CENTER FOR DRUG EVALUATION AND RESEARCH

Approval Package for:

Application Number: **020895**

Trade Name: **VIAGRA**

Generic Name: **Sildenafil Citrate**

Sponsor: **Pfizer**

Approval Date: **March 27,1998**

FEB 2 1998

DIVISION OF CARDIO-RENAL DRUG PRODUCTS

Joint Clinical Review

NDA: 20-895 (Sildenafil for male impotence).

Sponsor: Pfizer Pharmaceuticals, Inc.

Submission:

Submission	NDA 20-757	
	Volumes	Received
Electronic data (pre-NDA)	—	15 Aug 1997
Original NDA submission	1.1-1.150	30 Sep 1997
Biopharmaceutics data		3 Nov 1997
Biopharmaceutics data		5 Nov 1997
Safety update	4.1-4.5	8 Dec 1997
Biopharmaceutics data		18 Dec 1997
Biopharmaceutics data		22 Dec 1997
Biopharmaceutics data		8 Jan 1998

Review date: 22 January 1998.

Reviewers: K. Mahjoob, Ph.D., HFD-710

P. Marroum, Ph.D., HFD-860

N. Stockbridge, M.D., Ph.D., HFD-110

Concurrences: G. Chi, Ph.D., HFD-710

A. Parekh, Ph.D., HFD-860

Distribution:

NDA 20-895	HFD-19 (FOI)	
HFD-340/Viswanathan	HFD-110	HFD-110/CSO
HFD-710/Mahjoob	HFD-860/Marroum	HFD-110/Stockbridge
HFD-710/Chi	HFD-701/Anello	
HFD-860/Malinowsky	HFD-860/Mehta	HFD-860/Parekh

Center for Drug Evaluation and Research

Table of Contents

Viagra (Sildenafil)

"Joint Clinical Review" for NDA-20-895

Table of contents

List of tables

List of figures

Center for Drug Evaluation and Research

Viagra (Sildenafil)

"Joint Clinical Review" for NDA-20-895

Section 1, page 1 through Section 6.11, page 24

1. Materials utilized in review

1.1. Materials from NDA/IND

NDA submissions reviewed are listed on the front cover. The material included conventional study reports for a total of 71 clinical studies, most of which are individually reviewed in Appendix A. The sponsor also provided an electronic submission of study documents, all data from major placebo-controlled studies, and page-images of CRFs for deaths and withdrawals for adverse events. These materials were all reviewed.

The clinical development program for sildenafil was undertaken under IND
Some clinical study reports and clinical datasets were submitted to the IND in the weeks prior to full NDA submission.

1.2. Related reviews or consults

There are no other drugs of this pharmacological class previously reviewed. The Division has previously reviewed NDA 20-700, transurethral alprostadil for male impotence, and references are made to the clinical review of that NDA, dated 25 June 1996.

1.3. Other resources

An NLM search was conducted to look for publications involving clinical trials of sildenafil. The results of this search are discussed in section 5.2.3 on page 15.

2. Background

2.1. Indication

The proposed indication for sildenafil is for the treatment of male erectile dysfunction.

2.2. Information from related pharmacologically related agents

Sildenafil is a type V phosphodiesterase inhibitor. Other members of the class are under clinical and pre-clinical development. There are no previous NDAs for agents of this class.

2.3. Administrative history

The development program for sildenafil was managed under IND .led in The sponsor has met with DCRDP on several occasions; DCRDP encouraged changing the major efficacy end point to be sexual performance rather than erectile function. There have been no substantive administrative issues.

2.4. Proposed labeling

The proposed label is reviewed in section 9 on page 57.

2.5. Foreign marketing

As of the date of filing, sildenafil was not marketed in any country.

2.6. Other background information

None.

3. Chemistry, manufacturing, and controls[1]

3.1. Basis of review

This section of the review is based upon the Chemistry review dated 15 November 1997. This review is signed by Review Chemist J.V. Advani and Supervisory Chemist R. Wolters.

3.2. Structure

Sildenafil citrate is 1-[4-ethoxy-3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1 H-pyrazolo[4,3-d]pyrimidin-5- yl)phenylsulfonyl]-4-methylpiperazine citrate salt, with molecular formula $C_{29}H_{37}O_{11}N_6S$, molecular weight 666.7, and structure as shown in Figure 1 below.

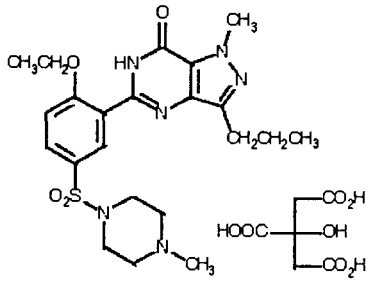


Figure 1. Structure of sildenafil.

3.3. Deficiencies

A small number of minor issues remained at the time of this review, the most notable of which was data to support a 24-month expiration date. Data had been provided in support of a 12-month expiration date.

[1] In some plases, the Chemistry Review refers to sildenafil as UK-92,480.

4. Animal pharmacology[1]

4.1. Basis of review

This section of the review is based upon draft pharmacology review (undated) by Drs. EA Barry, AF DeFelice, and T Papoian, but the interpretations are, unless otherwise noted, those of the clinical reviewers.

4.2. Mechanism of action

The data show that sildenafil is a potent, complete inhibitor of Type V phosphodiesterase. The distribution of various forms of phosphodiesterase and the relative selective selectivity of sildenafil for various forms is shown in Table 1 below.

Table 1. Inhibition of phosphodiesterase forms by sildenafil.

	Source	Mean IC_{50} (nM)		Source	Mean IC_{50} (nM)
PDE1	Human cardiac ventricle	280	PDE5	Human corpora cavernosa	3.5
	Rat kidney	430		Human platelet	6.1
	Rat diaphragm	218		Rabbit platelet	3.9
PDE2	Human corpora cavernosa	68000		Dog platelet	4.8
	Rat kidney	>100000		Rat diaphragm	1.8
	Rat diaphragm	33000			
PDE3	Human corpora cavernosa	16000	PDE6	Human retina—cone	34
	Human platelet	41000		Human retina—rod	38
	Rabbit platelet	48000		Dog retina—cone	27
PDE4	Human skeletal muscle	7200		Dog retina—rod	58
	Rat kidney	19000		Rat retina—cone	27
	Rat diaphragm	6300		Rat retina—rod	67

Sildenafil produced relaxation of corpora cavernosal smooth muscle pre-contracted by electrical stimulation, phenylephrine, or methacholine, in isolated tissues or intact animals. The effect of sildenafil appeared to be mediated by local liberation of nitric oxide, since the effects could be prevented by a nitric oxide synthase inhibitor.

Sildenafil alone had no effect on platelet aggregation, but it potentiated anti-aggregatory or dis-aggregatory effects of the nitric oxide donor, sodium nitroprusside.

4.2.1. Screening for other activities

Sildenafil had no effect on the dose-response curves for contraction of rabbit aorta by phenylephrine, constriction of rabbit coronary arteries by endothelin-1, tension in field-stimulated dog ventricular trabeculae.

Doses above that necessary to affect corpora cavernosa produced blood pressure reductions in spontaneously hypertensive rats.

Sildenafil had no effect on gastrointestinal tone in various mammalian species.

Effects of sildenafil on the electroretinogram in dogs were similar to those seen in humans[2].

Sildenafil displayed little affinity for adenosine A_1, A_{2b}, or A_3 receptors, α- and β-adrenoreceptors, dopamine D_1 or D_2 receptors, histamine H_1, 5-HT_1, 5-HT_2,

---

1. In some places, the Pharmacology Review refers to sildenafil as UK-92,480.
2. *Study 148-232: A randomised, double-blind, placebo-controlled, crossover pilot study to investigate the effects of a single oral tablet dose of sildenafil (200mg) on visual function (electroretinogram, photostress, visual field and colour discrimination tests) in healthy male volunteers and patients with diabetic retinopathy.* on page 177.

muscarinic, or opioid receptors, or for verapamil, dihydropyridine, or benzodiazepine binding sites.

In a study lacking a positive control, sildenafil had no effect on defibrillatory threshold in dogs.

Sildenafil was not found to show effects on the central nervous system—sedation, interaction with alcohol or barbiturates, or motor coordination.

Doses of irbesartan several-fold greater than necessary to antagonize angiotensin II receptor-mediated actions failed to produce central or autonomic effects in mice.

4.3. Pharmacokinetics

Single-dose pharmacokinetics are compared across species in Table 2 below, to facilitate interpretation of toxicity findings.

Table 2. Single-dose pharmacokinetics in mammals.

	Mouse	Rat (M)	Rat (F)	Rabbit	Dog	Man
Intravenous						
$t_{1/2}$ (h)	1.3	0.3	1.9	1.8	5.2	4.0
AUC (ng.h/mL/mg)	174	350	1280	—	1550	1990
CL/F (mL/min/kg)	91	48	13	—	12	9.8
V_d (L/kg)	1.0	1.1	2.0	—	5.2	1.5
Free (%)	6	5	5	9	14	4
Oral						
C_{max} (ng/mL/mg)	30	11	147	44	117	245
T_{max} (h)	0.5	1.0	3.0	2.0	1.1	1.5
AUC (ng.h/mL/mg)	31	51	252	190	842	815
Bioavail (%)	17	15	44	—	54	41
Cmax ratio (sildenafil/UK-103-320)	4.8	0.2	5.0	1.9	6.9	2.5

4.4. Toxicology

4.4.1. Genetic toxicity

A standard battery of genetic toxicity studies were performed. The results, discussed in detail in the pharmacologists' review, raise no clinical safety concerns.

4.4.2. Single-dose

Following oral administration, clinical signs were absent from mice and rats given 300 mg/kg. The minimum lethal dose is within an order of magnitude of this level. Intravenous administration was limited by solubility of sildenafil citrate; there were no clinical signs at 20 mg/kg in mice or 10 mg/kg in rats.

4.4.3. Sub-chronic/chronic

4.4.3.1. Oral

4.4.3.1.1. Rats

Ten-day oral exposure up to 500 mg/kg in rats was substantially unremarkable except for hepatic changes consistent with enzyme induction.

Thirty-day oral exposure up to 200 mg/kg in rats showed similar hepatic changes, but also showed testicular atrophy (level of seminiferous tubules; no gross effects) in most animals.

Six-month oral exposure up to 60 mg/kg in rats showed similar hepatic changes. Testicular changes are not mentioned in the review.

4.4.3.1.2. Dogs

Ten-day oral exposure up to 100 mg/kg in dogs showed dose-related decreases in blood pressure and increases in heart rate 2 hours after dosing. Dose-related increases in liver mass were also reported.

One-month oral exposure up to 80 mg/kg in dogs showed similar effects on blood pressure and heart rate.

Six-month oral exposure up to 50 mg/kg in dogs showed dose-related increases in liver mass and coronary periarteritis in the high-dose group.

Twelve-month oral exposure up to 50 mg/kg in dogs showed coronary periarteritis in the high-dose group.

The demonstrated "no-effect level" for periarteritis in these two studies of dogs was about 8 times the maximum recommended dose in man; periarteritis was observed at about 50 times the maximum recommended dose. Periarteritis has been described in animal toxicology studies of other vasodilators, and it is not believed to have any clinical significance.

4.4.3.1.3. Mice

Three-month oral exposure up to 200 mg/kg in mice revealed a minimum lethal dose of 50 mg/kg, with death attributed to gastrointestinal dilation. Increased liver mass was reported in a repeat study.

4.4.3.2. Intravenous

4.4.3.2.1. Rats

Thirteen-day intravenous exposure up to 60 mg/kg in rats showed acute vasodilation, but not other abnormalities were noted.

One-month intravenous exposure up to 4 mg/kg revealed no obviously treatment-related effects.

4.4.3.2.2. Dogs

Fourteen-day intravenous exposure up to 10 mg/kg in dogs showed increased liver mass.

One-month intravenous exposure up to 4 mg/kg in dogs showed no evidence of toxicity.

4.4.4. Chronic

4.4.4.1. Rats

Twenty-four-month oral exposure up to 60 mg/kg in rats revealed no carcinogenicity findings. Proliferative changes in thyroid follicular cells was thought to be the result of induction of the hepatic enzyme responsible for metabolism of T3 and T4. There were no significant treatment effects on body weight.

4.4.4.2. Mice

Twenty-four-month oral exposure up to 30 mg/kg in mice revealed no carcinogenicity findings and no effects on body weight. Observed mortality was largely attributed to gastrointestinal dilation.

4.4.5. Special toxicity studies

A positive-controlled antigenicity study in guinea pigs demonstrated no antigenic potential from sildenafil.

Intra-arterial injection of sildenafil in rabbits revealed no clinically significant effects.

Intestinal transit time and intestinal length were increased in mice administered oral sildenafil up to 200 mg/kg over 7 or 45 days. Similarly increased intestinal transit times were seen in mice given a single dose of 200 or 400 mg/kg.

A fairly standard battery of reproductive and neonatal studies were conducted and are described in the pharmacologists' review. There were no clinically relevant findings.

4.4.6. Safety margin

The sponsor calculated (and the pharmacologists reported) safety factors based on "no adverse effect levels" in animals and a maximum recommended dose in man, as shown in Table 3 below.

Table 3. Safety factors based upon animal toxicity and human and animal pharmacokinetics.

	Sildenafil				UK-103,320			
	Mice	Rat (M)	Rat (F)	Dog	Mice	Rat (M)	Rat (F)	Dog
Dose	2	42	42	10	—			
Free C_{max}	—	0.8	19	8	—	28	8	2
Free AUC	—	0.4	40	28	—	52	35	12

4.5. Summary of significant findings

Standard and entirely adequate animal toxicology, genetic toxicology, and reproductive toxicology studies were performed. For the most part, demonstrated effects in animals were clearly related to the specific effects of sildenafil on type 5 and 6 phosphodiesterase.

5. Description of clinical data sources

5.1. Primary source data

5.1.1. Study type and design and subject enumeration

5.1.1.1. Controlled studies Table 4 below lists controlled studies of effectiveness in subjects with erectile dysfunction. Each of these studies is the subject of a brief review in Appendix A. All such studies were randomized, double-blind, placebo-controlled studies in a population with mild-to-moderate erectile dysfunction of organic, psychogenic or mixed etiologies. There were no actively-controlled studies. In most of the larger studies, the primary end point was the answer to 2 IIEF questions pertaining to sexual performance.

Table 4. Controlled clinical trials of effectiveness.

	Review	Design	Weeks	Doses mg qd	N	Etiology (%)		
						Organ	Psych	Mixed
Fixed-dose								
Study 148-102: A double-blind, randomized, placebo-controlled, parallel group, fixed-dose, multicenter study to assess the efficacy and safety of UK-92,480 administered over six months to male patients with erectile dysfunction.	page 104	Parallel	24	25, 50, 100	532	78	9	13
Study 148-364: A double-blind, randomised, placebo-controlled, parallel group, multi-centre study to assess the efficacy and safety of fixed doses of sildenafil administered for three months to male patients with erectile dysfunction.	page 212	Parallel	12	25, 50, 100	514	43	32	25
Study 148-106: A double-blind, randomised, placebo controlled, parallel group, multicentre, fixed-dose study to assess the efficacy and safety of sildenafil administered as required to male subjects with erectile dysfunction.	page 126	Parallel	12	50, 100, 200	497	58	17	25
Study 148-101/101B: A randomised, double-blind, placebo controlled, parallel-group, fixed-dose, multicentre, long-term dose-response study to assess the efficacy and safety of sildenafil (UK-92,480) administered prior to sexual activity to male patients with erectile dysfunction.	page 99	Parallel	24	5, 25, 50, 100	416	74	8	18
Study 148-353: A randomised, double-blind, placebo controlled, parallel-group, multicentre, dose-response study to assess the efficacy and safety of sildenafil (UK-92,480) administered once daily for 28 days to patients with erectile dysfunction.	page 186	Parallel	4	10, 25, 50	351	—	58	42
Study 148-361: A 12-week, double-blind, placebo controlled, parallel group, multi-centre study followed by a 40 week open label extension to evaluate the efficacy and safety of UK-92,480 (sildenafil) in patients with erectile dysfunction.	page 203	Parallel	12	50, 100, 200	254	49	7	44
Titrated dose								
Study 148-103: A double-blind, randomized, placebo-controlled, parallel group, multicenter, flexible dose escalation study to assess the efficacy and safety of sildenafil administered as required to male patients with erectile dysfunction.	page 111	Parallel	12	25, 50, 100	329	59	15	26
Study 148-363: A double-blind, randomised, placebo-controlled, parallel group, multi-centre, flexible dose escalation study to assess the efficacy and safety of UK-92,480 administered over six months to male patients with erectile dysfunction.	page 206	Parallel	26	25, 50, 100	315	30	32	37
Study 148-104: A double-blind, randomized, placebo-controlled, parallel group, multicenter, flexible dose escalation study to assess the efficacy and safety of sildenafil administered as required to male diabetic patients with erectile dysfunction.	page 118	Parallel	12	50, 100	268	96	—	4

Table 4. Controlled clinical trials of effectiveness. *(Continued)*

	Review	Design	Weeks	Doses mg qd	N	Etiology (%)		
						Organ	Psych	Mixed
Study 148-356: A multi-centre study consisting of a 16-week open, dose-escalation phase followed by an 8-week randomised, double-blind, placebo controlled phase to assess the efficacy and safety of oral doses of UK-92,480 (sildenafil) taken as required by patients with erectile dysfunction.	page 193	Parallel	8	10-100	205	—	40	60
Study 148-367: A double-blind, randomised, placebo-controlled, two way cross-over, flexible dose study to assess the efficacy and safety of oral doses of sildenafil in patients with erectile dysfunction caused by traumatic injuries to the spinal cord.	page 218	Cross-over	6	50, 100	178	100	—	—
Study 148-359: A 12 week, double blind, placebo controlled, parallel group, multicentre study to evaluate a new sexual function questionnaire in the assessment of the efficacy of sildenafil (UK-92,480) in patients with erectile dysfunction.	page 199	Parallel	12	25, 50	111	40	39	8
Study 148-355: A double blind, randomised, placebo controlled, two way crossover study to investigate the efficacy of single doses of sildenafil (UK-92,480) (taken when required over a 28 day period) in patients with erectile dysfunction with no established organic cause.	page 191	Cross-over	4	25-75	44	—	100	0
Study 148-358: A two stage, double blind, placebo-controlled study to assess the efficacy and safety of oral doses of sildenafil (UK-92,480) in spinal cord injury patients with erectile dysfunction.	page 197	Parallel	4	50	27	100	—	—

This set of studies was the primary basis for the evaluation of effectiveness of sildenafil for the treatment of erectile dysfunction.

5.1.1.2. Clinical pharmacology

Studies of clinical pharmacology of sildenafil are listed in Table 5 below. Not included in this listing are those previously listed in Table 4 on page 8, but with pharmacokinetic data collected as well.

Table 5. Clinical pharmacology trials.

	Review	Design[a]	Doses mg qd	N	Purpose
Pharmacokinetics					
Study 148-001: Phase I single dose, open study of the clinical pharmacology of sildenafil in elderly and young healthy male volunteers.	page 89	OL	50	30	Effect of age
Study 148-003: Phase I open study to assess the effect of concomitant antacid administration on the absorption of sildenafil (UK-92,480) in normal, healthy male subjects.	page 94	R, OL, XO	50	12	Effect of gastric pH on absorption of sildenafil
Study 148-004: Phase I investigator-blind, placebo-controlled, evaluation of safety, toleration, and pharmacokinetics of sildenafil following escalating single oral doses in healthy male volunteers.	page 96	R, DB, PC, AD	100, 200, 300, 400, 600, 800	20	Single-dose pharmacokinetics
Study 148-203: A single blind, four way crossover study to investigate the pharmacokinetics of and assess the safety and tolerance of UK-92480 after administration of escalating intravenous doses in the fasted state.	page 131	R, SB, PC, XO	20, 40, 80 (i.v.)	8	Intravenous pharmacokinetics
Study 148-208: An open randomised, two way crossover study to investigate the pharmacokinetics of UK-92480 after oral administration and IV administration in the fasted state.	page 139	R, OL, XO	50	12	Comparison of oral and intravenous pharmacokinetics
Study 148-214: An open, parallel group study to determine the effects of impaired renal function on the pharmacokinetics, safety and toleration of sildenafil administered as a single 50 mg capsule dose.	page 142	OL	50	24	Effect of renal impairment
Study 148-215: An open, parallel group study to investigate the absorption, metabolism and excretion of a single oral and a single intravenous dose of radiolabeled [14C]-UK-92,480.	page 145	OL, II	25 (i.v.), 50 (p.o.)	6	^{14}C pharmacokinetics and metabolism
Study 148-221: An open, single dose study to compare the pharmacokinetics, safety and toleration of a single oral dose of sildenafil in patients with chronic stable hepatic cirrhosis to healthy subjects with normal hepatic function.	page 157	OL	50	12	Effect of hepatic impairment
Study 148-226: An open, randomised, single oral dose, three way crossover bioequivalence study to determine the pharmacokinetics of sildenafil in healthy male volunteers following administration of 100mg as capsules and tablets in the fasted state.	page 163	R, OL, XO	100	37	Comparative bioavailability for different formulations
Study 148-227: An open randomised, single oral dose, two way crossover study to determine the pharmacokinetics of sildenafil in healthy male volunteers following administration of 100 mg as commercial tablets in the fed and fasted state.	page 165	R, OL, XO	100	34	Comparison of fed and fasted pharmacokinetics

Table 5. Clinical pharmacology trials. *(Continued)*

	Review	Design[a]	Doses mg qd	N	Purpose
Study 148-228: An open, randomised, single oral dose, four way crossover study to determine the dose proportionality of the pharmacokinetics of sildenafil in healthy male volunteers over the dose range 25mg to 200mg.	page 167	R, OL, XO	25, 50, 100, 200	33	Single-dose pharmacokinetics
Pharmacokinetic drug interaction					
Study 148-002: Phase I open study to assess the potential of cimetidine to alter the pharmacokinetics of sildenafil (UK-92,480) in normal, healthy male subjects.	page 92	R, DB, PC, II	50	22	Interaction with cimetidine
Study 148-217: A double blind, randomised, placebo controlled, three way crossover study to investigate the haemodynamic and pharmacokinetic interactions of sildenafil and alcohol in healthy male volunteers.	page 151	R, DB, PC, XO	50	12	Interaction with ethanol
Study 148-218: A double blind, randomised, placebo controlled, two-way crossover study to investigate any pharmacokinetic or pharmacodynamic interaction between orally administered UK-92,480 and tolbutamide in healthy male volunteers.	page 153	R, DB, PC, XO	50	12	Interaction with tolbutamide
Study 148-219: A double-blind, randomised, placebo-controlled, two-way crossover study to assess the potential interaction between orally administered UK-92,480 (sildenafil) and warfarin in healthy male volunteers.	page 155	R, DB, PC, XO	50	12	Interaction with warfarin
Study 148-234: An open, randomised, placebo controlled, parallel group study to investigate the effects of multiple doses of erythromycin on the pharmacokinetics of a single 100mg dose of sildenafil.	page 179	R, OL, PC, II	100	24	Interaction with erythromycin
Pharmacodynamics					
Study 148-105: A double-blind, randomised, placebo controlled, four-way crossover study to investigate the efficacy, safety and toleration of single oral dose of sildenafil (25, 50, and 100 mg) in patients with male erectile dysfunction.	page 124	R, DB, PC, XO	25, 50, 100	54	Single-dose Rigiscan
Study 148-350: A double blind, randomised, placebo controlled, two way crossover pilot study to investigate the efficacy and safety of UK-92,480 (sildenafil, 25mg tid for 7 days) in patients with impotence.	page 183	R, DB, PC, XO	75	16	Multiple-dose Rigiscan
Study 148-351: A double blind, randomised, placebo controlled, four way crossover study followed by a double blind, randomised, placebo controlled, two way crossover study to investigate the efficacy of single doses of UK-92,480 (sildenafil) in patients with erectile dysfunction with no established organic cause.	page 184	R, DB, PC, XO	10, 25, 50	12	Single-dose Rigiscan
		R, DB, PC, XO	25	12	Multiple-dose erectile function
Study 148-357: A multi-centre, double blind, randomised, placebo controlled, three way crossover study to investigate the efficacy of single oral doses of sildenafil (UK-92,480) in diabetic patients with penile erectile dysfunction.	page 195	R, DB, PC, XO	25, 50	21	Single-dose Rigiscan in diabetics
		R, DB, PC, II	50	21	Multiple-dose erectile function in diabetics
Study 148-358: A two stage, double blind, placebo-controlled study to assess the efficacy and safety of oral doses of sildenafil (UK-92,480) in spinal cord injury patients with erectile dysfunction.	page 197	R, DB, PC, XO	50	27	Single-dose Rigiscan
		R, DB, PC, II	50	27	Multiple-dose erectile function in diabetics
Study 148-360: A double-blind, randomised, placebo controlled, two-way crossover study to investigate the onset of action of single oral doses of UK-92,480 (sildenafil) 50mg in patients with penile erectile dysfunction without an established organic cause.	page 201	R, DB, PC, XO	50	17	Single-dose Rigiscan in spinal cord injury
Study 148-369: A double blind, randomised, placebo controlled, sequential design, two way crossover study to investigate the duration of action of a single oral dose of sildenafil (100 mg) on penile erectile activity during visual sexual stimulation in patients with male erectile dysfunction without an established organic cause.	page 222	R, DB, PC, XO	100	16	Single-dose Rigiscan in psychogenic erectile dysfunction
Study 148-204: An open study in normal volunteers to investigate the effects of an escalating brachial artery infusion of UK-92,480 on forearm blood flow and forearm venous compliance.	page 133	OL, BL	0.003-1 (i.a.)	12	Forearm blood flow
Study 148-301: An open single intravenous dose study of the haemodynamic effects of UK-92,480 (sildenafil) in patients with stable ischaemic heart disease.	page 181	OL	40 (i.v.)	8	Invasive hemodynamics
Pharmacodynamic drug interaction					
Study 148-209: A double blind, randomised, placebo controlled, two-way crossover study to examine the effects of 25mg tid UK-92,480, administered as capsules, on the haemodynamic responses to glyceryl trinitrate in normal volunteers.	page 141	R, DB, PC, XO	75	12	Blood pressure response to nitroglycerin

Table 5. Clinical pharmacology trials. *(Continued)*

	Review	Design[a]	Doses mg qd	N	Purpose
Study 148-216: An open study to investigate the effects of a single dose of UK-92,480 (50mg) on bleeding time, followed by a double-blind, placebo-controlled, two-way crossover study to investigate the effects of a single dose of UK-92,480 (50mg) on aspirin-induced prolongation of bleeding time in healthy male volunteers.	page 150	R, DB, PC, XO	50	18	Bleeding time
Study 148-222: Single blind, placebo controlled, parallel group study to investigate the effects of a single oral dose of sildenafil (UK-92,480) (100mg) and isosorbide dinitrate (20mg) on aspirin-induced prolongation of bleeding time in healthy male volunteers.	page 159	R, RB, PC, ‖	100	45	Bleeding time
Study 148-225: A double-blind, placebo controlled, two way crossover study to investigate the effects of a single dose of sildenafil (100 mg) on blood pressure in subjects with essential hypertension being treated with amlodipine.	page 162	R, DB, PC, XO	100	16	Blood pressure response with amlodipine
Study 148-230: A double blind, placebo controlled, randomised, two way crossover study to investigate the effects of a single dose of sildenafil (50mg) in patients with stable angina taking isosorbide mononitrate oral therapy.	page 173	R, DB, PC, XO	50	16	Blood pressure response with isosorbide mononitrate
Study 148-231: A double blind, placebo controlled, randomised, two way crossover study to investigate the effects of a single dose of sildenafil (50mg) in patients with stable angina taking sublingual glyceryl trinitrate (GTN) therapy.	page 175	R, DB, PC, XO	50	16	Blood pressure response with glyceryl trinitrate
Special safety and other					
Study 148-206: A single blind, two way crossover, placebo controlled pilot study to investigate the effects of UK-92,480 (sildenafil) on platelet function in normal male volunteers.	page 134	R, SB, PC, XO	50	8	Platelet function
Study 148-207: A double blind, placebo controlled, single dose study followed by a double blind, placebo controlled 10-day multiple dose study to investigate the pharmacokinetics, platelet effects, safety and toleration of UK-92,480 (sildenafil) in healthy male volunteers.	page 135	R, DB, PC, ‖	25, 50, 75	38	Platelet function
Study 148-223: A double-blind, randomised, placebo controlled, four period crossover study to assess the effect of orally administered sildenafil (50, 100 and 200mg) on visual function in healthy male volunteers.	page 160	R, DB, PC, XO	50, 100, 200	16	Vision
Study 148-229: A double-blind, randomised, single oral dose, four period, two-way crossover pilot study to investigate the acute effects of sildenafil on sperm motility.	page 170	R, DB, PC, XO	100	17	Sperm motility
Study 148-232: A randomised, double-blind, placebo-controlled, crossover pilot study to investigate the effects of a single oral tablet dose of sildenafil (200mg) on visual function (electroretinogram, photostress, visual field and colour discrimination tests) in healthy male volunteers and patients with diabetic retinopathy.	page 177	R, DB, PC, XO	200	16[b]	Vision
Study 148-401: Statistical report a psychometric validation of the international index of erectile function (IIEF) in male patients with erectile dysfunction and age-matched controls.	page 223	ED vs normal control	None	58	IIEF validation
Study 148-451: A study to generate sexual function and quality of life data in male subjects who do not have a diagnosis of erectile dysfunction.	page 224	Normal control	None	109	IIEF validation

a. R=randomized; DB=double-blind; OL=open-label; PC=placebo-controlled; AC=active-control; AD=ascending dose; ‖=parallel; XO=cross-over.

b. Includes 8 subjects with diabetic retinopathy for whom no data were reported.

5.1.1.3. Open-label extensions

Other studies contributing to the safety assessment, generally long-term extensions to other studies listed here, are listed in Table 6 below. A few completed studies are subjects of individual study reports. Studies shown without a review were listed as being in progress as of the cut-off date for the NDA. All such contributed to the open-label safety experience, but studies in progress did not have complete reporting of adverse events.

Table 6. Long-term, open-label studies.

	Review	Extension to studies
Study 148-101C: An open, non-comparative study to assess the long-term safety of sildenafil in patients with erectile dysfunction.	page 102	148-101/101B
Study 148-354A: An open, non-comparative study to assess the efficacy and safety of UK-92,480 (sildenafil) taken over a 52-week period by patients with erectile dysfunction.	page 189	148-350, 148-351, 148-353, 148-355
Study 148-102C: An open, non comparative study to assess the long-term safety of sildenafil in patients with erectile dysfunction	—	148-102
Study 148-103C: An open, non comparative study to assess the Long-Term safety of sildenafil in patients with erectile dysfunction	—	148-103
Study 148-104C: An open, non comparative study to assess the long-term safety of sildenafil in diabetic patients with erectile dysfunction	—	148-104
Study 148-354B: An open non-comparative study to assess the efficacy and safety of UK-92,480 taken over a 52 week period by patients with erectile dysfunction	—	148-356
Study 148-354C: An open non-comparative study to assess the efficacy and safety of UK-92,480 taken over a 52 week period by patients with erectile dysfunction	—	148-355, 148-357, 148-358, 148-359, 148-360, 166-301
Study 148-366: An open, non comparative study to assess the long-term safety of sildenafil in patients with erectile dysfunction	—	148-363
Study 148-365: An open non-comparative study to assess the long term safety and efficacy of sildenafil (UK-92,480) in patients with erectile dysfunction	—	148-354A, 148-354B
Study 148-361OCS: A 12 week double blind placebo controlled parallel group multicentre study followed by a 40 week open label extension to evaluate the efficacy and safety of UK-92,480 in patients with erectile dysfunction	—	148-361

5.1.1.4. Studies not reviewed in detail

Some studies were not subjected to detailed clinical review. These are listed in Table 7 below. They contribute to the safety database, but they are not otherwise considered in this review document.

Table 7. Studies not reviewed in detail.

