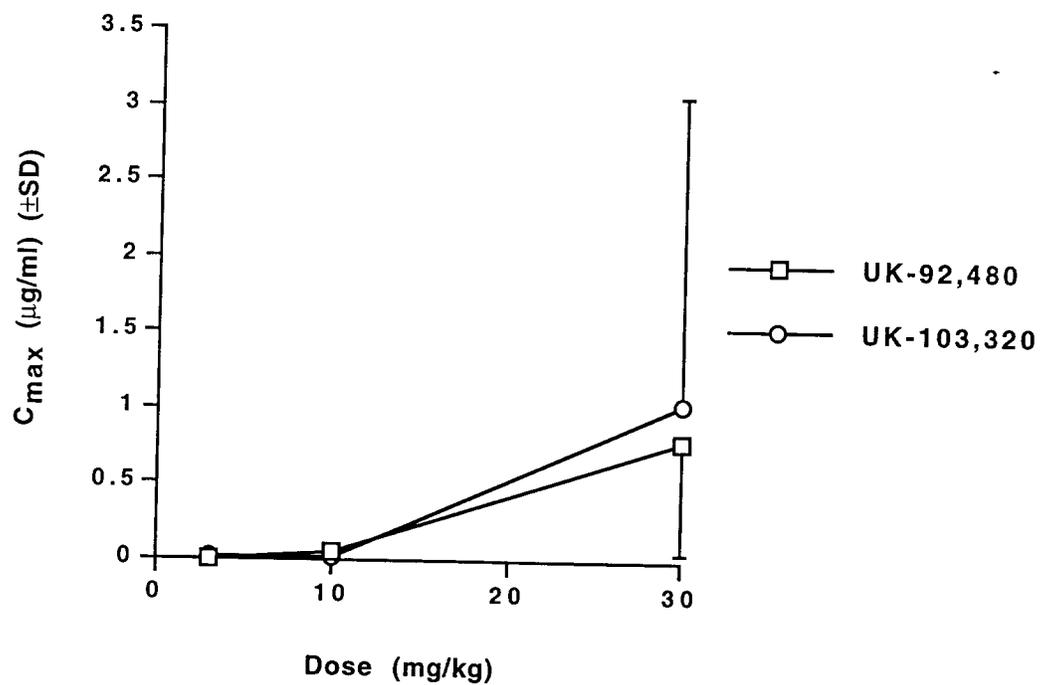
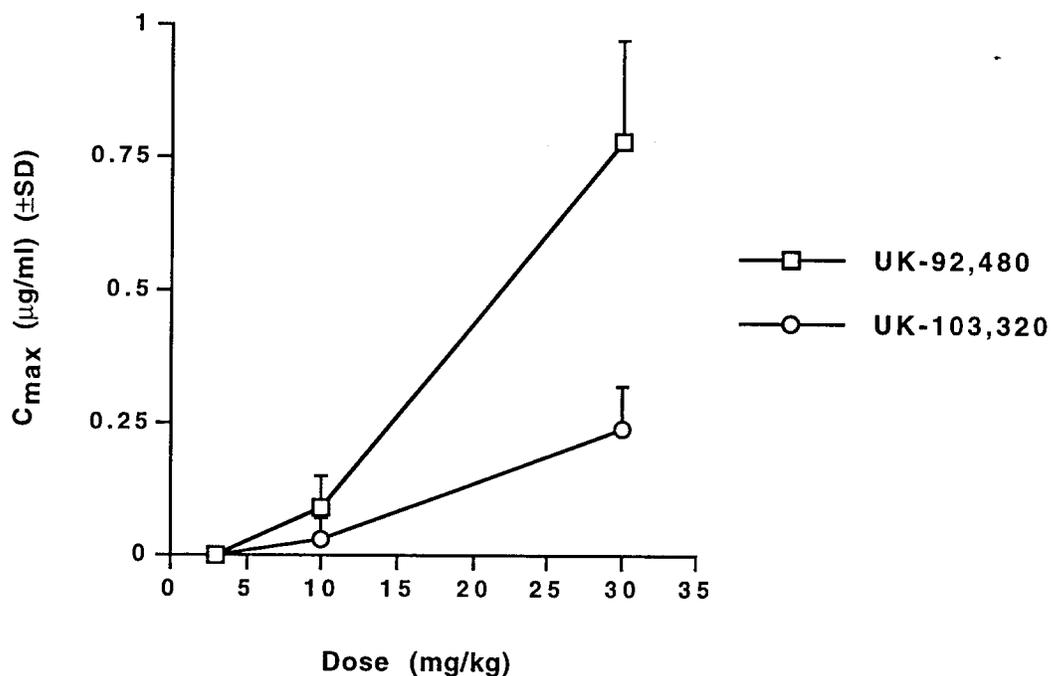


Figure 8B

Mean Drug Levels ( $C_{max}$ ) for UK-92,480 and UK-103,320 in Female Mice  
(One hour after dosing on Day 62)



As discussed above, male and female high dose groups were sacrificed early due to high mortality (15.1 and 13.5 months, respectively). The high dose groups, therefore, may not be appropriate for assessing carcinogenic risk when lifetime exposure to drug was less than 18 months.

