CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

PHARMACOLOGY REVIEWS

TOXICOLOGY PAGES 66-90

2.3.2. Mice

2.3.2.1. Twenty-four month oral toxicity and carcinogenicity study in CD1 mice (Study No. 95007; Vol. 1.25-1.30 pp. 5236-7327):

Testing Facility: Pfizer, Centre de Recherche, Amboise Cedex, France
Study Number: 95007
Study Date(s): 1/18/95 to 10/28/96
GLP Compliance: Yes

MOUSE STUDY DURATION: 104 weeks
MOUSE STRAIN: Crl: 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 17A) and in the mid and high dose females (Figure 17B).

Figure 17A (Sponsor's Figure 1)

Survival Plot in Male Mice

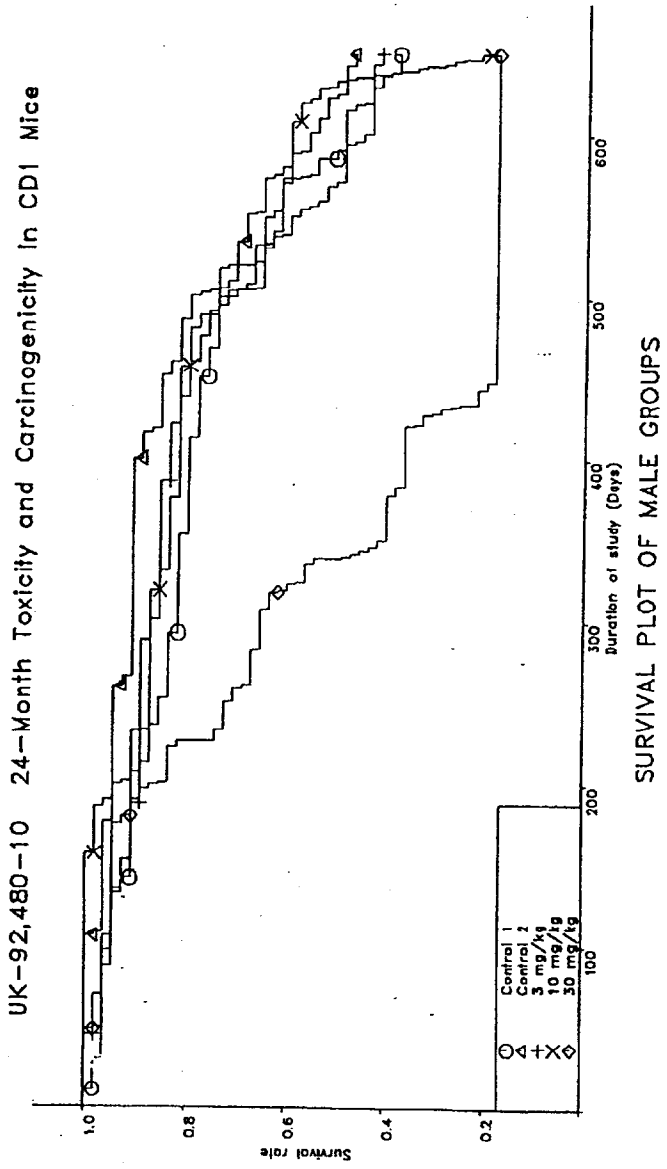
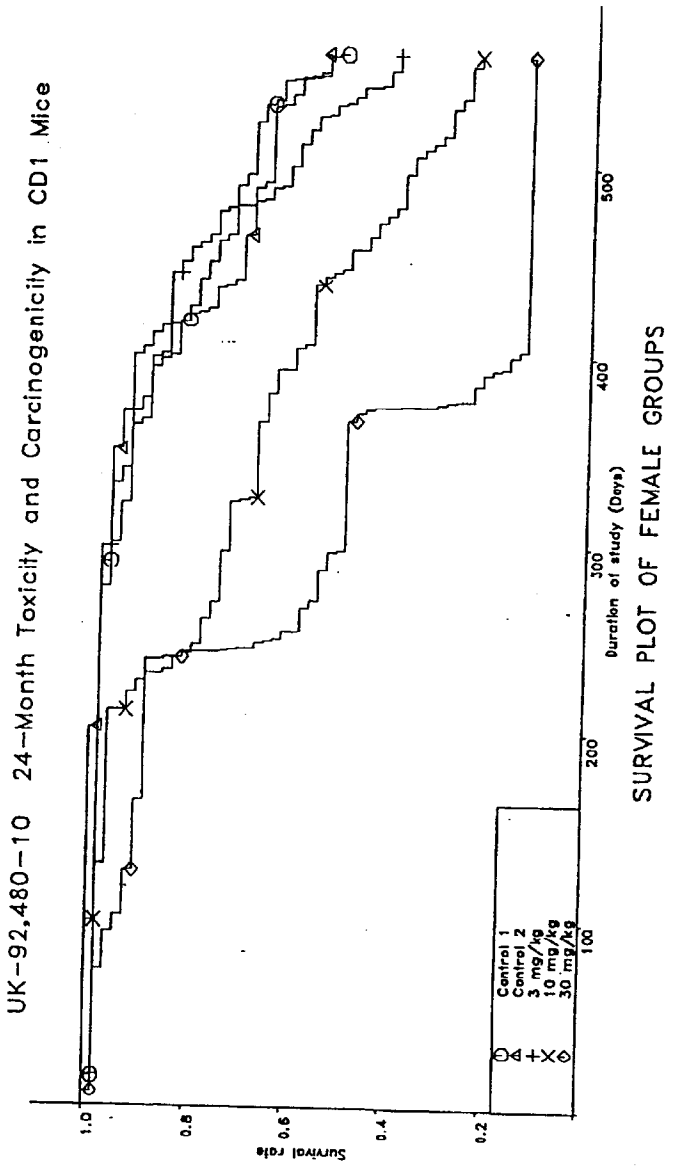


Figure 17B (Sponsor's Figure 2)

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 21.

Table 21

Times of Sacrifice and Percent Survival at Sacrifice in Mice

		Time of Sacrifice		
Sex	**Dose (mg/kg)**	**Days**	**Months**	**% Survival at Sac**
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 18A (males) and Figure 18B (females).