	Comment
Study 148-201: A single blind dose escalating single oral dose study to assess the safety, toleration and pharmacokinetics of UK-92,480	Single-dose pharmacokinetics covering 1.25 to 90 mg
Study 148-201A: An extension to a single blind dose escalating single oral dose study to assess the safety, toleration and pharmacokinetics of UK-92,480	Single-dose pharmacokinetics covering 100 to 200 mg
Study 148-202: An open randomized three way crossover study to investigate the pharmacokineticsof UK-92,480 after oral administration as a solution in the fasted state and as a capsule in the fed and fasted state.	Not relevant
Study 148-205: An open study in normal volunteers to compare the effects of escalating intravenous doses of UK-92,480 and GTN on human dorsal hand vein tone	Failed study
Study 148-210: An open randomized pilot study in normal subjects to assess the pharmacokinetics of UK-92,480 after oral administration of 50 mg as a solution and as three sustained release preparations	Not relevant
Study 148-211: A double blind double dummy randomized placebo controlled 8 day multiple dose study to investigate the pharmacokinetics, pharmacodynamics, safety and toleration of orally administered UK-92,480 in healthy male volunteers	Not relevant
Study 148-213: An open randomized three way crossover study to determine the pharmacokinetics of UK-92,480 in healthy male volunteers following oral administration of 50 mg as capsules and as tablets in the fasted state	Not relevant
Study 148-220: An open randomized single dose three way crossover bioequivalence study to determine the pharmacokinetics of UK-92,480 in healthy male volunteers following oral administration of 50 mg as capsules and as tablets in the fasted state	Not relevant
Study 166-301: A double-blind, randomized, placebo-controlled, three-way crossover study to investigate and compare efficacy of a single oral solution dose of UK-114,542 with that of sildenafil (UK-92,480) in patients with no established organic cause	N=10, comparison with a similar compound
Study JP-95-501: A phase I single dose study of UK-92,480 capsule	Japanese pharmacokinetics
Study JP-95-502: A phase I food interaction study of UK-92,480 capsule	Japanese food-effect
Study JP-95-503: A phase I day multiple dose study of UK-92,480 capsule	Japanese multiple-dose pharmacokinetics
Study JP-96-601: An early phase II study of UK-92,480 capsule in patients with male erectile dysfunction	Japanese pharmacokinetics

5.1.2. Enumeration

Table 8 below is a summary of the numbers of subjects exposed in the sponsor's development program.

Table 8. Subjects exposed in clinical studies.

	Pcbo	Sildenafil		
		Only	Pcbo ⇒	Total
Phase I				
IV	28	—	—	55
PO	215	—	—	533
Total	243	—	—	576
Phase II/III				
Single-dose		98	—	98
PRN		2600	769	3369
Multiple-daily		305	—	305
Total	1832	3003	769	3772
Japanese	16	—	—	178
Total	2091	—	—	4526

5.1.3. Demographics

Table 9 below shows the distribution of subjects' age in placebo-controlled and open-label studies of sildenafil. All subjects were males and exceedingly few subjects were non-Caucasian.

Table 9. Subjects by age in placebo-controlled and open-label studies.

	Age (years)								Tot
	18-28	28-38	38-48	48-58	58-68	68-78	78-88	88+	
Placebo-controlled	10	124	351	930	1373	993	152	2	3935
Open-label	2	33	154	545	790	595	79	1	2199

Table 10 below shows the distribution of etiologies of erectile dysfunction in placebo-controlled and open-label studies.

Table 10. Etiology of erectile dysfunction in placebo-controlled and open-label studies.

	Etiology					Total
	Organic	Psychogenic	Mixed	None	Other	
Placebo-controlled	2069	720	1016	117	13	3935
Open-label	1037	505	616	33	8	2199

Baseline diseases in placebo-controlled and open-label studies are described in Table 11 below.

Table 11. Baseline disease incidence (%) in placebo-controlled and open-label studies.

	PC N=3935	OL N=2199		PC N=3935	OL N=2199
Hypertension	25	27	Depression	5.1	5.4
Diuretics	4.4	2.5	Antidepressants	3.5	1.9
Other antihypertensives	24	14	Other psychiatric illness	2.9	3.2
Smoking	24	14	Spinal cord injury	4.5	0
Diabetes mellitus	18	19	Other neurological disease	1.7	1.4
Hyperlipidemia	14	15	Peripheral vascular disease	2.3	2.2
Cardiovascular disease	14	15	Peyronie's disease	1.9	1.5
Transurethral prostatectomy	5.5	4.2	Cerebrovascular disease	1.7	1.5
Radical prostatectomy	4.4	3.9	Chronic obstructive pulm disease	1.6	1.8

5.1.4. Extent of exposure

Characterizing exposure to study drug is complicated by two factors. The bulk of the experience was with p.r.n. dosing, so the period of exposure is a poor index, and the actual doses received were not captured in the sponsor's integrated clinical database.

5.1.4.1. Placebo-controlled experience

Study reports for only 2 fixed-dose trials were accompanied by full datasets permitting an assessment of drug exposure, as shown in Table 12 below.

Table 12. Study drug exposure (doses) in studies 148-102 and 148-361.

Study	Wks	Placebo		Sildenafil								
				25 mg		50 mg		100 mg		Any		
		N	Doses	N	Doses	N	Doses	N	Doses	N	Doses	Rate[a]
148-102	24	216	14004	102	7023	107	7795	107	7055	316	21873	2.9
148-364	12	127	3705	128	4313	132	4192	127	4062	387	12567	2.7

a. Per subject per week

Planned exposure in weeks for all fixed-dose studies in shown in Table 13 below. Because of subject withdrawals, actual exposure was 3.8% less (total of about 520 subject-years) in the active treatment groups.

Table 13. Planned exposure (weeks) in placebo-controlled fixed-dose studies.

Study	Weeks	Plcbo N	Sildenafil						
			5 mg N	10 mg N	25 mg N	50 mg N	100 mg N	200 mg N	Any N
148-102	24	216	—	—	102	107	107	—	316
148-364	12	127	—	—	128	132	127	—	387
148-106	12	122	—	—	—	127	124	124	375
148-101/101B	24	83	86	—	82	83	82	—	333
148-353	4	95	—	90	85	81	—	—	256
148-361	12	59	—	—	—	62	66	67	195
Subjects		702	86	90	397	592	587	191	1862
Subject-weeks		11252	2064	360	6292	8736	8340	2292	28084

Exposure to sildenafil in placebo-controlled, titrated-dose studies (148-363, 148-103, 148-104, 148-367, 148-359, 148-355, and 5 smaller studies) totalled 9090 subject-weeks (175 subject-years).

5.1.4.2. Open-label experience

Open-label experience in Studies 148-354A, 148-101C, 148-354B, 148-102C, 148-361O, 148-365, 148-103C, 148-366, 148-104C, and 148-354C contributed a total of almost 49000 subject-weeks (942 subject-years) of exposure to sildenafil. Although dose information does not appear in the sponsor's integrated database, by trial design little of this experience lies with doses other than 50 and 100 mg.

The sponsor indicates that 559 subjects received treatment over 1 year.

5.1.4.3. Safety updates

By agreement with the Division and in consideration of the compressed time frame for review, the sponsor's safety update was restricted to deaths and serious adverse events.

5.2. Secondary source data

5.2.1. Other studies

None applicable.

5.2.2. Post-marketing experience

Sildenafil is not approved for marketing in any country.

5.2.3. Literature

A search of the on-line catalog at NLM revealed only two published descriptions of clinical trials of sildenafil. Both publications appear to refer to the same small, pilot study of sildenafil in subjects with erectile dysfunction of psychogenic etiology. Neither publication was reviewed in detail.

5.3. Adequacy of clinical experience

Almost 1000 subjects have participated in fixed-dose, placebo-controlled studies for 6 months, and another 1500 have been in such studies for 1 to 3 months. Placebo-controlled titration studies of 1 to 6 months involved another 1500 subjects. Open-label studies contribute around 1000 subject-years of exposure to sildenafil.

Whether this level of safety assessment is adequate for a drug intended to provide benefit in some aspect of quality of life is a matter of judgement, but the degree of sampling in this development program is comparable to the typical database for a new antihypertensive agent, for which there is at least the expectation of an effect on morbidity and mortality.

5.4. Data quality and completeness

Full study reports have been provided for all pertinent clinical studies. Complete machine-readable data were provided for well-controlled studies.

DSI audit of a sampling of centers in major studies uncovered no problems of a material nature.

Outside of the DSI audit, there was no attempt made as part of this review to reconcile datasets with case report forms.

6. Clinical pharmacology and biopharmaceutics

6.1. Bioavailability/bioequivalence

6.1.1. Absolute bioavailability

The absolute bioavailability of a single 50-mg sildenafil dose, relative to an intravenous dose of 50 mg infused at a rate of 1 mg/min, was estimated to be 41%. These results are in good agreement with the results of the ^{14}C study[1] in which the absolute bioavailability was estimated to be 38%.

Study 148-215 showed that the absorption of sildenafil was approximately 90% calculated from the ratio of unchanged drug in the excreta (oral/intravenous). Sildenafil accounted for 60% of the total circulating radioactivity after intravenous administration and 32% after oral administration.

6.1.2. Food effects

The results of study 148-227[2] show that the co-administration of a high-fat breakfast with a single 100-mg commercial tablet decreased the rate of absorption of sildenafil. C_{max} decreased from 514 ng/mL in the fasted state to 364 ng/mL in the fed state. Sildenafil AUC in the fasted state was 1651 ng.h/mL compared to 1489 ng.h/mL in the fed state. The time to peak concentration was prolonged by 1 hour (from 1 to 2 hours) in the fed state. The same trends were observed for the metabolite, where C_{max} decreased from 215 to 137 ng/mL and AUC decreased from 729 ng.h/mL in the fasted state to 571 ng.h/mL in the fed state. This decrease in the rate of absorption is not expected to have any clinical consequences. The results of the above study were confirmed by the population pharmacokinetic study[3] where food was found to be a significant covariate on the absorption rate constant.

6.1.3. Bioequivalence

Study 148-226[4] showed that the 25-mg research capsule (given as 4x25 mg capsules), the 100-mg research tablet and the 100-mg commercial tablet formulations are bioequivalent to each other. Both sildenafil and its metabolite meet the 90% confidence intervals of 80 to 125%. Since the metabolite UK-103,320 has about 50% of the specific activity of sildenafil and its plasma levels are about 40% of sildenafil's, UK-103,320 contributes about 15% of the drug effect and so its bioequivalence is not considered important.

6.2. Pharmacokinetics

6.2.1. Single-dose pharmacokinetics

After single oral doses of sildenafil, absorption of sildenafil is rapid with mean T_{max} around 0.8 to 1 hour. Sildenafil plasma concentrations appear to decline in a bi-exponential manner with an apparent oral clearance of 41 L/h. Sildenafil's apparent steady-state volume of distribution was estimated to be 105 L. The elimination half-life was estimated to be around 3 to 4 hours.

For metabolite UK-103,320, the maximum observed concentrations occurred within 1 hour of dosing. The elimination half-life for this metabolite was of the same order as the parent drug—between 3 and 4 hours. The plasma concentrations of the active metabolite were approximately 40% of those seen for sildenafil after oral dosing and 15% after intravenous dosing.

---

1. *Study 148-215: An open, parallel group study to investigate the absorption, metabolism and excretion of a single oral and a single intravenous dose of radiolabeled [14C]-UK-92,480.* on page 145.
2. *Study 148-227: An open randomised, single oral dose, two way crossover study to determine the pharmacokinetics of sildenafil in healthy male volunteers following administration of 100 mg as commercial tablets in the fed and fasted state.* on page 165.
3. *Population pharmacokinetic and pharmacodynamic analysis of sildenafil phase III data.* on page 75.
4. *Study 148-226: An open, randomised, single oral dose, three way crossover bioequivalence study to determine the pharmacokinetics of sildenafil in healthy male volunteers following administration of 100mg as capsules and tablets in the fasted state.* on page 163.

6.2.2. Multiple-dose pharmacokinetics

Three-times daily administration of sildenafil for eight days produced slight accumulation at all the studied doses. The accumulation ratios between day 9 and single-dose administration, based on AUC_{0-8h}, for the 25-, 50- and 75-mg doses were 1.26, 1.59, and 1.32, respectively. The corresponding accumulation ratios, based on C_{max}, were 1.07, 1.55, and 1.09. The same degree of accumulation was also observed for the metabolite UK-103,320[5]. However, since the maximum recommended dosing frequency is once daily, no clinically significant accumulation is expected.

6.2.3. ADME

Five primary pathways have been identified for sildenafil namely demethylation at either the N methyl piperazine and/or N-methyl pyrazole moieties, oxidation of the piperazine ring, loss of a two-carbon piperazine ring and aliphatic hydroxylation. Secondary pathways include multiple combinations of the primary pathways in addition to further oxidation and demethylation of the piperazine ring. The metabolic profile of sildenafil is summarized in Figure 2 below.

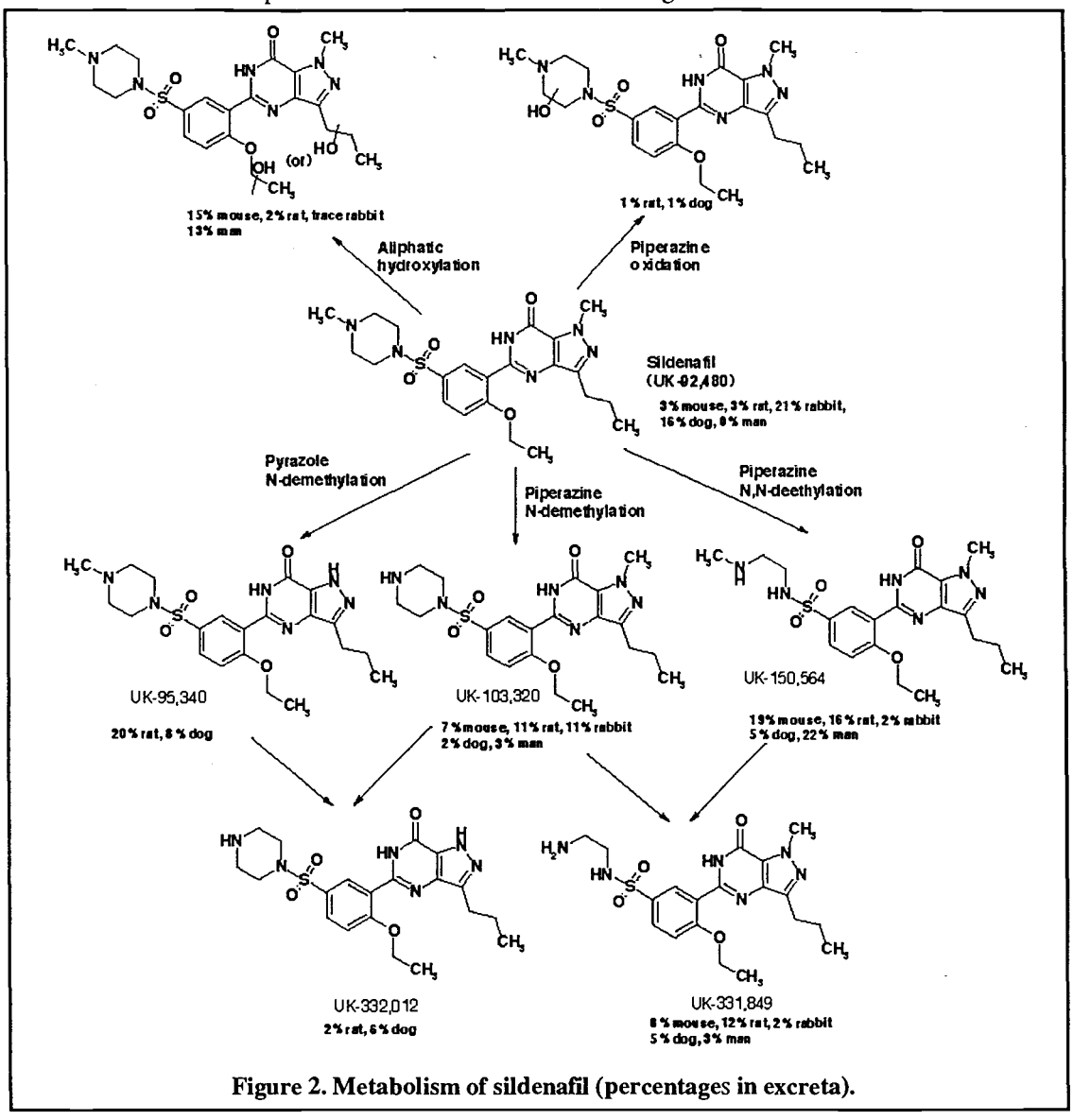


Figure 2. Metabolism of sildenafil (percentages in excreta).

[5] *Study 148-207: A double blind, placebo controlled, single dose study followed by a double blind, placebo controlled 10-day multiple dose study to investigate the pharmacokinetics, platelet effects, safety and toleration of UK-92,480 (sildenafil) in healthy male volunteers.* on page 135.

The metabolite profiles were qualitatively similar for the oral and intravenous route. The major component found in urine up to 24 hours was the aliphatic hydroxylated metabolite. The major component in fecal recoveries up to 72 hours post-dosing was UK-150,564. A further 16 metabolites were identified, individually accounting for less than 5% of the dose. There was no unchanged sildenafil detected in either the urine or feces.

In vitro studies have identified that the metabolism of sildenafil occurs in human hepatic microsomes and is mediated by two P450 isoforms, CYP2C9 and CYP3A4.

The major circulating metabolites after oral dosing were identified as UK-103,320, which was present at concentrations around 40% of the parent drug and UK-150,564, which was present at concentrations around 25% of those of the parent drug.

The majority of the radioactivity was excreted in the feces—76% of the intravenous dose and 79% of the oral dose. Less than 4% of the administered dose was excreted in the urine as sildenafil or UK-103,320[6].

In vitro studies have demonstrated that UK-103,320 has around 50% of the potency of sildenafil as a phosphodiesterase inhibitor. The major excretory metabolite UK-150,564 has 10% of the potency of sildenafil.

6.2.4. Plasma level-dose relationship

Study 148-228[7] showed that, in the dosing range between 10 and 200 mg, the pharmacokinetic parameters of sildenafil slightly deviate from proportionality. However, at doses above 200 mg, this non-proportionality becomes pronounced. At a dose of 800 mg, the AUC and C_{max} for sildenafil were 25686 ng.h/mL and 5834 ng/mL, respectively, while at a dose of 100 mg the values were 1691 ng.h/mL and 411 ng/mL, respectively. For an 8-fold increase in dose, there was a 15- and 14-fold increase in AUC and C_{max}, respectively. The same trend of results were observed for UK-103,320[8]. This pronounced nonlinearity is probably a result of saturation of the metabolic pathways of sildenafil and metabolite.

Study 148-203[9] showed that after intravenous administration, nonlinearity is evidenced at lower doses (in the range between 20 and 80 mg). The power functions indicated that non-proportionality was not extensive, with a 2-fold dose increase giving an approximately 2.5-fold increase in C_{max} and a 2.4-fold increase in AUC.

6.2.5. Concentrations in semen

The mean concentrations of sildenafil in the ejaculate, 1.5 and 4.5 hours post-dose were 18 and 17%, respectively, of those in plasma at the same time points. The concentrations of the metabolite at the same time points were 5 and 15% of those in the plasma. There were highly statistically significant relationships between the concentrations of sildenafil or UK-103,320 in semen and the total and free plasma concentrations[10].

6.2.6. Protein binding

The protein binding of sildenafil and metabolite UK-103,320 is high (96% bound) and is independent of the concentrations over the clinically relevant concentration range.

---

6. *Study 148-215: An open, parallel group study to investigate the absorption, metabolism and excretion of a single oral and a single intravenous dose of radiolabeled [14C]-UK-92,480.* on page 145.
7. *Study 148-228: An open, randomised, single oral dose, four way crossover study to determine the dose proportionality of the pharmacokinetics of sildenafil in healthy male volunteers over the dose range 25mg to 200mg.* on page 167
8. *Study 148-004: Phase 1 investigator-blind, placebo-controlled, evaluation of safety, toleration, and pharmacokinetics of sildenafil following escalating single oral doses in healthy male volunteers.* on page 96.
9. *Study 148-203: A single blind, four way crossover study to investigate the pharmacokinetics of and assess the safety and tolerance of UK-92480 after administration of escalating intravenous doses in the fasted state.* on page 131.
10. *Study 148-229: A double-blind, randomised, single oral dose, four period, two-way crossover pilot study to investigate the acute effects of sildenafil on sperm motility.* on page 170.

6.3. Special populations

6.3.1. Renal impairment

The pharmacokinetics of sildenafil and its active metabolite were significantly altered in subjects with severe renal impairment (CLcr <30 mL/min). Both the systemic exposure to sildenafil and metabolite and peak plasma concentrations were almost twice as high in this subject population compared to their healthy counterparts. The AUC for the parent drug was 1519 ng.h/mL, compared to 756 ng.h/mL, and C_{max} was 464 ng/mL, compared to 246 ng/mL for healthy volunteers. These findings were accompanied by an increased inter-subject variability in the severely impaired subjects. There were no significant differences across the various groups in protein binding (free fraction ranged from 2 to 2.7%). Moreover, there was a good correlation between C_{max}, AUC, or apparent oral clearance and the degree of renal impairment. The increased exposure is attributed to a reduction in apparent oral clearance which is directly related to the effects of renal impairment or to the effects of renal impairment on hepatic function or hepatic blood flow[11]. The effects of hemodialysis on the pharmacokinetics of sildenafil and its major metabolite was not investigated.

Based on the above results, consideration should be given to starting patients with severe renal impairment at a dose of 25 mg and titrating upwards as indicated.

6.3.2. Hepatic impairment

Study 148-221[12] showed that chronic stable hepatic cirrhosis alters the pharmacokinetics of sildenafil. The apparent clearance for the cirrhotic subjects was 46% lower leading to an 85% increase in exposure (AUC increased from 664 to 1226 ng.h/mL and C_{max} increased from 155 to 228 ng/mL after the administration of a single 50-mg dose). These results were confirmed by the population pharmacokinetic analysis that the sponsor undertook. A statistically significant relationship between SGOT (AST) levels and the sildenafil and UK-103,320 levels was found. There were 6% and 9% decreases in CL/F for every 10-unit increase of AST, for sildenafil and UK-103,320, respectively. Based on the above results, consideration should be given to starting patients with impaired liver function at a dose of 25 mg and titrating upwards as indicated.

6.3.3. Age

The results of study 148-001[13] showed that subjects of age greater than 65 years had a significantly reduced apparent clearance of sildenafil after a single 50-mg dose. CL/F was reduced about 50%, leading to an increase in AUC, compared to the young, from 586 to 1077 ng.h/mL and in C_{max} from 178 to 302 ng/mL. The differences in clearances between these two populations were attributed, by the sponsor, to differences in protein binding. The protein binding in the elderly was 96.6% compared to 95.7% in the young. Similar trends in the results were also observed for the metabolite where the plasma concentrations were doubled in the elderly compared to the young.

The population analysis confirmed the findings of study 148-001, suggesting an inverse relationship between age and apparent clearance of sildenafil. There was a 4% decrease in CL/F for each decade increase in age. Based on the above results, consideration should be given to starting patients of age greater than 65 years at a dose of 25 mg and titrating upwards as indicated.

---

[11] *Study 148-214: An open, parallel group study to determine the effects of impaired renal function on the pharmacokinetics, safety and toleration of sildenafil administered as a single 50 mg capsule dose.* on page 142.

[12] *Study 148-221: An open, single dose study to compare the pharmacokinetics, safety and toleration of a single oral dose of sildenafil in patients with chronic stable hepatic cirrhosis to healthy subjects with normal hepatic function.* on page 157.

[13] *Study 148-001: Phase I single dose, open study of the clinical pharmacology of sildenafil in elderly and young healthy male volunteers.* on page 89.

6.3.4. Diabetes Diabetes did not affect the pharmacokinetics of either sildenafil or metabolite UK-103,320[14].

6.3.5. Erectile dysfunction Subjects with erectile dysfunction achieved the same sildenafil plasma levels as their healthy counterparts.

6.4. Drug interactions

6.4.1. Tolbutamide The co-administration of a single 50-mg dose of sildenafil did not have any effect on the pharmacokinetics of a single 250-mg dose of tolbutamide[15].

6.4.2. Warfarin The co-administration of multiple 50-mg doses of sildenafil did not have any effect on either the bleeding time or prothrombin time associated with a single 40-mg dose of warfarin[16].

6.4.3. Ethanol The co-administration of a single 50-mg dose of sildenafil did not have any effect on the pharmacokinetics or hemodynamic effects of a single 0.5 g/kg dose of ethanol[17].

6.4.4. Calcium channel blockers The co-administration of a single 100-mg dose of sildenafil to mild hypertensive subjects stabilized on amlodipine 5 or 10 mg did not have any effects on either the pharmacokinetics or pharmacodynamic effects of amlodipine[18].

The population pharmacokinetic analysis showed that co-administration of calcium channel blockers did not have any effects on the pharmacokinetics of either sildenafil or its metabolite.

6.4.5. Cimetidine Study 148-002[19] investigated the effects of the co-administration of multiple 800-mg doses of cimetidine with a single 50-mg dose of sildenafil in healthy male volunteers. The results showed that cimetidine increased sildenafil's AUC by 56% and C_{max} by 54%. The AUC of UK-103,320 was increased by 30% without any increase in C_{max}. The magnitude of this pharmacokinetic interaction is not expected to have any clinical consequences.

6.4.6. Maalox The co-administration of maalox with sildenafil does not affect the pharmacokinetics of either sildenafil or UK-103,320[20].

6.4.7. Erythromycin The co-administration of multiple doses of erythromycin with a single 100-mg dose of sildenafil increased sildenafil's AUC 2.6-fold and C_{max} 2.1-fold. However, the same effect was not shown on metabolite UK-103,320, where the ratio of geometric AUC means was 1.2 and the ratio of geometric C_{max} means was 0.6. The sponsor attributed the increased sildenafil plasma levels to the inhibition of gastrointestinal CYP3A4 by erythromycin, thereby affecting the pre-systemic metabolism of sildenafil. The magnitude of increase in plasma levels suggests that one should consider starting

---

14. *Study 148-232: A randomised, double-blind, placebo-controlled, crossover pilot study to investigate the effects of a single oral tablet dose of sildenafil (200mg) on visual function (electroretinogram, photostress, visual field and colour discrimination tests) in healthy male volunteers and patients with diabetic retinopathy.* on page 177.
15. *Study 148-218: A double blind, randomised, placebo controlled, two-way crossover study to investigate any pharmacokinetic or pharmacodynamic interaction between orally administered UK-92,480 and tolbutamide in healthy male volunteers.* on page 153.
16. *Study 148-219: A double-blind, randomised, placebo-controlled, two-way crossover study to assess the potential interaction between orally administered UK-92,480 (sildenafil) and warfarin in healthy male volunteers.* on page 155.
17. *Study 148-217: A double blind, randomised, placebo controlled, three way crossover study to investigate the haemodynamic and pharmacokinetic interactions of sildenafil and alcohol in healthy male volunteers.* on page 151.
18. *Study 148-225: A double-blind, placebo controlled, two way crossover study to investigate the effects of a single dose of sildenafil (100 mg) on blood pressure in subjects with essential hypertension being treated with amlodipine.* on page 162.
19. *Study 148-002: Phase I open study to assess the potential of cimetidine to alter the pharmacokinetics of sildenafil (UK-92,480) in normal, healthy male subjects.* on page 92.
20. *Study 148-003: Phase I open study to assess the effect of concomitant antacid administration on the absorption of sildenafil (UK-92,480) in normal, healthy male subjects.* on page 94.

patients who are on known inhibitors of CYP3A4 on sildenafil 25 mg and titrating upwards as indicated[21].

6.4.8. CYP3A4 inducers The population pharmacokinetic analysis showed that co-administration of CYP3A4 inducers, such as rifampin, did not have any effects on the pharmacokinetics of either sildenafil or its metabolite. However, this analysis only included 31 subjects who were on these concomitant drugs and, therefore, these observations should be considered inconclusive. It is expected that since CYP3A4 is a major route of metabolism, co-administration of CYP3A4 inducers will lead to decreased sildenafil levels.

6.4.9. Diuretics The population pharmacokinetic analysis showed that loop and potassium-sparing diuretics decreased the apparent clearance of the metabolite UK-103,320 by 31%. This effect on the metabolite clearance is not expected to be of clinical significance. Moreover, this analysis showed that thiazide and related diuretics did not have any effects on the pharmacokinetics of sildenafil or its metabolite. Because there were only 36 subjects on these concomitant medications, the results of these analyses should be considered inconclusive.

6.4.10. β-blockers The population pharmacokinetic analysis revealed that nonspecific β-blockers decreased the apparent clearance of UK-103,320 by 51%. This decrease in clearance of the metabolite is not expected to be of clinical consequence. Cardio-selective β-blockers were found to have no effects.

6.4.11. CYP2C9 inhibitors The population pharmacokinetic analysis showed that co-administration of CYP2C9 inhibitors, such as tolbutamide or warfarin, did not have any effects on the pharmacokinetics of either sildenafil or its metabolite.

6.4.12. CYP2D6 inhibitors The population pharmacokinetic analysis showed that co-administration of CYP2D6 inhibitors did not have any effects on the pharmacokinetics of either sildenafil or its metabolite.

6.4.13. ACE inhibitors and angiotensin II antagonists The population pharmacokinetic analysis showed that co-administration of either ACE inhibitors or angiotensin II antagonists did not have any effects on the pharmacokinetics of either sildenafil or its metabolite.

6.4.14. Inhibition of CYP by sildenafil and UK-103,320 In vitro metabolic inhibition studies showed that, at the clinically relevant concentrations, neither sildenafil nor UK-103,320 had any inhibitory effects on any of the relevant CYP isozymes tested.

6.5. Population pharmacokinetic analysis

For sildenafil, the population-typical values (mean±SE) were 59±1.4 L/h for apparent clearance, 310±6.9 L for apparent volume of distribution, and 2.6±0.2 h^{-1} for K_a. The inter-individual variabilities (mean±SE of the variance) were 29±20% for CL/F, 20±50% for V/F, and 210±25% for K_a. The level of residual variability was 48±12%.

For UK-103,320, the population-typical values were 109±3.7 L/h for apparent clearance, 736±35 L for apparent volume of distribution, and 2.6±0.2 h^{-1} for the formation rate constant. The inter-individual variabilities were 49±21% for apparent clearance, 38±29% for apparent volume of distribution, and 292±21% for the formation rate constant. The level of residual variability was 46±12%.

6.6. Pharmacokinetic/pharmacodynamic relationships

Asymptotic E_{max} models with baseline and placebo components were used to describe the efficacy data. It was found that neither drug AUC nor metabolite AUC performed

[21] *Study 148-234: An open, randomised, placebo controlled, parallel group study to investigate the effects of multiple doses of erythromycin on the pharmacokinetics of a single 100mg dose of sildenafil.* on page 179.

better as a predictor of outcome than did dose. An additive model (i.e., an absolute change from baseline) was more appropriate than a relative change.

There was no obvious correlation between plasma concentrations of sildenafil and its metabolite and time to onset of erections or duration of rigidity. The mean duration of rigidity ≥60% at the base of the penis was 22 minutes for both 0- to 50- and 50- to 100-ng/mL ranges. At concentrations above 100 ng/mL, all responses exceeded 30 minutes.

The incidence of adverse events was much larger with the 200-mg dose than with lower doses. With the 200-mg dose, AUC values in excess of 2600 ng.h/mL and C_{max} values in excess of 500 ng/mL were associated with a 40% incidence of abnormal vision episodes, 15% incidence of gastrointestinal events, and 25% incidence of vascular events.

6.7. Formulations

The 25, 50 and 100 mg commercial tablet formulations are compositionally proportional, as shown in Table 14 below.

Table 14. Composition (mg) of sildenafil tablets.