Preliminary 3 month toxicity studies found a low incidence of mortality in mice given 40 mg/kg (1/20), but not in mice given 20 mg/kg. Although 30 mg/kg was selected as the high dose without FDA concurrence, it would have been difficult to predict the 78-87% mortality observed after 13-15 months of treatment with 30 mg/kg. Since the mid dose groups were treated for >18 months (21.7 and 18.6 months for males and females, respectively), plasma drug levels ( $C_{max}$ ) are given for both the high and mid dose groups and are compared to  $C_{max}$  values for normal male human volunteers given the maximum recommended human dose (MRHD) of 100 mg (=1.43 mg/kg based on a 70 kg man) (Table 10).

Table 10

Comparative  $C_{max}$  Values (Total Bound and Unbound) for UK-92,480 and UK-103,320 Between Male Humans and Male and Female Mice

Species	Dose	UK-92,480 $C_{max}$ ( $\mu\text{g/ml}$ )	UK-103,320 $C_{max}$ ( $\mu\text{g/ml}$ )
Man	100 mg/70 kg	0.561	0.254
Mouse (male)	10 mg/kg/day	0.05	0.01
	30 mg/kg/day	0.78	1.03
Mouse (female)	10 mg/kg/day	0.09	0.03
	30 mg/kg/day	0.78	0.24

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 mouse is shown in Table 11.

Table 11

Human and Mouse Plasma Protein Binding

Species	UK-92,480		UK-103,320	
	% Bound	Fraction Unbound	% Bound	Fraction Unbound
Man	96	0.04	95	0.05
Mouse	94	0.06	94	0.06

Comparison of the male and female mouse  $C_{max}$  for total drug exposure (sum of unbound UK-92,480 and UK-103,320  $C_{max}$ ) as a multiple of the maximum recommended human dose (MRHD) of 100 mg is shown in Table 12. The unbound  $C_{max}$  were calculated by multiplying the total bound and unbound  $C_{max}$  (Table 10) by the fraction unbound (Table 11). As shown, the total of unbound  $C_{max}$  in male and female mice given 30 mg/kg/day was 3.1X and 1.7X, respectively the  $C_{max}$  of men given a single dose of 100 mg. The multiple of the MRHD in male and female mice given the mid dose of 10 mg/kg was much less than the maximum recommended human exposure (0.1X and 0.2X, respectively).

Table 12

Mouse Multiple of MRHD as a Function of Total Drug Exposure  
(Sum of Unbound  $C_{max}$  of UK-92,480 and UK-103,320)

Species	Dose (mg/kg)	Unbound UK-92,480 $C_{max}$ ( $\mu\text{g/ml}$ )	Unbound UK-103,320 $C_{max}$ ( $\mu\text{g/ml}$ )	Total of Unbound $C_{max}$ ( $\mu\text{g/ml}$ )	Multiple of MRHD
Man	100 mg/70 kg (=1.43 mg/kg)	0.022	0.013	0.035	--
Mouse (male)	10	0.003	0.001	0.004	0.1
	30	0.045	0.062	0.107	3.1
Mouse (female)	10	0.005	0.002	0.007	0.2
	30	0.045	0.014	0.059	1.7

Since  $C_{max}$  may not be an appropriate value to determine the multiple of the human exposure for labeling purposes, additional multiples are given for body weight (mg/kg) and surface area ( $\text{mg/m}^2$ ) (Table 13). As shown, mice given the mid dose of 10 mg/kg/day for >18 months were exposed to 7.0X the MRHD of 1.43 mg/kg (= 100 mg/70 kg) when based on a mg/kg basis. However, when based on  $\text{mg/m}^2$ , this value was only 0.6X the MRHD. The high dose groups, in  $\text{mg/m}^2$ , would have been 1.9X the MRHD, if completed.

Table 13

Mouse Multiple of MRHD  
as a Function of Body Weight (mg/kg) and Surface Area ( $\text{mg/m}^2$ )

Species	Dose		Multiple of MRHD	
	mg/kg	$\text{mg/m}^2$	mg/kg	$\text{mg/m}^2$
Man	1.43	54.5	--	--
Mouse	10	35.0	7.0X	0.6X
	30	103.5	21.0X	1.9X

*Conclusions:* Results showed that no increases in neoplastic lesions were found that could be related to drug treatment. However, due to increased mortality (near or below 20% survival), 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 mice was calculated to be only 0.1X and 0.2X the maximum recommended human dose of 100 mg in male and female mice, respectively. 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.

10/22/97

Thomas Papoian, Ph.D.  
Pharmacologist

Concur: \_\_\_\_\_

10/22/97

CC:

ORIG. NDA

HFD-110

HFD-110 / T. PAPOIAN