Figure 18A (Sponsor's Figure 3)

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

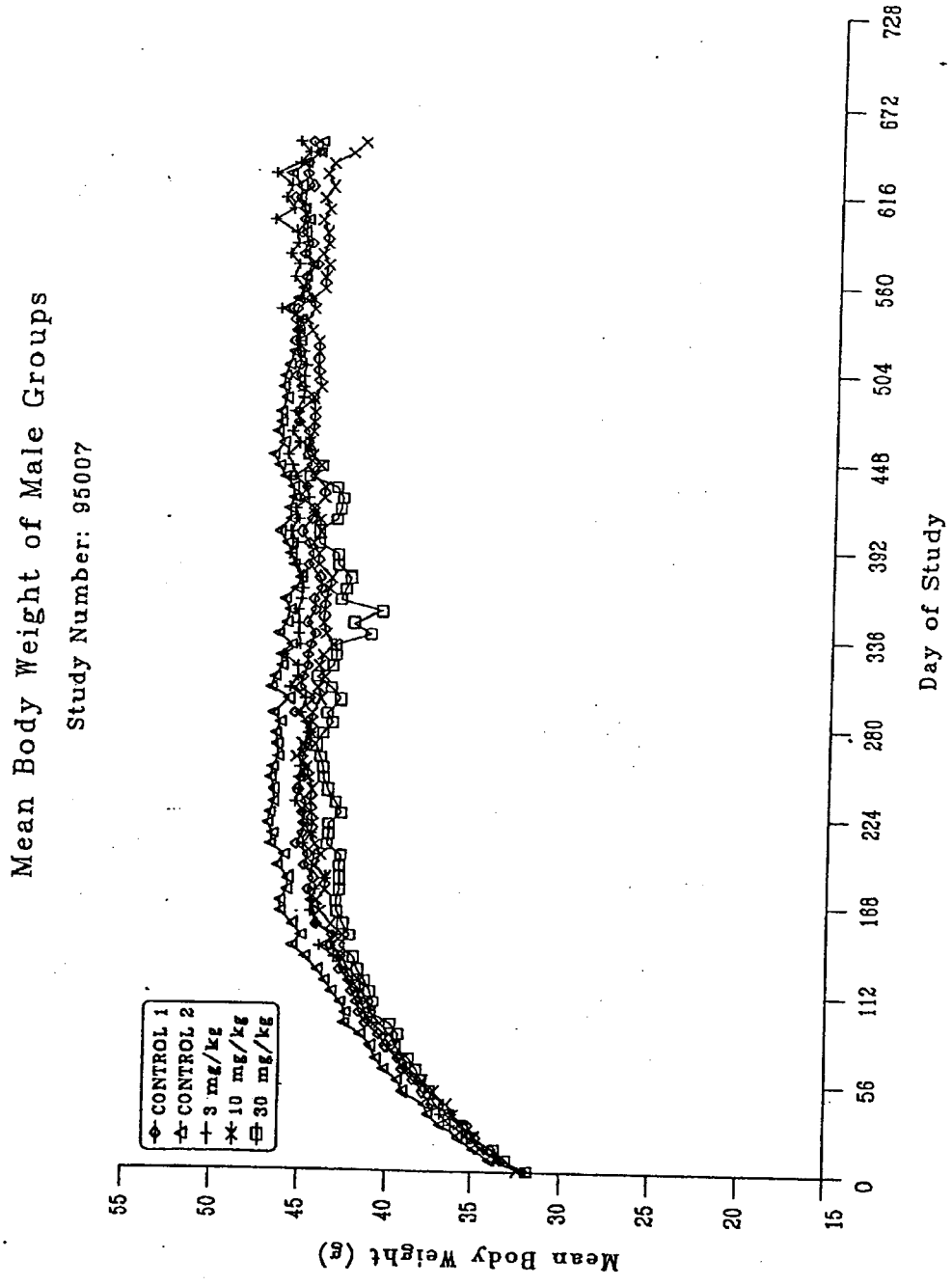
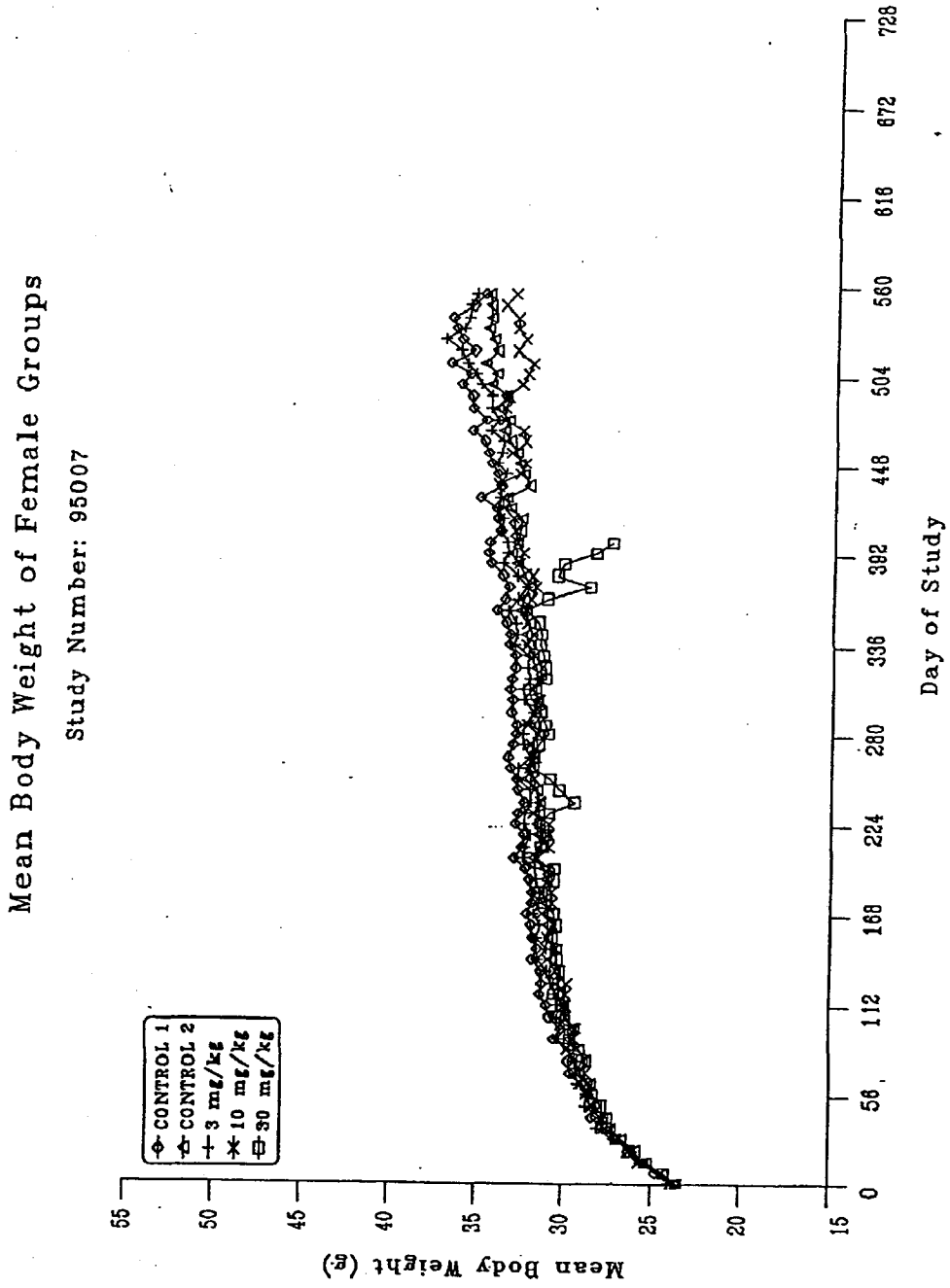


