CENTER FOR DRUG EVALUATION AND RESEARCH

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PHARMACOLOGY REVIEWS

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2.5.3. Definitive Embryo-Fetal Developmental Studies

2.5.3.1. Rat (Crl:CBS-VAF-CD(SD)BR

2.5.3.1.1. Study for Effects of UK-92,480 on Embryo-fetal Development in Sprague-Dawley Rats by the Oral Route." (**Studies No. 95058/95059** conducted with drug (Batch no. R110). Study dates: 11 July 95 to 3 August 1995)

Twenty inseminated F rats/group aged 9-14 wks at the start of the study weighing ~270 g bodyweight were used. The treatment was at 0 (vehicle), LD-10, MD-50 and HD-200 mg UK-92,480/kg by gavage on days 6 to 17 pi. UK-92,480 was suspended in aq. sol. of 0.5% methylcellulose containing 0.1% Tween 80. Drug samples were assayed once for homogeneity/concentration of each formulation and considered by drug sponsor to be "satisfactory".

The protocol submitted contains a detailed description of experimental conditions, procedures, and observations/measurements. Briefly, rats were housed individually and had free access to tap water/diet. Maternal observations/measurements included clinical signs, body weight/food consumption, hematology and clinical chemistry. Pregnant rats (18-20) were sacrificed on day 20 pi. Maternal postmortem observations included reproduction parameters (i.e. pregnancy rate; implantation rate and post-implantation rate) and at necropsy since no organs showed macroscopic abnormalities, no further measurements were done.

Observations in viable fetuses included numbers, sex, weight, and examination for external and buccal malformations. After sacrifice, alternate fetuses from each litter were prepared, their skeletons stained with alizarin red and their soft tissues were cleared.

Stained skeletons were examined under a dissecting microscope to determine the degree of ossification and for the presence of anomalies. Bone observations were coded; total and mean values of sternebral ossification were calculated for each dose level of UK-92,480.

Remaining fetuses were fixed before being serially sectioned. Slices were examined under a dissecting microscope for visceral abnormalities or anatomical variations. Drug sponsor stated that during the skeleton staining procedure some fetuses were damaged and these fetuses were excluded from the summary tables. A list of damaged fetuses/missing bones were listed in the submission.

Maternal plasma, amniotic fluid and fetal (homogenated) tissues were examined for content of UK-92,480 and metabolite UK-103,320 by HPLC. Determinations were done on day 17 p.i. for Study No. 95059.

Drug sponsor provided historical control values obtained in its laboratory for various reproductive and fetal measurements.

Various statistical analyses were applied to the data collected; the analyses used were explained and methods referenced in the submission. Drug sponsor asserted that statistical significance was not considered automatically to imply toxicologic significance and absence of statistically significant comparison was not considered to imply lack of a biologically important effect.

RESULTS

The following summary table of various rat maternal and fetal parameters was prepared by drug sponsor.*

Study Nos. 95058/59

UK-92,480-10
STUDY FOR EFFECTS ON EMBRYO-FOETAL DEVELOPMENT IN SPRAGUE-DAWLEY RATS BY THE ORAL ROUTE
(Reproductive Study III)

SUMMARY RESULTS

	CONTROL	10 MG/KG	50 MG/KG	200 MG/KG
REPRODUCTIVE VARIABLES FOR SACRIFICED FEMALES				
PREGNANCY RATE (%)	18/ 20 (90)	18/ 20 (90)	20/ 20 (100)	20/ 20 (100)
VIABLE LITTERS ON DAY 20 (%)	18/ 18 (100)	18/ 18 (100)	20/ 20 (100)	20/ 20 (100)
CORPORA LUTEA MEAN±S.D.	16.1± 2.21	16.2± 1.79	16.6± 2.26	15.9± 2.46
IMPLANTATION SITES MEAN±S.D.	15.0± 3.01	14.9± 1.91	14.8± 2.17	14.2± 3.37
NO FOETUSES MEAN±S.D.	13.8± 2.84	13.9± 2.05	14.1± 2.21	13.6± 3.41
IMPLANTATION RATE (%)	270/289(93.4)	268/291(92.1)	296/332(89.2)	284/317(89.6)
EMBRYOMORTALITY RATE (%)	22/270(8.1)	10/260(6.7)	15/296(5.1)	12/284(4.2)
FOETAL DEVELOPMENT				
SEX RATIO M/F(%)	117/131(89)	119/131(91)	124/157(79)	139/133(105)
MEAN FOET.WEIGHTS MALES (g)	3.82± 0.28	3.81± 0.33	3.84± 0.28	3.61± 0.27
MEAN FOET.WEIGHTS FEMALES (g)	3.54± 0.32	3.65± 0.24	3.60± 0.23	3.44± 0.28
GROWTH (Mean body weight gains or losses of pregnant females expressed in GRAMS)				
NO OF PREGNANT FEMALES	18	18	20	20
FROM DAY 1 TO DAY 20	130.5	132.3	127.9	123.6
FROM DAY 1 TO DAY 6	27.0	25.4	27.6	26.6
FROM DAY 6 TO DAY 18	82.1	83.3	79.8	74.1
FROM DAY 18 TO DAY 20	21.5	23.6	20.5	23.0

From the data reported, there is no clear indication that UK-92,480 induced no remarkable adverse effects on the mean number of corpora lutea, implantation sites/rates, viable fetuses or post-implantation loss.

Brief overview of maternal and fetal parameters:

No dams died. Drug sponsor reported as a remarkable clinical sign salivation in the HD group immediately after treatment. Other clinical signs noted included hair loss on forepaws and dyspnea were also reported mainly in some HD rats. Dose-related increase in body weight/body weight gain were reported for the MD and HD. At HD, bodyweight gain (days 6-9 p.i.) was significantly (P=

* Pregnancy rate: Ratio of # pregnant F to # of inseminated F.
Implantation rate: Ratio of # of implantation sites to # of corpora lutea.
Embryolethality rate: Ratio of # of dead implants to # implantation sites.

0.01) higher than control; this effect was considered by drug sponsor to be of toxicologic significance.

In the HD dams, mean food consumption tended to be reduced (9%) when compared to control; at the end of the treatment period mean food consumption was increased which is considered a sign of recovery.

Hematology showed a dose-related increase in reticulocyte count and minimal changes in red blood cell (RBC) parameters at the HD; drug sponsor asserted that this findings suggests an increase in RBC turnover that was compensated at the lower doses. Most remarkable finding in clinical chemistry consisted in a dose-related decrease in triglycerides reaching significance at the HD vs control.

At necropsy, no macroscopic abnormalities attributed to UK-92,480 were reported by drug sponsor.

M fetuses from HD dams showed lower mean body weight than the F. Only 1 LD fetus (1/250) showed craniorachischisis* with twisted tail complicated with anophthalmia** (noted at visceral examinations); this isolated finding was considered to be spontaneous in origin by drug sponsor.

No skeletal malformations or visceral abnormalities were reported that were considered related to the drug treatment. Although drug sponsor reported that the percentage of decreased skull ossification were higher in fetuses from the HD dams vs controls, and that these were still within historical control range, these data could not be verified by the reviewer at this time.

Overall drug sponsor concluded that "there were no treatment-related external, skeletal or visceral anomalies...[and] no teratologic effects at any dose." Although UK-92,480 produced only a slight maternal toxicity without embryotoxicity, M fetuses showed slight toxicity as reported above.

2.5.3.2. Rabbit (New-Zealand White)

2.5.3.2.1. Study for Effects of UK-92,480 (Batch No. R 112) on Embryo-fetal Development in NZW Rabbits by Oral Route. **Studies No. 95043/95044** conducted on dates: 19 May 95 to 06 June 95.

The NDA briefly reported on preliminary studies in rabbits treated with UK-92,480-10. In a pilot oral study (**No. 94110**) said to have been performed in non-pregnant rabbits treated with single oral doses ranging from 10 up to 500 mg/kg/day, signs reported in rabbits treated with 300 and 500 mg/kg consisted of tachypnea, and decrease in body weight/food consumption, and at the highest dose prostration and death.

In separate studies rabbit studies (**Nos. 95003/04**) conducted to identify maternal toxicity at doses ranging from 50 up to 200 mg/kg, the N-demethylated metabolite UK-103,320 was said to have been found in maternal plasma/amniotic fluid of these rabbits, and dams showed an increase in plasma glucose, and a decrease in plasma cholesterol. No embryotoxicity or adverse effect on fetal development were reported.