	25 mg	50 mg	100 mg
Sildenafil citrate	35.112	70.225	140.450
Microcrystalline cellulose			
Dibasic calcium phosphate, anhydrous			
Croscarmelose sodium			
Magnesium stearate			
Blue			
Purified water			
Clear			

6.8. Dissolution

The sponsor undertook a series of dissolution studies in order to select the best dissolution conditions for sildenafil. Figure 3 below shows the results of the dissolution of 100-mg tablets under various rotation speeds. The sponsor found that with the USP apparatus II the phenomenon of coning was occurring. Tablets coming to rest at the bottom dead center exhibited most coning and therefore had the lowest release. Moreover, the sponsor found that complete release of sildenafil from the tablet matrix was not possible at a paddle speed of 50 rpm even with extended dissolution times. For the above reasons the sponsor chose a speed of 100 rpm for the dissolution apparatus. Figure 4 below shows the dissolution of sildenafil 25-mg tablets in the various media tested in 900 mL at a basket speed of 100 rpm.

It was observed that in water, there was a small reduction in release after storage in high humidity environments. This phenomenon was eliminated by switching the dissolution medium to 0.9% saline. Moreover, it was the opinion of the sponsor that water was a hyper-discriminating medium and thus they opted to use 0.01 M hydrochloric acid as the dissolution medium. The proposed specification was Q= in 30 minutes.

Figure 5 below shows the dissolution profiles, obtained with the proposed dissolution method, for the 25-, 50-, and 100-mg tablets, with and without the cosmetic coat. The results show that dissolution of sildenafil tablets, with and without the cosmetic coat, are similar and very rapid. Based on these results, the following dissolution method is

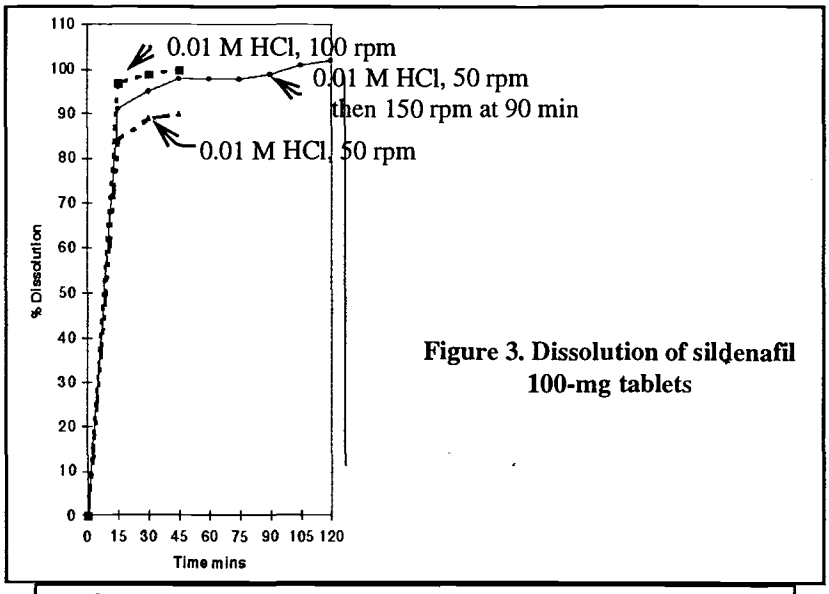


Figure 3. Dissolution of sildenafil 100-mg tablets

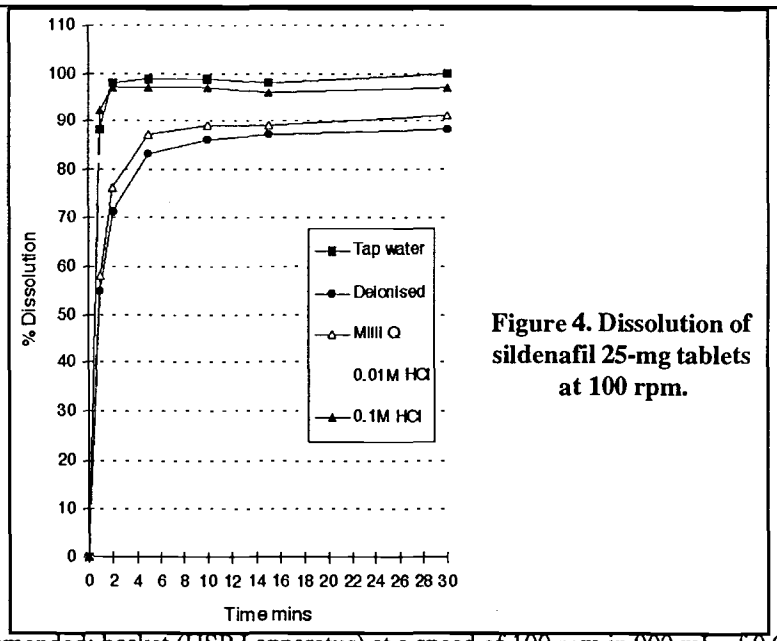


Figure 4. Dissolution of sildenafil 25-mg tablets at 100 rpm.

recommended: basket (USP I apparatus) at a speed of 100 rpm in 900 mL of 0.01 M HCl, and Q of ı 15 minutes.

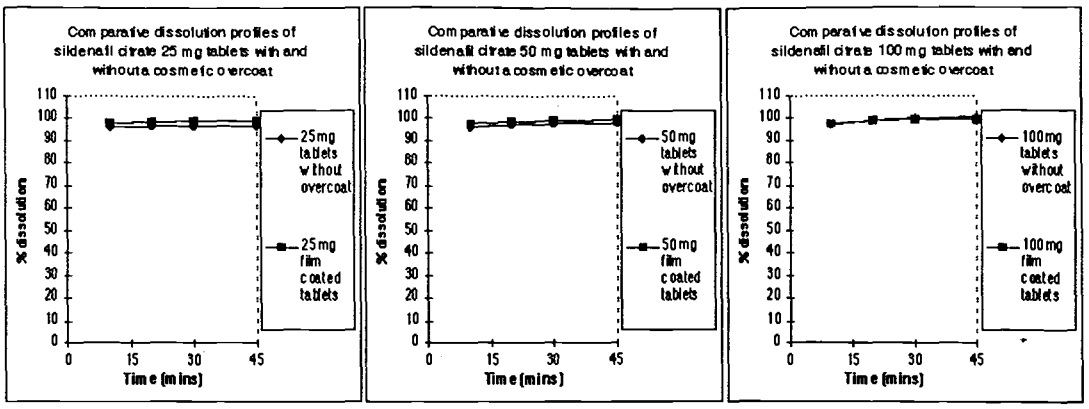


Figure 5. Dissolution of 25-, 50-, and 100-mg tablets with and without film coating.

6.9. Assay

In the vast majority of studies, an was used to measure the plasma concentrations of sildenafil and its metabolite. In a few studies a assay was used. Overall the analytical validation was satisfactory.

6.10. Comments

- The sponsor investigated the effects of sildenafil on the pharmacokinetics of tolbutamide. Valuable additional information on the pharmacokinetics of sildenafil in the presence of a competitive inhibitor CYP2C9 could have been obtained if they also assayed for sildenafil and its metabolite.
- The population pharmacokinetic analysis showed that concomitant administration of CYP3A4 inhibitors decreased the apparent oral clearance of sildenafil by only 14%. However, Study 148-234 showed there was a 182% increase in sildenafil exposure upon concomitant administration of erythromycin. These results would not have been predicted from the population analysis. The sponsor is requested to resolve the discrepancy between the results of the two analyses.
- In clinical study 148-102, the sponsor used a tablet formulation that is different in shape from the tablet used in the pivotal bioequivalence study to link the commercial tablet formulation with the formulations used in the pivotal clinical trials. The sponsor submitted dissolution data in 900 mL of 0.01 M HCl using a paddle speed of 50 rpm to show that the dissolution of this formulation is very rapid (>90% in 15 minutes) and is very similar to the commercial tablet formulation. Based on the submitted dissolution data, an in vivo bioavailability waiver is granted for the above clinical formulation (S00406AC and S00394AD).

6.11. Recommendation

The following dissolution method and specifications are recommended for sildenafil tablets: USP Apparatus I (basket) at a speed of 100 rpm and Q of n 15 minutes.

Center for Drug Evaluation and Research

Viagra (Sildenafil)

"Joint Clinical Review" for NDA-20-895

Section 7, pages 25-39

7. Integrated review of effectiveness

7.1. Mechanism of action

Clinical pharmacology studies are listed in Table 5, "Clinical pharmacology trials.," on page 9. The sponsor has cited studies indicating that 60% rigidity on penile plethysmography is adequate for penetration. This has been the basis for evaluation of effects of sildenafil in the clinic.

7.1.1. Single-dose studies

7.1.1.1. Mixed etiology

Study 148-105[1] was a randomized, double-blind, 4-period (placebo, 25 mg, 50 mg, and 100 mg) single-dose crossover study in 54 subjects organic or psychogenic erectile dysfunction (but not spinal cord injury). During each period, subjects underwent penile plethysmography during a 20-minute videotape of sexual activity (beginning 30 minutes after dosing) and for a 1-hour period following it. The mean duration of 60% rigidity was 0.06 minutes on placebo, 0.53 minutes on 25 mg, 0.39 minutes on 50 mg, and 0.95 minutes on 100 mg[2].

7.1.1.2. Psychogenic etiology

Study 148-351[3] was a randomized, double-blind, 4-period (placebo, 10 mg, 25 mg, and 50 mg) single-dose crossover study in 12 subjects with psychogenic erectile dysfunction. During each period, subjects underwent penile plethysmography during presentation of visual sexual stimulation (beginning 30 minutes after dosing) and for a 2.5-hour period following it. The mean duration of 60% rigidity at the tip of the penis was 2.9 minutes on placebo, 19 minutes on 10 mg, 26 minutes on 25 mg, and 27 minutes on 50 mg.

Study 148-360[4] was a randomized, double-blind, 2-period (placebo and 50 mg) single-dose crossover study in 17 subjects with psychogenic erectile dysfunction. Subjects underwent a 1-hour penile plethysmography, accompanied by visual sexual stimulation, beginning 10 minutes after dosing. Although the mean duration of erection was several-fold greater after sildenafil, the difference was not statistically significant.

Study 148-369[5] was a randomized, double-blind, 2-period (placebo and 100 mg) single-dose crossover study in 16 subjects with psychogenic erectile dysfunction. Two separate crossovers studies were conducted in the same subjects. Subjects underwent penile plethysmography, accompanied by visual sexual stimulation, 4 hours after dosing and then 2 hours after dosing. Erections of 60% rigidity lasted 3-times as long with sildenafil at 2 hours, and 2-times as long as placebo at 4 hours. Duration of erections correlated poorly with plasma levels of sildenafil or UK-103,320.

---

1. *Study 148-105: A double-blind, randomised, placebo controlled, four-way crossover study to investigate the efficacy, safety and toleration of single oral dose of sildenafil (25, 50, and 100 mg) in patients with male erectile dysfunction.* on page 124.
2. Analyses focussed on means may have been sub-optimal in this and the other clinical pharmacology studies described here. Many subjects, particularly on placebo, had no erections, and, as a consequence, the means do not represent a typical response.
3. *Study 148-351: A double blind, randomised, placebo controlled, four way crossover study followed by a double blind, randomised, placebo controlled, two way crossover study to investigate the efficacy of single doses of UK-92,480 (sildenafil) in patients with erectile dysfunction with no established organic cause.* on page 184.
4. *Study 148-360: A double-blind, randomised, placebo controlled, two-way crossover study to investigate the onset of action of single oral doses of UK-92,480 (sildenafil) 50mg in patients with penile erectile dysfunction without an established organic cause.* on page 201.
5. *Study 148-369: A double blind, randomised, placebo controlled, sequential design, two way crossover study to investigate the duration of action of a single oral dose of sildenafil (100 mg) on penile erectile activity during visual sexual stimulation in patients with male erectile dysfunction without an established organic cause.* on page 222.

7.1.1.3. Diabetes

Study 148-357[6] was a randomized, double-blind, 3-period (placebo, 25 mg, and 50 mg) single-dose crossover study in 21 subjects with erectile dysfunction and diabetes. Subjects underwent penile plethysmography from 15 minutes prior to dosing, through presentation of visual sexually stimulating materials, for a total of 2 hours after dosing. The mean duration of 60% rigidity at the tip of the penis was 1.3 minutes on placebo, 2.7 minutes on 25 mg, and 4.3 minutes on 50 mg.

7.1.1.4. Spinal cord injury

Study 148-358[7] was a randomized, double-blind, 2-period (placebo and 50 mg) single-dose crossover study in 27 subjects with erectile dysfunction and spinal cord injury. Subjects underwent penile plethysmography in association with 4-minute periods of vibratory stimulation 0.5, 1, and 1.5 hours after study drug administration. Although there were statistically significant sildenafil-placebo differences claimed for median durations of erections, estimates of mean effects, by time after dosing, were analyzed neither by the sponsor nor by the reviewers.

7.1.2. Multiple-dose studies

7.1.2.1. Psychogenic etiology

Study 148-350[8] was a randomized, double-blind, 2-period (placebo and sildenafil 25 mg tid for 7 days) multipile-dose, crossover study in 16 subjects with psychogenic erectile dysfunction. On day 7, subjects underwent penile plethysmography during and for 10 hours following presentation of visual sexual stimulation. The mean duration of 60% rigidity at the tip of the penis was 7.4 minutes on placebo and 36 minutes on sildenafil.

7.1.3. Effects by etiology of erectile dysfunction

The sponsor studied populations with erectile dysfunction presumably resulting from psychogenic (no known organic) causes, diabetes mellitus, spinal cord injury, and mixed organic and psychogenic etiology[9], similar to populations in principal effectiveness studies discussed in section 7.2 on page 27. The results are consistent with a beneficial effect of sildenafil on the ability of subjects with erectile dysfunction to attain an erection suitable for intercourse, regardless of the etiology of the disease. However, the data showing an effect in subjects with diabetes and spinal cord injury are much less compelling than are the data in erectile dysfunction of psychogenic and mixed etiologies.

7.1.4. Time course of effects after a dose

The time course of effects on erectile function has not been well studied. A single study in subjects with psychogenic erectile dysfunction showed greater sildenafil-placebo differences at 2 hours than at 4 hours. Most studies evaluated erectile function in the first hour after study drug administration.

7.1.5. Time course of effects with repetitive dosing

There was only one study, in subjects with psychogenic erectile dysfunction, with penile plethysmography after multiple daily dosing. This study did not have an evaluation of erectile function after the first dose, so it is difficult to interpret with respect to the development of effects with successive dosing. In this population, sildenafil was associated with erections of longer duration than was placebo, and the range of durations was not materially different than that seen in single-dose studies with subjects having psychogenic erectile dysfunction.

7.1.6. Relationship between dose and erectile function

Single-dose studies in erectile dysfunction of either psychogenic or mixed etiologies explored the dose range from 25 to 100 mg. The results suggest that 25 mg is substantially better than placebo and that 100 mg is not likely to be on the plateau of the dose-response curve.

---

6. *Study 148-357: A multi-centre, double blind, randomised, placebo controlled, three way crossover study to investigate the efficacy of single oral doses of sildenafil (UK-92,480) in diabetic patients with penile erectile dysfunction.* on page 195.
7. *Study 148-358: A two stage, double blind, placebo-controlled study to assess the efficacy and safety of oral doses of sildenafil (UK-92,480) in spinal cord injury patients with erectile dysfunction.* on page 197.
8. *Study 148-350: A double blind, randomised, placebo controlled, two way crossover pilot study to investigate the efficacy and safety of UK-92,480 (sildenafil, 25mg tid for 7 days) in patients with impotence.* on page 183.
9. The term 'mixed etiology' should be interpreted with respect to the population. Individual subjects could have organic, psychogenic, or combined causes for their erectile dysfunctions.

7.1.7. Relationship between plasma levels and erectile function

Most studies were inadequately designed (admittedly difficult) or were under-powered to assess the relationship between plasma levels of sildenafil or metabolite UK-103,320 and erectile function. The data are suggestive that plasma levels are not highly predictive of response.

7.2. Effects on sexual performance

7.2.1. Methods of assessment

7.2.1.1. Primary

The sponsor developed a standard questionnaire for obtaining information pertaining sexual function. Although some early studies were performed with end points pertaining to the ability to attain erections or some measure of subject satisfaction, the standard questionnaire, and in particular 2 questions, pertaining to sexual performance, were the primary end points of most studies of effectiveness.

> *[3] Over the past 4 weeks, when you attempted sexual intercourse, how often were you able to penetrate (enter) your partner?*
>
> *[4] Over the past 4 weeks, during sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?*

Responses to these questions (and the other 13 on the IIEF) were categorical and the sponsor's analyses assigned the categories naturally ordered integral values. Responses to IIEF questions 3 and 4 were scored as 0 for no attempts, 1 for never or rarely successful, etc., up to 5 for always or almost always successful. All randomized subjects with a post-randomization assessment were included in the sponsor's ITT analyses. The sponsor's analyses were LOCF, which tends to make placebo, which had a higher withdrawal rate, better than it otherwise would be.In analyzing studies that used these questions as the primary end point, the sponsor prospectively stated that a study would be considered 'positive' only if the p-value associated with both questions was <0.05.

For fixed-dose studies, the null hypothesis was that the slope of the dose-response curve was zero. All analyses were intent-to-treat, with last observation carried forward. Since withdrawal rates were always higher on placebo, carrying forward the last observation is more conservative than assigning a worst rank to withdrawals.

Validation of the sponsor's sexual function questionnaire is reviewed in Appendix A3 *Development and validation of the primary efficacy instrument (International Index of Erectile Function; IIEF).* on page 87. The validation procedure appears to have established this instrument as being specific for sexual function, but the relationship between responses to the questionnaire and actual performance is nowhere addressed.

7.2.1.2. Supportive

The other IIEF questions, generally treated by the sponsor as supportive, addressed other aspects of sexual function or sense of well-being, and these were analyzed in a manner similar to the primary questions.

Many studies incorporated a global assessment question, pertaing to satisfaction with treatment, a general quaility of life questionnaire, and a partner questionnaire, to which a minority of partners responded.

All studies included an event log wherein subjects reported taking doses of study drug, attempted intercourse, and successful intercourse.

7.2.2. Dose dependence

7.2.2.1. Common characteristics of fixed-dose studies

There were 6 randomized, double-blind, parallel, placebo-controlled, fixed-dose studies, evaluating doses in the range from 5 to 200 mg in the home setting. Some characteristics of these studies are shown in Table 4 on page 8. Four of these studies used the IIEF—Study 148-102, 148-106, 148-361, and 148-364—although questions 3 and 4 were the primary end points in only 3 of them.

These studies recruited men age >18, with erectile dysfunction[10] of >6 months' duration, and in a heterosexual relationship for >6 months. Subjects were excluded for (1) anatomical deformities such as severe penile fibrosis, (2) other sexual disorders such as hypoactive sexual desire, (3) elevated prolactin (3x ULN) or low free testosterone (20% below LLN), (4) major, uncontrolled psychiatric disorders, (5) history of alcohol or drug abuse, (6) history of major hematologic, renal, or hepatic disorder, (7) erectile dysfunction following spinal cord injury, (8) uncontrolled diabetes or diabetic retinopathy, (9) stroke or myocardial infarction within 6 months, (10) cardiac failure, unstable angina, ECG ischemia, or life-threatening arrhythmia within 6 months, (11) blood pressure outside 90/50 to 170/100 mmHg, (12) active peptic ulcer disease or bleeding disorder, (13) any clinically significant baseline laboratory abnormality, (14) need for anticoagulants, nitrates, androgens, or trazodone, (15) need for aspirin or NSAIDs and a history of peptic ulcer disease, (16) unwillingness to cease use of vacuum devices, intracavernosal injection, or other therapy for erectile dysfunction, (17) other experimental drug use within 3 months, or (18) history of retinitis pigmentosa.

Studies had a 4-week treatment-free run-in period during which baseline sexual performance data were collected, after which subjects were randomized and followed for 12 or 24 weeks.

7.2.2.2. Fixed-dose studies assessed by IIEF

Four fixed-dose studies assessed using the IIEF are described in Table 15 below. All of these studies excluded subjects with erectile dysfunction attributable to spinal cord injury.

Table 15. Fixed-dose studies utilizing the IIEF.

Study	Doses (mg)				N	Weeks	Etiology (%)			Diabetes (%)
	25	50	100	200			Organic	Psychogenic	Mixed	
148-102	✓	✓	✓		532	24[a]	78	9	13	15
148-106		✓	✓	✓	497	12	58	17	25	17
148-361[b]	✓	✓	✓	✓	254	12	49	7	44	?
148-364	✓	✓	✓		514	12	43	32	25	9

a. Primary end point was at week 12, but the double-blind period was 24 weeks.
b. Primary end point was IIEF question 1: ability to attain erection.

7.2.2.2.1. Analyses of sexual performance by IIEF

The sponsor's analyses of the sexual performance questions in these 4 studies are shown in Table 16 below.

[10] 'the inability to attain and/or maintain penile erection sufficient for satisfactory sexual performance'

Table 16. ITT analyses of IIEF questions 3 and 4 (fixed-dose studies).

	Study	Baseline Q[a]	Placebo		Sildenafil								P^b
					25 mg		50 mg		100 mg		200 mg		
			n	Q	n	Q	n	Q	n	Q	n	Q	
How often were you able to penetrate your partner?	148-102	2.0	190	2.3	95	3.3	100	3.7	96	4.0	—	—	<0.0001
	148-106	1.8	109	2.2	—	—	116	3.5	112	3.7	112	3.5	<0.0001
	148-361	1.9	58	1.9	61	3.4	64	3.7	66	3.7	—	—	<0.0001
	148-364	2.2	117	2.2	121	3.2	123	3.7	120	3.8	—	—	<0.0001
How often were you able to maintain your erection after penetration?	148-102	1.6	189	2.2	95	3.2	100	3.5	96	3.9	—	—	<0.0001
	148-106	1.5	109	1.7	—	—	115	3.2	112	3.6	112	3.4	<0.0001
	148-361	1.7	58	1.9	61	3.3	64	3.7	65	3.7	—	—	<0.0001
	148-364	1.8	115	2.0	119	3.0	122	3.4	118	3.6	—	—	<0.0001

a. Mean value for question.
b. P-value for non-zero slope to dose-response.

None of the sponsor's analyses were recapitulated by the reviewers. However, for 2 studies for which the complete SAS datasets were provided by the sponsor, the reviewers performed sub-group analyses based upon baseline characteristics likely to have some effect on disease severity. These results are summarized in Table 17 below. These results are entirely consistent with the sponsor's highly statistically significant treatment effect, strongly support effectiveness in subjects with erectile dysfunction of organic, psychogenic, or mixed etiology, strongly support effectiveness with or without a history of nocturnal erections, strongly support effectiveness in erectile dysfunction of relatively short or long duration (less than or greater than 3 years), and strongly support effectiveness with or without a history of previous medical or mechanical treatment of erectile dysfunction. Less compelling is the evidence from these studies that sildenafil is effective in subjects with diabetes mellitus.

Table 17. Sub-group analyses of IIEF questions 3 and 4[a] (Studies 148-102 and 148-364).

	N		How often were you able to penetrate your partner?						How often were you able to maintain your erection after penetration?					
	148-		148-102			148-364			148-102			148-364		
	102	364	Intcpt	Slope	P^b	Intcpt	Slope	P	Intcpt	Slope	P	Intcpt	Slope	P
Etiology														
Organic	411	165	0.2±0.1	16±2	0.0001	0.1±0.2	13±3	0.0001	0.4±0.1	15±2	0.0001	0.4±0.2	12±3	0.003
Psychogenic	50	129	0.2±0.2	23±5	0.0001	0.3±0.2	15±3	0.0001	0.1±0.2	28±5	0.0001	0.6±0.2	15±3	0.0001
Mixed	70	219	0.7±0.3	19±6	0.003	0.5±0.2	11±3	0.001	1.1±0.3	16±5	0.005	0.6±0.2	12±3	0.003
Nocturnal erections														
Yes	308	335	0.5±0.1	18±2	0.0001	0.2±0.1	16±2	0.0001	0.7±0.1	18±2	0.0001	0.4±0.1	17±2	0.0001
No	175	151	0.0±0.1	16±3	0.0001	0.4±0.2	11±3	0.002	0.1±0.2	15±3	0.0001	0.7±0.2	8±3	0.02
Duration														
<3 years	325	177	0.1±0.1	19±2	0.0001	0.6±0.2	8±3	0.008	0.5±0.1	17±2	0.0001	0.7±0.2	11±3	0.0007
>3 years	206	336	0.5±0.1	13±3	0.0001	0.2±0.1	15±2	0.002	0.5±0.1	17±3	0.0001	0.5±0.1	13±2	0.002
Previous treatment														
Yes	284	375	0.4±0.1	16±3	0.0001	0.4±0.1	12±2	0.0001	0.6±0.1	15±3	0.0001	0.6±0.1	12±2	0.0001
No	247	138	0.1±0.1	17±2	0.0001	0.3±0.2	15±4	0.0001	0.3±0.1	18±3	0.0001	0.5±0.2	14±3	0.0001
Diabetes mellitus														
Yes	72	44	0.4±0.2	5±5	0.29	0.3±0.3	2±5	0.72	0.5±0.2	8±5	0.11	0.4±0.2	5±4	0.26
No	459	469	0.3±0.1	18±2	0.0001	0.4±0.1	14±2	0.0001	0.5±0.1	17±2	0.0001	0.6±0.1	13±2	0.0001

a. Reviewers' LOCF analyses; slope of dose-response (change in score per g)

b. P-value for non-zero slope to dose-response analysis of treatment alone.

7.2.2.2.2. Analyses of other IIEF questions

Other aspects of the IIEF were consistent with the effectiveness of sildenafil, as shown in Table 18 below. On all questions except the one pertaining to frequency of desire, the individual studies are consistent and highly statistically significant, so appropriate adjustments for multiple end points are not at issue. For the frequency of desire question, the 2 US studies (148-102 and 148-106) show at trend toward a treatment benefit and the 2 European studies show high statistical significance.

Table 18. ITT analyses of supportive IIEF questions at week 12 (fixed-dose studies)[a].

Question	Study	Base-line	Placebo		Sildenafil								P^b
					25 mg		50 mg		100 mg		200 mg		
			n	Q	n	Q	n	Q	n	Q	n	Q	
Able to get erection	148-102	2.5	189	2.9	95	3.8	100	4.0	97	4.4	—	—	<0.0001
	148-106	2.3	108	2.3	—	—	116	3.3	112	3.7	112	3.7	<0.0001
	148-361	2.1	58	2.1	—	—	61	3.8	64	3.8	66	3.9	<0.0001
	148-364	2.5	118	2.4	123	3.4	125	3.7	120	3.9	—	—	<0.0001
Erections hard enough	148-102	2.1	190	2.1	95	3.3	99	3.8	97	4.0	—	—	<0.0001
	148-106	1.8	110	2.0	—	—	116	3.3	112	3.6	112	3.4	<0.0001
	148-361	1.9	58	2.0	—	—	61	3.5	64	3.8	66	3.8	<0.0001
	148-364	2.2	118	2.2	123	3.3	125	3.6	117	3.9	—	—	<0.0001
Difficulty maintaining erection	148-102	1.5	190	2.1	95	3.2	100	3.6	97	3.9	—	—	<0.0001
	148-106	1.5	110	1.7	—	—	116	3.4	113	3.6	112	3.4	<0.0001
	148-361	1.6	58	1.7	—	—	61	3.2	64	3.5	66	3.6	<0.0001
	148-364	1.7	114	1.9	118	3.1	124	3.6	118	3.6	—	—	<0.0001
Confidence in erection	148-102	1.6	190	2.1	95	2.7	98	3.3	96	3.4	—	—	<0.0001
	148-106	1.6	108	1.8	—	—	113	3.0	111	3.2	110	3.1	<0.0001
	148-361	1.7	57	1.9	—	—	61	3.2	64	3.4	65	3.4	<0.0001
	148-364	2.0	117	2.3	120	3.0	123	3.2	117	3.5	—	—	<0.0001
Attempted intercourse	148-102	1.9	191	2.7	95	3.1	100	3.0	97	3.6	—	—	<0.0001
	148-106	2.0	110	2.7	—	—	116	3.3	113	3.3	112	3.2	0.001
	148-361	1.5	58	1.8	—	—	61	2.6	64	2.8	66	2.9	<0.0001
	148-364	2.0	114	2.4	120	3.0	123	3.1	117	3.3	—	—	<0.0001
Satisfaction of intercourse	148-102	1.8	191	2.3	94	3.4	100	3.7	97	3.9	—	—	<0.0001
	148-106	1.6	110	1.9	—	—	116	3.2	112	3.5	112	3.6	<0.0001
	148-361	1.8	58	1.9	—	—	61	3.4	64	3.7	66	3.6	<0.0001
	148-364	1.9	114	2.1	118	3.1	122	3.5	116	3.8	—	—	<0.0001
Enjoyment of intercourse	148-102	1.8	191	2.3	94	3.0	100	3.6	97	3.8	—	—	<0.0001
	148-106	1.8	110	1.9	—	—	115	3.1	112	3.2	112	3.2	<0.0001
	148-361	1.8	58	1.8	—	—	61	2.8	64	3.1	66	3.3	<0.0001
	148-364	2.0	113	2.2	118	2.9	123	3.4	117	3.4	—	—	<0.0001
Frequency of ejaculation	148-102	3.1	189	3.2	93	4.0	97	4.2	97	4.3	—	—	<0.0001
	148-106	2.7	108	2.9	—	—	116	3.6	110	3.7	111	3.6	0.0002
	148-361	2.8	55	2.8	—	—	61	3.8	63	4.2	65	4.0	<0.0001
	148-364	3.0	118	3.2	118	3.5	121	3.9	120	4.0	—	—	<0.0001

Table 18. ITT analyses of supportive IIEF questions at week 12 (fixed-dose studies)[a].*(Continued)*

Question	Study	Baseline	Placebo		Sildenafil								P^b
					25 mg		50 mg		100 mg		200 mg		
			n	Q	n	Q	n	Q	n	Q	n	Q	
Frequency of orgasm	148-102	3.0	190	3.2	94	3.5	100	4.2	97	4.1	—	—	<0.0001
	148-106	2.7	109	2.9	—	—	116	3.6	113	3.7	112	3.5	0.0002
	148-361	2.8	55	2.7	—	—	61	3.7	63	4.1	65	3.9	<0.0001
	148-364	2.8	118	2.8	117	3.4	121	3.6	119	3.8	—	—	<0.0001
Frequency of desire	148-102	3.5	190	3.3	95	3.3	100	3.5	97	3.6	—	—	0.2
	148-106	3.3	109	3.3	—	—	115	3.5	112	3.5	111	3.5	0.4
	148-361	3.0	55	3.0	—	—	61	3.3	63	3.6	65	3.7	0.0005
	148-364	3.3	116	3.2	120	3.2	123	3.5	119	3.6	—	—	0.001
Rating of desire	148-102	3.2	190	3.2	95	3.3	100	3.4	97	3.3	—	—	0.2
	148-106	3.1	110	3.1	—	—	116	3.3	112	3.3	111	3.4	0.008
	148-361	2.8	56	2.8	—	—	61	3.0	63	3.4	66	3.5	0.0004
	148-364	3.1	116	3.1	118	3.2	123	3.3	119	3.4	—	—	0.01
Satisfaction with sex life	148-102	1.9	190	2.4	95	3.1	100	3.4	97	3.6	—	—	<0.0001
	148-106	1.9	109	2.1	—	—	116	3.2	112	3.4	111	3.5	<0.0001
	148-361	1.9	56	2.0	—	—	61	3.2	63	3.4	66	3.5	<0.0001
	148-364	2.1	118	2.3	118	3.1	123	3.4	117	3.6	—	—	<0.0001
Satisfaction with relationship	148-102	2.7	187	3.1	94	3.7	100	3.8	95	4.1	—	—	<0.0001
	148-106	2.5	108	2.6	—	—	116	3.6	111	3.8	110	3.8	<0.0001
	148-361	2.5	56	2.7	—	—	61	3.7	63	3.9	66	4.0	<0.0001
	148-364	2.6	118	2.9	116	3.3	122	3.7	116	3.8	—	—	<0.0001

a. Sponsor's analyses.
b. P-value for non-zero slope to dose-response.