Figure 18B (Sponsor's Figure 4)

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



Percent changes in mean body weight gains in male mice are shown in Table 22A. 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 22A

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 22B. 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 22B

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 23). 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 23

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

Sex	Dose (mg/kg)	Incidence (%)
Male	0	0/55 (0)
	3	2/55 (4)
	10	1/55 (2)
	30	18/55 (33)
Female	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 19), and the extent of slowed intestinal transit correlated with the incidence of death due to gastro-intestinal dilation in both male and female mice (Figures 20A and 20B).

Figure 19

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

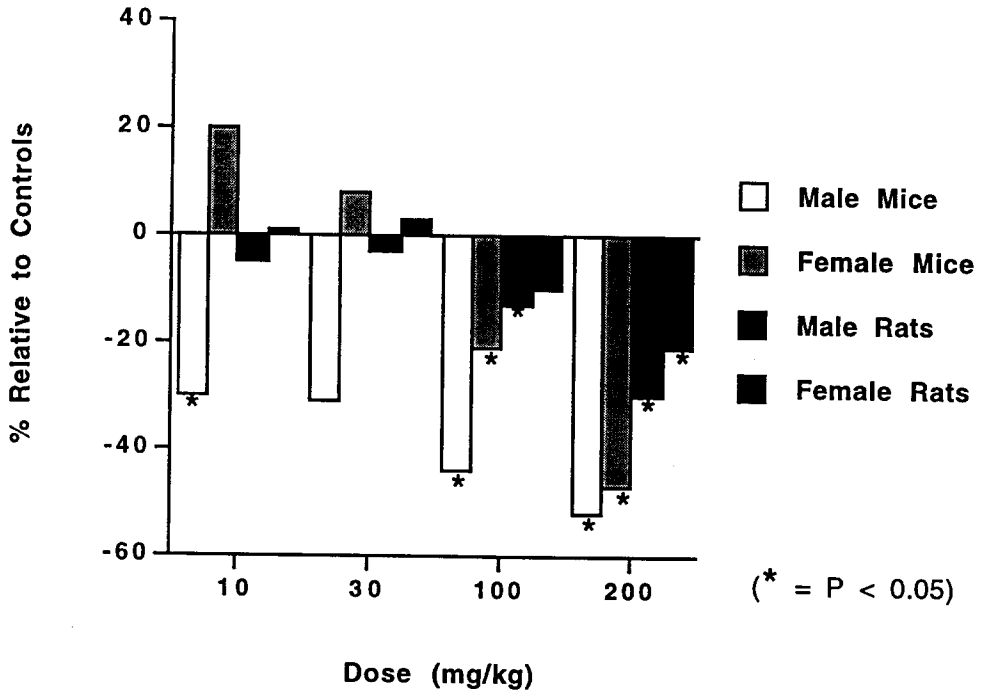


Figure 20A

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

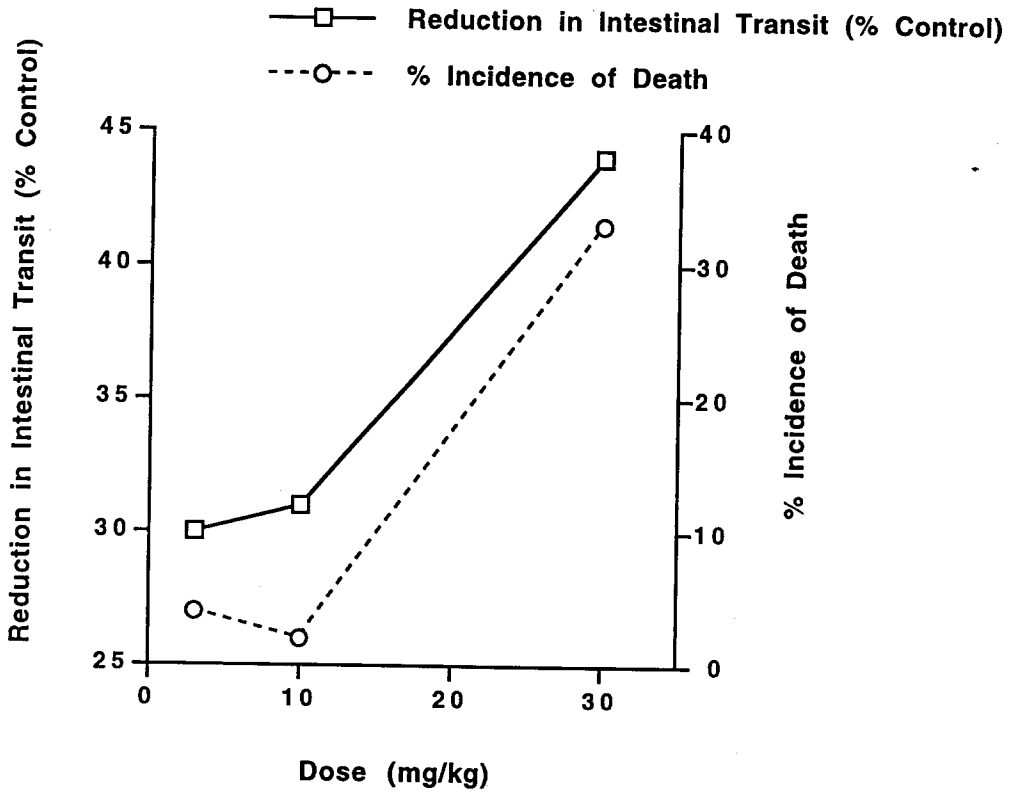
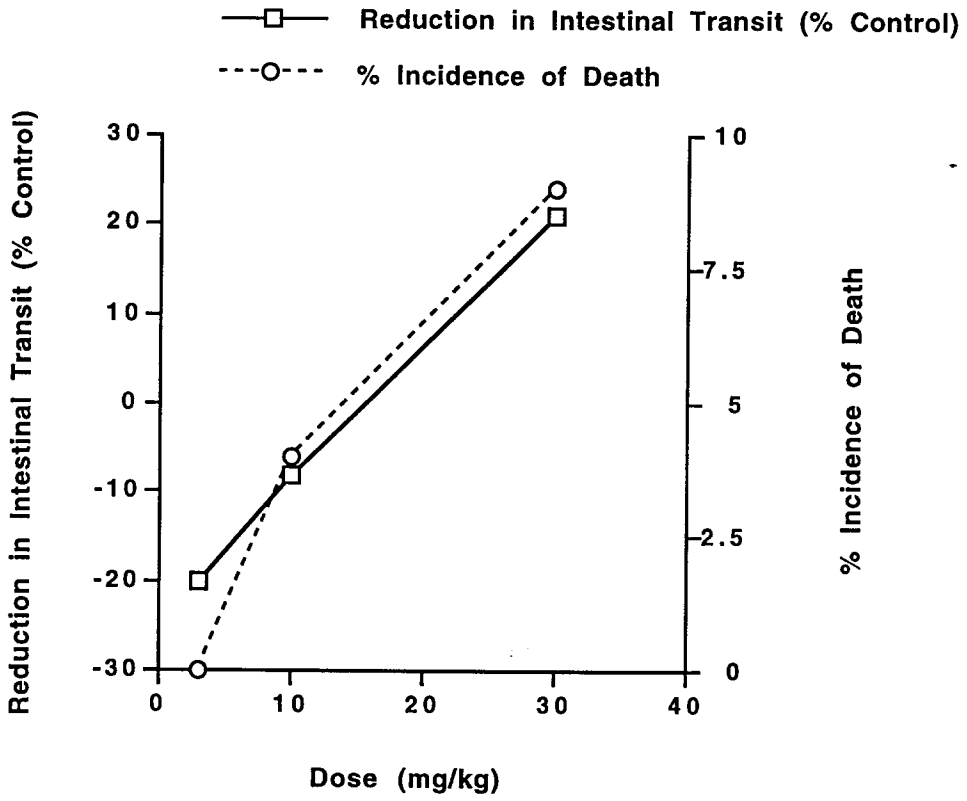


Figure 20B

Correlation Between Reduction in Intestinal Transit
and Death Due to Gastro-Intestinal Dilation (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 21A (males) and Figure 21B (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 21A

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

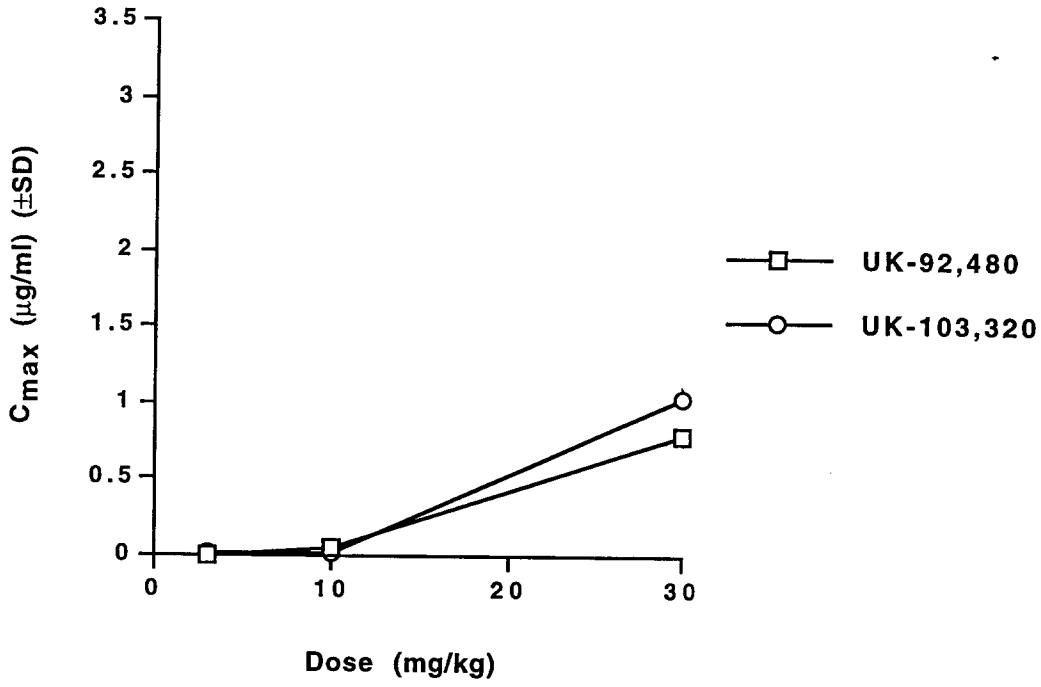
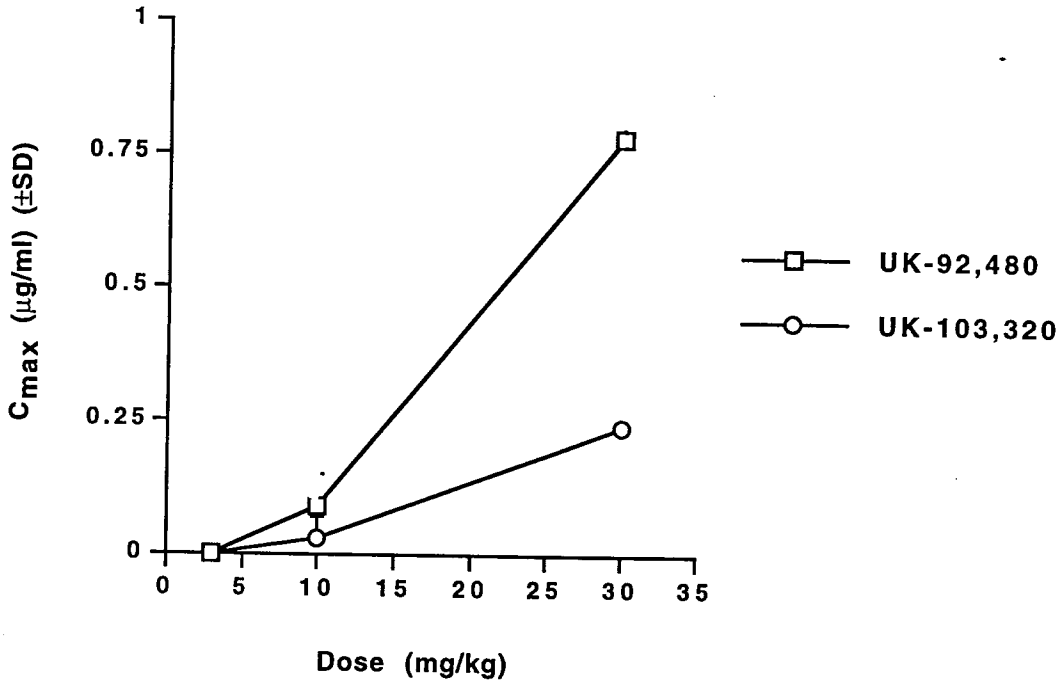