The purpose of the definitive (**No. 95043**, and **No. 95044** for the pharmacokinetic portion) was to assess the potential toxic effects of UK-92,480-10 on the dams and the embryo-fetal

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* Congenital fissure of the skull/vertebral column.

** Complete absence of eyes or vestigial eyes.

development in artificially inseminated rabbits (20/group, 19-23 weeks at the start of study with a mean body weight ~3.5 kg). The doses selected were based on the preliminary studies. The gavage doses tested ranged from 0 (vehicle), LD-10, MD-50 and HD-200 mg UK-92,480-10/kg given on days 6 to 18 pi; the animals were sacrificed on day 28 pi.

The drug sponsor submitted a detailed description of experimental conditions, procedures, and observations/measurements. Briefly, rabbits were housed individually and had free access to diet/filtered tap water. Maternal observations/measurements included clinical signs, body weight/food consumption, hematology and clinical chemistry. Maternal postmortem observations included reproduction parameters (i.e. pregnancy rate; implantation rate and post-implantation rate). At necropsy if adrenal/liver showed abnormalities, these organs were taken and weighed and relative weight calculated. All fetuses were numbered/weighed and examined for external, buccal, visceral anomalies and degree of ossification. Skeletons were stained and soft tissues cleared; stained skeletons were examined for degree of ossification and presence of abnormalities.

The parallel pharmacokinetic study (No. 95044) was performed with 3 inseminated rabbits treated with a LD of 10 mg/kg/po on days 6-18 p.i. On day 18 pi maternal blood/amniotic fluid and fetuses were taken/homogenized to determine their drug/metabolite contents according to protocol.

Various statistical analyses were performed in rabbit data and drug sponsor provided historical controls values obtained in its laboratory for various reproductive and fetal measurements.

RESULTS

Maternal Parameters:

No dams died. Reproductive parameters showed no effect of treatment on numbers of corpora lutea, implantation sites, viable fetuses and pregnancy and implantation rates.

One MD and 1 HD dam aborted on day 21 and 19 p.i., respectively; at hysterectomy, 1 HD F had evidence of implantation but no viable litters. The incidence of abortion (1 each at MD and HD) was reported as being below that recorded in drug sponsor's laboratories.

MD and HD dams showed reduced body weight gain on days 15 and 21 and on day 28 of study. The controls, LD and MD groups gained (absolute) weight throughout the study, and HD dams lost weight from day 19 p.i. which was sustained until the end of the study; this body weight reduction was regarded as toxicologically significant by drug sponsor.

Changes in body weight (adjusted for covariance to day 6 p.i.) and body weight gain relative to day 6 p.i.

(% change relative to controls)

Dose (mg/kg)	Body weight Day (p.i.) 9	15	21	28	Body weight gain Day (p.i.) 9	15	21	28
10	0	0	0	0	+7	+4	-6	+3
50	0	-1	0	0	+24	-16	-10	+4
200	+1	0	-2	-1	+77	-10	-36 ***	-12

*** : statistically significant at p=0.001.

Food consumption was reduced ~9.2% at the HD on day 15 p.i. vs controls.

Compared to controls, minimal hematologic changes were reported at HD (e.g., 6% decrease in RBC count, Hgb, and HCT), and at LD decreases in monocytes (45%), and at MD basophils decreased by 27%. Plasma chemistry changes were reported also as minimal decreases at MD and HD in alanine aminotransferase and alkaline phosphatase.

Maternal post-mortem observations were unremarkable.

Fetal Observations:

External abnormalities reported included abdominal wall malformations in 2/17 MD litters (e.g., 1 omphalocele* and 1 gastroschisis** each, in 2 fetuses). Drug sponsor stated that omphalocele had not been previously reported in drug sponsor's laboratories; drug sponsor considered these abnormalities as "isolated anomalies".

Remarkable skeletal abnormalities reported included scoliosis, and rib alterations linked to scoliosis in LD fetuses; these were considered spontaneous since they did not occur at higher rates at the higher doses of UK-92,480.

* Omphalocele- Protrusion of part of the intestine through defect in the abdominal wall.

** Gastroschisis- Congenital fissure of abdominal wall usually accompanied by part of the intestine.

Incidence of grouped skeletal anomalies and anatomical variants (%)

(Number of foetuses (F) and litters (L) affected)

	Control		10 mg/kg		50 mg/kg		200 mg/kg	
	F	L	F	L	F	L	F	L
Number examined	136	19	136	18	139	17	123	16
Vertebral alterations			1[a] 0.7%	1 5.6%				
Rib alterations			2[a] 1.5%	2 11.1%				
Sternebral alterations	11 8.1%	7 36.8%	13 9.6%	9 50.0%	10 7.2%	8 47.1%	5 4.1%	4 25.0%
Digit alterations	1 0.7%	1 5.3%						

[a]: associated observations found in one foetus

Notable visceral malformations included ventricular septal defects and in 1 case complicated with left ventricular hypertrophy and atrophy of the right ventricle and pulmonary artery. The summary table below was provided by drug sponsor.

Incidence of foetuses (F) and litters (L) with a visceral malformation (%)

	Control		10 mg/kg		50 mg/kg		200 mg/kg	
	F	L	F	L	F	L	F	L
Number examined	136	19	136	18	139	17	123	16
Ventricular septal defect	1 0.7%	1 5.3%	1 0.7%	1 5.6%	2 1.4%	2 11.8%	2 1.6%	2 12.5%

Drug sponsor asserted that the highest incidence of ventricular septal defect in 2 fetuses from the MD and HD dams vs 1 fetus from each of the LD and control dams. At HD (1.6%) the incidence was marginally above the highest incidence reported in historical controls for this laboratory; however drug sponsor does not consider this malformation to be related to drug treatment.

Other visceral anomalies were reported in control and treated groups; remarkable anomalies reported for the HD group only consisted of atrophies of left or right ventricle and pulmonary artery, enlarged aortic arches.

Incidence of foetuses (F) and litters (L) with at least one malformation or minor anomaly

Control		10 mg/kg		50 mg/kg		200 mg/kg	
F	L	F	L	F	L	F	L
22/136	12/19	26/136	11/18	25/139	14/17	16/123	10/16
16.2%	63.1%	19.1%	61.1%	18.0%	82.3%	13.0%	62.5%

* this analysis excluded variations (persistence of the left cardinal vein, agenesis of the innomina artery and displaced inferior vena cava).

2.5.3.2.2. Study for Effects of UK-92,480 on Embryo-fetal Development in NZW Rabbits by Oral Route: **Pharmacokinetic Study No. 95044** (At the LD-10 mg/kg, plasma/tissue levels of UK-92,480 and metabolite UK-103,320).

UK-92,480 is demethylated to **UK-103,320**; this metabolite is reported to be _ 50% as active as the parent drug as a PDE5 inhibitor. Levels these 2 compounds were determined using an HPLC method.

The following table of maternal plasma and amniotic fluid levels, and fetal levels of **UK-92,480** and **UK-103,320** were provided by drug sponsor:

Pfizer Central Research
Drug Safety Evaluation
Centre de Recherche - Amboise - France
Rabbit/New Zealand White

Drug Concentrations in maternal plasma, amniotic fluid and fetuses
Study number: 95044
Data for scheduled date : 06-Jun-95
Study start date: 16-May-95

Dose	Animal Number	Maternal plasma				AUC	Amniotic fluid	Fetuses	
		Pre-dose	1H	3H	5H	1-5h		Right	Left
mg/kg		µg/ml	µg/ml	µg/ml	µg/ml	µg.h/ml	µg/ml	µg/g	µg/g
					UK-92,480 concentrations				
10	651								
10	652								
10	653								
Group means:		0.023	0.297	0.167	0.097	0.727	0.020		
Standard deviations:		0.021	0.099	0.032	0.021	0.137	0.000		
					UK-103,320 concentrations				
10	651								
10	652								
10	653								
Group means:			0.377	0.153	0.083	0.767			
Standard deviations:			0.159	0.032	0.051	0.273			

AUC 1-5h: area under plasma concentration versus time curve calculated by the trapezoidal rule using 1-5 hours data

BLD: below the limit of determination of the assay :
0.03 µg/ml (plasma)
0.02 µg/ml (amniotic fluid)
0.10 µg/g (fetuses)
BLD was assumed to be 0 in calculating mean values

The drug concentration data reported above indicate that the highest maternal mean plasma levels of both compounds occurred between 1-3 hrs post dose (10 mg/kg po), by 3 hrs post drug levels were low.