For the frequency of desire question, the 2 US studies (148-102 and 148-106) show at least a trend toward a treatment benefit and the 2 European studies show high statistical significance in favor of a benefit.

7.2.2.2.3. Analyses of event logs

The sponsor's analyses of event logs were based upon the proportion of all attempts that were successful. These results are included in some of the study reports.

The reviewers' analyses of event logs, derived from fixed-dose studies for which full SAS datasets were available, are summarized in Table 19 below. The results illustrate that subjects in these trials were not profoundly incapacitated. One-third to one-half of subjects had successful intercourse during a treatment-free run-in period. The number of attempts at intercourse was not much affected by the treatment, so the sponsor's analyses of success rates was valid and informative. Whether assessed by the number of successful attempts per subject per week, the proportion of attempts that were successful, or the proportion of subjects who were successful at least once during the study, sildenafil treatment groups had markedly better sexual performance success than did placebo. However, there appeared to be very little to distinguish among the doses (25 to 100 mg).

Table 19. Successful intercourse by event logs (fixed-dose studies).

		Study #	Placebo	Sildenafil		
				25 mg	50 mg	100 mg
Attempts	Total	148-102	14004	7023	7795	7055
		148-364	3705	4313	4192	4062
	Per subject mean	148-102	65	69	73	66
		148-364	29	34	32	32
	Per subject per week	148-102	2.7	2.9	3.0	2.8
		148-364	2.4	2.8	2.7	2.7
Successes	Total	148-102	3388	2701	3994	3627
		148-364	481	1635	1808	1879
	Per subject mean	148-102	16	26	37	34
		148-364	3.8	13	14	15
	Per subject per week	148-102	0.7	1.1	1.5	1.4
		148-364	0.3	1.1	1.2	1.3
Success by attempts	(%)	148-102	24	38	51	51
		148-364	13	38	43	46
Success by subjects (%)	During run-in	148-102	43	33	47	48
		148-364	32	42	33	31
	Double-blind period	148-102	69	86	89	92
		148-364	53	79	91	82

7.2.3. Titration studies

7.2.3.1. Common characteristics of titration studies

There were 8 randomized, double-blind, parallel or crossover, placebo-controlled studies in which subjects' dose of randomized treatment could be modified, as considered appropriate by the investigator, to achieve maximum benefit or to avoid adverse effects. These studies evaluated doses in the range from 25 to 100 mg in the home setting. Some characteristics of these studies are shown in Table 4 on page 8. Five of these studies used the IIEF, although questions 3 and 4 were the primary end points in only 3 of them[11].

The inclusion and exclusion criteria in these studies were similar to those in fixed-dose studies, except that Study 148-104 was restricted to subjects with diabetes mellitus and Study 148-367 was restricted to subjects with spinal cord injury.

7.2.3.2. Titration studies assessed by IIEF

The 4 titration studies are described in Table 20 below.

Table 20. Titration studies utilizing the IIEF.

Study #	Doses (mg)			N	Weeks	Etiology (%)			Diabetes (%)
	25	50	100			Organic	Psychogenic	Mixed	
148-103	✓	✓	✓	329	12	59	15	26	10
148-104		✓	✓	268	12	96	—	4	100
148-363	✓	✓	✓	315	26[a]	30	32	37	16
148-367[b]		✓	✓	178	6	100	0	0	<1

a. Primary analysis at week 12.
b. Crossover design, subjects with spinal cord injury, 6 weeks per arm.

[11] A fourth study was part if the IIEF validation program and is discussed elsewhere.

7.2.3.2.1. Analyses of sexual performance by IIEF

The sponsor's analyses of the sexual performance questions in these 4 studies are shown in Table 21 below.

Table 21. ITT analyses of IIEF questions 3 and 4 (titration studies).

	Study	Pop'n	Baseline	Placebo		Sildenafil		P[a]
				n	Q	n	Q	
How often were you able to penetrate your partner?	148-103	Mixed	2.0	138	2.3	138	3.9	<0.0001
	148-363	Mixed	1.9	101	2.2	124	3.5	<0.0001
	148-104	Diabetes	1.7	126	2.0	131	3.2	<0.0001
	148-367	Cord	2.0	158	2.2	155	3.8	<0.0001
How often were you able to maintain your erection after penetration?	148-103	Mixed	1.5	138	1.8	137	3.6	<0.0001
	148-363	Mixed	1.6	116	2.1	136	3.5	<0.0001
	148-104	Diabetes	1.4	125	1.6	131	2.9	<0.0001
	148-367	Cord	1.5	158	1.7	155	3.6	<0.0001

a. P-value from two-sample t-test.

None of the sponsor's analyses were recapitulated by the reviewers. However, for the two of these studies for which unabridged SAS datasets were provided, the reviewers performed sub-group analyses, based upon baseline characteristics likely to have some relationship to disease severity. These results are summarized in Table 22 below. For most sub-groups, the sildenafil-placebo differences were highly statistically significant for both effectiveness questions. This was true for diabetic subjects in Study 148-363; in Study 148-103, the difference was less statistically compelling, although entirely consistent with Study 148-363.

Table 22. Sub-group analyses of IIEF questions 3 and 4[a] (Studies 148-103 and 148-363).

	N		How often were you able to penetrate your partner?						How often were you able to maintain your erection after penetration?					
	148-103	148-363	148-103			148-363			148-103			148-363		
			Pcbo	Sil	P	Pcbo	Sil	P	Pcbo	Sil	P	Pcbo	Sil	P
Etiology														
Organic	193	91	0.1	1.6	0.0001	0.7	1.5	0.0001	0.2	1.9	0.0001	0.3	2.0	0.0001
Psychogenic	49	100	0.2	2.3	0.0001	0.3	1.5	0.0001	0.3	2.4	0.0001	0.3	1.7	0.0001
Mixed	87	114	0.4	1.9	0.0001	0.7	1.7	0.0001	0.4	2.2	0.0001	0.2	1.8	0.0001
Nocturnal erections														
Yes	202	181	0.2	1.9	0.0001	0.3	1.7	0.0001	0.3	2.2	0.0001	0.3	1.8	0.0001
No	102	112	0.6	2.0	0.0001	0.1	1.7	0.0001	0.4	2.0	0.0001	0.3	1.9	0.0001
Duration														
<3 years	132	134	0.4	1.7	0.0001	0.1	1.9	0.0001	0.3	2.1	0.0001	0.5	1.6	0.0001
>3 years	197	180	0.1	1.9	0.0001	0.3	1.4	0.0001	0.2	2.1	0.0001	0.1	1.9	0.0001
Previous treatment														
Yes	230	255	0.1	1.9	0.0001	0.2	1.7	0.0001	0.2	2.1	0.0001	0.2	1.8	0.0001
No	99	59	0.3	1.5	0.0001	0.3	1.5	0.002	0.3	1.9	0.0001	0.4	1.6	0.02
Diabetes mellitus														
Yes	31	43	0.6	1.6	0.07	0.1	2.1	0.0001	0.3	1.6	0.02	0.0	1.8	0.0001
No	298	269	0.2	1.8	0.0001	0.2	1.6	0.0001	0.3	2.1	0.0001	0.3	1.8	0.0001

a. Reviewers' LOCF analyses; sildenafil-placebo difference in score, after adjustment for baseline and age, classified as <55 or >55.

7.2.3.2.2. Analyses of other IIEF questions

Other aspects of the IIEF were consistent with the effectiveness of sildenafil, as shown in Table 23 below. On all questions except the one pertaining to frequency of desire, the individual studies are consistent and highly statistically significant, so appropriate adjustments for multiple end points are not at issue. with the fixed-dose studies, the sildenafil-placebo difference was much more compelling in the European studies than in the US studies.

Table 23. ITT analyses of supportive IIEF questions at week 12 (titration studies)[a].

Question	Study	Pop'n	Base-line	Placebo		Sildenafil		p[b]
				n	Q	n	Q	
Able to get erection	148-103	Mixed	2.4	138	2.4	138	3.9	<0.0001
	148-363	Mixed	2.2	120	2.4	139	3.8	<0.0001
	148-104	Diabetes	2.0	126	1.8	131	3.1	<0.0001
	148-367	Cord	2.4	156	2.4	153	4.0	<0.0001
Erections hard enough	148-103	Mixed	2.0	138	2.1	138	3.8	<0.0001
	148-363	Mixed	1.9	117	2.1	138	3.6	<0.0001
	148-104	Diabetes	1.6	126	1.8	131	3.1	<0.0001
	148-367	Cord	2.3	155	2.2	154	3.7	<0.0001
Difficulty maintaining erection	148-103	Mixed	1.6	138	1.9	138	3.7	<0.0001
	148-363	Mixed	1.7	113	2.3	134	3.6	<0.0001
	148-104	Diabetes	1.3	127	1.6	131	2.7	<0.0001
	148-367	Cord	1.4	157	1.6	155	3.5	<0.0001
Confidence in erection	148-103	Mixed	1.6	137	1.9	136	3.3	<0.0001
	148-363	Mixed	2.0	120	2.2	137	3.4	<0.0001
	148-104	Diabetes	1.5	127	1.6	131	2.5	<0.0001
	148-367	Cord	1.9	156	1.9	155	3.5	<0.0001
Attempted intercourse	148-103	Mixed	2.2	139	2.9	138	3.5	<0.0001
	148-363	Mixed	2.1	121	2.7	139	2.9	0.4
	148-104	Diabetes	2.0	126	2.7	131	3.4	<0.0001
	148-367	Cord	1.6	158	2.6	155	3.2	<0.0001
Satisfaction of intercourse	148-103	Mixed	1.8	139	2.0	138	3.7	<0.0001
	148-363	Mixed	1.7	121	1.9	136	3.4	<0.0001
	148-104	Diabetes	1.5	127	1.7	131	2.7	<0.0001
	148-367	Cord	1.6	158	1.9	155	3.5	<0.0001
Enjoyment of intercourse	148-103	Mixed	1.9	139	2.2	138	3.6	0.0001
	148-363	Mixed	1.9	121	2.2	138	3.0	<0.0001
	148-104	Diabetes	1.7	126	1.8	131	2.8	<0.0001
	148-367	Cord	1.8	158	2.1	155	3.2	<0.0001
Frequency of ejaculation	148-103	Mixed	2.8	139	2.8	134	3.9	<0.0001
	148-363	Mixed	2.9	120	2.9	138	3.8	<0.0001
	148-104	Diabetes	2.9	127	3.3	131	3.9	0.0006
	148-367	Cord	1.9	155	1.8	152	2.1	0.001
Frequency of orgasm	148-103	Mixed	2.7	139	2.9	138	3.8	<0.0001
	148-363	Mixed	2.6	119	2.7	137	3.7	<0.0001
	148-104	Diabetes	2.9	127	3.3	131	3.7	0.02
	148-367	Cord	1.8	155	1.8	152	2.5	<0.0001

Table 23. ITT analyses of supportive IIEF questions at week 12 (titration studies)[a].*(Continued)*

Question	Study	Pop'n	Baseline	Placebo		Sildenafil		P[b]
				n	Q	n	Q	
Frequency of desire	148-103	Mixed	3.6	138	3.5	138	3.5	0.7
	148-363	Mixed	3.2	120	3.4	136	3.6	0.02
	148-104	Diabetes	3.6	127	3.7	131	3.7	0.7
	148-367	Cord	3.7	158	3.3	155	3.7	<0.0001
Rating of desire	148-103	Mixed	3.3	139	3.3	138	2.5	0.006
	148-363	Mixed	3.0	120	3.2	135	3.4	0.08
	148-104	Diabetes	3.3	127	3.4	131	3.5	0.2
	148-367	Cord	3.7	158	3.3	155	3.6	<0.0001
Satisfaction with sex life	148-103	Mixed	1.8	138	2.0	138	3.7	<0.0001
	148-363	Mixed	1.9	120	2.4	138	3.6	<0.0001
	148-104	Diabetes	1.8	127	2.1	131	2.9	<0.0001
	148-367	Cord	2.6	157	2.5	155	3.8	<0.0001
Satisfaction with relationship	148-103	Mixed	2.6	138	2.8	137	4.0	<0.0001
	148-363	Mixed	2.4	117	2.9	137	3.7	<0.0001
	148-104	Diabetes	2.5	127	2.8	130	3.3	0.001
	148-367	Cord	2.9	157	2.9	155	3.9	<0.0001

a. Sponsor's analyses.
b. P-value for non-zero sildenafil-placebo difference.

7.2.3.2.3. Analyses of event logs

The sponsor's analyses of event logs were based upon the proportion of all attempts that were successful. These results are included in some of the study reports.

The reviewers' analyses of event logs, derived from titration studies for which full SAS datasets were available, are summarized in Table 24 below. The results illustrate that subjects in these trials were not profoundly incapacitated. One-third to one-half of subjects had successful intercourse during a treatment-free run-in period. The number of attempts at intercourse was not much affected by the treatment, so the sponsor's analyses of success rates was valid and informative. Whether assessed by the number of successful attempts per subject per week, the proportion of attempts that were successful, or the proportion of subjects who were successful at least once during the study, sildenafil treatment groups had markedly better sexual performance success than did placebo.

Table 24. Successful intercourse by event logs (titration studies).

		Study	Placebo	Sildenafil
Attempts	Total	148-103	5645	5971
		148-363	6984	8978
	Per subject mean	148-103	34	37
		148-363	45	56
	Per subject per week	148-103	2.8	3.1
		148-363	1.7	2.2

Table 24. Successful intercourse by event logs (titration studies).*(Continued)*

		Study	Placebo	Sildenafil
Successes	Total	148-103 148-363	732 1780	2792 5284
	Per subject mean	148-103 148-363	4.4 11	17 33
	Per subject per week	148-103 148-363	0.4 0.4	1.4 1.3
Success by attempts	(%)	148-103 148-363	13 25	47 59
Success by subjects (%)	During run-in	148-103 148-363	37 33	32 38
	Double-blind period	148-103 148-363	55 63	87 89

7.3. Summary of key effectiveness findings

7.3.1. Mechanism of action

In the absence of (intentional) excitatory sensory stimulation, penile erections were only infrequently reported in association with sildenafil. Studies of erectile function by penile plethysmography showed that sildenafil administration, accompanied by visual sexual stimulation or mechanical stimulation, was associated with more frequent and longer duration erections than was placebo. These studies do not address the molecular or receptor-mediated mechanisms of sildenafil, but they provide a plausible basis for findings in studies of effectiveness with respect to sexual intercourse.

7.3.2. Dose-dependent effects

Multiple-single-dose crossover studies of erectile function by penile plethysmography showed that doses of 25 to 100 mg were more effective in producing erections than was placebo. The data suggest that 25 mg is not the smallest dose with a detectable effect in a small study, and that 100 mg is not associated with the largest attainable effect.

Parallel, placebo-controlled studies of sexual function leave no doubt that 25 to 100 mg are effective doses, as assessed by a validated sexual function questionnaire. Further, these studies strongly support a monotonic relationship to dose: placebo < 25 mg < 50 mg < 100 mg. One study is consistent with 200 mg being not differentiable from 100 mg. The 25-mg-placebo difference is more than half of the 100-mg-placebo difference; this suggests that the 25-mg dose is already fairly high on the dose-response curve.

Event log data, analyzed by various means, and IIEF questions relating to erectile function and (male) sexual satisfaction are highly internally consistent with findings pertaining to sexual performance. Quality-of-life questions afield of sexual performance tended to show no effect.

In titration studies, subjects generally took the first opportunity to migrate from a starting dose of 25 or 50 mg to a higher dose. Few subjects discontinued use of sildenafil for lack of effectiveness. The proportion of subjects remaining on various available dose levels varied for study to study, quite likely dependent upon the etiology of the erectile dysfunction.

7.3.3. Time course of effects

7.3.3.1. Time course after a dose

This was not well studied. In principle, it should have been possible to estimate the success rate as a function of time after dosing in titration studies (148-103 and 148-363). However, the case report forms captured neither the time of dosing nor the time of sexual activity.

7.3.3.2. Time course with repeated dosing

Studies 148-102 and 148-363 had evaluations of IIEF questions at 3 and 6 months. The sponsor's LOCF analyses (although not optimal for this assessment) do not suggest a waning of effectiveness over this interval.

The long-term open-label experience demonstrates a low rate of withdrawal for any reason.

7.3.4. Effectiveness in sub-groups

7.3.4.1. Non-specific organic etiology

In general, this category included vascular and neurological etiology, including diabetes, but not spinal cord injury or anatomical defects. The reviewers carried out analyses of the primary effectiveness questions in the subset of subjects with 'organic' erectile dysfunction in 4 studies, as shown in Table 25 below. Although the 'organic' category is not well characterized, the effectiveness of sildenafil is not in doubt.

Table 25. Effectiveness in organic erectile dysfunction.

	Fixed-dose studies				Titration studies					
	148-102 N=411		148-364 N=165		148-103 N=193			148-363 N=91		
	Slope	*P*	Slope	*P*	Pcbo	Sil	*P*	Pcbo	Sil	*P*
How often were you able to penetrate your partner?	16±2	0.0001	13±3	0.0001	0.1	1.6	0.0001	0.7	1.5	0.0001
How often were you able to maintain your erection after penetration?	15±2	0.0001	15±3	0.0001	0.2	1.9	0.0001	0.3	2.0	0.0001

7.3.4.2. Psychogenic etiology

Study 148-355[12] was a randomized, double-blind, 4-week, 2-period, placebo-controlled, flexible-titration, crossover study in 44 subjects with erectile dysfunction of no established organic cause. The primary end points included the *fraction* of erections adequate for intercourse. The *number* of such erections, analyzed by the sponsor, was highly statistically significantly greater on sildenafil.

The reviewers carried out analyses of the primary effectiveness questions in the subset of subjects with psychogenic erectile dysfunction in 4 studies, as shown in Table 26 below. There can be little doubt that sildenafil is effective in treating erectile dysfunction of psychogenic etiology.

Table 26. Effectiveness in psychogenic erectile dysfunction.

	Fixed-dose studies				Titration studies					
	148-102 N=50		148-364 N=129		148-103 N=49			148-363 N=100		
	Slope	*P*	Slope	*P*	Pcbo	Sil	*P*	Pcbo	Sil	*P*
How often were you able to penetrate your partner?	23±5	0.0001	15±3	0.0001	0.2	2.3	0.0001	0.3	1.5	0.0001
How often were you able to maintain your erection after penetration?	28±5	0.0001	15±3	0.0001	0.3	2.4	0.0001	0.3	1.7	0.0001

[12] *Study 148-355: A double blind, randomised, placebo controlled, two way crossover study to investigate the efficacy of single doses of sildenafil (UK-92,480) (taken when required over a 28 day period) in patients with erectile dysfunction with no established organic cause.* on page 191.

7.3.4.3. Diabetes

Study 148-104[13] was a randomized, double-blind, parallel, placebo-controlled, flexible-titration study in 268 subjects with well-controlled type I or type II diabetes and erectile dysfunction. The primary end points were the 2 IIEF questions pertaining to sexual performance. Subjects began on sildenafil 50 mg and at the first opportunity, mre than 75% migrated to the 100-mg dose. The results of the primary effectiveness analyses are reproduced in Table 27 below[14].

Table 27. ITT analyses of IIEF questions 3 and 4 (Study 148-104).

		Placebo N=132		Sildenafil N=136		*P*
		n	Q	n	Q	
How often were you able to penetrate your partner?	Baseline	—	1.7[a]	—	—	
	Week 12	126	2.0	131	3.2	<0.0001
How often were you able to maintain your erection after penetration?	Baseline	—	1.4	—	—	
	Week 12	125	1.6	131	2.9	<0.0001

a. Pooled baseline value for all subjects.

The reviewers carried out analyses of the primary effectiveness questions in the subset of subjects with erectile dysfunction and diabetes in 4 studies, as shown in Table 28 below. There were fewer such subjects than subjects with psychogenic dysfunction, but not many fewer. Both of the questions for all 4 studies (8 comparisons) lean in the direction of showing a benefit to sildenafil, but the magnitude of the effect is clearly not very large[15].

Table 28. Effectiveness in diabetics with erectile dysfunction.

	Fixed-dose studies				Titration studies					
	148-102 N=72		148-364 N=44		148-103 N=31			148-363 N=43		
	Slope	*P*	Slope	*P*	Pcbo	Sil	*P*	Pcbo	Sil	*P*
How often were you able to penetrate your partner?	5±5	0.29	2±5	0.72	0.6	1.6	0.07	0.1	2.1	0.0001
How often were you able to maintain your erection after penetration?	8±5	0.11	5±4	0.26	0.3	1.6	0.02	0.0	1.8	0.0001

7.3.4.4. Spinal cord trauma

Study 148-358[16] was 2 studies conducted in the same population of 27 subjects with a history of spinal cord trauma preserving an erectile response to a vibrator. The first phase was a randomized, double-blind, 2-period (placebo and sildenafil 50 mg), single-dose crossover study in which subjects underwent penile plethysmography in the clinic. The proportion of subjects attaining an erection with >60% rigidity was 4% on placebo and 46% on sildenafil. The second phase was a randomized, double-blind, parallel, placebo-controlled study over 4 weeks of use at home, with interest in continued use of the drug being the primary end point. The proportion of successful intercourse attempts, as assessed by the sponsor, was 38% on placebo vs. 67% on sildenafil.

---

13. *Study 148-104: A double-blind, randomized, placebo-controlled, parallel group, multicenter, flexible dose escalation study to assess the efficacy and safety of sildenafil administered as required to male diabetic patients with erectile dysfunction.* on page 118
14. Same as Table 92 on page 121.
15. 'Large' being difficult to interpret where numerical values have been assigned to categorical responses.
16. *Study 148-358: A two stage, double blind, placebo-controlled study to assess the efficacy and safety of oral doses of sildenafil (UK-92,480) in spinal cord injury patients with erectile dysfunction.* on page 197.

Study 148-367[17] was a randomized, double-blind, 2-period (placebo and sildenafil 50-100 mg), 6-week, crossover study in 89 subjects with traumatic spinal cord injury. The primary end point was election of the preferred treatment, but subjects also filled out the IIEF. For both IIEF questions pertaining to sexual performance, highly statistically significant improvements were observed on sildenafil, and all other IIEF supporting questions showed similar, highly internally consistent effects.

Spinal cord injury was a specific exclusion from other studies of effectiveness.

7.3.4.5. Blacks

The sponsor performed analyses of IIEF sexual performance questions by race and found a significant interaction (p=0.005 and p=0.02[18]) in flexible-dose studies (148-103 and 148-363), in which about 6% of subjects were non-Caucasian. Similar analyses in fixed-dose studies (148-102 and 148-364) showed no statistically significant effect (p=0.18[19]). The sponsor's larger meta-analyses of 8 studies[20], reviewed in no detail, also showed no significant effect of race (p=0.87 and p=0.92).

The reviewers performed no analyses of effectiveness by race.

The sponsor's meta-analyses of 8 studies[21], reviewed in no detail, showed no significant effect of race (p=0.31 and p=0.91).

7.3.4.6. Elderly

The reviewers' sub-group analyses of IIEF sexual performance questions in studies 148-102, 148-103, 148-363, and 148-364 showed nominally statistically significant interactions with age in about half of the comparisons.

The reviewers conclude that if there is an effect of age, it is not of clinically significant magnitude.

---

17. *Study 148-367: A double-blind, randomised, placebo-controlled, two way cross-over, flexible dose study to assess the efficacy and safety of oral doses of sildenafil in patients with erectile dysfunction caused by traumatic injuries to the spinal cord.* on page 218.
18. These are the p-values for the IIEF questions. The sponsor's presentation does not indicate in which treatment group sildenafil appears to be more effective.
19. Applies to 'frequency of penetration' question only.
20. Studies 148-101/101B, 148-102, 148-103, 148-104, 148-106, 148-359, 148-363, and 148-364.
21. Studies 148-101/101B, 148-102, 148-103, 148-104, 148-106, 148-359, 148-363, and 148-364.

Center for Drug Evaluation and Research

Viagra (Sildenafil)

"Joint Clinical Review" for NDA-20-895

Section 8, pages 40-56

8. Integrated review of safety

8.1. Methodology

8.1.1. Deaths

For each death reported by the sponsor, the sponsor's narrative summary was compared with the case report form. The case report form was combed for indications of drug-relatedness of the immediate cause of death or associated problems.

8.1.2. Serious adverse events

The sponsor identified serious adverse events as those that were fatal, life-threatening, permanently disabling, resulted in or prolonged hospitalization, associated with congenital anomaly, cancerous, or resulted from overdose of study drug.

8.1.3. Withdrawals and other significant adverse events

8.1.3.1. Overall profile of withdrawals

Withdrawals were characterized by the primary associated event, as identified by the sponsor. The reviewers made no attempt to characterize other adverse events occurring in this sub-group, nor was any attempt made to recategorize the causes for withdrawal.

8.1.3.2. Adverse events associated with withdrawal

Adverse events associated with withdrawal were analyzed separately for the double-blind and open-label periods. Case report forms were examined for all adverse events identified by the sponsor as leading to withdrawal, to look for drug-relatedness or associated problems. Tables were generated by the reviewers for adverse events leading to withdrawal, organized by content-dependent categories, and including dosing exposure and demographic data.

8.1.3.3. Other significant adverse events

Common adverse events were analyzed separately for the double-blind and open-label periods. Dose-relatedness was assessed for common adverse events in placebo-controlled studies for which dosing information was available[1].

8.1.4. Other search strategies

Adverse events were reviewed on case report forms for deaths and withdrawals.

8.1.5. Adverse event incidence

8.1.5.1. Approach to eliciting adverse events in the development program

Protocols all contained provision for the periodic ascertainment of adverse events from subjects through non-directive questioning. There is no reason to suspect bias or inadequate reporting of adverse events with this methodology.

8.1.5.2. Appropriateness of adverse event categorization and preferred terms

Rates for common adverse events were determined solely by the sponsor's categorization. There is, therefore, some possibility that some events were scattered among terms allocated, perhaps inappropriately, to multiple body systems. Little effort was spent searching the safety data systematically for such abuses. Categorization of events by body system and by likelihood of being related to study drug was performed by the reviewers.

8.1.5.3. Identifying common and drug-related adverse events

Common adverse events in placebo-controlled studies were derived from the experience available electronically with the original submission.

8.1.5.4. Additional analyses

No additional analyses of adverse events were performed.

8.1.6. Laboratory findings

8.1.6.1. Extent of laboratory testing

Clinical chemistry, hematology, urinalysis, and ECG were all routinely performed at the beginning and end of blinded treatment periods in major studies. Testing was also performed at follow-up visits during open-label studies. This level of monitoring should be considered adequate for a drug for use in this population.

8.1.6.2. Selection of studies and analyses for drug-control comparisons

While serious event-related analyses covered all trial experience, analyses of trends in laboratory data focussed on previously identified collections of double-blind. placebo-controlled parallel-group studies.

---

[1] Unabridged datasets were supplied only for Studies 148-102, 148-103, 148-363, and 148-364.

8.1.6.3. Standard analyses

8.1.6.3.1. Analyses focussed on central tendency and outliers

The reviewers undertook a staged approach to examination of laboratory data. The sponsor identified no clinically significant changes in central tendency. The reviewers screened data for subjects experiencing a significant shift from a normal baseline value. In a few cases, all lab values were reviewed for the few subjects demonstrating at least an excursion to a clinically significant value. Specific details are described in the various sections dealing with laboratory findings.

8.1.6.3.2. Withdrawals for laboratory abnormalities

Withdrawals for laboratory abnormalities are listed among withdrawals for other adverse events.

8.1.7. Vital signs

Vital signs were analyzed in the same manner as clinical laboratory data.

8.1.8. ECGs

ECGs were analyzed in the same manner as other clinical laboratory data.

8.1.9. Special studies

The sponsor conducted studies of effects of sildenafil on vision, on platelet aggregation and bleeding, and on blood pressure response when co-administered with nitroglycerin.

Drug interaction studies are discussed elsewhere.

8.1.10. Withdrawal phenomena

Withdrawal effects have not been studied formally.

8.1.11. Abuse potential

Although there are no evident effects on the central nervous system indicative of a risk of physical dependence, it can be supposed that sildenafil will be prescribed in a population with little objective need. Sildenafil is also likely to be see use in a population outside of the one for which it is prescribed.

8.1.12. Human reproduction data

All studies were conducted in a population at low risk of pregnancy.

There are no reports of women conceiving with partners on sildenafil.

8.1.13. Overdose experience

The sponsor's recommendation is for dosing not to exceed once daily, but in studies, some subjects exceeded that by amounts difficult to ascertain. Single doses up to 800 mg have been studied formally in normal volunteers.

8.2. Safety results

8.2.1. Deaths

Deaths occurring on study drug or within 30 days of receipt of study drug are listed in Table 29 below. There was only one death on placebo. Of 8 deaths on sildenafil, all were of a cardiovascular nature and most occurred in a setting of risk factors and medical history making the observed events plausibly not related to study drug. In no case did an investigator attribute death to study drug.

Table 29. Deaths on or within 30 days of study drug.

Study	Subject	Age	Race	Dose mg	Description
148-102	95220372	76	Cauc	Pcbo	History of diabetes and CHF. He died of self-inflicted gunshot wound on day 51.
148-104C	95110230	67	Cauc	100	History of diabetes, CHF, s/p aortic valve replacement. He died of multiple organ failure on day 51.
148-301	0090004	70	Cauc	40	Subject in angina study received 1 dose 11 days prior to emergency CABG. He died on day 14.
148-356	0780131	66	Cauc	10	History of smoking. He died on day 9 of presumed myocardial infarction. There was no autopsy.
148-363	1090178	66	Cauc	25	History of alcohol abuse, hypertension, hypercholesterolemia. He died of probable myocardial infarction on day 89.

Table 29. Deaths on or within 30 days of study drug. *(Continued)*

Study	Subject	Age	Race	Dose mg	Description
148-365	0950016	53	Cauc	100	No pertinent history. Died of myocardial infarction on day 17.
148-370	1390461	64	Cauc	Unk	History of arrhythmia (unspecified). He fell and suffered a cerebral hemorrhage and died of pulmonary sepsis on day 155.
148-365	0270007	62	Cauc	50	History of coronary artery disease. He died of myocardial infarction on day 104.
148-370	1540729	58	Cauc	50	He died of cardiac failure secondary to metastatic bladder carcinoma an unknown time after receiving study drug.
148-102C	95220009	57	Cauc	100	History of hypertension. He completed 6 months open-label and died of myocardial infarction 1 or 13 days after the last dose.

8.2.2. Withdrawals

8.2.2.1. Withdrawals for any cause

8.2.2.1.1. Withdrawals from placebo-controlled studies

Reasons for withdrawal from placebo-controlled studies are shown in Table 30 below. Lack of effectiveness (LOE) was the most common cause for withdrawal from placebo groups, particularly in longer-running studies. Adverse events (AE) and withdrawal of consent (With) were less than half as common in the placebo group. Lack of effectiveness was a much less common cause for withdrawal from sildenafil treatment groups (about 27% as common). Adverse events as reasons for withdrawal had the same incidence in placebo and sildenafil treatment groups. Laboratory abnormalities, loss to follow-up, 'other' (Oth), protocol violations, and withdrawal of consent were all more common on placebo than on sildenafil. There were 3 deaths on sildenafil versus none on placebo, and 3 subjects withdrawn from sildenafil because they were randomized in error versus none on placebo. Overall, 16% of subjects randomized to placebo and 9% of subjects randomized to sildenafil withdrew for any reason from placebo-controlled studies, with much of the difference between groups being attributable to differences in rates of withdrawal for lack of effectiveness, particularly in longer-running studies.

Table 30. Withdrawals from placebo-controlled studies[a].