Figure 21B

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 24).

Table 24

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 Cmax (µg/ml)	UK-103,320 Cmax (µg/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 25.

Table 25

Human and Mouse Plasma Protein Binding

	UK-92,480		UK-103,320	
Species	% 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 26. The unbound C_{max} were calculated by multiplying the total bound and unbound C_{max} (Table 24) by the fraction unbound (Table 25). 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 26

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} (µg/ml)	Unbound UK-103,320 C_{max} (µg/ml)	Total of Unbound C_{max} (µg/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 (mg/m^2) (Table 27). 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 mg/m^2, this value was only 0.6X the MRHD. The high dose groups, in mg/m^2, would have been 1.9X the MRHD, if completed.

Table 27

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

	Dose		Multiple of MRHD	
Species	mg/kg	mg/m^2	mg/kg	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.

2.4. Special Toxicity Studies

2.4.1. Antigenicity study (Study No. 95-29-81; Vol. 1.33 pp. 9078-9095):

Testing Facility:
Study Number: 95-29-81
Study Date(s): 2/3/95 to 3/29/95
GLP Compliance: Yes

The antigenicity of UK-92,480 was evaluated in the following immediate type allergic reactions in guinea pigs: (1) active systemic anaphylaxis (ASA) and (2) homologous passive cutaneous anaphylaxis (PCA) with sera isolated from immunized guinea pigs.

ASA test: Male Hartley guinea pigs (5/group; 360-416 gms) were sensitized by both oral and subcutaneous (s.c.) routes (Table 28). The oral dose was given by gavage at 4 mg/kg/day for 21 days. The s.c. dose was given by injection at 4 mg/kg in Freund's complete adjuvant (FCA) 1X/week for 4 weeks. Each animal received an i.v. challenge with either UK-92,480, vehicle (saline plus 10% DMSO), or BSA (positive control) 19 days after the last oral sensitizing dose or 16 days after the last s.c. sensitizing dose. Animals were observed for 30 minutes for signs of anaphylaxis. Deaths within 24 hours were also recorded.

Table 28 (Sponsor's Table)

The composition of test groups

Group	Sensitizing condition	Challenge antigen	No. of animals
1	UK-92,480 P.O. 4 mg/kg	UK-92,480 (20 mg/animal)	5
2	UK-92,480 P.O. 20 mg/kg	UK-92,480 (20 mg/animal)	5
3	UK-92,480 + FCA S.C. 2 mg/animal	UK-92,480 (20 mg/animal)	5
4	UK-92,480-OVA + FCA S.C. 10 mg/animal	UK-92,480 (20 mg/animal)	5
5	Vehicle[1] + FCA S.C. 0.5 ml/animal	Vehicle[2] (1 ml/animal)	5
		UK-92,480 (20 mg/animal)	5
6	BSA + FCA S.C. 1 mg/animal	BSA (10 mg/animal)	5

1) 0.5% methyl cellulose (MC)

2) Physiological saline containing 10% dimethyl sulfoxide (DMSO)

Results showed that no guinea pigs sensitized with UK-92,480 showed signs of systemic anaphylaxis after i.v. injection with UK-92,480 as challenge antigen. Negative controls (vehicle group) were also negative. The BSA positive control group all died within 10 min of challenge due to a marked systemic anaphylactic reaction.

PCA test: Blood was withdrawn from the sensitized guinea pigs in the ASA test two days before the challenge. Sera dilutions (50 µl) were injected intradermally (i.d.) into the shaved back of recipient guinea pigs. Four hours later, either UK-92,480, vehicle (negative control), or BSA (positive control) was injected i.v. together with Evans blue dye. After 30 min, animals were killed and the backs measured for extravasation of dye. A positive PCA response was a diameter of >5 mm. PCA titers were expressed as the ratio of highest serum dilution giving a positive response.

Results showed that no positive PCA reactions occurred in guinea pigs given UK-92,480 antisera and challenged with UK-92,480. Negative (vehicle) controls were also negative. Guinea pigs given BSA antisera then challenged with BSA gave marked positive responses with PCA titers of 10,000-20,000.

These results showed that UK-92,480 did not produce a systemic anaphylactic reaction or a passive cutaneous anaphylactic reaction in guinea pigs (negative allergic reaction).

2.4.2. Intra-arterial irritation in rabbits (Study No. 91073; Vol. 1.33 pp. 9096-9125):

Two groups of 4 female New Zealand white rabbits (3.363 kg ± 0.245 kg) received on day 1 of study a single injection of 0 (vehicle - aqueous solution containing 5% mannitol) or 1 mg of UK-92,480-10 in 0.5 ml into the median artery of the left ear. The animals were examined daily for clinical signs and their food consumption was evaluated.

The injection site and the corresponding area of the right ear were examined 1, 3 and 5 hours post dosing on day 1, then once a day up to sacrifice. Body weight was recorded weekly. Two animals of each of the control and treated groups were sacrificed on day 3. The other animals were sacrificed on day 21. The injection sites and corresponding areas of the right ear were sampled for histopathological examination.