The table also shows that maternal mean plasma levels of UK-92,480 and the metabolite **UK-103,320** were highest at 1 hr post dose (the levels of the metabolite were higher that the parent compound.) By 3 hrs post dose mean values of UK-92,480 and metabolite were not remarkable different from each other.

Samples taken 24 hrs after the dose on day 17 pi yielded mean concentrations of 0.02 µg/ml of **UK-92,480, and below limit of determination for the UK-103,320.** AUC_{1-5h} values was 0.73 µg.h/ml and 0.77 µg.h/ml, respectively.

Since only one dose was tested, it is unknown whether mean AUC_{1-5h} values would have increased with increasing dose levels as noted in the rat.

In the amniotic fluid and fetal homogenates the mean concentrations of **UK-92,480 and UK-92,480** were low and in the case of the metabolite below the limit of determination.

In conclusion, (rabbit/Study 95043/044) drug sponsor considers that at the HD of UK-92,480 there was minimal maternal toxicity (ie, decrease in mean body weight gain, and body weight by the end of the treatment period.) Decrease in food consumption may have contributed to these last mentioned changes.

Remarkable visceral anomalies reported was noted in 2 fetuses each from the MD and HD dams were ventricular septal defect consisting of left ventricular hypertrophy and atrophy of the right ventricle and pulmonary artery. These anomalies were also reported in 1 fetus each from the control and LD dams.

The data showed that low levels of the drug was detected in the amniotic fluid, and barely detected in the fetuses. The principal metabolite was barely detectable in the maternal plasma, amniotic fluid and fetal homogenates at all dose levels.

2.5.4. Pre- And Post-Natal Development, Including Maternal Function. (Vol. 1.32)

2.5.4.1. Rat [(Crl:COBS-VAF-CD(SD)BR]

2.5.4.1.1. Study for Effects on Pre- and Post-Natal Development, Including Maternal Function, in Sprague-Dawley Rats by the Oral Route with UK-92,480 (Batch No. R202).

(**Studies No. 95068 & 95095** conducted from 5 August '95 to 04 December '95, and 4 December '95 to 22 March '96, respectively.

The purpose of this study was to evaluate the potential adverse effects of UK-92,480-10 in pregnant/lactating rats (28/group, 12-15 weeks old weighing ~257 g) and on the development of the conceptus/offspring following exposure from implantation through weaning.

In previous rat studies summarized above, data reported indicate that UK-92,480-10 was maternotoxic to pregnant rats at 200 mg/kg, and in M/F rats the drug decreased the levels of plasma triglycerides but did not interfere with their fertility at 60 mg/kg. In the rat reproduction studies, the **NOAEL** for maternal/fetal/embryotoxicity appears to be
below 3 mg/kg (in the fertility study) and up to 50 mg/kg (in the maternal toxicity study).

In this pre- and postnatal development/maternal function study (**No. 95068**), rats were treated by gavage with 0, LD-10, MD-30 and HD-60 mg/kg UK-92,480-10 (Batch No. R202) starting on day 6 post insemination (pi) and ended on day 20 post partum (pp).

The materials/methods used in this study were fully described in the NDA. Briefly, the F_0 dams were observed for mortality/clinical signs twice a day during treatment period and once a day on week-ends. Body weight/food consumption were determined at protocol designated time intervals during gestation. Other observations included gestation parameters (e.g., implantation sites, length of gestation); parturition (e.g., dystocia); lactation (e.g., examination of teats, stomachs of neonates/growth of neonates), maternal care of the pups. Hematology (7 parameters), clinical chemistry (3 parameters) were performed at day 20 pp prior to treatment. F_0 were euthanized/necropsied at weaning (day 21 pp), and macroscopic anomalies were collected/fixed, to be examined microscopically, if needed.

At birth, the F_1 pups were counted (dead/viable)/sexed/weighed/examined and external abnormalities recorded and pups culled. Observations in culled pups, 1/sex/litter included, prior to weaning, and all pups assessing for surface-righting/air-righting reflexes, for the appearance of superior incisors and opening of palpebral fissures; after weaning, at various different times for each type of observation such as ophthalmologic examination (2 pups/sex/group at ~day 22 pp), spontaneous motor activity (2M/2F/litter) and determination of vaginal opening (~day 28 pp) or the separation of prepucial fissure (~ 37 pp), and a battery of functional tests (over days 26-29 pp which included behavior while being handled, when placed on and stimulus reactivity in the open field, etc.).

At 11 weeks of age, some F_1 rats were euthanized and some necropsied. Two F_1 generation rats/sex/litter were selected for reproduction function studies. In the F1 generation/their progeny study (**No. 95095**), at the age of 12 weeks, the F_1 F rats were caged with M (not brothers/sisters) for 2 weeks. F were checked for the presence of spermatozoa/monitored for estrus cycle. F positive with a vaginal smears were then housed individually, weighed twice a week and later, after the birth of the F_2 offspring, during days 1 and 4 pp.

The F_2 pups were examined for external abnormalities; viable pups were counted/sexed on days 1 and 4, and the mean body weight of pups from each litter was determined. F_2 pups found dead were necropsied/examined for visceral abnormalities.

F_1 pups (2M, 2F/litter) that were chosen for further developmental studies were mated at 12 wks of age. Pregnant F_1 rats were weighed weekly and on days 1/4 pp. Their offspring (F_2 generation), dead/alive pups were examined/counted/sexed on days 1/4 pp. Then both F_2 pups and F_1 F were killed on day 4 pp and fully necropsied/examined macroscopically. Macroscopic anomalies of the mothers were sampled/fixed for microscopic examination if needed.

The fertility/developmental parameters assessed were copulation rate, pregnancy rate and post-implantation loss. Peri and post-natal viability of pups was estimated by the following indices: 24-hr survival, 4-day survival and lactation index.

The drug sponsor provided detailed information and reference citations on statistical methods used to analyses the data collected in this study.

RESULTS

Maternal Observations (F_0):

No F_0 dams died. No noteworthy signs of toxicity were reported with the exception of hypogalactia in 1 HD dam.

At the end of treatment period, there were minimal dose-related increases in RBC parameters which were not considered by drug sponsor to be of toxicologic importance.

Clinical chemistry findings compared to controls included increases in glucose (~ 6-8%) at all dose levels, and of cholesterol at the MD and HD only (statistically significant at the HD). Although not statistically significant vs controls, increases up to 14% in blood cholesterol were reported.

The reproduction parameters reported for F_0 dams appeared unremarkable affected by the drug treatment. The summary table below was prepared by drug sponsor.

- Summary reproduction data of F_0 females

UK-92,480-10
STUDY FOR EFFECTS ON PRE- AND POST-NATAL DEVELOPMENT, INCLUDING MATERNAL FUNCTION, IN SPRAGUE-DAWLEY RATS BY THE ORAL ROUTE

REPRODUCTION DATA FOR F_0 FEMALES

	Control	10 mg/kg	30 mg/kg	60 mg/kg
Pregnancy rate (%)	28/28 (100.0)	26/28 (92.8)	28/28 (100.0)	28/28 (100.0)
Number of implantation sites (Mean ± S.D.)	15.8 ± 1.9	16.2 ± 1.3	15.7 ± 1.5	15.7 ± 1.5
Number of viable litters at birth	28	26	28	27#
Viable pups at birth (%)	405/416 (97.4)	385/398 (96.7)	403/416 (96.9)	346/400 (86.5)*
Mean litter size of viable pups at birth (Mean ± S.D.)	14.5 ± 1.9	14.8 ± 1.7	14.4 ± 1.5	12.8 ± 2.9*
Post-implantation loss (%)	25/441 (5.7)	24/422 (5.7)	23/439 (5.2)	24/424 (5.7)
Duration of gestation in days (Mean ± S.D.)	21.6 ± 0.50	21.5 ± 0.51	21.5 ± 0.51	21.6 ± 0.49

* $p < 0.05$ # F816 was excluded as she bore no viable young.

Pregnancy rate = Ratio of the number of pregnant females (those showing implants or traces of implants), including those that died before littering, to the number of inseminated females.

Post implantation loss = Ratio of the number of implantation sites minus the total number of pups at birth, including dead ones, to the number of implantation sites.