	Placebo											Sildenafil										
	N	AE	Death	LOE	Lab	LFU	Oth	Viol	With	Rand	Any	N	AE	Death	LOE	Lab	LFU	Oth	Viol	With	Rand	Any
148-101/101B	83	6	—	1	1	—	2	—	6	—	16	333	5	1	6	3	3	8	4	11	—	41
148-102	216	5	—	11	1	6	3	4	6	—	36	316	8	—	5	2	6	3	4	3	—	31
148-103	166	4	—	3	—	2	2	1	1	—	13	163	3	—	1	—	3	—	—	2	—	9
148-104	132	1	—	1	3	—	1	—	5	—	11	136	1	—	1	—	1	—	—	2	—	5
148-106	122	4	—	8	2	2	1	1	—	—	18	375	16	—	7	2	5	5	3	4	1	43
148-353	95	4	—	—	—	4	—	—	—	1	9	256	13	—	4	3	2	3	—	—	—	25
148-355	43	1	—	—	—	1	—	1	—	—	3	44	—	—	—	—	1	—	1	—	—	2
148-359	54	2	—	1	—	2	4	1	1	—	11	57	—	—	1	—	—	—	—	—	—	1
148-361	59	1	—	—	—	—	—	2	2	—	5	195	2	—	1	1	—	—	2	3	1	10
148-363	156	5	—	54	3	5	4	2	4	—	77	159	8	2	13	2	4	2	3	—	1	35
148-364	127	1	—	6	—	2	—	—	2	—	11	387	6	—	3	1	3	1	2	3	—	19
148-367	89	2	—	—	1	—	1	—	—	—	4	89	5	—	—	—	1	—	—	—	—	6
Total	1342	36	0	85	11	24	18	12	27	1	214	2510	67	3	42	14	29	22	19	28	3	227
Incidence (%)	—	2.7	—	6.3	0.8	1.8	1.3	0.9	2.0	—	16	—	2.7	0.1	1.7	0.6	1.2	0.9	0.8	1.1	0.1	9.0

a. AE = adverse event; LOE = lack of effectiveness; Lab = laboratory abnormality; LFU = loss to follow-up; Viol = protocol violation; With = withdrawal of consent; Rand = randomization in error.

8.2.2.1.2. Withdrawals from open-label extensions

Reasons for withdrawal from open-label studies are shown in Table 31 below. The incidence of withdrawals for lack of effectiveness was higher during open-label studies than during placebo-controlled studies, but the incidence is very low and the period of exposure is much longer for open-label studies. Overall, only 10% of subjects withdrew for any reason from open-label studies.

Table 31. Withdrawals from open-label studies.

	Sildenafil										Sildenafil								
	N	AE	Death	LOE	Lab	LFU	Oth	Viol	With		N	AE	Death	LOE	Lab	LFU	Oth	Viol	With
148-101C	337	14	1	29	2	7	7	1	7	148-354B	148	6	—	2	—	5	5	—	1
148-102C	402	—	—	15	1	1	1	1	4	148-354C	32	1	—	6	2	8	—	—	4
148-103C	225	5	—	8	1	—	—	1	3	148-361O	227	1	—	—	4	—	—	—	2
148-104C	185	3	—	16	—	—	1	1	1	148-366	203	—	—	7	1	1	—	—	—
148-105	54	1	—	—	—	—	—	—	—										
148-350	16	1	—	—	—	—	—	—	—	Total	2137	41	1	94	13	27	19	7	26
148-354A	308	9	—	11	2	5	5	3	4	Incidence (%)		1.9	—	4.4	0.6	1.3	0.9	0.3	1.2

8.2.2.2. Withdrawals for adverse events

8.2.2.2.1. Withdrawals from placebo-controlled studies

Subjects who discontinued from double-blind, placebo-controlled studies because of adverse event or adverse laboratory values are shown in Table 32 below. Dosing and evaluation of events as serious or not was available from all studies; where the dose of a subject randomized to sildenafil was not available from the sponsor's integrated safety database, the value is shown below as '>0' and where the evaluation of seriousness was unavailable, the table shows '?'. Event categories were established by clinical importance or apparent frequency in the data; some subjects have events match more than one category and are listed multiple times. Lines are not replicated in the 'other' category. For some categories, the available CRFs were scanned for pertinent baseline findings or medical history.

Table 32. Adverse events leading to withdrawal from placebo-controlled studies.

Study	PID	Dose	Age	Race	Days	SAE	Related	Description
Cardiovascular—arrhythmia								
101	95000036	0	68	White	151	?	N	Deep thrombophlebitis, atrial fibrillation
101	95030028	0	62	White	50	?	Y	Palpitation; history of MI
102	95270566	0	64	Black	43	Y	N	Atrial fibrillation; no relevant history
353	630409	0	43	White	1	?	Y	Palpitation, anxiety; no relevant history
363	1260330	0	53	Asian	81	N	N	Ventricular extrasystoles; diabetes diagnosed at screening
106	95670251	>0	70	White	57	?	N	Atrial fibrillation; history of MI, hypertension, arrhythmia
363	1160280	>0	70	White	81	?	N	Cardiomyopathy; arrhythmia; agitation, arthralgia
363	1090178	25	65	White	92	Y	N	Cardiac arrest; history of hypertension
Cardiovascular—blood pressure								
101	95020385	0	64	White	30	?	N	Hypertension
106	95720414	>0	63	Other	41	?	N	Hypotension; asthenia; vomiting; diarrhea
353	550371	>0	49	White	27	?	N	Vasodilation
363	1260328	>0	62	White	58	?	Y	Vasodilation; abdominal pain
363	1320081	100	59	White	126	N	N	Hypertension; history of same
Cardiovascular—myocardial infarction								

Table 32. Adverse events leading to withdrawal from placebo-controlled studies. *(Continued)*

Study	PID	Dose	Age	Race	Days	SAE	Related	Description
103	95000163	0	63	White	29	Y	N	Myocardial infarction, stroke; history of CABG, peripheral vascular disease, hypertension
103	95070035	0	64	White	56	Y	N	Myocardial infarction, angioplasty; no relevant history
106	95610018	0	55	White	68	?	N	Myocardial infarct; history of hypertension, CAD, angina, diabetes, hyperlipidemia
106	95780138	0	59	White	59	?	N	Respiratory tract infection, MI; history of hypertension and smoking
355	850113	0	62	White	29	?	Y	Respiratory tract infection, MI; no relevant history
361	1020009	>0	68	White	45	?	N	Myocardial infarct; history of diabetes
102	95050319	25	64	White	86	Y	N	Myocardial infarction; no relevant history
102	95170401	50	63	White	192	Y	N	Myocardial infarction; no relevant history
Cardiovascular—other								
102	95220372	0	76	White	15	N	N	Shortness of breath, heart failure; history of myocardial infarction, CABG, and diabetes
106	95740242	0	62	White	32	?	N	Anxiety, CAD, dyspnea
101	95210164	>0	49	White	113	?	N	Hemoptysis; cardiomyopathy; infection
106	95670251	>0	70	White	57	?	N	Worsening heart failure; pharygitis; history of hypertension, MI, arrhythmia, CHF
363	1190322	>0	64	White	92	?	N	Cerebrovascular disorder; history of diabetes and TIA
364	1440268	>0	76	White	73	?	N	Cerebrovascular disorder, AV block; history of hypertension
363	1120007	50	74	White	48	N	Y	ECG changes; no relevant history
102	95390139	100	63	White	1	Y	N	Coronary artery disease; history of hypertension, myocardial infarction
103	95450321	100	57	White	57	Y	N	Angina; history of peripheral vascular disease, hypertension
363	1160280	100	70	White	81	Y	N	Cardiomyopathy, arrhythmia; history of peripheral vascular disease
LFT								
101	95080249	0	47	Black	36	?	Y	SGOT, SGPT increased; no relevant history
101	95100088	0	68	White	114	?	N	Hepatoma, back pain
102	95350454	0	43	White	21	N	Y	Increased LFTs; no relevant history
106	95810153	0	65	White	31	?	Y	SGOT, SGPT increased; no relevant history
363	1250374	0	60	White	73	?	Y	SGPT, SGOT, AlkP increased; no relevant history
363	1250374	0	60	White	73	N	Y	Liver enzyme elevation; no relevant history
363	1270108	0	58	White	143	N	N	Liver enzyme elevation; no relevant history
367	1630409	0	36	White	25	?	Y	Skin disorder; SGOT, SGPT increased; no relevant history
101	95190375	>0	55	White	13	?	Y	Liver function tests abnormal; headache
353	640358	>0	63	White	3	?	N	BUN, AlkP, SGPT, SGOT increased; no relevant history
102	95290191	25	50	White	98	N	N	Increased LFTs; history of peripheral vascular disease
102	95370156	50	55	Black	95	N	Y	Increased LFTs; no relevant history
364	1460269	100	53	White	70	N	N	Liver enzyme elevation, attributed to alcohol; history of manic-depression, but not alcohol abuse
Headache								
102	95220247	0	70	White	18	N	Y	Nausea, headache
103	95000202	0	66	White	30	N	Y	Headache, dizziness; no relevant history
353	100180	0	59	White	28	?	Y	Headache
363	1060054	0	58	White	28	N	Y	Headache; no relevant history
367	1650393	0	44	White	25	?	Y	Headache, myalgia
101	95190375	>0	55	White	13	?	Y	Liver function tests abnormal; headache
101	95200154	>0	73	White	4	?	Y	Emotional lability; headache; nervousness
106	95410089	>0	58	White	13	?	Y	Diarrhea; headache; abnormal vision

Table 32. Adverse events leading to withdrawal from placebo-controlled studies. *(Continued)*

Study	PID	Dose	Age	Race	Days	SAE	Related	Description
106	95670264	>0	62	White	14	?	Y	Headache; asthenia; abdominal pain
106	95680385	>0	79	White	6	?	Y	Bloody diarrhea; headache
106	95680417	>0	66	White	15	?	Y	Headache
106	95690312	>0	68	White	5	?	Y	Abnormal vision; vomiting; lacrimal disorder; headache
106	95740247	>0	62	White	28	?	Y	Headache; asthenia; dyspepsia; vasodilation
106	95740297	>0	46	White	28	?	Y	Anorexia; headache; abnormal vision; rhinitis; eructation
106	95800083	>0	65	White	29	?	N	Headache; hyperglycemia
106	95800142	>0	54	White	8	?	N	Gum hemorrhage; vasodilation; headache; nausea
353	260186	>0	56	White	29	?	N	Flu syndrome; headache
353	270034	>0	24	White	28	?	Y	Headache; hypertonia; dyspepsia; pain
353	310101	>0	54	White	15	?	Y	Headache; vasodilation
353	310104	>0	49	Asian	10	?	Y	Respiratory disorder; headache
353	360021	>0	54	White	14	?	Y	Eye pain; myalgia; headache
353	640354	>0	44	White	29	?	Y	Vasodilation; headache; pharyngitis; rhinitis; epistaxis
353	670390	>0	54	White	14	?	Y	Nausea; headache; somnolence; respiratory disorder
363	1080247	>0	70	White	13	?	Y	Headache; abnormal vision
363	1110213	>0	55	White	48	?	Y	Insomnia; headache
363	1220302	>0	52	White	8	?	Y	Dizziness; headache
364	1460269	>0	53	White	70	?	N	Liver function tests abnormal; headache
367	240105	>0	32	White	18	?	N	Headache
367	1650391	>0	40	White	7	?	Y	Nausea; headache
367	1800058	>0	48	White	9	?	Y	Nausea; dyspepsia; vasodilation; headache
364	160490	>0	70	White	5	?	Y	Headache
364	750613	>0	65	Black	2	?	N	Dyspepsia; diarrhea; flatulence; headache; vomiting; nausea; abdominal pain
363	1080247	25	70	White	13	N	Y	Headache, visual disturbance; no relevant history
363	1220302	25	52	White	8	N	Y	Headache, dizziness; no relevant history
363	1110213	50	55	White	48	N	Y	Headache; no relevant history
363	1190322	50	64	White	92	Y	N	CVA, headache; no relevant history
102	95190117	100	41	White	94	N	Y	Headache, heartburn; no relevant history
102	95370159	100	72	White	4	N	Y	Headache
103	95070087	100	72	White	50	N	Y	Headache, flushing; history of hypertension
364	160490	100	70	White	5	N	Y	Headache, new onset diabetes; no relevant history
Visual disturbance								
102	95330219	0	73	White	34	N	N	Retinal changes; history and in-study atrial fibrillation
106	95690312	>0	68	White	5	?	Y	Abnormal vision; vomiting; lacrimal disorder; headache
106	95740297	>0	46	White	28	?	Y	Anorexia; headache; abnormal vision; rhinitis; eructation
363	1080247	>0	70	White	13	?	Y	Headache; abnormal vision
106	95800084	>0	59	White	15	?	N	Retinal hemorrhage
106	95800088	>0	53	White	8	?	Y	Abnormal vision
353	310102	>0	29	White	8	?	N	Abnormal vision
363	1080247	25	70	White	13	N	Y	Headache, visual disturbance; no relevant history
Other								
101	95000038	0	60	White	120	?	N	Chest pain

Table 32. Adverse events leading to withdrawal from placebo-controlled studies. *(Continued)*

Study	PID	Dose	Age	Race	Days	SAE	Related	Description
101	95210255	0	42	Black	28	?	Y	Pain
102	95250463	0	66	White	57	Y	N	Mesothelioma, stroke; history of hypertension, diabetes
103	95400300	0	67	White	71	Y	N	Possible TIA; no relevant history
104	95360115	0	40	White	1	?	N	Npn increased
104	95480148	0	70	White	17	?	N	Hyperglycemia
106	95770197	0	54	White	66	?	N	Varicose vein, bilirubinemia
353	100177	0	54	White	14	?	Y	Diarrhea
353	330089	0	35	White	19	?	Y	Psoriasis
359	270032	0	54	White	28	?	N	Procedure
359	600070	0	63	White	62	?	N	Pancreatitis, abdominal pain
361	1030253	0	62	White	68	?	N	Procedure (medical/surgical/health service)
363	1130297	0	57	White	30	?	N	Arthralgia, anemia, bronchitis, gastritis
363	1080245	0	59	White	73	Y	N	<<<AE page missing.>>
363	1150254	0	60	White	17	Y	N	Prostate cancer
363	1180309	0	33	White	1	N	N	"Aggravation of" alcohol abuse; no relevant history
363	1130297	0	57	White	30	N	N	Anemia; present from baseline
364	1520233	0	58	White	23	Y	N	Major depression; history of depression
101	95000039	>0	65	White	96	?	N	Carcinoma
101	95030030	>0	60	Black	65	?	N	Leukopenia
101	95170123	>0	46	White	87	?	N	Respiratory disorder, arthralgia, esoghageal ulcer
101	95170355	>0	66	White	94	?	N	Thrombocytopenia
101	95200155	>0	72	White	76	?	N	Allergic reaction
101	95200157	>0	52	White	112	?	N	Respiratory tract infection; lymphoma-like reaction; abscess; lymphadenopathy
106	95670241	>0	78	White	11	?	Y	Asthenia; myalgia; tachycardia; pain
106	95810144	>0	59	White	13	?	N	Arteriosclerosis
353	240171	>0	60	White	15	?	Y	Arthralgia
353	240171	>0	60	White	15	?	Y	Abdominal pain
353	290055	>0	63	White	29	?	Y	Dyspepsia
353	340113	>0	49	White	14	?	N	Asthenia; back pain; weight loss; nausea
353	360026	>0	61	White	15	?	Y	Pain
353	520211	>0	63	White	14	?	Y	Abdominal pain; leg cramps
353	560279	>0	69	White	22	?	Y	Kidney function abnormal; insomnia; anxiety
361	1020009	>0	68	White	45	?	N	Joint disorder
361	1030286	>0	61	White	59	?	N	Dyspepsia
363	1090177	>0	68	White	195	?	N	Accidental fall
363	1210354	>0	65	White	4	?	N	Hyperglycemia
363	1220382	>0	57	White	4	?	Y	Nausea; diarrhea
364	630018	>0	54	White	3	?	Y	Abdominal pain
364	630018	>0	54	White	3	?	Y	Vomiting; dyspepsia
364	1440254	>0	79	White	58	?	N	Chest pain
367	240105	>0	32	White	18	?	N	Skin ulcer
367	240104	>0	45	White	4	?	Y	Conjunctivitis; headache
367	1590133	>0	38	White	20	?	N	Abdominal pain; hypesthesia

Table 32. Adverse events leading to withdrawal from placebo-controlled studies. *(Continued)*

Study	PID	Dose	Age	Race	Days	SAE	Related	Description
102	95110054	25	48	White	174	Y	N	Need for concomitant medication; history of hypertension, myocardial infarction, and angioplasty
102	95270399	25	67	White	1	N	Y	Nausea, vomiting
363	1220382	25	57	White	4	N	Y	Nausea, diarrhea
363	1210354	25	65	White	4	N	N	Hyperglycemia; history of diabetes
102	95170103	50	64	White	13	N	Y	Back and leg ache; no relevant history
103	95400284	50	58	White	10	Y	N	Cerebral infarct; no relevant history
363	1090177	50	68	White	195	Y	N	Accidental death
364	1440268	50	76	White	73	Y	N	TIA; history of hypertension
363	1260328	100	62	White	58	N	Y	Hot flushes, stomach ache
364	630018	100	54	White	3	N	Y	Abdominal pain, vomiting
364	750613	100	65	Black	2	N	N	Subacute intestinal obstruction; no relevant history
364	1380438	100	64	White	58	Y	N	Hepatic metastasis of bladder cancer
364	1440254	100	79	White	58	N	N	Chest (muscle) pain; history of asymptomatic supraventricular arrhythmia

8.2.2.2.2. Withdrawals from open-label extensions

Adverse events or laboratory findings associated with or contributing to withdrawal from open-label studies are shown in Table 33 below. This table is similar to Table 32 on page 43, but for none of these trials was the dosing information or an evaluation of seriousness easily determined from the electronic data, so these fields are omitted.

Table 33. Adverse events leading to withdrawal from open-label studies

Study	PID	Age	Race	Days	SAE	Description
Cardiovascular						
101C	95080079	60	White	148	Yes	Abnormal vision; dyspnea; vasodilation
101C	95070326	60	White	158	Yes	Rhinitis; dyspepsia; vasodilation
101C	95110232	58	White	83	No	Conjunctivitis; angina pectoris; insomnia; rectal hemorrhage; vasodilation
105	95060053	24	Black	1	Yes	Vertigo; vasodilation
104C	95470185	57	White	12	Yes	Dizziness; hypertension; headache
101C	95230285	60	White	185	No	Procedure (medical/surgical/health service); CAD
354B	00956162	68	White	355	No	Myocardial infarct
LFT						
101C	95210169	68	White	149	No	SGOT, AlkP increased
103C	95000157	49	White	30	No	Alkaline phosphatase increased
354A	00290057	61	White	336	No	Arthritis; convulsion; AlkP increased
354C	00248018	63	White	61	Yes	Liver function tests abnormal
354C	00608071	48	White	179	No	Liver function tests abnormal; flu syndrome
366	1150260	47	White	24	Yes	Liver function tests abnormal
Headache						
101C	95050318	73	Asian	79	Yes	Headache, dizziness, prostatic disorder, abnormal vision
101C	95110237	61	White	89	Yes	Headache; rhinitis
102C	95000286	75	White	15	No	Sweating; dizziness; lab test abnormal; headache
103C	95070065	64	White	28	Yes	Headache
103C	95110155	64	White	57	Yes	Herpes simplex; headache
103C	95430169	68	White	28	Yes	Headache; abdominal pain
104C	95470185	57	White	12	Yes	Dizziness; hypertension; headache

Table 33. Adverse events leading to withdrawal from open-label studies

Study	PID	Age	Race	Days	SAE	Description
354A	00240020	42	White	6	Yes	Dyspepsia; headache; lacrimation disorder; voice alteration; respiratory disorder
354A	00580141	53	White	49	Yes	Headache; dyspepsia; respiratory tract infection
354C	00608067	55	White	110	Yes	Headache
Visual disturbance						
101C	95050318	73	Asian	79	Yes	Headache, dizziness, prostatic disorder, abnormal vision
101C	95080079	60	White	148	Yes	Abnormal vision; dyspnea; vasodilation
103C	95180011	56	White	54	Yes	Abnormal vision; vasodilation; headache
104C	95070012	75	White	10	Yes	Pain; abnormal vision
354A	00670400	36	White	50	Yes	Diarrhea; abnormal vision; abdominal pain
Other						
101C	95020018	67	White	82	No	Carcinoma
101C	95090208	60	White	124	No	Lymphadenopathy; lymphoma-like reaction
101C	95100418	77	White	121	No	Procedure (medical/surgical/health service)
101C	95130101	72	White	175	No	Carcinoma; accidental injury
101C	95140113	64	Other	169	No	Respiratory tract infection
101C	95140113	64	Other	169	No	Hyperglycemia; insomnia; constipation; pancreatitis; gastrointestinal carcinoma; dyspepsia; anorexia; hernia; cholelithiasis
101C	95150198	65	White	192	No	Dyspnea; lung edema
101C	95220177	72	White	148	Yes	Dizziness; pain
101C	95220276	73	White	10	No	Deafness; encephalopathy; dizziness
350	100008	42	White	2	Yes	Dyspepsia
354A	00240020	42	White	6	Yes	Dyspepsia; headache; lacrimation disorder; voice alteration; respiratory disorder
354A	00242119	63	White	73	Yes	Flu syndrome; hemorrhage
354A	00290056	57	White	66	No	Dizziness
354A	00480226	45	White	261	No	Seborrhea
354A	00620334	66	White	130	No	Skin melanoma
354A	00620415	45	Other	110	No	Leukopenia; gastritis; dyspepsia
354A	00640360	61	White	113	No	Cerebral thrombosis
354A	00690250	65	White	24	Yes	Abdominal pain
354B	00776040	57	White	352	Yes	Flu syndrome; somnolence; angina pectoris; headache; respiratory tract infection
354B	00776246	51	White	56	Yes	Rhinitis; pain
354B	00786106	47	White	239	No	Abdominal pain
354B	00786106	47	White	239	No	Testis disorder; carcinoma; back pain
354B	B0087620	69	White	266	No	Carcinoma
361O	01030249	49	White	84	Yes	Sinusitis; vasodilation

8.2.3. Common adverse events

8.2.3.1. Relationship to dose

Dose-relatedness of common adverse events was investigated for the 4 placebo-controlled studies for which dosing information was available—Studies 102, 103, 363, and 364. The denominators for incidence were the numbers of subjects exposed to a dose; because three of these studies were titration studies, many subjects contributed to the denominators for more than one active dose level.

Dose-relatedness of adverse events is shown in Table 34 below. Headache, dyspepsia, rhinitis, vision abnormalities, arthralgia, pain, abdominal pain, and rash were plausibly dose-related.

Table 34. Dose-relatedness of common adverse events in placebo-controlled studies.

	Placebo N=665		Sildenafil 25 mg N=394		50 mg N=519		100 mg N=439			Placebo N=665		Sildenafil 25 mg N=394		50 mg N=519		100 mg N=439	
	n	%	n	%	n	%	n	%		n	%	n	%	n	%	n	%
Headache	58	8.7	81	21	122	24	126	29	Dizziness	13	2.0	15	3.8	16	3.1	14	3.2
Vasodilation	9	1.4	69	18	103	20	89	20	Arthralgia	17	2.6	6	1.5	9	1.7	14	3.2
Dyspepsia	16	2.4	15	3.8	42	8.1	51	12	Pain	10	1.5	6	1.5	9	1.7	19	4.3
Respir tract infect	48	7.2	14	3.6	18	3.5	18	4.1	Sinusitis	23	3.5	4	1.0	6	1.2	11	2.5
Back pain	18	2.7	16	4.1	21	4.0	11	2.5	Abdominal pain	11	1.7	4	1.0	6	1.2	14	3.2
Accident	27	4.1	10	2.5	17	3.3	12	2.7	Diarrhea	14	2.1	9	2.3	6	1.2	9	2.1
Rhinitis	17	2.6	7	1.8	16	3.1	23	5.2	Rash	13	2.0	4	1.0	7	1.3	12	2.7
Flu syndrome	23	3.5	17	4.3	1	2.1	11	2.5	Nausea	4	0.6	8	2.0	9	1.7	12	2.7
Abnormal vision	5	0.8	5	1.3	12	2.3	38	8.7									

8.2.3.2. Overall incidence

The sponsor and the reviewers agree that the description of the incidence of adverse events most likely to mirror any subsequent experience in clinical practice would be derived from placebo-controlled flexible-dosing studies[2]. Table 35 below lists events by body system with an incidence of at least 1% in the combined sildenafil treatment group.

Table 35. Percentage of subjects with common adverse events in flexible-dosing studies.

	Placebo N=725	Sildenafil N=734		Placebo N=725	Sildenafil N=734
Any	41	60	Musculoskeletal	4.5	5.4
General	15	26	Arthralgia	1.5	2.0
Headache	3.9	16	Myalgia	1.0	1.2
Flu syndrome	2.9	3.3	Nervous system	5.2	9.3
Back pain	1.7	2.2	Dizziness	1.2	2.2
Abdominal pain	1.0	1.5	Nervousness	—	1.8
Asthenia	0.6	1.4	Hypertonia	0.6	1.6
Infection	0.2	1.2	Respiratory	11	13
Pain	1.0	1.1	Rhinitis	1.5	4.2
Accident	1.7	1.1	Respir tract infec	5.4	4.2
Cardiovascular	4.1	14	Respiratory disorder	0.8	1.9
Vasodilation	0.7	11	Pharyngitis	1.4	1.4
Gastrointestinal	6.6	14	Skin	4.1	5.4
Dyspepsia	1.7	6.5	Rash	1.4	2.2
Diarrhea	1.0	2.6	Special sense	1.4	6.0
Nausea	0.4	1.2	Abnormal vision	0.4	2.7
Tooth disorder	0.8	1.1	Urogenital	5.1	7.1
Hematologic	0.8	0.8	Urinary tract infec	1.5	3.1
Metabolic/nutritional	1.9	2.2	Prostate disorder	1.0	1.2

[2] Studies 148-103, 148-104, 148-355, 148-359, 148-363, and 148-367.

8.2.4. Laboratory data

8.2.4.1. Hepatic function

Laboratory abnormalities pertaining to hepatic function were screened by identification of subjects in placebo-controlled studies who had bilirubin, SGOT, SGPT, or alkaline phosphatase within normal limits at baseline but increased 2-fold from baseline at study end. For such subjects, bilirubin, SGOT, SGPT, alkaline phosphatase, albumin, and total protein values were abstracted for further examination. Subjects were then classified as having abnormalities limited to bilirubin only, or abnormalities (greater than 2-fold increase from baseline) in enzymes as well.

The incidence of hepatic function abnormalities is shown in Table 36 below. Most of the recorded abnormalities were isolated increases in bilirubin, SGOT, or SGPT. Only 2 subjects[3], both on sildenafil, had as much as a doubling of alkaline phosphatase. In placebo-controlled studies, the rates and severity of laboratory abnormalities pertaining to hepatic function were similar in placebo and active treatment groups.

Table 36. Hepatic function abnormalities in placebo-controlled studies.

	Placebo N=1342			Sildenafil N=2510		
	n	%	-fold max	n	%	-fold max
Bilirubin only	22	1.6	2.1	35	1.4	4.0
Enzymes						
1	23	1.7	19	46	1.8	17
2	6	0.4		9	0.4	
3	—	—		—	—	

8.2.4.2. Renal function

Laboratory abnormalities pertaining to renal function were screened by identification of subjects in placebo-controlled studies who had BUN or creatinine within normal limits at baseline but increased 1.2-fold from baseline at study end. For such subjects, BUN and creatinine values were abstracted for further examination.

Only 2 subjects were identified as having potentially clinically significant increases in BUN or creatinine during placebo-controlled studies. One subject on placebo[4] had no change in creatinine (0.81 mg/dL at baseline and 0.83 at study end) but BUN went from 50 to 114. One subject on sildenafil[5] progressed from a creatinine of 1.1 to 1.5 mg/dL; BUN was 20 at study end.

8.2.4.3. Electrolytes, hematology, etc.

Significant changes in electrolytes, creatine kinase, and hematologic parameters were assessed by counting subjects on placebo and sildenafil with fractional shifts from baseline exceeding a parameter-specific amount. The thresholds were chosen, in part to detect a change of potential clinical significance and in part to select a small percentage of placebo subjects to optimize detecting a sildenafil-placebo difference.

Potentially clinically significant shifts in electrolytes and creatine kinase during placebo-controlled studies are shown in Table 37 below. Shifts in hematologic parameters are shown in Table 38 below. Of these, the only laboratory parameter for which further investigation appeared to be appropriate was calcium: there is at least a trend for subjects on sildenafil to be less likely to have an increase in calcium and more likely to show a decrease.

Because of the apparent trend for a reduction in calcium in the preceding analysis, a second analysis was undertaken to look for systematic difference in calcium levels as

[3] Study 101 subject 95020019 and study 102 subject 95000286.
[4] Study 359 subject 700140.
[5] Study 101 subject 95110096.

Table 37. Potentially clinically significant shifts in electrolytes in placebo-controlled studies.

		Placebo N=1342		Sildenafil N=2510				Placebo N=1342		Sildenafil N=2510	
		n	%	n	%			n	%	n	%
Sodium	↑5%	7	0.5	5	0.2	Calcium	↑10%	40	3.0	51	2.0
	↓5%	7	0.5	10	0.4		↓10%	17	1.3	68	2.7
Potassium	↑10%	126	9.4	252	10	Creatine kinase	↑10%	192	14	364	15
	↓10%	137	10	268	11		↓10%	191	14	401	16

Table 38. Potentially clinically significant shifts in hematologic parameters in placebo-controlled studies.

		Placebo N=1342		Sildenafil N=2510				Placebo N=1342		Sildenafil N=2510	
		n	%	n	%			n	%	n	%
Hematocrit	↑10%	34	2.5	63	2.5	WBC	↑50%	74	5.5	114	4.5
	↓10%	59	4.4	134	5.3		↓50%	9	0.5	31	1.2
							↓75%	2	0.1	3	0.1
Hemoglobin	↑10%	30	2.2	71	2.8	Neutrophil	↑50%	114	8.9	217	8.6
	↓10%	21	1.6	34	1.4		↓50%	25	1.9	47	1.9
							↓75%	5	0.4	7	0.3
RBC	↑10%	78	5.8	291	12	Platelets	↓50%	2	0.1	2	0.1
	↓10%	41	3.1	65	2.6		↓75%	—	—	—	—

a function of dose. Because dose information was not available in the integrated datasets, this analysis was performed on a dataset formed from laboratory data from the 4 placebo-controlled studies[6] for which full data were available. The analysis used the sponsor-identified baseline values and the last on-treatment value available for each subject, ignoring differences in the period of follow-up specified in the protocol design. The result is shown in Table 39 below. There is no evidence of an effect on mean calcium levels at any dose, and no dose-related trend.

Table 39. Relationship between dose at calcium level (Studies 102, 103, 363, and 364).

	Placebo N=1052	Sildenafil (mg) 25 N=288	50 N=360	100 N=608
Baseline	9.1±0.5	9.1±0.5	9.0±0.4	9.1±0.5
On treatment	9.2±0.4	9.1±0.5	9.2±0.4	9.2±0.4
Range	8.2–11.7	6.9–10.6	8.1–10.2	8.4–10.4

8.2.4.4. Vital signs

The sponsor evaluated median changes in vital signs in placebo-controlled phase II and II studies together and then looking at median changes in subjects on anti-anginal drugs—nitrates, β-blockers, calcium channel blockers, and potassium channel activators. These results are summarized in Table 40 below.

The reviewers computed mean changes in systolic and diastolic pressures and in pulse rate, for subjects in placebo-controlled studies. They also plotted vital signs at study

6. Studies 102, 103, 363, and 364.

Table 40. Summary of median changes in vital signs in placebo-controlled studies.