RESULTS

CLINICAL OBSERVATIONS/MEASUREMENTS:

Mortality/injection site examination and clinical signs:

All the animals survived throughout the study. Hematoma or redness around the site of injection were seen in all the treated and control animals. In both groups the changes disappeared within 4 to 6 days. No other clinical signs were observed.

Food consumption and body weight:

No remarkable effect of treatment was reported.

POST-MORTEM OBSERVATIONS

Necropsy findings:

No remarkable changes were reported.

Microscopic findings:

Following the single intra-arterial administration of the injectable solution of UK-92,480-10 or of the vehicle alone, peri-arterial hemorrhage and acute inflammation were present at day 3 while a few peri-arterial hemosiderin laden macrophages were the only indicator of the administration at day 21. These changes were recorded with similar incidence and severity in control and treated groups.
No other lesions were considered to be related to the injection.

Study No. 91073

UK-92,480-10 INTRA-ARTERIAL IRRITANCY IN RABBITS

HISTOLOGY SUMMARY TABLE - ALL FINDINGS

	0.00 M	0.00 F	1.00 M	1.00 F
INJECTION SITE				
HAEMORRHAGE	2	0	2	0
INFLAMMATION, ACUTE	2	0	2	0
INFLAMMATION, CHRONIC, FOCAL	2	1	0	2
HYPERKERATOSIS	1	0	0	0
INTIMAL PROLIFERATION	1	0	1	2
HAEMOSIDEROSIS	0	1	0	2
# Examined	2	2	2	2
# ON TEST	2	2	2	2
# AT RISK	2	2	2	2

2.4.3. Intestinal transit in mice after repeat dose administration of UK-92,480-10 by the oral route (Study No. 96068; Vol. 1.33 pp. 9126-9149):

[Note: The following five intestinal transit studies were performed as a result of the early deaths found in mice during the two year carcinogenicity studies described above. The cause of death was attributed to gastrointestinal dilation which was 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.]

Male and female Crl-COBS-VAF-CD1 (ICR) BR mice (20/sex/group; 29 gms for males and 24 gms for females) were given UK-92,480 (lot R202) orally by gavage at 200 mg/kg/day for either 7 days or 44-45 days. Controls received vehicle (0.5% methylcellulose with 0.1% Tween 80). One hour after the last dose, mice received a 0.3 ml suspension of charcoal. Twenty minutes later mice were sacrificed and the small intestine removed. Gastrointestinal transit was expressed as the ratio of the distance traveled by the charcoal relative to the total length of the small intestine. A necropsy was performed to determine the presence of gastrointestinal dilation.

Results showed a statistically significant slowing of the intestinal transit in both male and female mice at both time points (7 and 44-45 days) (Table 29). Intestinal length increased less than 10% indicating a small degree of intestinal dilation.

Table 29

Decreased Intestinal Transit in Mice Treated with UK-92,480
(% Change from Controls)

	Males	Females
7 Days	-42***	-18**
44-45 Days	-30***	-36***

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

It was concluded that UK-92,480 caused a marked slowing of intestinal transit in mice after repeat dosing with 200 mg/kg/day for 7-45 days.

2.4.4. Intestinal transit time in mice after single dose administration of UK-92,480-10 by the oral route (Study No. 96094; Vol. 1.33 pp. 9150-9169):

Male and female Crl-COBS-VAF-CD1 (ICR) BR mice (5/sex/group; 33 gms for males and 28 gms for females) were given a single dose of UK-92,480 (lot R202) orally by gavage at 200 or 400 mg/kg. Controls received vehicle (0.5% methylcellulose with 0.1% Tween 80). One hour after the last dose, mice received a 0.3 ml suspension of charcoal. Twenty minutes later mice were sacrificed and the small intestine removed. Gastrointestinal transit was expressed as the ratio of the distance traveled by the charcoal relative to the total length of the small intestine. A necropsy was performed to determine the presence of gastrointestinal dilation.

Results showed a statistically significant slowing of the intestinal transit in both male and female mice at both doses (Table 30). Also, intestinal length increased, particularly at the high dose of 400 mg/kg.

Table 30

Decreased Intestinal Transit and Increased Intestinal Length
in Mice Treated with UK-92,480
(% Change from Controls)

	Intestinal Transit		Intestinal Length	
Dose (mg/kg)	Males	Females	Males	Females
200	-52**	-47**	0	+7*
400	-55**	-39**	+13*	+14*

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

It was concluded that UK-92,480 caused a marked slowing of intestinal transit and increased intestinal length in mice treated with single oral doses of 200 or 400 mg/kg.

2.4.5. Intestinal transit time in mice after single dose administration of UK-92,480-10 by the oral route (Study No. 97056; Vol. 1.33 pp. 9170-9188):

Male and female Crl-COBS-VAF-CD1 (ICR) BR mice (10/sex/group; 32 gms for males and 27 gms for females) were given a single dose of UK-92,480 (lot R202) orally by gavage at 1 or 3 mg/kg. Controls received vehicle (0.5% methylcellulose with 0.1% Tween 80). One hour after the last dose, mice received a 0.3 ml suspension of charcoal. Twenty minutes later mice were sacrificed and the small intestine removed. Gastrointestinal transit was expressed as the ratio of the distance traveled by the charcoal relative to the total length of the small intestine.

Results showed that there was no effect on intestinal transit in mice given a single oral dose of UK-92,480 at up to 3 mg/kg.

2.4.6. Intestinal transit in rats after single dose administration of UK-92,480-10 by the oral route (Study No. 97027; Vol. 1.33 pp. 9189-9208):

Male and female Crl-COBS-VAF-CD (SD) BR rats (5/sex/group; 216 gms for males and 177 gms for females) were given a single dose of UK-92,480 (lot R202) orally by gavage at 10, 30, 100, or 200 mg/kg. Controls received vehicle (0.5% methylcellulose with 0.1% Tween 80). One hour after the last dose, mice received a 0.3 ml suspension of charcoal. Twenty minutes later

mice were sacrificed and the small intestine removed. Gastrointestinal transit was expressed as the ratio of the distance traveled by the charcoal relative to the total length of the small intestine.

Results showed a statistically significant slowing of intestinal transit at the 100 and 200 mg/kg doses in males and at the 200 mg/kg dose in females (Table 31).