Few macroscopic changes were reported for necropsy.

Pups Observations (F_1):

At the MD and HD, the 4-day survival index* of F_1 pups was decreased vs. controls 97% vs. 99.3 % and 92.1% vs 99.3%, respectively; based on these findings drug sponsor asserted that there was no important toxicologic effect noted.

There was only a decrease (~ 9% vs controls) on day 1 pp body weight of HD pups, however; this finding was reversible.

F_1 pups were observed submitted to a battery of functional assays. HD F pups were slower than the correspondent M in the appearance of upper incisors, and in the their ability to succeed in the air righting and surface righting reflexes. Although drug sponsor did not considered this developmental finding to be of toxicologic significance, reviewer considers that these findings to be gender related.

* Ratio of the # of surviving pups alive 24 hrs to the # of pups born alive.

Notable external and visceral observations reported in the drug treated F_1 pups found dead at birth/dying shortly after birth during lactation, were increases in dilatation of ureters and hydroureters mainly from the HD dams. However, similar findings were reported in F1 pups sacrificed at culling in all treatment groups. Drug sponsor did not consider these findings to be related to treatment.

When F_1 generation was mated, at wk 12 of age, fertility/reproduction was reported as unaffected.

Maternal Observations (F_1 adults):

No remarkable effects noted.

Pups Observations (F_2):

No treatment related effects were reported on the 24-hr and 4-day survival indices of the F_2 pups. There was a slight decrease in the F body weights on day 4 pp in the LD/HD groups vs. controls; but these findings were considered fortuitous by drug sponsor.

A noteworthy finding in the HD F_2 pups sacrificed at day 4 pp was again dilatation or deformed ureters or distended bladder alone or in combination (8M, 6F). Some pups (from control/drug treatment groups) dying before day 4 pp showed some of the same kidney visceral findings. Drug sponsor considered these minor anomalies and unrelated to the treatment.

In conclusion, in study, the NOAEL for maternal toxicity for UK-92,480-10 may be considered to be the MD or ~ 30 mg/kg/day administered to the pregnant rats for 36 days (starting on day 6 pi through day 20 pp) because no dams died, the duration of pregnancy/parturition were unaffected, ratio of viable pups at birth, and mean litter size. Although these dams showed a dose-related increase in RBC, this and other hematologic findings reported (increased Hct/Hgb concentrations) were not reported a being drug related.

Although the 1st/2nd generation pups (F_2 and F_3) from drug treated F_0 generation did not reveal abnormalities, remarkable findings reported were dilatation or deformed ureters or distended bladder alone or in combination. Regarding functional observational battery and spontaneous motor activity assessments, data reported no adverse effects on the parameters examined.

2.6. Genetic Toxicity Studies

Drug sponsor reported the results of 3 *in vitro* and 1 in vivo assays (1 bacterial and 3 mammalian cells) conducted to detect the genotoxic potential UK-92,480. This battery of tests is in keeping with published guidelines on studies to be conducted for registration of pharmaceuticals.

The assays are reported as conducted in compliance with GLP regulations at the drug sponsor's facilities in Groton, Conn. All 4 assays were grouped/reported under GLP Study Nos. 90-817-01/90-817-02 using different batches of UK-92,480. The criteria for acceptable assays, and for determining positive/negative response were fully described in the NDA.

2.6.1. *In Vitro*

2.6.1.1. Bacterial Cells

2.6.1.1. *In vitro* test with UK-92,480 (Lot No. 1150/262/C) for gene mutation in bacterial cells

(Ames test conducted May-July 1990)

Briefly, studies examined reverse mutation at the histidine (his) operon of Salmonella typhimurium strains TA 1537, TA 1535, TA 100 and TA 98* . The assay measures his^- to his^+ reversion induced by chemicals which cause base changes or frameshift mutation in the genome of these bacteria.

The method used in these assays was that of "direct plate incorporation", and the procedure was fully described in the NDA. A dose-related, reproducible three-fold increase over control value was considered a positive response, but drug sponsor pointed out that responses up to two-fold are occasionally noted in some assays and are not considered indicative of mutagenicity.

S. typhimurium cultures were assayed in with/without S9 fraction prepared from livers of M rats** or mice*** which had been dosed 5 days with a single i.p. injection of 500 mg/kg of Aroclor 1254. The S9 fractions used were incomplete S9 mixture (with glucose-6-phosphate) or S9 mixture (NADP).

A preliminary assay showed insoluble UK-92,480 the overlay after incubation at 0.5 up to 10 mg/plate and none at 0.1 mg/plate.

In the definitive assays, the bacterial cells were exposed to UK-92,480* (conc. ranging from 0.002 to 1 mg/plate) with and without metabolic activation of S9 fraction.

Plates were incubated for at least 60 hrs at 37o C and the average number of revertant colonies/plate recorded. Average number of revertant colonies/plate treated with the drug were compared to the spontaneous revertant colonies in control plates.

Mutagenic activity is detected by reversion to prototrophy (histidine independence.)

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* Lot No. 1150/262/C
** Rat (Crl:COBS CD(SD) BR
*** Mouse (Crl:COBS CD-1 (ICR) BR

S. Typhimurium Strains and Types of Mutations Detected

Strain Designation	Gene Affected	Additional Mutations			Mutation Type Detected
		Repair	LPS	R Factor	
TA 1535	his G46	uvrB	rfa	—	Base-pair substitution
TA 1537	his C3076	uvrB	rfa	—	Frameshift
TA 98	his D3052	uvrB	rfa	pKM101	Frameshift
TA 100	his G46	uvrB	rfa	pKM101	Base-pair & Frameshift

Positive Controls

Indicator Strain	Plate Incorporation Assay Without S9	Plate Incorporation Assay With S9
TA 1535	Sodium nitrite	2-Anthramine
TA 1537	9-Aminoacridine	2-Anthramine
TA 98	2-Nitrofluorene	2-Anthramine
TA 100	Nitrofurantoin	2-Anthramine

Each assay includes plates with an overlay of each indicator strain on which a disk with ampicillin is placed to check for the absence or presence of the R factor pKM101.

Compound	Source	Lot Number
Sodium nitrite	Mallinckrodt	KPEL
9-Aminoacridine	Sigma	96F-05641
2-Nitrofluorene	Aldrich	2610-PE
Nitrofurantoin	Sigma	114F-0512
2-Anthramine	Sigma	58F-3462

Brief Overview of Study Design: Typhimurium Reverse Mutation Assay
(Study Nos. 90-817-01/90-817-02)

Species/ Strain	Metabolic Activator System	Drug/Concen-tration Range (mg/plate)	Replicates	Positive Controls (With/without S9)	Negative Control	Laboratory and Date
S. typhimurium strains TA1537 TA1535 TA100 TA98	Aroclor-induced **mouse** or **rat** liver microsomal enzyme mix (S9 fraction)	UK-92,480 dissolved in DMSO at 0.002, 0.02, 0.05, 0.2, 1.0. (This last conc. precipitated)	3	Sodium nitrite 9-Aminoacridine 2-Nitrofluorene Nitrofurantoin 2-Anthramine	DMSO	May/July 1990 Sponsor's labs.

RESULTS

The concentrations of UK-92,480 tested in each assay included a level that was insoluble. Drug sponsor asserts that in all assays, negative and positive controls performed within expected historical ranges, and provided historical control data from its laboratories between January 1982 to July 1990.

There was no evidence that UK-92,480 at any level tested increased the number of revertant colonies per plate in the with and without metabolic activation S9 from either rat or mouse. In all assays, negative and positive controls performed within the expected historical control ranges.

Results reported indicate that UK-92,480 did not induce reversion in frameshift in TA 1537, TA100 and TA 98 strains or base substitution in TA 1535 and TA 100 in these Salmonella strains tested directly with the drug or after metabolic activation by liver S9 fraction from Aroclor-induced mice or rats.

2.6.1.2. Mammalian Cells

2.6.1.2.1. *In vitro* test with UK-92,480 (Lot R-1) for evaluation of gene mutation in mammalian cells.

(Studies were conducted from October 1990 and completed March 1991.)