		All placebo-controlled				All placebo-controlled				All placebo-controlled			
		Placebo		Sildenafil		Placebo		Sildenafil		Placebo		Sildenafil	
		Base	Δ	Base	Δ	Base	Δ	Base	Δ	Base	Δ	Base	Δ
Sitting	N	1187		2200		187		353		471		847	
	Sys	130	0	130	0	140	0	140	-4	135	0	137	0
	Dias	80	0	80	0	82	0	82	0	80	0	80	0
	Pulse	72	0	72	0	72	0	72	0	72	0	74	0
Standing	N	16		16		—		—		—		—	
	Sys	116	0	118	-6	—	—	—	—	—	—	—	—
	Dias	80	-1	80	0	—	—	—	—	—	—	—	—
	Pulse	72	7	79	4	—	—	—	—	—	—	—	—
Supine	N	54		55		—		—		—		—	
	Sys	125	0	128	-3	—	—	—	—	—	—	—	—
	Dias	80	0	80	0	—	—	—	—	—	—	—	—
	Pulse	70	0	68	-1	—	—	—	—	—	—	—	—

end against baseline values, for these same studies, to look for outliers. These analyses (not shown here) are consistent with the sponsor's analyses of median changes; any such effects are small. This result however, must be interpreted in the context that end-of-study measurements were likely often made many hours or days after the last dose of study drug. Other data, discussed in section 8.2.5.1 on page 54, is indicative of a potentially clinically significant effect of sildenafil on blood pressure.

8.2.4.5. ECG

Electrocardiograms were obtained within 24 hours of a dose in phase III Studies 148-102 and 148-106, which exposed subjects to doses from 25 to 200 mg. ECGs were obtained 40 minutes after dosing subjects receiving intravenous sildenafil in Study 148-203. Studies 148-001, 148-004, 148-201, and 148-201A exposed 4 to 10 subjects per dose to doses ranging from 1.25 to 800 mg, with ECGs obtained 1 hour after dosing, near the time of expected peak plasma levels. Data from the latter collection of studies are shown in Figure 6 below. The figure and the data from the study with intravenous administration are consistent with there being a small, clinically insignificant, dose-related effect of sildenafil on heart rate. None of the study designs permit comment upon the duration of this effect, other than it being undetectable at 24 hours after dosing.

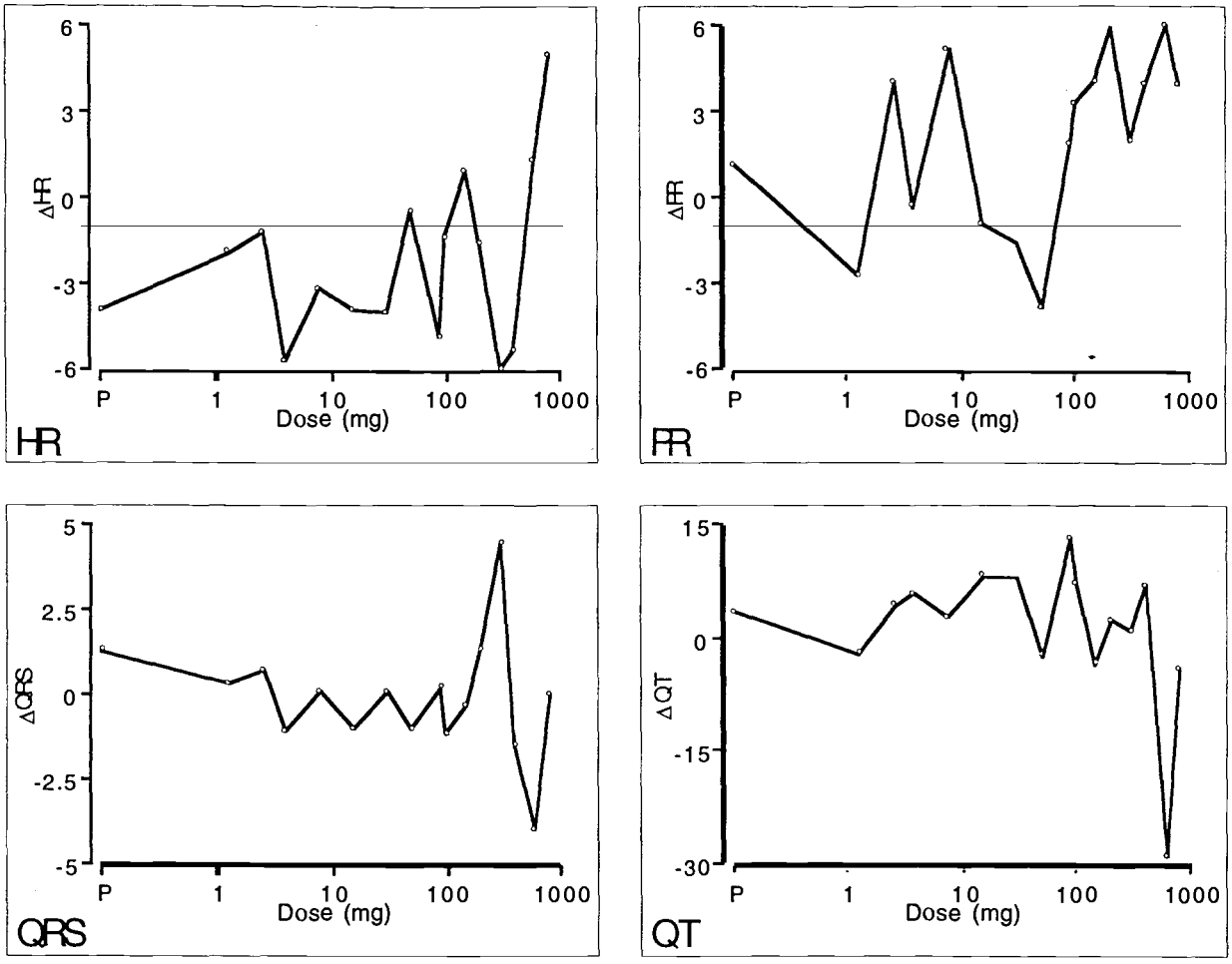


Figure 6. ECG data (Studies 148-001, -004, -201, and -201A.

8.2.5. Pharmacologic basis for safety issues

Sildenafil is a selective inhibitor of phosphodiesterase, an enzyme that catalyzes the cleavage of cAMP or cGMP. Different tissues have different forms of this enzyme, and these different forms have different affinities for sildenafil.

Sildenafil has the lowest IC_{50} for PDE5—about 3 nM. PDE5 is found in the corpus cavernosum, platelets, skeletal muscle, and vascular and visceral smooth muscle. The IC_{50} for PDE6, found in the retina, is about 30 nM. The IC50 for other phosphodiesterases is 7 μM (skeletal muscle PDE4) to 68 μM (corpus cavernosum PDE2), with cardiac muscle PDE3 (involved in the action of milrinone and other inotropes) somewhere in between. Thus, sildenafil has a high degree of selectivity (~1000-fold) for PDE5 and PDE6.

The role of phosphodiesterase is illustrated (for vascular smooth muscle) in Figure 7 below. Endothelial receptors mediate activity of nitric oxide synthase. NO diffuses from endothelial cells (or comes from organic NO donors like glyceryl trinitrate) to smooth muscle cells where it activates guanylate cyclase. Guanylate cyclase catalyzes the formation of cGMP from GTP. Cyclic GMP is, in turn, broken to GMP by the action of phosphodiesterase. An increase in cGMP, resulting from inhibition of phosphodiesterase, causes a reduction in intracellular calcium, and that leads to relaxation of the smooth muscle cells. Similar mechanisms of control of intracellular calcium probably mediate actions of this system in other cells.
On the basis of the proposed mechanism of action, relative affinities of sildenafil for different forms of phosphodiesterase, and the distribution of phosphodiesterase in

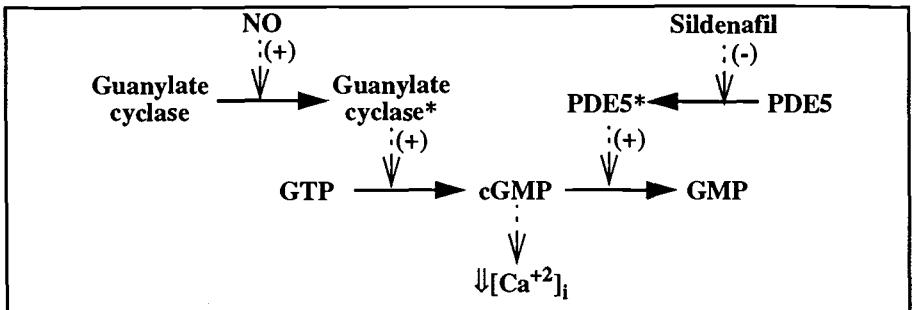


Figure 7. Presumed pharmacological basis for actions of sildenafil.

different tissues, there are effects of sildenafil that can be predicted. These effects include (a) penile erection resulting from relaxation of smooth muscle controlling inflow of blood to the corpus cavernosum, (b) systemic vasodilation and, possibly, hypotension, (c) inhibition of platelet aggregation and increased risk of hemorrhage, (d) skeletal muscle weakness, (e) reduced activity of the gastrointestinal tract, and (f) interference with vision. Each of the potential safety concerns is considered separately.

8.2.5.1. Vital signs

As shown in Study 148-204[7], sildenafil produces peripheral vasodilation, and in phase III studies, where vital signs were not recorded in close association with dosing, sildenafil was associated with vasodilation as an adverse event more commonly than was placebo. Study 148-301[8] was a study of acute hemodynamics of intravenous sildenafil in subjects with chronic ischemic heart disease (not erectile dysfunction). There, sildenafil administration was associated with a blood pressure reduction from baseline of 12/11 mmHg, accompanied by a 10% increase in heart rate[9]. Study of sildenafil (25 mg tid) with glyceryl trinitrate[10] showed a baseline- and placebo-subtracted effect on blood pressure attributable to sildenafil of -15/-5 mmHg with little effect on heart rate. Study of sildenafil (single 100-mg dose) and amlodipine[11] produced peak placebo- and baseline-subtracted effects on blood pressure of -8/-6 mmHg supine and -11/-9 mmHg standing, again with little effect on heart rate.

Sildenafil doses of 800 mg were associated with transient, potentially clinically significant reductions in blood pressure and increases in heart rate in normal male volunteers, as shown in Study 148-004[12]. There was one case of syncope in this study. Available data are not adequate to establish the full dose-response relationship for the effects of sildenafil on blood pressure; hypotension may be the most serious potential adverse effect associated with overdosage.

Sildenafil was otherwise uncommonly associated with symptoms of orthostatic hypotension or with syncope.

---

7. *Study 148-204: An open study in normal volunteers to investigate the effects of an escalating brachial artery infusion of UK-92,480 on forearm blood flow and forearm venous compliance.* on page 133.
8. *Study 148-301: An open single intravenous dose study of the haemodynamic effects of UK-92,480 (sildenafil) in patients with stable ischaemic heart disease.* on page 181.
9. It was also accompanied by a 20% reduction in cardiac output. Systemic vascular resistance was not calculated, but, by implication, it must have increased. Most likely the cardiac output values are in error, since there is other evidence of systemic vasodilation.
10. *Study 148-209: A double blind, randomised, placebo controlled, two-way crossover study to examine the effects of 25mg tid UK-92,480, administered as capsules, on the haemodynamic responses to glyceryl trinitrate in normal volunteers.* on page 141.
11. *Study 148-225: A double-blind, placebo controlled, two way crossover study to investigate the effects of a single dose of sildenafil (100 mg) on blood pressure in subjects with essential hypertension being treated with amlodipine.* on page 162.
12. *Study 148-004: Phase I investigator-blind, placebo-controlled, evaluation of safety, toleration, and pharmacokinetics of sildenafil following escalating single oral doses in healthy male volunteers.* on page 96.

8.2.5.2. Hemorrhage

The sponsor undertook several clinical trials pertinent to assessing the risk of hemorrhage with sildenafil. Bleeding time and platelet aggregation[13] in response to ADP was assessed in Study 148-206[14], 2, 8, and 12 hours after placebo or sildenafil 50 mg. There was no effect bleeding time and no direct effect of sildenafil on platelet aggregation. However, sildenafil produced a 10-fold decrease in the IC_{50} for sodium nitroprusside.

8.2.5.3. Weakness

Weakness was not formally assessed in the clinical development program (and perhaps it is not true that inhibition of PDE5 lowers intracellular calcium in skeletal muscle or that lowering intracellular calcium leads to weakness. Weakness was reported as an adverse event by fewer than 1% of subjects on sildenafil.

8.2.5.4. Decreased gastrointestinal motility

Gastrointestinal motility was not formally assessed in the clinical development program (and perhaps it is not true that inhibition of PDE5 lowers intracellular calcium in visceral muscle or that lowering intracellular calcium leads to decreased motility. Gastrointestinal symptoms were more commonly reported on sildenafil (14%) than on placebo (6.6%) in studies with flexible-dosing schedules. Specific symptoms more common on sildenafil than on placebo included dyspepsia (6.5 vs. 1.7%), diarrhea (2.0 vs 1.0%), nausea (1.2 vs. 0.4%), and tooth disorder (1.1 vs. 0.8%), none of which are easy to associate with the pharmacological mechanism.

8.2.5.5. Vision abnormalities

Because of novel adverse events pertaining to abnormalities in vision, the sponsor performed 2 studies with a battery of tests of visual acuity, contrast sensitivity, color discrimination, pupillometry, electroretinography, photostress, and visual fields. Study 148-223[15] was a single-dose crossover study with doses 50, 100, and 200 mg, and placebo. Study 148-232[16] was a crossover study of placebo and sildenafil 200 mg. These studies demonstrated a dose-related effect of sildenafil on color discrimination, probably restricted to the blue-green part of the spectrum. The effect was manifest particularly 1 to 2 hours after dosing, around the time of the peak plasma levels. Electroretinograms demonstrated a reduced amplitude response to blue light coincident with the color discrimination impairment and presumably a manifestation of the same defect. Study 232 included a cohort of subjects with diabetic retinopathy, but these results were not reported with the NDA.

The clinical significance of effects on vision cannot be unambiguously determined from these data. The effects appear to be short-lived and without collateral effects on aspects of vision which might lead to accidents.

8.3. Summary of key safety findings

- The safety data collected with the sildenafil development program was adequate in terms of doses studied, dosage schedules, duration of exposure, total number of subjects exposed, and special studies performed, to allow the usual level of confidence in an assessment of the risks of sildenafil for its proposed indication.
- There was one death, a suicide, on placebo. There were 8 deaths on sildenafil or within 30 days of treatment. There were 2 deaths of non-cardiovascular causes: One was a metastatic bladder carcinoma—in which a role of study drug is not plausible. The other was a death associated with a fall, cerebral hemorrhage, and pulmonary sepsis, in a subject with pre-existing arrhythmia; a role of study

---

[13] Platelets can be induced to aggregate in vitro with ADP. ADP-mediated platelet aggregation can be inhibited in a dose-related manner by the nitric-oxide donor sodium nitroprusside. The effect of sildenafil was assessed by the effect on the dose-response curve for sodium nitroprusside.

[14] *Study 148-206: A single blind, two way crossover, placebo controlled pilot study to investigate the effects of UK-92,480 (sildenafil) on platelet function in normal male volunteers.* on page 134

[15] *Study 148-223: A double-blind, randomised, placebo controlled, four period crossover study to assess the effect of orally administered sildenafil (50, 100 and 200mg) on visual function in healthy male volunteers.* on page 160.

[16] *Study 148-232: A randomised, double-blind, placebo-controlled, crossover pilot study to investigate the effects of a single oral tablet dose of sildenafil (200mg) on visual function (electroretinogram, photostress, visual field and colour discrimination tests) in healthy male volunteers and patients with diabetic retinopathy.* on page 177.

drug cannot be excluded. Four subjects died from myocardial infarctions, 3 of whom had pertinent histories or evident risk factors. One was a post-operative death in a subject with pre-existing heart failure, and one was a peri-operative death in a study of chronic stable angina. Thus, a small number of deaths might represent increased cardiovascular risk associated with sildenafil.

- Increased cardiovascular risk was not apparent from withdrawals from studies for arrhythmias or myocardial infarctions—despite smaller numbers of subjects and lower periods of exposure, there were more such withdrawals on placebo. Nor was risk evident in comparisons of overall cardiovascular event rates, ECG data, or vital signs.
- Rates of withdrawal from placebo-controlled studies were lower for subjects randomized to sildenafil than for subjects randomized to placebo, with most of the difference attributable to withdrawals for lack of effectiveness. Rates of withdrawal for adverse events were similar on placebo and sildenafil.
- Rates of withdrawal from open-label extensions were quite low—about 10%, with lack of effectiveness being the most common cause.
- The most common adverse event resulting in withdrawal was headache, and this was much more common on sildenafil than on placebo. Adverse events were an uncommon cause for withdrawal from open-label extensions, but among such withdrawals, headache was not uncommon.
- Adverse events reported 'significantly' more commonly on sildenafil than on placebo included headache, vasodilation, dyspepsia, and visual disturbances. All of these can plausibly be linked to pharmacological properties of sildenafil. For the most part, they appear to be dose-related.
- Careful study of blood pressure in the setting of another blood pressure-lowering agent—nitroglycerin or amlodipine—reveals a potentially clinically significant further reduction of blood pressure caused by sildenafil. This effect develops and declines with a time course of hours, similar to the time course of plasma levels of sildenafil after a dose. Although this effect was not associated with symptomatology in trials that included subjects on antihypertensive agents, it remains a safety concern.
- Other adverse events bearing apparent relationship to sildenafil—headaches, dyspepsia, vasodilation, and vision abnormalities—appear to be nuisances of short duration, sufficient to be dose-limiting, but they are not safety concerns. In particular, the vision disturbance has been fairly well characterized, and it does not appear to pose a risk to men operating motor vehicles or other heavy equipment.
- A few effects of sildenafil were expected on the basis of the distribution of phosphodiesterase or observed effects in animals but were missing from the safety experience. Phosphodiesterase inhibition in visceral smooth muscle predictably produced lower gastrointestinal motility in animals, but was without obvious symptomatology, except, perhaps, dyspepsia, in man. Phosphodiesterase inhibition in platelets did not appear to increase the risk of hemorrhage in man or animals.

Center for Drug Evaluation and Research

Viagra (Sildenafil)

"Joint Clinical Review" for NDA-20-895

Section 9, page 57 through Section 10.7, page 70

Pages 57-68

Deleted

as Draft Labeling

exemption (b)(4)

10. Summary and recommendations

10.1. Chemistry

The only outstanding issue pertains to assignment of an expiration date of 12 or 24 months.

10.2. Pharmacology and toxicology

There are no outstanding issues or concerns.

10.3. Biopharmaceutics

There are somewhat conflicting data concerning the effect of CYP 3A4 inhibitors on the pharmacokinetics of sildenafil. Recommended labeling reflects the larger observed effect. The sponsor might reasonably elect to attempt to resolve the discrepancy with additional data.

The adequacy of proposed methods for dissolution testing cannot be evaluated until the sponsor provides dissolution profiles for all proposed tablet strengths using the proposed methods.

The sponsor should be requested to provide dissolution data comparing formulations S00406AC and S00394AD, used in key clinical studies, to the formulation proposed for marketing.

10.4. Effectiveness

Sildenafil 25 to 100 mg was associated with dose-related improvements in erectile function and sexual performance in subjects with erectile dysfunction of various (often ill-characterized) etiologies, including subjects with mild to fairly severe impairment. Improvement in sexual performance was assessed in placebo-controlled studies utilizing a retrospective questionnaire, and it was confirmed using subject diaries.

The studies are sufficient to indicate the drug for use in patients with 'organic' etiologies, including diabetes and spinal cord injury, and in patients with psychogenic erectile dysfunction. Diabetics generally showed a smaller benefit than did other groups.

The primary effect of sildenafil is to *enable* an erection sufficient for sexual intercourse, in the setting of appropriate sexual stimulation. In this respect, it differs from intracavernosal or transurethral alprostadil which *produce* an erection. It seems likely, then, that these other treatments may be effective in some patients for whom sildenafil is ineffective, but this has not been addressed in any trial.

In studies where dose-titration was permitted, most subjects ended study on the highest available dose (usually 100 mg).

The time course of sildenafil's effects has not been well-characterized, but it probably corresponds roughly with the time course of plasma levels of sildenafil and its active metabolite, UK-103,320—0.5 to several hours after dosing.

10.5. Safety

There were 9 cardiovascular deaths among subjects receiving sildenafil, versus one non-cardiovascular death on placebo. Only 2 of the deaths on sildenafil occurred in subjects without substantial risk factors. In no case was sildenafil considered—by the investigator, the sponsor, or the clinical reviewers—to have played a likely role.

Withdrawals for adverse events were no more common on sildenafil than on placebo. Overall, withdrawals from placebo were substantially higher, largely attributable to differences in withdrawal for lack of effectiveness.

Many common treatment-related adverse events—notably headache, vasodilation, dyspepsia, and vision disturbance—were clearly dose-related. At least small clinical studies explored doses up to 800 mg. Doses above the recommended maximum (100 mg) produced higher incidences of events seen at lower doses, but no new phenomena. These adverse events were dose-limiting, but they were rarely serious.

Sildenafil produces a peak reduction in blood pressure of about 8/6 mmHg accompanied by an increase in heart rate, around the time of the peak plasma concentration. Co-administration of a nitric oxide donor results in a substantially larger effect on blood pressure and heart rate.

Effects of sildenafil on vision appear to be restricted to aberrations in color discrimination which are ascertainable by careful testing much more often than they result in subjective complaints. Available evidence suggests that effects of sildenafil on vision do not represent a safety hazard.

10.6. Recommendation

SIldenafil should be approved for the treatment of erectile dysfunction.

10.7. Comments on label

Section 9 of this review contains the clinical reviewers' recommendations for the sponsor's label.

The reviewers removed the quantitative description of the results from the sexual function questionnaire because it would not communicate anything useful to the prescribing physician.

The reviewers added a statement concerning sexual performance to the indication for use, comparable to the statement in the MUSE label.

The reviewers concurred with the sponsor's plan to list common adverse events from the titration studies, since that trial design probably better reflects clinical practice.

Center for Drug Evaluation and Research

Viagra (Sildenafil)

"Joint Clinical Review" for NDA-20-895

Appendix A, page 71 through Appendix A4.6, page 91

A. Study reports

A1. In vitro metabolism studies

A1.1. Report DM2: In vitro metabolism of UK-92480 in hepatic microsomes from rat, dog, rabbit, and man.

A1.1.1. Methods

This study investigated the in vitro metabolism of sildenafil by hepatic microsomes of rat, dog, rabbit, and man. The cytochrome content was determined spectrophotometrically. Incubations were carried out in sildenafil 1 µM and cytochrome P450 0.4 mM in a total volume of 12 mL.

A1.1.2. Results

The disappearance half-life values are shown in Table 41 below. The disappearance of sildenafil was accompanied by the formation of a metabolite identified as UK-103,320.

Table 41. Disappearance half-lives for sildenafil in hepatic microsomes (Report DM2).

	$t_{1/2}$ min			$t_{1/2}$ min
Rat			Dog	38
Male	2		Rabbit	113
Female	129		Human	45

A1.2. Report DM3: In vitro metabolism of UK-92480 in human liver microsomes enzymology of UK-103,320 formation.

A1.2.1. Source documents

NDA 20-895, vol 1.34; electronic document 46815069.pdf.

A1.2.2. Objectives

The aim of this study was to identify the enzymes responsible for the N-demethylation to UK 103,320.

A1.2.3. Methods

Hepatic microsomes were prepared from individual human livers or a combination of 4 human livers by the process of differential centrifugation. For each of the assays used, the final incubation volume was 1 mL. A sildenafil substrate concentration of 0 to 750 µM was chosen to look at the kinetics of UK-103,320 formation, and the effects of specific inhibitors of various P450 isoforms on the metabolism of sildenafil were investigated. Table 1 shows the concentrations of inhibitors used, the isoform which they specifically inhibit and the percentage of inhibition of probe substrate for that isoform. These inhibitors were co-incubated with sildenafil at 2.5 and 250 µM.

Table 42. P450 inhibitors (Report DM3).

P450	Inhibitor	µM	Inhibition (%)	P450	Inhibitor	µM	Inhibition (%)
CYP1A2	Furafylline	1	59	CYP2D6	Quinidine	2.5	82
		10	95			25	95
CYP2C9	Sulphaphenazole	2.5	53	CYP3A4	Ketoconazole	2.5	79
		25	85			25	88

In addition, a bank of 14 human livers was used to assess sildenafil metabolism. Microsomes from these livers had been previously characterized for P450 isoform activity. A correlation was performed between sildenafil metabolism at 2.5 and 250 µM and each isoform activity across the human liver bank. The correlation was weighted by using the logarithm of the sildenafil rates and P450 probe substrate activities to minimize the influence of high-activity livers.

The formation of the metabolite UK-103,320 from sildenafil (2.5 and 250 µM) was assessed in microsomes from AHH-1 TK+/- cells engineered to express one of CYP1A2, CYP2C9, CYP2D6, CYP2E1, or CYP3A4 as the cell's only P450. The kinetics of UK-103,320 formation were investigated in cells expressing CYP3A4 and CYP2C9.

A1.2.4. Results

The rate of formation of UK-103,320 in human liver microsomes was linear with time up to 60 minutes and protein up to 0.1 mg/mL microsomal protein. The mean kinetic parameters for 3 livers are given in Table 43 below. Figure 8 below shows the effect of specific CYP inhibitors on the metabolism of sildenafil. The results of the inhibition study show that CYP3A4 and CYP2C9 are involved in the formation of UK-103,320. Table 44 below shows the results of analyses of the correlation across a bank of 14 human livers which indicate a strong correlation between the formation of UK-103,320 and the activity of CYP3A4 and CYP2C9. This is further illustrated in Figure 9 below. Figure 10 below shows the results of the study done with cells lines expressing CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A4. At a concentration of 2.5 µM of sildenafil both CYP2C9 and CYP3A4 mediated UK-103,320 formation with CYP3A4 producing a rate 20 times that of CYP2C9. At 250 µM CYP2D6, CYP2C9 and CYP3A4 mediated UK-103,320 formation. However, the rate produced by CYP2D6 was considered negligible at the concentration level studied. This rate increased 20 fold for CYP3A4 and 13 fold for CYP2C9 when going from a 2.5 µM to 250 µM.

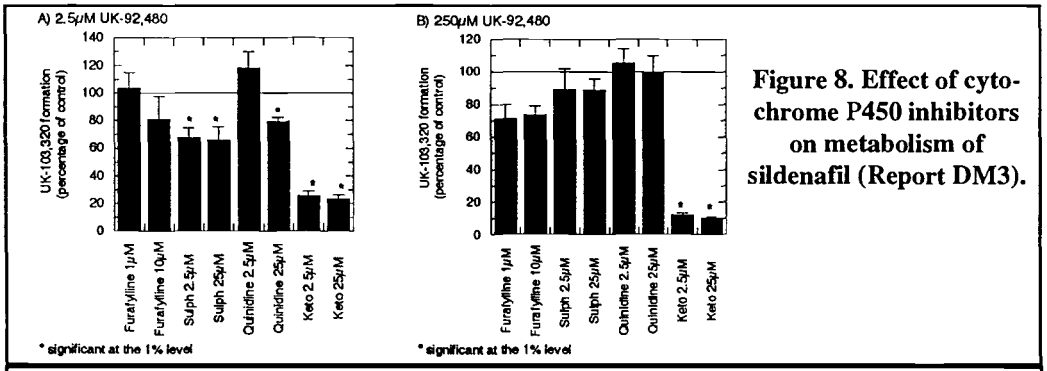


Figure 8. Effect of cytochrome P450 inhibitors on metabolism of sildenafil (Report DM3).

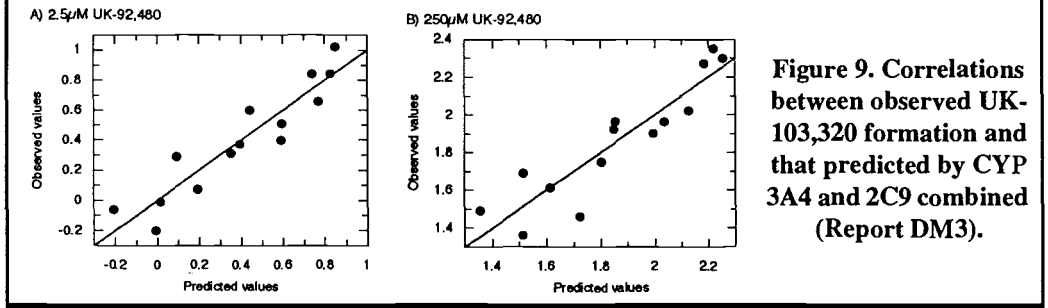


Figure 9. Correlations between observed UK-103,320 formation and that predicted by CYP 3A4 and 2C9 combined (Report DM3).

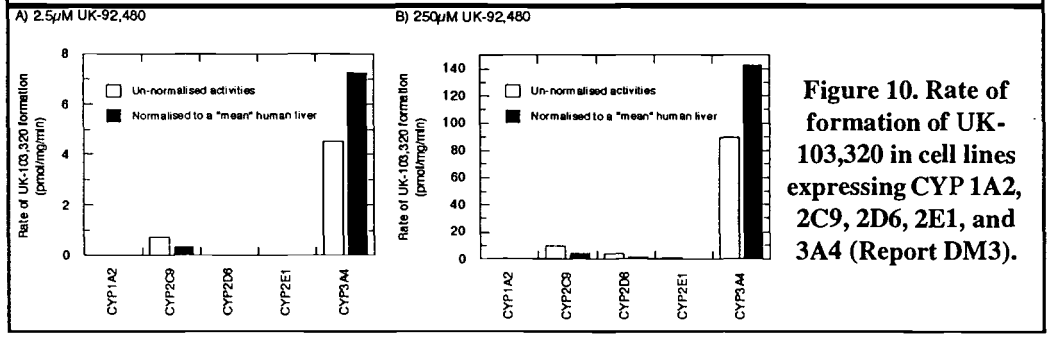


Figure 10. Rate of formation of UK-103,320 in cell lines expressing CYP 1A2, 2C9, 2D6, 2E1, and 3A4 (Report DM3).

Table 43. UK-103,320 formation in human liver microsomes (Report DM3).

	Affinity	
	High	Low
K_m (μM)	6.0±2.5	81±45
V_{max} (pmol/mg/min)	22±9.7	138±77

Table 44. Correlation between rate of UK-103,320 formation and P450 isoform activity (Report DM3).

Isoform	Substrate	Sildenafil μM[a]		Isoform	Substrate	Sildenafil μM	
		2.5	250			2.5	250
CYP1A2	Caffeine	0.49	0.53	CYP2D6	Bufuralol	0.34	0.33
CYP2A6	Coumarin	0.24	0.44	CYP2E1	Chlorzoxazone	0.36	0.49
CYP2C9	Phenytoin	0.77*	0.80*	CYP3A4	Testosterone	0.87*	0.84
CYP2C19	S-mephenytoin	0.48	0.31				

a. *P<0.01 by sponsor's analysis.

A1.2.5. Conclusion

Sildenafil is metabolized to UK-103,320 in human liver microsomes by two enzymes. CYP2C9 was the high affinity enzyme with a K_i of 6 μM. CYP3A4 was considered to be the low affinity enzyme with a K_i of 81 μM.

A1.3. Report DM4: In vitro inhibition studies on UK-92480 in human liver microsomes.

A1.3.1. Source documents

NDA 20-895, vol 1.34.

A1.3.2. Objectives

The objective of the study was to investigate the potential of sildenafil to inhibit 6 cytochrome P450 isoforms considered to be of general importance in drug metabolism. These isoforms are CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4. The study was conducted in vitro with human liver microsomes using probe substrates for these isoforms.

A1.3.3. Results

The estimated IC_{50} for the various isoforms tested are summarized in Table 45 below.

Table 45. Inhibition of CYP isoforms by sildenafil (Report DM4).

Isoform	Substrate	IC_{50} μM	Isoform	Substrate	IC_{50} μM
CYP1A2	Phenacetin	~300	CYP2D6	Bufuralol	>300
CYP2C9	Phenytoin	150	CYP2E1	Chlorzoxazone	>1000
CYP2C19	S-mephenytoin	~300	CYP3A4	Felodipine	>300

A1.3.4. Conclusion

Since the expected peak plasma concentrations in the clinically relevant dosing range (25 to 100 mg) is around 1 μM, it is unlikely that sildenafil will inhibit any of the relevant CYP isoforms to any significant extent, and thus no drug-drug interactions are expected based on inhibition of the P450 system.