Table 31

Decreased Intestinal Transit in Rats Treated with UK-92,480
(% Change from Controls)

Dose (mg/kg)	Males	Females
10	-5	+1
30	-3	+3
100	-13*	-10
200	-30*	-21*

(*=P<0.05)

2.4.7. Intestinal transit in mice after single dose administration of UK-92,480-10 by the oral route (Study No. 97028; Vol. 1.33 pp. 9209-9227):

Male and female Crl-COBS-VAF-CD1 (ICR) BR mice (5/sex/group; 29 gms for males and 24 gms for females) were given a single dose of UK-92,480 (lot R202) orally by gavage at 10, 30 or 100 mg/kg. Controls received vehicle (0.5% methylcellulose with 0.1% Tween 80). One hour after the last dose, mice received a 0.3 ml suspension of charcoal. Twenty minutes later mice were sacrificed and the small intestine removed. Gastrointestinal transit was expressed as the ratio of the distance traveled by the charcoal relative to the total length of the small intestine.

Results showed a statistically significant slowing of intestinal transit in at the low and high dose in male and at only the high dose in female mice (Table 32). These results correlated with the increased mortality found in male mice when compared to female mice during the course of the two year carcinogenicity studies described above. Also, male mice appear to be more sensitive than male rats and may explain the lack of mortality due to intestinal dilation in the two year rat carcinogenicity studies.

Table 32

Decreased Intestinal Transit in Mice Treated with UK-92,480
(% Change from Controls)

Dose (mg/kg)	Males	Females
10	-30*	+20
30	-31	+8
100	-44*	-21*

(*=P<0.05)

2.5. Reproduction and Neonatal Studies

In these GLP reproduction studies, UK-92,480 citrate (as aq. sol in 0.5% methylcellulose containing Tween 80) was given by gavage because oral administration is the intended route for humans. Reproduction studies were conducted in Amboise, France at laboratories previously mentioned. Drug was formulated in drug sponsor's laboratories in the UK, and samples were assayed once for homogeneity/concentration of each formulation and reported by drug sponsor to be "satisfactory". Dose levels of the drug are expressed as the base.

2.5.1. Fertility And Early Embryonic Development To Implantation

2.5.1.1. Rat (Sprague-Dawley)

2.5.1.1.1. Fertility and Early Embryonic Development to Implantation in Rats. (**Study N° 94081**) conducted in France. Study dates for M: 12-09-94 to 21-12-94; for F: 31-10-94 to 19-12-94.

The purpose of this study was to evaluate the effects of UK-92,480-10 (Batch No. R 103) on the fertility of adult M and F rats, and to assess the development of pre-implantation stages of embryos as well as changes in fetal body weight, external and buccal anomalies resulting from a treatment lasting to the implantation time point.

The doses in this fertility study were based on doses used in a 6-mo rat repeat dose oral toxicity study at LD-3, MD-12 and HD-60 UK-92,480 mg/kg/day in which the HD was associated with pathologic changes in liver, thyroid and adrenal gland hypertrophy.
In this fertility/early embryonic development study, the methods were described in detail in the NDA. Briefly, UK-92,480 was administered to 20 rats/sex/group at 0 (vehicle- aq. sol. of methylcellulose containing Tween-80), LD-3, MD-12 and HD-60 UK-92,480-10 mg/kg/day; controls were treated concurrently with drug treated animals. M were treated 9 weeks prior to mating and during mating (2 wks); F were treated 2 wks prior to mating, throughout mating (1 to 14 days), and during early gestation until day 6 post-insemination (pi). Hysterectomy of the F took place on day 20 pi. The total duration of treatment was ~ 102 days for M, and 23-36 days for F.

All rats were observed for clinical signs twice a day during the treatment period. The estrus cycle of F rats was monitored during the mating period.

The body weights/food consumption of all rats were recorded regularly during the study at protocol designated times. At the end of the mating period, the M were sacrificed 24 hours after the last drug treatment and submitted to a full necropsy. The testes and epididymides, and any macroscopic anomalies were sampled and archived.

Blood samples were taken for hematology and biochemistry analyses at the end of the treatment period of both M and F rats.
All dams were subjected to full necropsy. All macroscopic anomalies noted, the ovaries and uteri, were taken, fixed, and archived. Relevant reproductive parameters were recorded (e.g., number of corpora lutea, implantation sites and viable fetuses, and rates of pregnancy, implantation, and embryolethality were calculated). All fetuses were sexed, weighed and examined for external buccal anomalies.

RESULTS

Dam Observations and Measurements:

Clinical signs and mortality: No deaths or drug-related clinical signs were reported. A few LD F, and some M from all groups showed scabs and/or alopecia.

Estrus cycle in F was not affected. Body weight in HD F slightly decreased (- 1.7% vs controls) and only a significant reduction in the mean body weight was noted on day 7 of study; this finding was **not** considered of biological significance by drug sponsor.

MD M showed a minor change in hematology as an increase (+3%) in hematocrit (HCT). Pregnant and non-pregnant dams, showed variations in red blood cells, white blood cells, platelet variations and differential counts between individual animals, but these were **not** considered by drug sponsor to be drug-related effects.

The only drug-related clinical chemistry finding reported in M was a dose-related decrease in ALT (up to a decrease of 30% at HD); this finding was not considered by drug sponsor to be of biological importance. Only in pregnant F, there were no significant differences between control and the groups given 3 and 12 mg/kg/day. The HD-60 mg/kg/day produced a moderate decrease in triglycerides (- 30% vs controls) together with minor decreases in proteins, AP, and both ALT and AST.

Maternal Fertility and Reproduction parameters:

Copulation (indicated by the presence of spermatozoa in the vaginal smears), occurred during the first 4 days of the mating period for most animals. At the end of the 14-day mating period, the number of mated F in each group of 20 was C-18, LD-18, MD-18 and HD-20. Of these F, the corresponding number of pregnant animals was C-16, LD-15, MD-18 and HD-19.

There were no significant differences between the control and drug treated groups in either copulation or pregnancy rates, in the number of corpora lutea, implantation* sites or viable fetuses, and in embryomortality rate. Significant increases in the implantation rate observed at all dose levels were considered by the drug sponsor to be coincidental.

The most remarkable gross finding in dams reported was hydrometra in non-pregnant control. Drug sponsor asserts that the nature and distribution of the few macroscopic abnormalities noted at necropsy did **not** suggest any treatment-related effect.

Fetal Observations/Measurements:

Only 2 fetuses from the HD dams showed minor changes (1 small fetus showed edema and pale skin, and the other showed violet color abdomen and cyanosis in hind part of the body.