The potential of UK-92,480* to induce mammalian cell gene mutation (forward gene mutation) was examined using the Chinese hamster ovary (CHO) cells. Forward gene mutation in mammalian DNA refers to a change in the composition or sequence of a base pair of a codon that results in either an altered gene product or a gene product that is non-functional. To be detected, this change must be heritable, non-lethal and phenotypically expressed. The cell line used was hypoxanthine guanine phosphoribosyl transferase$^+$ (HGPRT$^+$) subclone CHO-K_1BH_4 to detect gene mutation at the x-linked HGPRT loci coding for the enzyme HGPRT. The CHO-K_1BH_4 cell line was used because it has been reported as being karotypically stable with a low spontaneous mutant frequency at the HGPRT locus.

Highly mutagenic to CHO-K_1BH_4 cells were the 2 positive controls used- ethyl methanesulfonate (EMS) without metabolic activation at 50 and 400 µg/ml, and with metabolic activation 3-methylcholanthrene (3-MCA) at 5 and 10 µg/ml.

HGPRT is a nonessential enzyme in mammalian cells which catalyzes inosine monophosphate (IMP) or GMP synthesis from hypoxanthine or guanine and 5-PRPP. The heritable loss of HGPRT activity through gene mutations confers cellular resistance to the cytotoxic purine analog, 6-thioguanine (6TG) indicating missing or deficiency in HGPRT enzyme protein.

The following is a brief overview of the study design of the assay.

Mammalian Cell Gene Mutation Assay*
Using **CHO-K_1BH_4** Cells

Cell Line	Activator Used	Concentration of UK-92,480	Replicates	Negative Control	Positive Control
HGPRT$^+$ Subclone CHO-K^1-BH^4	Aroclor-induced rat liver microsomal mix (S-9 fraction)	In preliminary tests: (14 conc. levels) ~2 up to 2000 µg/ml In definitive test: (6 conc. levels) 65 up to 240 µg/ml	- 2	1% DMSO	EMS (50 and 400 µg/ml) 3-MCA (5 and 10 µg/ml)

Methods used in these studies were fully reported in the NDA. The solvent of choice was DMSO and a 1% sol. was used as the negative control in this study. Preliminary cytotoxicity studies were performed on UK-92,480 to determine the appropriate conc. for the definitive assay. Results are based on colony formation ability of cells treated (5 hr) with a range of UK-92,480 concentrations, both directly or with metabolic activation (S9 fraction from livers of Aroclor induced rats). Cytotoxicity was determined by the percentage (%) of colony counts in treated cultures compared to control cultures. The HGPRT+ subclone CHO-K_1BH_4 in logarithmic growth was used for testing UK-92,480. Briefly, cells were plated in flasks at a predetermined density (1.25×10^6 cells) per flask in media containing 10% fetal bovine serum (FBS), and 24 hours later (Day 1) the monolayers were washed/prepared for direct experiments. Cultures were exposed to selected concentrations of UK-92,480 (with/without S9) or to positive controls (at conc. mentioned above) or negative control (DMSO).

Following treatment, the cells were prepared/plated in petri containing complete medium. Colonies were fixed/stained/counted following an 8-day incubation period. Cytotoxicity (day 1) was determined by the % of colony counts in treated cultures vs control cultures.

To allow for the expression of induced mutations, a known number of cells per culture were plated/subcultured at different times in complete medium containing 6TG to detect mutant cells and medium without 6GT to determine viable counts (cloning efficiency.) All plates were then incubated, cell colonies stained/counted. Mutant per survivors (mutant frequency) were then calculated. Statistical analyses were performed on the data using the SAS System.

RESULTS

In the preliminary cytotoxicity assay (with/without metabolic activation) at drug concentrations ranging from ~2.2 up to 2000 _g/ml, precipitation of UK-92,480 occurred in medium at ~141 µg/ml and above. Without metabolic activation (direct), the greatest level of cytotoxicity observed was at 2000 µg/ml with an absolute cloning efficiency of ~34% vs ~74% of the negative control DMSO; with metabolic activation greatest level of cytotoxicity observed was also at 2000 µg/ml with an absolute cloning efficiency of 48% vs ~81 for DMSO solvent control.

In the definitive mutagenicity study, without metabolic activation (direct), UK-92,480 was tested at 6 duplicate concentrations of 65, 84, 109, and 240 µg/ml UK-92,480, precipitation of the drug in medium occurred at 84 µg/ml and above. On Day 1 of test without metabolic activation, cytotoxicity was not observed in this range of UK-92,480 conc. tested. On Day 15 mutagenicity test without metabolic activation, UK-92,480 at the concentrations tested absolute cloning efficiency was unaffected and was within the range of the negative control DMSO. On Day 1 of test with S-9 metabolic activation, cytotoxicity was not observed in this range of UK-92,480 conc. tested. On Day 15 mutagenicity test with metabolic activation, UK-92,480 at the concentrations tested absolute cloning efficiency was unaffected and was within the range of the negative control DMSO. Mutants cells per 10^6 survivors of UK-92,480 and positive controls drugs treated cells were within the range of the historical controls.

From the data collected it may be concluded that the mutagenicity tests with metabolic activation and direct assay (without metabolic activation), provided acceptable results for the evaluation of UK-92,480 in the CHO/HGPRT assay. The results reported indicate that no significant dose-dependent relationships exists between concentration of UK-92,480 tested and transformed cells thus, UK-92,480 does not appear to induce forward mutation at the HGPRT locus in these CHO ($CHO\text{-}K_1BH_4$) cells.

The tables below were selected from numerous tables provided by drug sponsor to show the results of in vitro assays for evaluation of potential genetic damage in mammalian cells by UK-92,480.

CYTOTOXICITY AND MUTAGENICITY
UK-92,480
IN THE CHO/HGPRT ASSAY

Culture Number	Dose Level (µg/ml)	*Relative Cloning Eff (%)	**Absolute Cloning Eff (%)	***Mutants per 10^6 Survivors	Regression Analysis
Negative Control: 1% DMSO					
14.	—	100	97	4	
13.	—	100	94	6	
Historical Solvent Control(x ± SD)				7 ± 5	
Test Article: UK-92,480					
12.	65	109	103	7	Slope of dose-response curve is not significant ($p > 0.05$)
11.	65	100	103	3	
10. ppt	84	107	99	2	
9. ppt	84	115	103	4	
8. ppt	109	109	102	4	
7. ppt	109	111	104	8	
6. ppt	142	105	102	0	
5. ppt	142	99	101	1	
4. ppt	185	98	102	11	
3. ppt	185	108	101	3	
2. ppt	240	101	102	12	
1. ppt	240	100	104	12	
Positive Control: EMS (a direct positive mutagen)					
16.	50	93	95	42	
15.	400	51	61	240	

Experiment Number: 267
Legal Book Number: 20757-25
Cmpd. Lot Number: R-1
Test Date: 1/10/91

* Relative Cloning Efficiency (RCE) = $\frac{\text{ACE per culture}}{\text{600 cells plated Day 10}} \times 100$ Day 1

** Absolute Cloning Efficiency (ACE) = $\frac{\text{Total VC per culture}}{\text{average ACE of negative control cultures}} \times 100$ Day 15

*** Mutants per 10^6 Survivors = $\frac{\text{Mutant Counts per culture}}{\text{ACE per culture}} \times 100$ Day 15

CYTOTOXICITY AND MUTAGENICITY
UK-92,480 PLUS AROCLOR-INDUCED S9
IN THE CHO/HGPRT ASSAY

Culture Number	Dose Level (µg/ml)	*Relative Cloning Eff (%)	**Absolute Cloning Eff (%)	***Mutants per 10^6 Survivors	Regression Analysis
Negative Control: 1% DMSO					
14.	—	100	97	3	
13.	—	100	84	2	
Historical Solvent Control(x ± SD)				9 ± 6	
Test Article: UK-92,480					
12.	65	86	98	13	Slope of dose-response curve is not significant ($p > 0.05$)
11.	65	106	93	5	
10. ppt	84	99	86	11	
9. ppt	84	107	91	9	
8. ppt	109	95	93	10	
7. ppt	109	97	94	7	
6. ppt	142	99	97	9	
5. ppt	142	99	94	4	
4. ppt	185	97	101	5	
3. ppt	185	93	101	2	
2. ppt	240	90	103	2	
1. ppt	240	90	101	5	
Positive Control: 3-MCA(a positive mutagen in the presence of rat liver S9)					
10.	5	82	83	65	
15.	10	93	94	90	

Experiment Number: 266
Legal Book Number: 20757-12
Cmpd. Lot Number: R-1
Test Date: 1/8/91
S9 Source: R901016 Aroclor-Induced

ppt: precipitation occurred in media

* Relative Cloning Efficiency (RCE) = $\frac{\text{ACE per culture}}{\text{600 cells plated Day 15}} \times 100$ Day 1

** Absolute Cloning Efficiency (ACE) = $\frac{\text{Total VC per culture}}{\text{average ACE of negative control cultures}} \times 100$ Day 15

*** Mutants per 10^6 Survivors = $\frac{\text{Mutant Counts per culture}}{\text{ACE per culture}} \times 100$ Day 15

2.6.2.2. *In vitro* cytogenetic assay of UK-92,480 (Lot No. R-1) for detection of clastogenic activity using human lymphocytes.