A1.4. Report DM5: In vitro metabolism and P450 inhibition studies of UK-103,320 in human liver microsomes.

A1.4.1. Source documents

NDA 20-895, vol 1.34; electronic document 46815067.pdf.

A1.4.2. Objectives

The objectives of this study were to investigate whether UK-103,320 is metabolized by cytochrome P450 and also to determine the its potential to inhibit 6 cytochrome

P450 isoforms considered of general importance in drug metabolism (viz CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4).

A1.4.3. Methods

The studies were conducted in vitro in human liver microsomes.

A1.4.4. Results

The results suggest that UK-103,320 is metabolized by the CYP450 system. Two putative metabolites were identified—UK-321,120 (N-desmethyl) and UK-331,849 (removal of two-carbon fragment from piperazine ring). Scheme 1 shows the partial metabolic scheme for UK 103,320 in human liver microsomes.

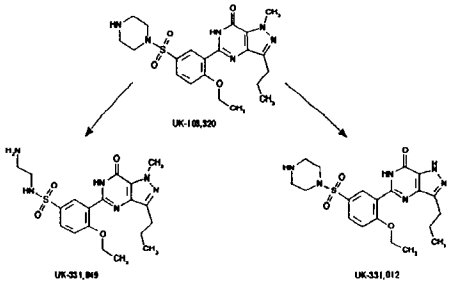


Figure 11. Partial metabolic scheme for UK-103,320 in human liver microsomes (Report DM5).

Moreover, the results of this study indicate that UK-103,320 is a very weak inhibitor of the CYP isoforms investigated, as shown in Table 46 below. The IC_{50}'s are well above the expected peak plasma concentrations for this metabolite.

Table 46. Inhibition of CYP isoforms by UK-103-320 (Report DM5).

Isoform	Substrate	IC_{50} µM	Isoform	Substrate	IC_{50} µM
CYP1A2	Phenacetin	>1000	CYP2D6	Bufuralol	71
CYP2C9	Diclophenac	>1000	CYP2E1	Chlorzoxazone	>1000
CYP2C19	S-mephenytoin	>300	CYP3A4	Felodipine	>300

A1.5. Report DM34: In vitro interaction between UK-92480 and the CYP3A4 substrates terfenadine and testosterone in human liver microsomes.

A1.5.1. Source documents

NDA 20-895, vol 1.34.

A1.5.2. Objectives

The objective of this study was to investigate the potential of sildenafil to inhibit the metabolism of terfenadine and testosterone in human liver microsomes.

A1.5.3. Results

Figure 12 below shows the effect of sildenafil on the activity of testosterone 6-ß-hydroxylase and terfenadine hydroxylase.

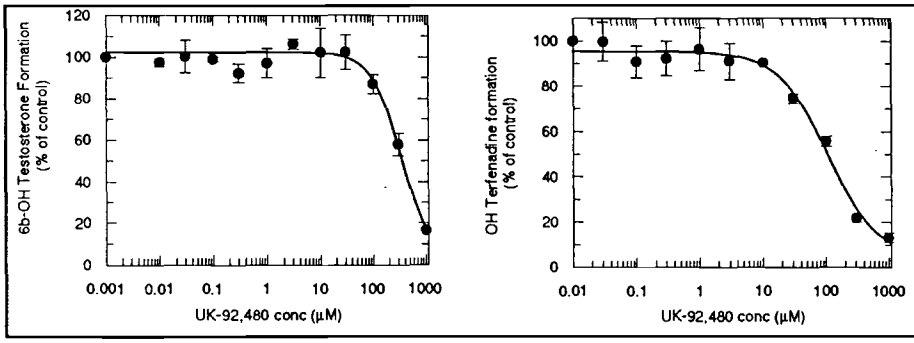


Figure 12. Inhibition of metabolism of testosterone and terfenadine by sildenafil (Report DM34).

The IC_{50} of sildenafil was greater than 300 µM for testosterone and around 100 µM for terfenadine. The K_i was estimated to be 50 µM. Since the peak plasma sildenafil concentrations are expected to be around 3 µM, these results suggest there will be no clinically significant interactions between sildenafil and CYP3A4 substrates.

A2. Population pharmacokinetic and pharmacodynamic analysis of sildenafil phase III data.

A2.1. Methods

A2.1.1. Data collection

A population pharmacokinetic strategy was incorporated into 5 phase III clinical study protocols (studies 148-102, 148-103, 148-104, 148-106 and 148-364). Two thousand seventy-seven subjects (1335 on sildenafil) were asked to take an additional dose of study drug prior to their scheduled clinic visits on 4 or 5 occasions throughout the study. A single plasma sample was obtained at random times after dosing and assayed for both drug and metabolite UK-103,320 concentrations. A total of 4582 samples were assayed (3.4 samples per individual). Data from individual studies were combined into one dataset.

A2.1.2. Pharmacokinetic modeling

Formal population pharmacokinetic analysis was performed using the nonlinear mixed effects modeling approach. The software package NONMEM version IV, level 2.2 was used to derive the population mean (and variance) values for specific pharmacokinetic parameters, such as apparent clearance and apparent volume of distribution, and these were subsequently used to derive estimates of exposure (AUC). Appropriate structural pharmacokinetic models were fitted to both the parent drug and metabolite using standard population pharmacokinetic methodology.

Both linear and nonlinear relationships between the individual parameter estimates and the various covariate indices for demography, biochemistry and concomitant medication were explored and, where indicated, used to refine the population model and characterize sources of inter-individual and intra-individual variability. Covariates were added to the model if they significantly decreased the objective function by 0.1% level of significance. Covariates were removed from the model if ±2 SE of the resultant parameter estimate encompassed 0. The significance of each of the covariates in the fully developed model was further tested by fixing each structural model parameter used to characterize the covariate relationship to a null value and performing reduced-versus-full model pair comparisons. The resultant final model only contained covariates that met the pre-defined statistical criteria. The clinical relevance of any relationship was also considered.

For the drug pharmacokinetic model, model building was initiated on a test database which comprised studies 148-102, 148-103 and 148-104. A validation dataset comprised of studies 148-106 and 148-364 was used to test the predictive performance of the derived population model from the test data. These datasets were subsequently combined and the resultant population model refined and finalized. As part of the model-building process, the validity of the population model was assessed via deletion diagnostics; i.e., the population model parameter values were re-estimated following sequential removal and replacement of individual study data.

A2.1.3. Pharmacokinetic-pharmacodynamic modeling

The relationship between the AUC of the parent drug and metabolite to questions 3 (*How often were you able to penetrate your partner?*) and 4 (*How often were you able to maintain your erection after you had penetrated your partner?*) of the International Index of Erectile Function (IIEF) sexual function questionnaire were investigated. The responses to these effectiveness questions were recorded as follows: (0) did not attempt an intercourse, (1) almost never or never, (2) a few times, (3) sometime, (4) most times, or (5) almost always or always.

Asymptotic E_{max} models with baseline and placebo components were used in these analyses. A number of independent variables were incorporated in these models including dose, drug AUC, metabolite AUC and both drug and metabolite AUC concurrently.

Relationships between sildenafil dose and the individual estimates of both AUC and C_{max} to the various adverse events were explored graphically.

A2.2. Results

Figure 13 below shows the drug concentrations for parent drug and metabolite. Insets show log-plots of the same data, omitting concentration values below 1 ng/mL.

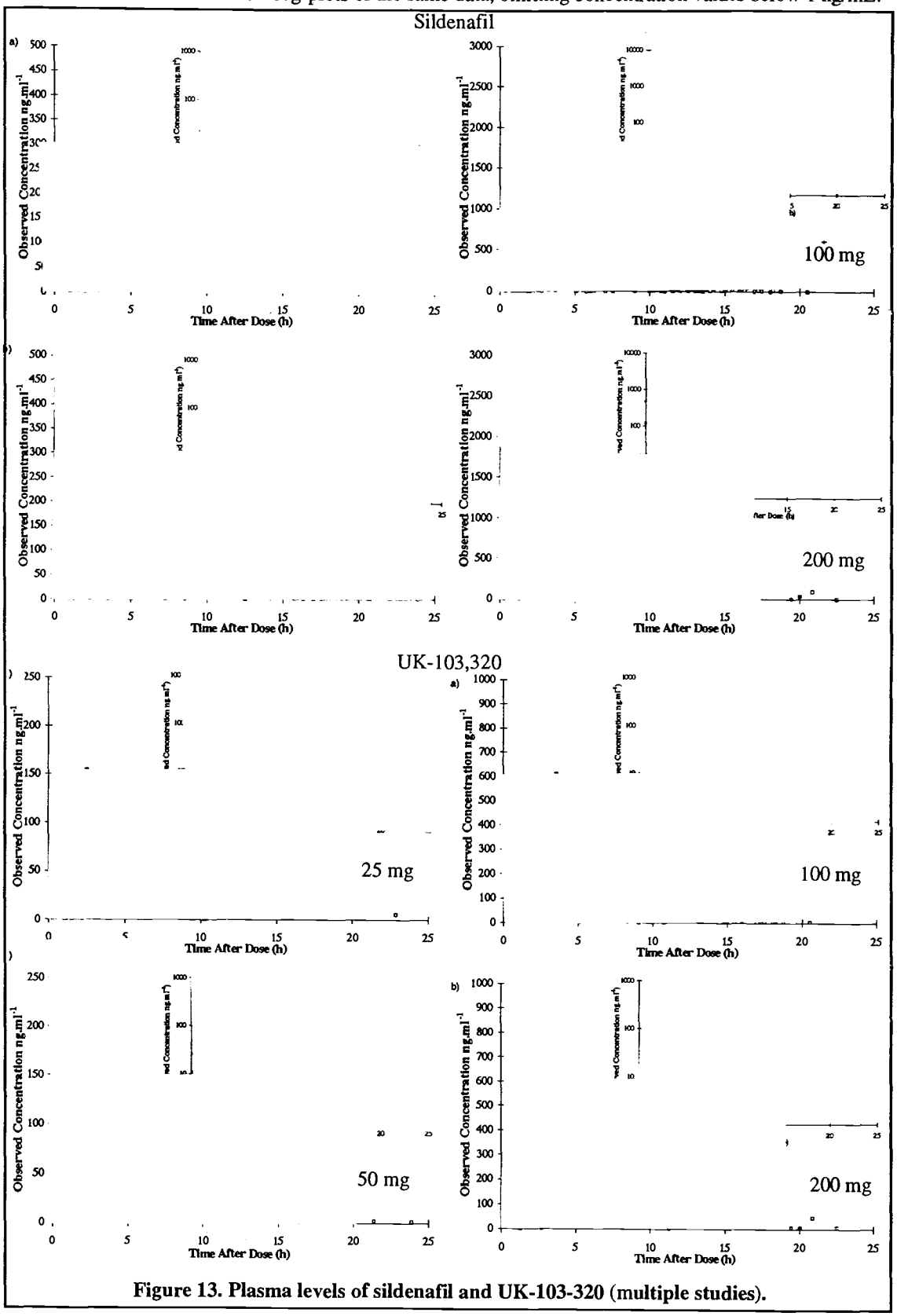


Figure 13. Plasma levels of sildenafil and UK-103-320 (multiple studies).

A2.2.1. Sildenafil pharmacokinetics

According to the sponsor, the sildenafil plasma concentration-versus-time data were appropriately described by a 1-compartment disposition model with first-order input. There was no evidence in the goodness-of-fit plot (Figure 14 below) that a more complicated structural model would be required.

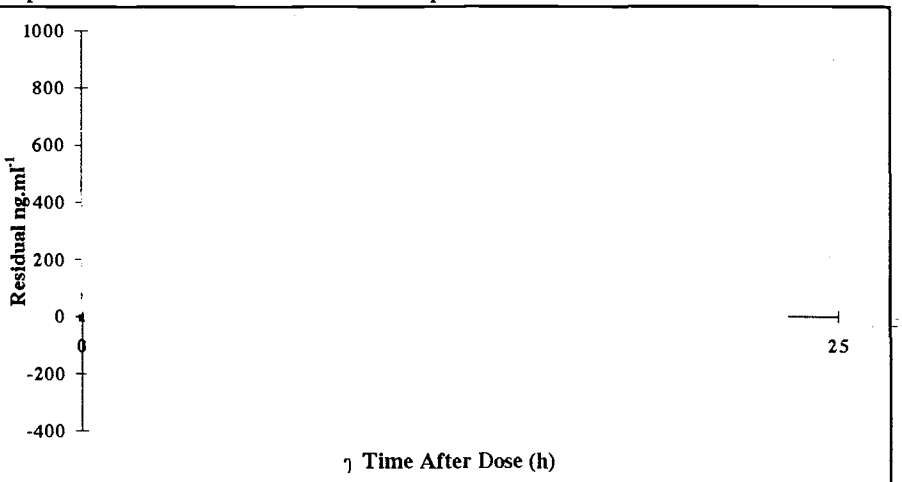


Figure 14. Residuals from final model of sildenafil pharmacokinetics.

Three covariates considered to statistically and clinically influence the apparent clearance of sildenafil were age, AST concentration, and whether patients were receiving CYP3A4 inhibitors. There was a 4% decrease in Cl/F for every decade increase, a 6% decrease in Cl/F for every 10-unit increase in AST, and a 14% decrease with co-administration of CYP3A4 inhibitors. For apparent volume of distribution, weight was considered to be a significant covariate. There was a 6% increase in V/F for every 10-kg increase. Figure 15 below shows the relationships between some of these continuous covariates and the structural model parameters they influence.

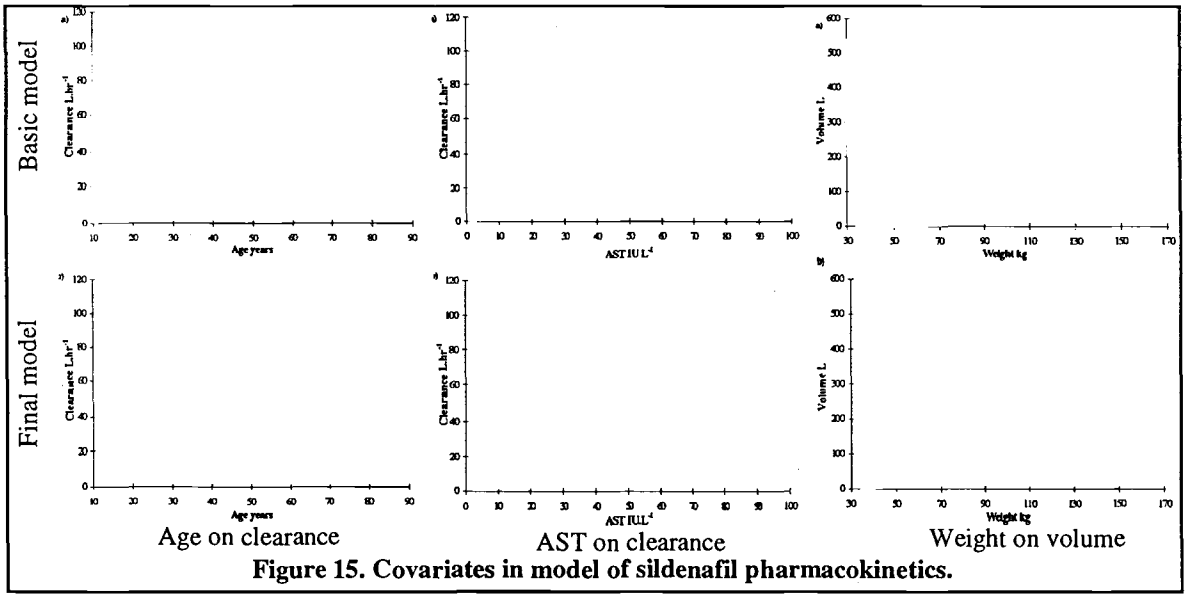


Figure 15. Covariates in model of sildenafil pharmacokinetics.

The model included 2 absorption rates, representing the fasted and fed states.

Non-proportionality of plasma levels of sildenafil with dose was best described by a 40% increase in bioavailability associated with the 200-mg dose.

Figure 16 below gives the goodness of fit plots for the population predicted concentrations and the individual predicted concentrations versus observed final data

set concentrations. Table 47 below shows the estimated pharmacokinetic parameters following administration of sildenafil 25 to 200 mg. Table 48 below shows the effects of addition of covariates to the basic pharmacokinetic model for sildenafil, using the test dataset. Table 49 below shows the effect of additions and deletions in the covariates to the full pharmacokinetic model for sildenafil, using the final dataset. Table 50 below gives the structural and covariate parameter estimates for the population pharmacokinetic model for sildenafil after removal of component studies.

Table 47. Pharmacokinetic parameters (mean±SD) for final sildenafil model.

	25 mg	50 mg	100 mg	200 mg		25 mg	50 mg	100 mg	200 mg
AUC (ng.h/mL)	464±175	950±345	1963±859	5485±1964	CL/F (L/h)	60±18	58±17	58±18	41±12
C_{max} (ng/mL)	84±81	156±49	327±236	902±287	V/F (L)	302±76	299±75	309±88	210±55
T_{max} (h)	1.1±0.9	1.0±0.8	1.2±1.0	1.0±0.8	K_a (h^{-1})	13±26	15±28	14±25	21±37
$T_{1/2}$ (h)	3.6±0.7	3.7±0.7	3.8±0.8	3.7±0.7	K_e (h^{-1})	0.20±0.04	0.20±0.04	0.19±0.04	0.19±0.03

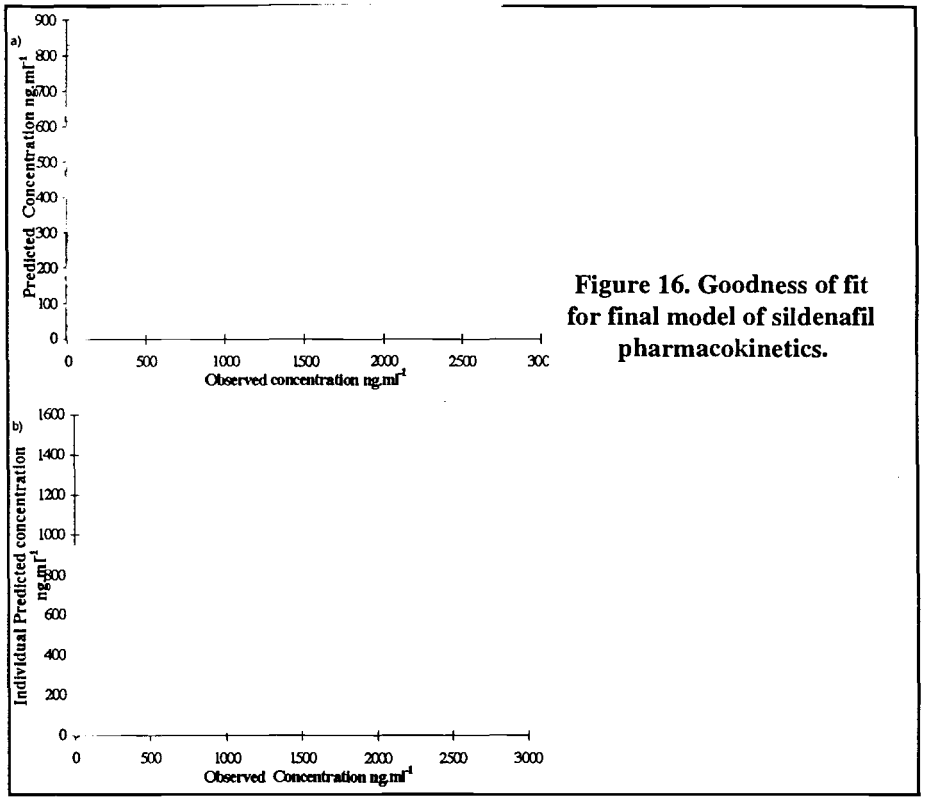


Figure 16. Goodness of fit for final model of sildenafil pharmacokinetics.

Table 48. Effect of addition of covariates to basic sildenafil model (test data).

Covariate model for CL/F				Covariate model for V/F			
Covariate	ΔFit	Covariate	ΔFit	Covariate	ΔFit	Covariate	ΔFit
Age	-48	Nitrate	-15	BSA	-56	MI	-16
CLcr	-29	AST	-15	Weight	-48	Subj	-16
ACEI	-28	Race	-11	Height	-29	Age	-14
Dose	-27			CCK	-22	CLcr	-13
				Dose	-21	AlkP	-12
				Bili	-20		

Table 49. Effect of changes in covariates on pharmacokinetic model for sildenafil (final dataset).

Covariate change	ΔFit	Decision	Covariate change	ΔFit	Decision	Covariate change	ΔFit	Decision
-Age on CL/F	+18	Keep	-Nitrate on CL/F	+3	Drop	-CCK, +Log(CCK)	+1	Drop
-CCK on CL/F	+1	Drop	-Race on CL/F	+23	Keep	-AST, +Log(AST)	-10	Log(AST)?
-AST on CL/F	+30	Keep	-Weight on V/F	+45	Keep	-AST, +ALT	-20	ALT?
-CCB[a] on CL/F	0	Drop	-MI on V/F	+20	Keep	-AST, +Log(ALT)	+10	Keep AST
-ACEI on CL/F	+7	Drop	-25/50mg on Bioavail	+5	Drop	-AST, +AlkP	+26	Keep AST
-3A4 inhib on CL/F	+15	Keep	-200mg on Bioavail	+32	Keep	-AST, +Log(AlkP)	+20	Keep AST
-Diuretics on CL/F	+2	Drop	-2 parameters[b] on K_a	+37	Keep	+CLcr	+7	Drop
-β-block on CL/F	+13	Keep						

a. Calcium channel blockers
b. No idea

Table 50. Effect of deletion of studies from final pharmacokinetic model for sildenafil.

	Study deleted							Study deleted					
	None	-364	-106	-104	-103	-102		None	-364	-106	-104	-103	-102
CL/F	1	1.02	1.04	0.98	0.98	0.98	3A4 inhib	1	1.01	1.01	0.97	1.02	1.01
V/F	1	1.00	1.03	0.95	0.99	1.04	β-block	1	1.16	0.92	0.99	1.01	0.96
K_a	1	0.94	0.98	1.00	1.04	1.07	Race	1	1.00	1.06	0.98	1.02	0.96
Resid	1	1.01	1.02	0.99	0.98	0.99	Weight	1	1.19	1.21	0.70	0.97	1.13
Age	1	1.52	1.38	0.96	0.95	0.27	MI	1	0.99	0.97	1.05	0.99	3.98
AST	1	0.59	1.29	1.16	0.85	0.95	200 mg	1	1.00	0	0.95	0.99	1.06

The population typical values were 59±1.4 L/h for clearance, 310±7 L for apparent volume of distribution, and 2.6± 0.2 h^{-1} for K_a. The inter-individual variability (mean±SE) was 29±20% for clearance, 20±50% for apparent volume of distribution, and 210±25% for K_a. The level of residual variability (CV±SE) was 48±12%.

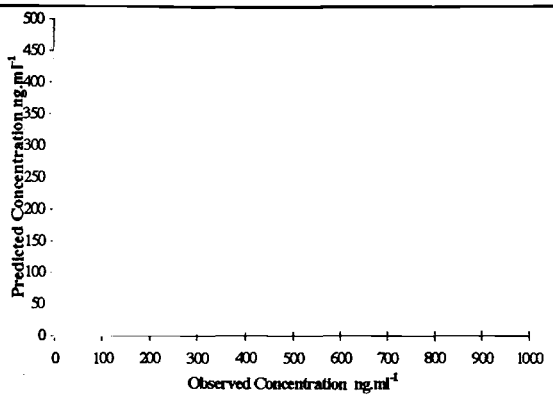
A2.2.2. UK-103,320 pharmacokinetics

Figure 17 below shows the goodness-of-fit plot for the final population model for UK-103,320 for the population-predicted concentrations and individual predicted concentrations versus observed concentrations in the final dataset. Figure 18 below shows selected covariate relationships for the final population pharmacokinetic model. Table 51 below shows the effects of addition of covariates to the basic pharmacokinetic model for UK-103,320, using the test dataset. Table 52 below shows the effect of deletions of covariates to the full pharmacokinetic model for UK-103,320, using the final dataset. Table 53 below gives the structural and covariate estimates for the population pharmacokinetic model after removal of component studies.

Table 51. Effect of addition of covariates to basic UK-103,320 model (test data).

Covariate model for CL/F				Covariate model for V/F			
Covariate	ΔFit	Covariate	ΔFit	Covariate	ΔFit	Covariate	ΔFit
Dose	-105	3A4 inhib	-23	AST	-54	3A4 inhib	-19
CCB	-57	CCK	-20	CCB	-40	Diuretics	-17
CLcr	-52	ACEI	-15	Bili	-40	CS β-blocker[a]	-15
AST	-34	ALT	-13	CLcr	-29	Subject	-11
β-blocker	-29	BSA	-11	ALT	-25		
Subject	-29	AlkP	-11				

a. Cardio-selective β-blocker.



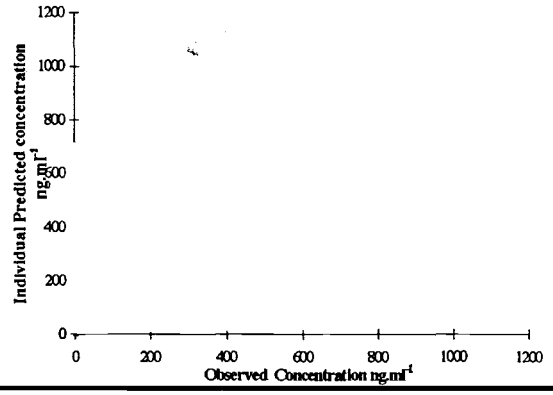


Figure 17. Goodness of fit for final model of UK-103,320 pharmacokinetics.

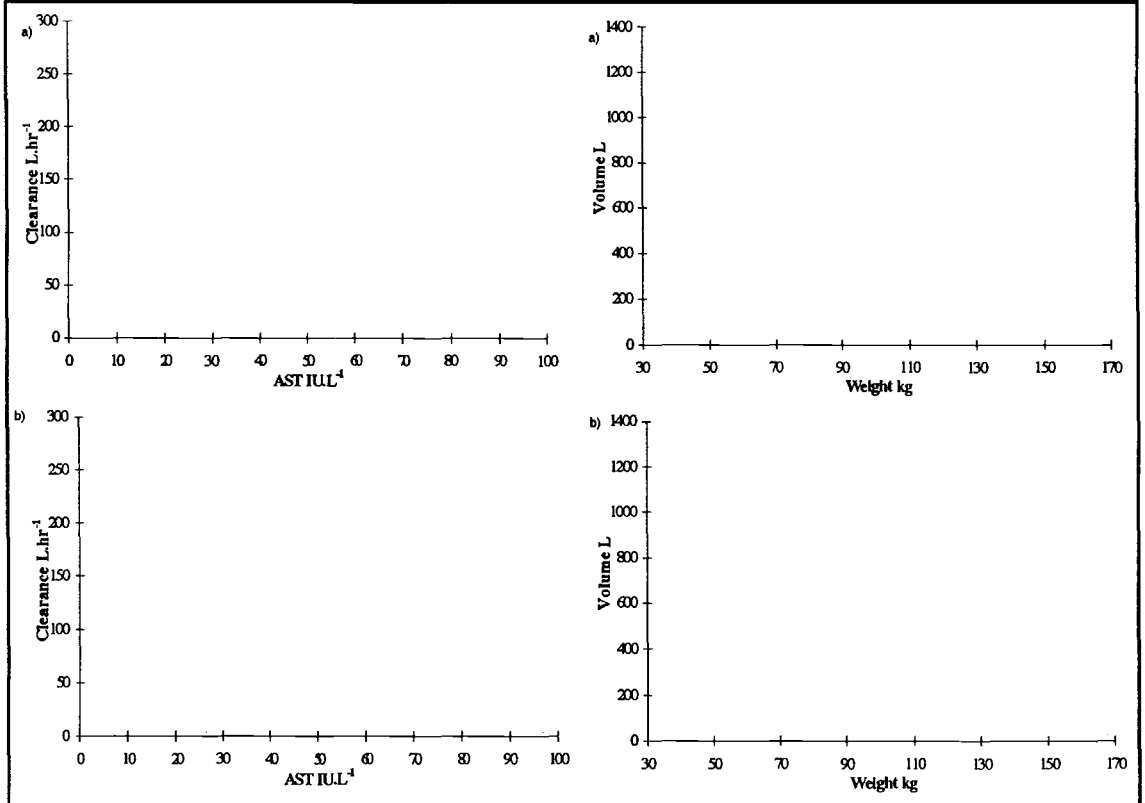


Figure 18. Selected covariate relationships in the final model of UK-103,320 pharmacokinetics.

Table 52. Effect of changes in covariates on pharmacokinetic model for UK-103,320 (final dataset).

Covariate change	ΔFit	Decision	Covariate change	ΔFit	Decision	Covariate change	ΔFit	Decision
-25/50mg	+48	Keep	-AST	+9	AST?	-2C4 inhib	+2	Drop
-200mg	+17	Keep	-ALT	+2	Drop	-Age	0	Drop
-CLcr	+3	Drop	-AlkP	+3	Drop	-Weight	+15	Keep
-CCK	+8	CCK?	-CCB	+21	Keep	-Diuretics	+12	Keep
-Bili	0	Drop	-ACEI	+3	Drop	-β-blockers	+96	Keep

Table 53. Effect of deletion of studies from final pharmacokinetic model for UK-103,320.

	Study deleted							Study deleted					
	None	-364	-106	-104	-103	-102		None	-364	-106	-104	-103	-102
CL/F	1	1.01	1.03	0.99	0.99	0.99	AST	1	0.56	1.00	0.75	1.00	1.04
V/F	1	1.02	0.97	0.98	0.97	1.05	CCB	1	0.96	0.90	1.03	1.12	0.97
K_a	1	0.97	0.96	1.04	1.04	0.99	Race	1	1.12	1.13	0.94	0.98	0.90
Resid	1	1.00	1.04	0.98	0.98	1.02	Weight	1	0.50	1.01	1.16	1.27	1.20
25/50 mg	1	0.76	1.29	1.03	0.91	1.10	Diuretic	1	0.96	1.17	0.88	1.07	0.93
200 mg	1	1.07	1.36	0.96	1.12	1.02	β-blocker	1	0.86	1.41	0.94	1.25	0.79

Three covariates were found to have a significant effect on the apparent clearance of UK-103,320 There was a 9% decrease in clearance for each 10-unit increase in AST. Loop diuretics and nonspecific β-blockers decreased CL/F by 31% and 54%, respectively. The relationship between weight and apparent volume of distribution predicted a 3% change in V/F for every 10-kg change in weight. The non-proportionality in bioavailability predicted a 13% decrease with the 25- and 50-mg doses and a 14% increase with the 200-mg dose, relative to the 100- mg dose. The population-typical values were 109±3.7 L/h for Cl/F, 736±35 L for V/F, and 2.6±0.2 h^{-1} for input rate. The inter-individual variability (mean±SE) were 49±21% for apparent clearance, 38±29% for V/F, and 292±21% for input rate. The level of residual variability was 48±12%.

A2.2.3. Pharmacodynamics

Asymptotic E_{max} models with placebo and baseline components were used in the efficacy analyses. A number of independent variables were incorporated into these models including dose, drug AUC, metabolite AUC, and both drug and metabolite AUC concurrently. The predicted parameters were the baseline value, a component for placebo response, the maximum response (E_{max}) and the value of the dependent variable that was associated with 50% of the E_{max} (D_{50}).

Effectiveness question 3[1]: Table 54 below shows that irrespective of the estimation method adopted with NONMEM and the independent variable used, an additive model performed better than a proportional model (an absolute change in response from baseline was more appropriate than a relative change). The table also shows that neither drug nor metabolite AUC performed any better as a predictor of outcome than simply using the administered dose value.