The following summary table on maternal and fetal findings in this fertility/early embryonic development to implantation study with UK-92,480 citrate was prepared by drug sponsor. The results reported indicate that the fertility of F rats was not affected by the method of treatment and doses of the drug used; neither was the fertility of M affected in this study.

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* Implantation rate: Ratio of number of implantation sites to the number of corpora lutea.

Embryomortality rate: Ratio of number of dead implants to the number of implantation sites. Data were analyzed using a chi-square test or a Fisher's exact test.

The HD-60 mg/kg in M rats, when using a conversion factor (km)* of 7 results in an estimated dose of ~429 mg/M^2. Calculating for the proposed maximum recommended human dose (MRHD) of 100 mg UK-92,480 citrate (~ 1.66 mg/kg/po assuming a 60 kg man) and using a km of 37 results in ~61.42 mg/M^2 dose of the drug. Using the obtained values doses for both rat and man, it may be concluded that HD in M rats that did not affect fertility represents ~ 7X the MRHD.

Study No. 94081

UK-92,480-10

STUDY OF FERTILITY AND EARLY EMBRYONIC DEVELOPMENT TO IMPLANTATION IN SPRAGUE-DAWLEY RATS BY THE ORAL ROUTE

	CONTROL	3 MG/KG	12 MG/KG	60 MG/KG
REPRODUCTIVE VARIABLES FOR SACRIFICED FEMALES				
COPULATION RATE(%)	18/ 20 (90)	18/ 20 (90)	18/ 20(90)	20/20 (100)
PREGNANCY RATE (%)	16/ 18 (89)	15/ 18 (83)	18/ 18 (100)	19/ 20 (95)
VIABLE LITTERS ON DAY 20 (%)	16/ 16 (100)	15/ 15 (100)	17/ 18 (94)	19/ 19 (100)
CORPORA LUTEA MEAN±S.D.	18.9± 4.17	19.3± 2.05	18.4± 3.32	19.4± 3.04
IMPLANTATION SITES MEAN±S.D.	16.1± 3.64	18.1± 1.39	17.1± 4.42	17.9± 2.05
NO FOETUSES MEAN±S.D.	15.4± 4.24	16.5± 3.16	16.1± 4.08	17.0± 1.97
IMPLANTATION RATE (%)	257/303(84.8)	271/289(93.8)	290/312(92.9)	340/368(92.4)
EMBRYOMORTALITY RATE (%)	10/257(3.9)	23/271(8.5)	17/290(5.9)	17/340(5.0)
FOETAL DEVELOPMENT				
SEX RATIO M/F(%)	117/130(90)	123/125(98)	131/142(92)	165/158(104)
MEAN FOET.WEIGHTS MALES (g)	3.69± 0.26	3.74± 0.27	3.74± 0.31	3.67± 0.38
MEAN FOET.WEIGHTS FEMALES (g)	3.51± 0.29	3.53± 0.31	3.58± 0.25	3.43± 0.38
MEAN BODY WEIGHT GAINS OF PREGNANT FEMALES(grams)				
NO OF PREGNANT FEMALES	16	15	17	19
FROM DAY 1 TO DAY 20	159.8	169.3	167.7	169.3
FROM DAY 1 TO DAY 7	28.8	30.0	28.5	23.2
FROM DAY 7 TO DAY 20	131.0	139.4	139.2	146.1

From the findings reported, drug sponsor concluded that UK-92,480 citrate given at doses of 3, 12 and 60 mg/kg p.o. to adult M and F rats prior to- and during mating period, and during gestation induced **no** adverse effects on fertility of either sex, and no maternal, embryo- or fetotoxicity.

Reviewer considers that treatment with UK-92,480 was associated with some <u>maternal toxicity</u> at the HD 60 mg/kg/day 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, the drug is not intended for human F <u>by oral</u> administration.

* Cancer Chemotherapy Reports, 50(4):219, May 1966

2.5.2. Dose-Range Finding Studies

2.5.2.1. Pregnant Rat (Crl:COBS-VAF-CD(SD)BR)

2.5.2.1.1. Preliminary Fetotoxicity Study with UK-92,480 (Batch No. R 103) in Pregnant Rats. (**Study No. 92020**, conducted between Feb. 11 and March 1992. Vol. 1.31; p. 7939).

The purpose of the preliminary study was to collect data to aid with the selection of doses for a definitive teratology study.

In this preliminary study, pregnant rats (7/dose group of artificially inseminated animals weighing ~280 g) were treated during organogenesis (days 7 through 17 of pregnancy) to determine maternal toxicity with doses of 0, LD-10, MD-50 and HD-200 mg/kg UK-92,480 given by gavage and then sacrificed on day 20 of gestation and necropsied.

Briefly, remarkable maternal findings were reported in the dams treated with the HD-200 mg/kg/day dose. When HD dams were compared to controls, clinical hematology showed moderate decreases: -12.5% Hgb, -15% in red blood cell (at LD -6%) and clinical chemistry showed a marked decrease in plasma triglycerides (-62%).* Mean platelet values at the HD were minimally elevated (+4%) vs controls.

Although no dams died, at the HD signs of maternal toxicity reflected on reproductive parameters included statistically significant decrease in implantation rate when compared to controls (76.0% vs. 85.9%, respectively). Histopathologic changes reported included an increase in the liver weight (absolute/relative hepatic weights of 27% and 26%, respectively) accompanied by centrilobular hypertrophied hepatocytes exhibiting rounded cytoplasmic borders, slightly pale cytoplasm, and loss or margination of cytoplasmic basophilic stippling (considered related to the induction of xenobiotic-metabolizing enzymes). One control and one LD dam had a few small foci of subcapsular necrosis in the liver.

Although M embryolethality was dose-related vs control, this was not statistically significant. The most remarkable fetal change reported was a slight reduction in mean body weight of M fetuses from the HD group. The adjusted mean body weight of the M fetuses was significantly decreased at HD (- 7%) vs. controls.

Based on the reported decrease in RBC at LD-10 mg/kg in these pregnant rats (interpreted by drug sponsor as spurious), reviewer considers that the **NOAEL** of UK-92,480 may be considered to be < 10 mg/kg dose given to dams during the period of organogenesis. However, drug sponsor considered that the NOAEL to be higher based on plasma chemistry as 50 mg/kg, and on fetal body weights as 200 mg/kg. No statements were found regarding what doses of UK-92,480 would be used in the definitive study.

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* Drug sponsor reported that a marked decrease in triglycerides was noted in rats treated with 45 and 200 mg/kg/day UK-92,480 for 1-mo.