(Study conducted between September 1990 and January 1991).

Methods used in this assay are fully described in the NDA. Briefly, sponsor states that a complete in vitro cytogenetics assay consists of a preliminary toxicity (dose-ranging) test using the mouse lymphoma cells (L5178Y) with a series of conc. of the test drug with/without metabolic activation to assess cytotoxicity (relative to survival measured by trypan blue exclusion). This is followed by the definitive test for chromosome damage using duplicate primary cultures of mitogen-stimulated human lymphocytes over a narrower range of conc. of the test drug. Prior to harvesting cell cultures are treated with the spindle inhibitor Colcemid to accumulate cell in metaphase. These mitogen-stimulated primary cultures of the human lymphocytes provide a population of actively dividing cells which are useful for detecting clastogenic activity of the test drug added to the culture medium. Toxicity of the test drug may also be monitored as a reduction in the mitotic index of the cultures.
The table below is a brief overview of the study design.

***In Vitro* Cytogenetics: Human Lymphocyte Assay**
(To detect chromosomal aberrations which were classified as chromatid or chromosome breaks and rearrangements.)

Cell Line	Metabolic Activation	Concentration of UK-92,480 (µg/ml) suspended in 1% DMSO	Replicates	Controls		Laboratory and Date of Study
				Negative	Positive	
In preliminary test: Mouse lymphoma cells (L5178Y) In definitive test: Human lymphocytes*	Aroclor-induced rat liver microsomal enzyme mix (S9 fraction)**	In preliminary test: Range finding- 7.8 up to 500*** In definitive test: 1 up to 250 with S9, and 1 up to 250 without S9.***	2 4	DMSO 1%	A direct acting mutagen: **Mitomycin-C** 0.05 _g/ml With metabolic activation the clastogen: **Cyclophosphamide** 10 µg/ml	Sponsor's labs? Sept. '90 - Jan '91

* Human lymphocytes were cultured with the mitogen M-phytohemagglutinin at 1%.
Following exposure to UK-92,480, the human lymphocytes were treated with the spindle inhibitor Colcemid to arrest cells in metaphase-like phase.
** The metabolic activator (liver S9 fraction) was obtained from M rats (Crl:COBS CD, SD) pretreated for 5 days with single **ip** doses of Aroclor 1254.
***For the preliminary toxicity test using L5178Y cells, these were treated with 7 doses of UK-92,480 (ranging from 7.8 up to 500 µg/ml suspended ~1% DMSO) assessed cytotoxicity. In the definitive test, human lymphocytes are treated with a narrower conc. range of UK-92,480.

Drug sponsor followed the method briefly described above, using UK-92,480, to detect potential for in vitro clastogenic activity in human lymphocyte cultures with/without exogenous metabolic activation.

In the preliminary toxicity study in mouse lymphoma cell a drug conc. range of 7.8 to 500 µg/ml was used.

In the definitive assay (with/without metabolic activation) human lymphocytes cultures were treated with 1 up to 250 µg/ml UK-92,480 dissolved in DMSO. Concurrent positive controls cultures were treated with the direct acting mutagen mitomycin C without metabolic activation, and with cyclophosphamide with the required metabolic activation for clastogenicity. In the assay, cell exposed for 3 hrs to the drug were, then centrifuged, resuspended and incubated for and additional 24 hrs, with Colcemid present for the last 3 hrs.

After treatments cells were harvested/stained, and the frequency of mitosis (defined as the number of metaphase figures/total nuclei) was determined for each culture of 1000 cell samples. At least 100 metaphase figures were analyzed for chromosome aberration from each culture and aberrations were classified as either chromatid or chromosome breaks and rearrangements. Pulverized chromosome were collected in the total tally as abnormal cells. Polyploid cells and cells that contained gaps were recorded but not included in the total abnormal cell tally.

RESULTS:

Overall, in the preliminary assays (dose-ranging/cytotoxicity) with mouse lymphoma cells, sponsor reports that UK-92,480 at conc. ranging from 7.8 up to 500 µg/ml (without metabolic activation vs vehicle control DMSO, produced marginal reductions in cell viability (determined by trypan blue exclusion) up to 62.5 µg/ml. No substantial reduction in cell viability was noted with metabolic activation. Viability was not determined in cultures treated at the higher conc. of the drug (with/without metabolic activation) because crystal precipitates made the plates difficult to score.

In the definitive assays with human lymphocytes, cultures treated with UK-92,480 conc. of 100 up to 250 µg/ml with metabolic activation gave conflicting findings. In one study only at 100 µg/ml level was there a significant difference ($p \leq 0.05$) from solvent control in the number of cells with multiple aberrations; in repeat study at the same levels of the drug, **no** significant difference in the number of abnormal cells between the treated and either the concurrent/or historical controls were reported. In both of these assays, there was a nominal to substantial in mitotic index suppression* (range from 18 to 48% decreased) compared to the solvent controls suggesting toxicity; a mitotic index suppression index of 48% was reported for the positive control Mitomycin-C chromatid or chromosome breaks, rearrangements and multiple aberrations.

In the definitive assay without metabolic activation, compared to concurrent DMSO control/historical controls, UK-92,480 (10 up to 25 µg/ml) produced **no** significant increase in the number of abnormal cells. The mitotic index suppression was reported as 22-49% compared to solvent control; 66% reduction for Cyclophosphamide-the concurrent positive control.

From the data, drug sponsor asserted that although statistically significant elevations in the number of abnormal cells compared to concurrent controls were noted at 100 up to 250 µg/ml UK-92,480 in the first definitive study with metabolic activation, the number of abnormal cells at the same drug conc. range were noted in the repeat assay and these were no different from concurrent or historical negative control values. Thus, the data from the activation assay of UK-92,480 in human lymphocytes for chromosome damage did not meet the drug sponsor's reproducibility criterion for a positive response. In absence of exogenous metabolic activation, when compared to concurrent or historical control values, none of those same drug conc. levels of

* Mitotic index suppression (%)- Expressed as the % reduction of the treated mean mitotic index vs the mean mitotic index of the solvent control.

UK-92,480 tested caused an increase in the number of abnormal cells.

2.6.2. ***In Vivo***

2.6.2.3. In vivo test for chromosome damage using rodent hematopoietic cells (Micronucleus test).

(Study conducted during Oct. to Nov. 1990).

Drug sponsor asserted that the induction of chromosome damage *in vivo* in erythrocyte precursors can be detected either by direct observation of the damage in bone marrow cells undergoing mitosis (metaphase analysis) or by observation of elevated numbers of micronuclei in interphase of the daughter cells. These micronuclei represent chromosomes (intact or structurally altered) that are not incorporated into the main nucleus during telophase (last stage cell divisions).

Quantitation of micronuclei frequency in the resultant daughter cells gives evidence of clastogenic activity and/or aneuploidy induction in the parental cells. Although micronuclei can be detected in any cell type, the mechanics of erythropoiesis provides a cell population in which micronuclei can be easily detected/quantitated.

After the final mitotic cycle of the erythroblast, the immature erythrocyte extrudes its nucleus and is expelled into the peripheral blood. This process takes ~ 24 to 48 hrs after the final mitosis. The cytoplasm of the resultant immature erythrocyte contains significant amounts of RNA which dissipate over the next 24 to 48 hours. This cell type is called polychromatic erythrocyte (**PCE**) because of the presence of RNA, and can be identified by several selective staining procedures.

The presence of micronuclei in PCE can be observed/quantitated either in bone marrow or in peripheral blood. The method used to detect potential damage by UK-92,480 to the chromosome or mitotic apparatus of mice was described in detail in the NDA.

The following is an brief overview of the study design.

In Vivo Cytogenetics Assay in Mammalian Bone Marrow

(Micronucleus Assay)

Cell Type	Number, Species and Strain	Dose Range (mg/kg p.o.)	Replicates	Controls		Laboratories and Date of Study
				Negative	Positive	
Bone marrow from femur of UK-92,480 treated mice	5 M, 5 F Mice [Crl:COBS CD(1CR) BR] per group	UK-92,480 susp. in 0.1% methylcellulose at 0, 250, 1000 and 2000 for 3 days	2	Solvent 0.1% methyl-cellulose	Mitomycin-C 0.5 mg/kg/day i.p.	Sponsor's labs. Nov.-Dec. '90

Briefly, 5 mice/sex/group received treatment regimen described in the above table. UK-92,480 was suspended in distilled water containing 0.1% methylcellulose. All concentrations showed evidence of insolubility.
Positive control used was Mitomycin-C (2 mg i.p.) which was given to M/F mice. Samples of bone marrow were obtained 24 hrs after the final treatment. Smears were made from the bone marrow from the femur of each mouse. At least 2 slides were prepared from each tissue. Slides were fixed/stained with acridine orange.

Using this procedure, micronuclei stain bright yellow, normochromatic erythrocytes (**NCE**) stain dark green and **PCE** are bright red. For each preparation, 1000 PCE were scored for the presence of micronuclei. The proportion of PCE : NCE in 200 erythrocytes was determined as an index of cytotoxicity (a decrease in this proportion indicates a slowdown of erythrocyte production).

A positive response was defined as a substantial, dose-related and reproducible elevation in the number of micronucleated PCE in the treated animals. Results from a test group were considered acceptable if data is available from at least three of the mice in the group and the negative and positive controls are consistent with historical negative and positive controls. Statistical analysis was performed on data.

RESULTS:

Drug sponsor reports that in the M mouse bone marrow studies, frequencies of micronucleated PCE (% MNPCE) at all dose levels of UK-92,480 treatment were within the limits of the negative controls (solvent) with **no** evidence of clastogenicity. Negative control showed expected results which were within the range of historical controls.

Also in M, positive controls showed an elevation in % MNPCE in accordance with historical control data for positive controls.

In F mice treated with UK-92,480, there was **no** remarkable change in % MNPCE compared to solvent control (no evidence of clastogenicity); positive control responded as expected with an increase in % MNPCE, which was within historical range.

Regarding polychromatic erythrocytes (%PCE) in positive controls and drug treated mice, these values appeared to be within the same range and tended to decrease in a dose-related manner in the UK-92,480 treated groups. These decreases in UK-92,480 treated mice were interpreted by sponsor as drug-related cytotoxicity.

The results from these studies gave no clear evidence that treatment with UK-92,480 induced micronuclei in the polychromatic bone marrow erythrocytes of M or F mice. Reduction in the % of polychromatic erythrocytes was noted in these mice at all dose levels of the drug suggesting that the doses of UK-92,480 used were near the maximum tolerated dose.

The following summary tables were submitted by drug sponsor showing the findings in the mice in vivo cytogenic micronucleus test with UK-92,480.

Effect of UK-92,480 on micronucleus frequency in male mouse bone marrow.

	Animal No.	% MNPCE	% PCE[a]
Solvent Controls	1	0.1	41.0
	2	0.1	44.0
	3	0.2	35.0
	4	0.2	41.0
	5	0.1	37.5
Mean (± SD)		0.14 ± 0.05	39.7 ± 3.5
Positive Controls Mitomycin C 0.5mg/kg/d)	1	2.4	30.0
	2	1.8	27.0
	3	2.6	26.0
	4	3.0	24.0
	5	Died	
Mean (± SD)		2.5 ± 0.33	26.8 ± 2.45
Low Dose 500 mg/kg/d	1	0.1	34.0
	2	0.2	31.0
	3	0.1	37.0
	4	0.2	37.0
	5	0.0	36.0
Mean (± SD)		0.12 ± 0.08	35.0 ± 2.
Mid Dose 1000 mg/kg/d	1	0.1	31.0
	2	0.1	33.0
	3	0.3	34.0
	4	0.1	34.0
	5	0.1	33.0
	6		
Mean (± SD)		0.14 ± 0.09	33.0 ± 1.2
High Dose 2000 mg/kg/d	1	0.1	33.0
	2	0.0	30.0
	3	0.3	29.0
	4	0.1	28.5
	5		30.0
Mean (± SD)		0.14 ± 0.11	30.1 ± 1.7

Data location:18883:7:9

a. Based on the ratio of PCE to NCE in a sample population of 200 erythrocytes.

Statistical analysis of the 0, 500, 1000 and 2000 mg/kg/day groups by linear con indicated a p-value of 0.8918.

Effect of UK-92,480 on micronucleus frequency in female mouse bone marrow.

	Animal No.	% MNPCE	% PCE[a]
Solvent Controls	1	0.2	14.0
	2	0.1	48.0
	3	0.0	44.0
	4	0.2	47.0
	5	0.1	40.0
Mean (± SD)		0.12 ± 0.08	42.2 ± 12.3
Positive Controls (Mitomycin C 0.5 mg/kg/d)	1	2.2	32.0
	2	1.8	32.0
	3	2.2	31.0
	4	1.8	34.0
	5	1.9	28.0
Mean (± SD)		1.94 ± 0.26	31.4 ± 2.2
Low Dose 500 mg/kg/d	1	0.1	29.0
	2	0.3	31.0
	3	0.1	34.0
	4	0.2	35.0
	5	0.1	36.0
Mean (± SD)		0.16 ± 0.09	33.0 ± 2.9
Mid Dose 1000 mg/kg/d	1	0.2	27.5
	2	0.1	30.0
	3	0.0	42.5
	4	0.1	32.0
	5	0.3	29.0
Mean (± SD)		0.14 ± 0.11	32.2 ± 6.2
High Dose 2000 mg/kg/d	1	0.1	28.0
	2	0.1	28.0
	3	0.2	34.0
	4	0.1	28.0
	5	0.1	34.0
Mean (± SD)		0.14 ± 0.05	28.6 ± 2.0

From the data submitted, UK-92,480 did not induce genotoxicity in bacterial or mammalian cells assayed in vitro, neither did the drug induce clastogenic activity in vivo or in vitro assays. Positive controls and negative controls performed as expected within a reasonable range of laboratories historical control values.

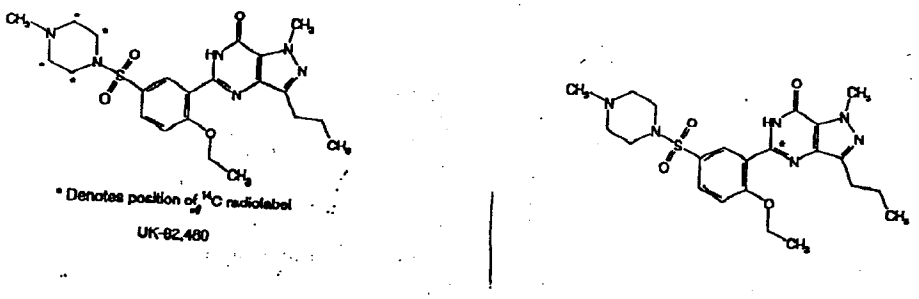
3. PHARMACOKINETICS (ADME)

3.1. Overview Of Pharmacokinetics/Toxicokinetics Of UK-92,480. (V. 1.34)

The selective CGMP-phosphodiesterase$_V$ inhibitor UK-92,480 is a lipophilic compound, with weakly basic and acidic functions and Pk_a values of 5.7 and 8.7, respectively.

The pharmacokinetics, metabolism and bioavailability of UK-92,480 have been studied in four (4) animal species of the same strains of those used in the toxicology studies: mouse (CD-1), rat (Sprague-Dawley), rabbit (New Zealand White) and beagles, and in M humans. An HPLC method was used for assaying the drug and metabolites in biologic fluids. *In vitro* methods used to investigate the metabolism of UK-92,480 included hepatic microsomes (closed vesicles of endoplasmic reticulum) from rat, dog, rabbit and man. Actual studies were performed in various laboratories and reports prepared in drug sponsor's labs in the UK.

When radiolabelled UK-92,480 were used, one form was ^{14}C labeled in the piperazine ring, and the 2nd form on the pyrimidine ring of the molecule. The figure below provided in the NDA shows positions of ^{14}C label of the drug molecule.



U-piperazinyl-14C-UK-92,480
(Nominal specific activity (NSA) of 55 µCi/mg)

Pyrimidine ring-2-14CUK-92,480 citrate
(NSA of 24.5 µCi/mg)

Briefly, in animals the drug is **absorbed** from the g.i. tract with T_{max} values reported at ~ 3 h in all species studied. In mouse, rat, dog and human, oral bioavailability is attenuated by pre-systemic hepatic metabolism (first-pass effect), the extent is greater in mouse/M rat > F rat/dog and man. The attenuated bioavailability in considered to be in agreement with the species differences in plasma clearance (ml/min/kg) values. UK-92,480 clearance (when normalized for body weight) decreases with increasing body weight across the species studied, and is the principal determinant of the shorter half-life (T_) in rodents relative to dog and man. The lower clearance, longer elimination T_ and higher bioavailability of the drug in F vs M rats is considered by the drug sponsor to reflect a species-specific gender difference in metabolic clearance. Volume of distribution (V_d; L/kg) is reported to be similar in rodents and humans but higher in dog, which is considered to be reflecting the lower plasma protein binding in the dog (_<90% v >90% in rodents, lagomorph and man). Tissue distribution using whole body autoradiography following single iv doses of ^{14}C-labeled drug in rat showed radioactivity in all tissues with residual radioactivity after 24 hrs in retina, substantia nigra and pigmented skin. The drug is extensively metabolized (17 metabolites identified in human) and drug/metabolites excreted in feces and urine

(the drug and at least one metabolite was identified in human semen.) No effect on human sperm motility was noted.

3.2. Single Dose Pharmacokinetics

3.2.1. Mouse (5M) (DM-96-148-06)

In mice, after 1 mg/kg **iv**, plasma samples per time point were pooled/analyzed for UK-92,480. The drug UK-92,480 was eliminated rapidly with an initial T 1/2 of 0.1 h. Plasma clearance was 91 ml/min/kg and V_d was 1.0 L/kg. In this study, after 0.5h post-dose the parent compound was undetectable.

After 10 mg/kg po, C_{max} of 298 ng/ml was reached at 0.5h post-dose, after which plasma conc. exhibited a biphasic decline with an apparent terminal T 1/2 of 1.3 h. Absolute bioavailability was reported as ~17%.

Plasma conc. of the N-demethylated **metabolite UK-103,320** was detected after both routes of administration. After the oral route, the plasma C_{max} ratio UK-92,480 : UK-103,320 was reported as ~ 4.8.

After a single **oral** dose of 10 mg/kg [pyrimidine-2-^{14}C]-UK-92,480 to both M/F mice, ~93 % of the dose radioactivity was recovered in **excreta** (~ 85% in feces, ~6% in urine and ~3% in cagewash) over 120 h, most radioactivity was excreted within the first 24 hrs post dosing. Conc. of radioactivity was ~40% > in plasma vs whole blood.

3.2.2. Rat (3M/3F)

UK-92,480 was given to groups of 3M/3F rats, the animals having been surgically catheterized for serial blood sampling (Study No. DM-96-148-11). Pharmacokinetic parameters were determined for each rat and mean values were calculated. Following 4 mg/kg iv, elimination T 1/2 values were 0.3h and 1.9 h in M and F rats, respectively. This gender difference in T 1/2 is considered from drug sponsor to result primarily from the higher plasma clearance in M (48 ml/min/kg) vs F (13 ml/min/kg). V_d in both sexes of rats was similar.

After 45 mg/kg po, C_{max} was higher in F rats (6.6 µg/ml) vs. M rat (477 ng/ml), reflecting the gender difference in clearance. After normalization for differences in iv and oral dose levels, absolute bioavailability was calculated for M as 15% and for F as 20%. The latter figure suggests some saturation of elimination in F at the 45 mg/kg oral dose level.

The metabolite **UK-103,320** was detected circulating after both routes of administration. After oral dosing, the plasma C_{max} ratio UK-92,480 : UK-103,320 was 0.2 in M and 5.0 in F. These findings suggest that the gender difference in elimination of the parent drug in the rat (also seen in toxicology studies) reflect more rapid formation of UK-103,320 in M animals. This findings was also supported by results from *in vitro* metabolism studies.

In 1M/1F rat at 1 mg/kg both **iv** and **po** and in which plasma samples were collected revealed a similar gender difference in T 1/2 and clearance. Although pharmacokinetic parameters varied somewhat from those reported in study DM-96-148-11 above, this change may reflect, according to the drug sponsor, differences in methods and doses tested. In this study, after oral dosing Cmax was only reported for F as 136 ng/ml at 0.5 hr; plasma concentrations of the metabolite **UK-103,320** were detectable after both routes of administration, but always at lower levels than the parent compound. A lower oral bioavailability reported in M was 23% and in F as 44%, at a higher oral dose of 45 mg/kg, suggesting according to drug sponsor that elimination is saturable in F rats at high doses UK-92,480.

3.2.3. 10 F Rabbit (DM-97-148-18)

This study was conducted to determine the pharmacokinetics of [pyrimidine 2-^{14}C]-UK-92,480 and its N-desmethyl metabolite UK-103,320. Rabbits were treated with a single oral dose of 50 mg/kg of the drug. Blood samples were collected from 3/10 animals 120 h post dose, and from 2/7 (up to 13 samples at various times intervals ranging from 0.25 up to 24 hrs so that each rabbit provided a total of 4 samples of plasma). Drug sponsor reported that plasma C_{max} for the drug as 2.19 µg/ml at 2 h post-dose, the AUC_{max} 9.50 µg.h/ml, and elimination T 1/2 1.8 h.

A plasma C_{max} 1.18 µg/ml for the metabolite UK 103,320 (AUC 5.82 µg.h/ml) was reported by 3 h post-dosing with the parent drug UK-92,480 and, T 1/2 of 4.5h for the metabolite. In these rabbits, the ratio of the parent compound: UK-103,320 was reported as ~ 1.9.

3.2.4. 5 M Beagles (DM-97-148-19)

The pharmacokinetic parameters of single **iv/oral** (oral sol. by gavage) doses of UK-92,480 were determined in M beagles, and mean values calculated. After 1 mg/kg **iv**, plasma conc. of the drug declined in a biphasic manner with a terminal elimination T 1/2 of 5.2 h. Plasma clearance was 12 ml/min/kg and V_d was 5.2 L/kg.

After 1 mg/kg **po** (gavage), plasma C_{max} for UK-92,480 was 117 ng/ml at ~ 1.1 h post-dose in all dogs, and the absolute bioavailability was calculated to be ~54%.
In this study, plasma conc. of the **metabolite UK-103,320** were detected after both routes of administration. Determined in 2 dogs, a mean plasma C_{max} ratio of
UK-92,480 : UK-103,320 after oral dosing was reported as ~ 6.9.

3.2.5. M Human (In the clinical portion of the NDA, the Human Pharmacokinetics and Bioavailability is reported in detail.)

Briefly, pharmacokinetic parameters for UK-92,480 from an **iv/oral** were reported in crossover study No. 148-208.

Repeated administration of the drug (25 and 50 mg, TID for 10 days) caused only small increases in C_{max}. The drug is rapidly metabolized after oral absorption. Elimination appeared to be biphasic with an initial rapid decline followed by a mean terminal elimination T 1/2 of 4.0 to 6 h and a plasma clearance of 9.8 ml/min/kg, and a V_d of 1.5 L/kg.

In separate clinical studies, close to linear increases were observed in AUC values and C_{max} over an the **oral** dose range of 1.25 mg to 800 mg (**Studies 148-201, 148-201A and 148-004**) and an **iv** dose range of 20 to 80 mg UK-92,480 (**Study 148-203**). Plasma C_{max} was attained by 1 h post-dose in the oral studies, and at the end of infusion (40 min) study. The metabolite UK-103,320 circulates in humans, and the plasma C_{max} ratio UK-92,480 : UK-103,320 from a range of oral studies was reported as ~ 2.5.

In an iv/oral capsule study conducted at 50 mg (~ 1 mg/kg), mean values for plasma clearance and V_d at steady state (Vss) were 41 L/h and 105 L, respectively. Mean C_{max} after oral dosing was 245 ng/ml and absolute bioavailability was 41%. Human data are compared with the pharmacokinetic parameters for animals in Table 1 prepared by sponsor.