Figure 19 below shows the summary of week 12 response scores for question 3 by dose and by treatment. These figures show that all doses were superior to placebo.

The analyses showed that three covariates appear to influence the baseline value. There was a 12% decrease in baseline for each decade increase in age, a 3% decrease for every 10-kg increase, and a 17% increase for subjects with psychogenic etiology.

[1]. How often were you able to penetrate your partner?

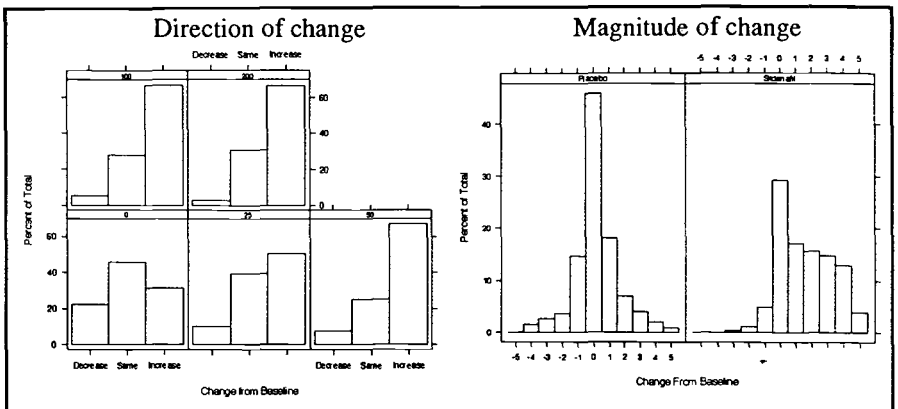


Figure 19. Change in response to question 3.

Table 54. Effect of additive or proportional E_{max} and estimation method on fit to pharmacodynamic model.

	Objective function			
	1st order		1st order conditional	
	Add	Prop	Add	Prop
Dose	7894	8022	7621	8150
Sildenafil AUC	7905	8025	7648	8221
UK-103,320 AUC	7909	8026	7665	8156

No significant covariate relationships could be discerned for E_{50}. Table 55 below shows the results of fitting the final covariate model to a series of datasets with one of the five datasets sequentially removed and replaced.

Table 55. Effect of deletion of studies from final pharmacodynamic model[a].

	Study deleted							Study deleted					
	None	-364	-106	-104	-103	-102		None	-364	-106	-104	-103	-102
Base	1	1.13	0.90	0.98	1.03	—	Add	1	1.11	1.09	1.02	1.04	—
D_{50}[b]	1	1.14	1.00	0.83	1.10	—	Age	1	1.04	0.98	1.02	1.08	0.86
Placebo	1	1.04	0.83	1.13	1.02	—	Subject	1	0.97	1.11	0.83	0.99	1.08
Pcbo ETA[c]	1	0.91	0.91	1.06	1.01	—	Weight	1	1.16	0.95	0.56	0.86	1.52

a. For question 3, how often were you able to penetrate your partner?
b. Dose producing half-maximal response.
c. No idea

For a patient of average age and weight, and with organic etiology for sexual dysfunction, the mean baseline value was 1.51, E_{max} was 5, the mean±SE D_{50} was estimated to be 36±6 mg, and the placebo response was 0.45±0.08. As these components were modeled in an additive manner, the average maximum drug response was 3.05. The inter-individual variability on E_{50} was estimated to be 331±11% and on placebo 148±31%. The level of additive residual variability (SD±SE) was estimated to be 0.46±17%. Figure 20 below gives the results of the simulation that the sponsor performed over a dosing range from 0 to 200 mg.

Table 56 below gives the estimated D_{50} (mg) for each of the five clinical studies used in the population model. These results suggest that subjects in study 104, who were

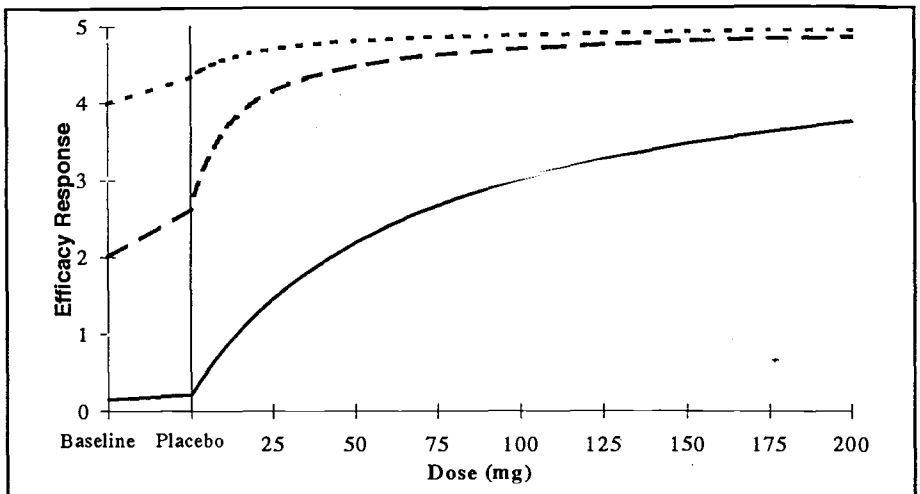


Figure 20. Final dose-response model by baseline for question 3.

mostly diabetics, were less responsive to the effects of sildenafil compared to the other subjects.

Table 56. Estimated D_{50} for question 3, by study.

	148-102	148-103	148-106	148-364	148-104
D_{50}	26± 7.7	20±7.7	40±11	29±7.8	182±67

Effectiveness question 4[2]: A similar model to that developed for question 3 was used to model the effectiveness scores in response to question 4. Table 57 below gives the results of fitting the final model to a series of data sets with one of the data sets removed and replaced. Similar results to those of question 3 were obtained There was an 11% decrease in baseline values for each decade increase in age, a 4% decrease for each 10-kg increase in weight, and a 17% increase for psychogenic etiology for sexual dysfunction.

Table 57. Effect of deletion of studies from final pharmacodynamic model[a].

	Study deleted							Study deleted					
	None	-364	-106	-104	-103	-102		None	-364	-106	-104	-103	-102
Base	1	1.09	0.97	1.01	1.01	—	Add	1	1.12	1.09	1.02	1.05	—
D_{50}[b]	1	1.08	0.98	0.85	1.13	—	Age	1	1.03	1.05	1.03	1.01	0.83
Placebo	1	1.12	1.07	1.13	1.12	—	Subject	1	0.84	1.06	0.88	0.98	1.21
Pcbo ETA[c]	1	0.90	0.92	1.03	1.01	—	Weight	1	0.99	0.90	0.68	1.15	1.30

a. For question 4, how often were you able to maintain an erection after penetration?
b. Dose producing half-maximal response.
c. No idea

For a subject of average weight and age and with organic etiology, the mean baseline value was 0.9, E_{max} was 5, (mean±SE) E_{50} was 41±5.6 mg, and the mean placebo response was 0.4±0.07. Therefore, the average maximum drug response was estimated to be 3.7. The inter-individual variability on E_{50} was 316±15% on active treatment and 119±31% on placebo. The level of additive variability (SD±SE) was 0.5±20%.

[2] How often were you able to maintain an erection after penetration?

Table 58 below gives the estimated D_{50} (mg) for each of the five clinical studies used in the population model.

Table 58. Estimated D_{50} for question 4, by study.

	148-102	148-103	148-106	148-364	148-104
D_{50}	28± 6.7	22±7.6	49±12	35±8.3	199±66

These results confirm the findings of the responses to question 3, that the drug seems to be less effective in diabetic subjects. Similarly, Figure 21 below shows the summary of week 12 response scores for question 4 by dose and by treatment. These figures confirm the previous finding that all doses were superior to placebo. Figure 18 gives the results of the simulation that the sponsor performed over a dosing range from 0 to 200 mg.

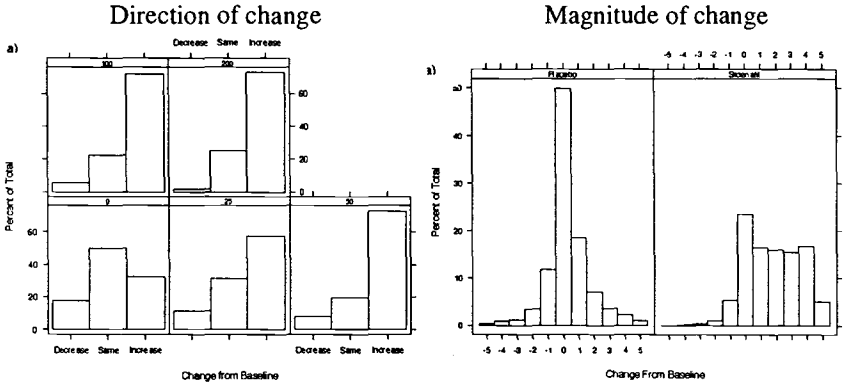


Figure 21. Change in response to question 4.

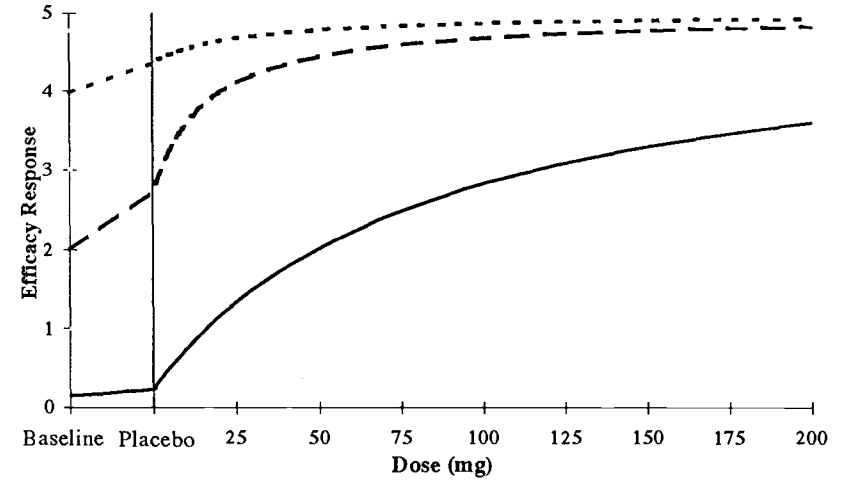


Figure 22. Final dose-response model by baseline for question 4.

A2.2.4. Adverse events

Figure 23 below shows the modeled incidence of specific classes of adverse events by severity (solid bars show moderate or severe events). The incidences of visual, gastrointestinal, vascular, and pain adverse events are all shown to be related to dose, drug AUC, and C_{max}. As with the efficacy analysis, it seems that relationships to dose appear to be as predictive as drug AUC or C_{max}. The incidence of adverse events rose steeply in association with doses of 200 mg, AUC >2600 ng.h/mL, or C_{max} >500 ng/mL. At this highest exposure level, the modeled incidence of abnormal vision was 40%, the incidence of gastrointestinal events was 15%, and the incidence of vascular events was about 25%.

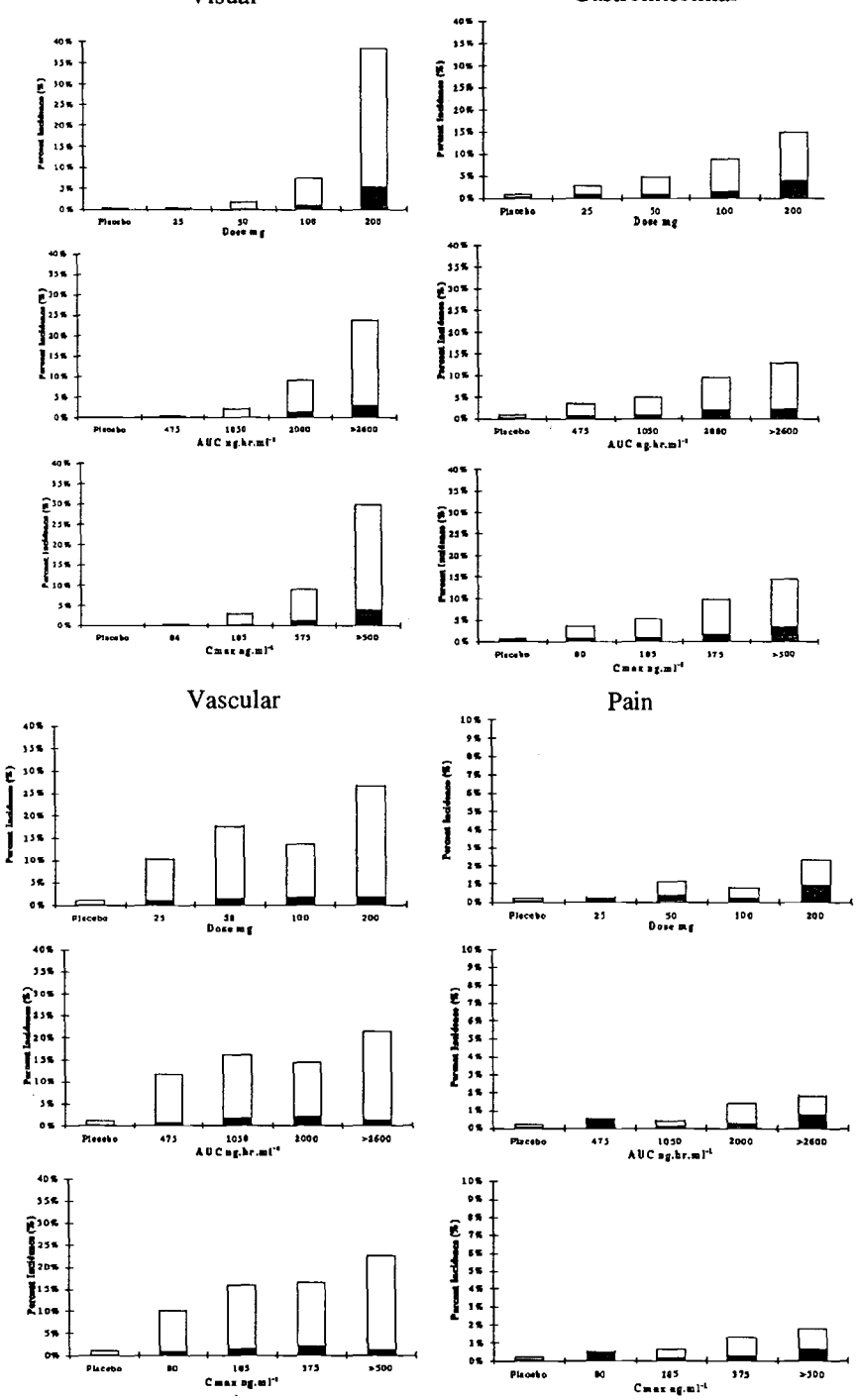


Figure 23. Modeled dependence of selected adverse events on dose, AUC, and C_{max}.

A2.3. Summary

This population analysis showed that hepatic transaminase elevation, aging, and the concomitant administration of CYP3A4 inhibitors can each reduce the clearance of sildenafil. Hepatic transaminase elevation and co-administration of loop diuretics and nonspecific β-blockers were associated with reduced clearance (CL/F) for the metabolite UK-103,320. Increased weight was associated with increased volume of distribution of both sildenafil and its metabolite.

Population effectiveness analyses showed that the responses to questions 3 and 4 can be described by an E_{max} model with terms for baseline values and placebo response. Baseline values were affected by age, weight, and etiology of erectile dysfunction. The analyses showed that sildenafil was effective regardless of such factors, but probably less effective in subjects with diabetes mellitus than with other etiologies. Dose was as good a predictor of effectiveness as drug AUC or metabolite AUC.

Population analyses of adverse events showed an increase in the incidence of adverse events at a dose of 200 mg.

A3. Development and validation of the primary efficacy instrument (International Index of Erectile Function; IIEF).

A3.1. Source documents Study report: INL vol 17.1.

A3.2. IIEF development The IIEF questionnaire was developed by the sponsor from a variety of sources including published literature and interviews conducted in multiple countries. A pilot version of the questionnaire was utilized in study 148-353 (n=351). A near-final version was subsequently developed and subjected to a validation program in 10 languages in 12 countries (US and Europe). Minor revisions resulted in the IIEF sexual function scale utilized in the phase III program[1].

A3.3. IIEF validation studies The IIEF was validated based upon data obtained in three clinical studies. Study 148-359[2] involved 111 subjects with erectile dysfunction studied at baseline and after 4 weeks of treatment and study 148-451[3] involved 109 age-matched normal male volunteers studied once.

A "principal components analysis with varimax rotation" and some clinical judgement applied to the data in study 148-359 allowed the 15 questions to be allocated to domains pertaining to erectile function, ejaculatory function, sexual desire, satisfaction with intercourse, and overall satisfaction. Generally questions related well to a single domain ("independent factor structure"). Inter-domain correlations ranged from 0.30 to 0.76.

Comparison of IIEF responses with the independent evaluation of each domain by the clinician allowed assessment whether the IIEF measured what it was supposed to measure ("convergent validity"). Such measures were reported as highly statistically significant. The same study compared results of the IIEF with those of related, but distinctly different, domains: marital adjustment (Locke-Wallace[4]) and social

---

1. *Study 148-102: A double-blind, randomized, placebo-controlled, parallel group, fixed-dose, multicenter study to assess the efficacy and safety of UK-92,480 administered over six months to male patients with erectile dysfunction.* on page 104, *Study 148-103: A double-blind, randomized, placebo-controlled, parallel group, multicenter, flexible dose escalation study to assess the efficacy and safety of sildenafil administered as required to male patients with erectile dysfunction.* on page 111, *Study 148-104: A double-blind, randomized, placebo-controlled, parallel group, multicenter, flexible dose escalation study to assess the efficacy and safety of sildenafil administered as required to male diabetic patients with erectile dysfunction.* on page 118, *Study 148-106: A double-blind, randomised, placebo controlled, parallel group, multicentre, fixed-dose study to assess the efficacy and safety of sildenafil administered as required to male subjects with erectile dysfunction.* on page 126, *Study 148-363: A double-blind, randomised, placebo-controlled, parallel group, multi-centre, flexible dose escalation study to assess the efficacy and safety of UK-92,480 administered over six months to male patients with erectile dysfunction.* on page 206, *Study 148-364: A double-blind, randomised, placebo-controlled, parallel group, multi-centre study to assess the efficacy and safety of fixed doses of sildenafil administered for three months to male patients with erectile dysfunction.* on page 212, and *Study 148-367: A double-blind, randomised, placebo-controlled, two way cross-over, flexible dose study to assess the efficacy and safety of oral doses of sildenafil in patients with erectile dysfunction caused by traumatic injuries to the spinal cord.* on page 218.
2. *Study 148-359: A 12 week, double blind, placebo controlled, parallel group, multicentre study to evaluate a new sexual function questionnaire in the assessment of the efficacy of sildenafil (UK-92,480) in patients with erectile dysfunction.* on page 199.
3. Study 148-451, "A study to generate sexual function and quality of life data in male subjects who do not have a diagnosis of erectile dysfunction" was conducted at 3 sites in the UK between February 1996 and July 1996. Subjects (n=109) who were normal, age-match controls for subjects in study 148-359 received no treatment; they merely filled out the IIEF and quality of life questionnaires.
4. This is a 15-question survey with defined point-values for various responses. Higher values indicate a harmonious relationship. Reference is Journal of Consulting Psychology (1960) 24:349-354.

desirability (Marlowe-Crowne[5]). For these, the correlations were poor and not statistically significant ("divergent validity").

Large and highly statistically significant differences were seen between the responses of subjects with the presumed condition (study 148-359) and normal volunteers (study 148-451), indicating that the IIEF reliably distinguishes subjects with erectile dysfunction ("discriminant validity").

Subjects in study 148-359 self-rated as treatment responders showed large and statistically significant improvements from baseline in each domain ("sensitivity"), while subjects self-rated as non-responders showed no significant changes in scores ("specificity").

Study 148-401[6] used the final version of the IIEF was a 4-week study in 37 subjects with erectile dysfunction and 21 age-matched control subjects. Both groups were evaluated using the IIEF, at baseline and after 4 weeks (no treatment). Pearson product-moment correlation coefficients, by domain, ranged from 0.64 to 0.84, indicating a relatively high reproducibility ("test-retest repeatability").

All three studies evaluated the correlation among questions within a domain using Cronbach's alpha. Values ranged from 0.73 to 0.96, indicative of highly consistent responses ("internal consistency").

5. This is a 33-true/false question survey of personal attitudes on social issues. Reference is Marriage and Family Living (1959) 21:251-255.

6. Study 148-401, "A psychometric validation of the International Index of Erectile Function (IIEF) in male patients with erectile dysfunction and age-matched controls" was conducted between February and May 1996 at one site in the US. Subjects with erectile dysfunction (n=37) or normal volunteers (n=21) completed surveys at baseline and 4 weeks later. There was no study treatment.

A4. Study 148-001: Phase I single dose, open study of the clinical pharmacology of sildenafil in elderly and young healthy male volunteers.

A4.1. Source documents

Study protocol NDA 20-895, vol 1.39; study report: NDA vol 1.39; electronic document: 46917384.pdf.

A4.2. Investigators

A4.3. Study dates

9 January 1995 to 2 February 1995.

A4.4. Study design

This study description was based upon the final study report, dated 2 June 1997.

A4.4.1. Objectives

The objectives were

- To assess the side effect and laboratory test safety profile of a single dose of sildenafil in elderly males (age 65 years or older) and young males (age 18 to 45 years inclusive).
- To compare the disposition of a single dose of sildenafil administered orally between elderly and young male subjects.

A4.4.2. Formulation

Drug supplies were 25-mg capsules, lot ED-S-347-994.

A4.4.3. Population

The intent was to enroll 15 young normal male volunteers, age 18 to 45, and 15 elderly male volunteers, age >65.

A4.4.4. Procedures

This was an open, parallel, single-dose study in 15 young and 15 elderly male volunteers. In the morning, following an overnight fast, each subject received sildenafil 50 mg with 240 ml of water. During each treatment period, 3-ml blood samples were collected at the following times: 0, 0.25, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 32, 40, and 48 hours post-dose.

A4.4.5. Assay

A4.4.6. Analysis

Pharmacokinetic parameters were calculated using standard non-compartmental techniques. Confidence intervals were calculated on the ratios of the log-transformed C_{max} and AUC for the 2 age groups. Untransformed AUC for sildenafil and its metabolite was subjected to regression analysis to determine whether there was a linear relationship between AUC and age or creatinine clearance that would explain any changes in AUC. Two regression models were fitted using type I sums of squares. The first analysis tested if creatinine clearance alone significantly influenced variability in AUC and if age (age/Clcr) significantly further influenced variability in AUC. In the second analysis, age was fitted first followed by creatinine clearance. This analysis tested if age alone significantly influenced variability in AUC and if creatinine clearance (Clcr/age) significantly further influenced variability in AUC.

A4.4.7. Safety

Routine safety data were recorded.

A4.5. Results

A4.5.1. Conduct

All 30 subjects completed both study phases. Protocol violations appear to have been minor.

A4.5.2. Pharmacokinetics

Mean plasma concentration-time profiles for sildenafil and its metabolite for elderly and young subjects are shown in Figure 24 below. The corresponding parameters summarized in Table 59 below. Figure 25 below shows relationships among selected pharmacokinetic parameters for young and elderly subjects. The left side of Figure 25.

shows, for sildenafil, AUC as a function of age, Cl/F as a function of creatinine clearance, and C_{max} as a function of age. The right side of Figure 25. shows, for metabolite UK-103,320, AUC as a function of age, AUC as a function of creatinine clearance, and C_{max} as a function of age.

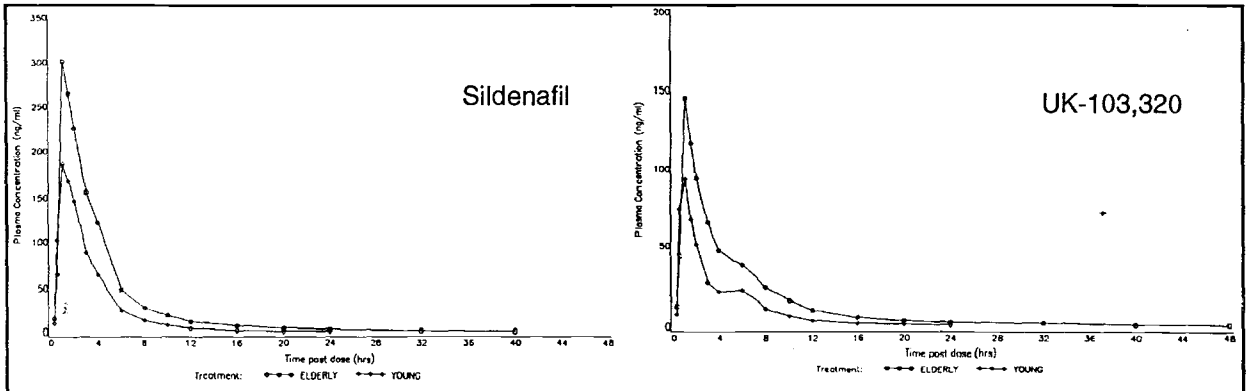


Figure 24. Plasma sildenafil and UK-103,320 levels (Study 148-001).

The results of the study seem to indicate that the influence of age on the pharmacokinetics of sildenafil and its metabolite is marked, since elderly subjects showed almost doubling in their AUC and C_{max}. This difference in plasma levels could be partially attributable to differences in oral clearance. Moreover, the fraction of unbound drug was smaller in the elderly compared to the young (3.4 vs. 4.3%), and this difference in protein binding might result in differences in volume of distribution leading to elevated plasma levels in the elderly compared to the young. The relationships between AUC and age for both sildenafil and its metabolite were not attributable to age-related differences in creatinine clearance. Inclusion of age and creatinine clearance in the regression model showed that the effect of age was statistically significant (p=0.0055), but the effect of creatinine clearance was not (p=0.93).

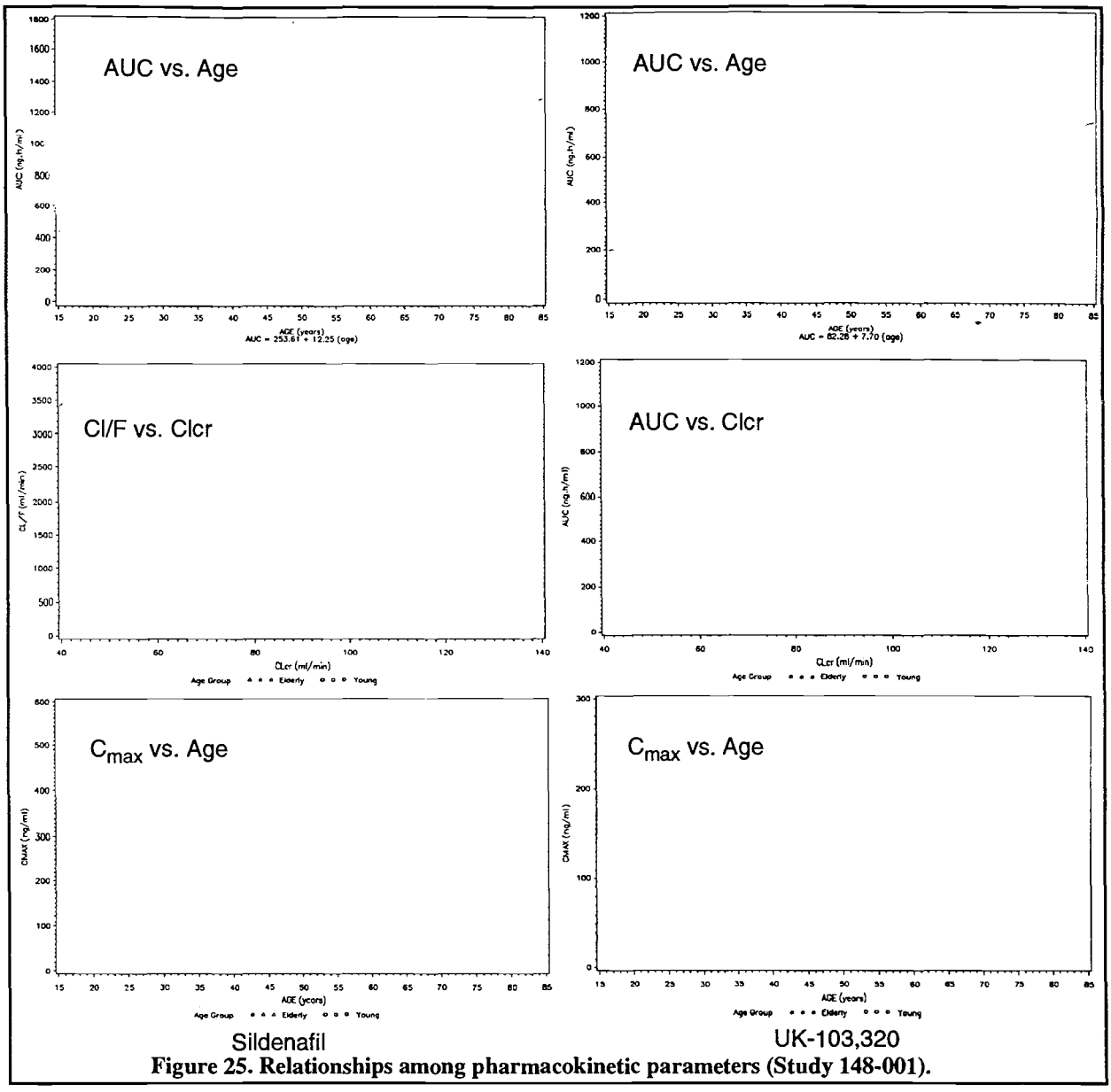


Figure 25. Relationships among pharmacokinetic parameters (Study 148-001).

Table 59. Pharmacokinetic parameters for sildenafil and UK-103,320 (Study 148-001).

	Sildenafil		UK-103,320			Sildenafil		UK-103,320	
	Young	Elderly	Young	Elderly		Young	Elderly	Young	Elderly
C_{max} (ng/mL)	178	303	90	146	$t_{1/2}$ (h)	2.6	3.8	3.1	5.2
T_{max} (h)	1.1	1.2	0.9	1.0	Unbound (%)	4.3	3.4	4.9	3.8
AUC (ng.h/mL)	586	1077	282	582	Cl/F (mL/min)	1537	800	—	—
k_{el} (h^{-1})	0.27	0.18	0.2	0.13					

A4.5.3. Safety

There were no serious or severe adverse events reported.

A4.6. Summary

The results of the study showed that elderly subjects had about twice as high AUC and C_{max} for sildenafil and metabolite UK-103,320 compared to young subjects. The difference was not attributable to differences in creatinine clearance.

Center for Drug Evaluation and Research

Viagra (Sildenafil)

"Joint Clinical Review" for NDA-20-895

Appendix A5, page 92 through Appendix A9.6, page 103

A5. Study 148-002: Phase I open study to assess the potential of cimetidine to alter the pharmacokinetics of sildenafil (UK-92,480) in normal, healthy male subjects.

A5.1. Source documents Study protocol NDA 20-895, vol 1.40; study report: NDA vol 1.40; electronic document: 46917592.pdf.

A5.2. Investigators

A5.3. Study dates 18 November 1995 to 29 January 1996.

A5.4. Study design This study description was based upon the final study report, dated 2 May 1997.

The objective was to determine whether multiple-dose cimetidine administration alters the pharmacokinetics of sildenafil.

Drug supplies are shown in Table 60 below.

Table 60. Drug supplies (Study 148-002).

	Lot		Lot
Placebo for cimetidine	ED-G-222-891	Sildenafil 25 mg capsules	ED-S-347-994
Cimetidine (Tagamet)	7694T26		

A total of 20 health male volunteers, age 18 to 45, were to be recruited.

All subjects received a single oral dose of sildenafil 50 mg after overnight fast on day 1. On days 3 to 6, subjects received either double-blind placebo or cimetidine 800 mg. On day 5, subjects received randomized treatment followed 2 hours later by sildenafil 50 mg. Subjects fasted another 4 hours and refrained from caffeine after each drug administration.

Blood samples for determination of plasma levels of sildenafil and UK-103,320 were obtained on days 1 and 5 pre-dose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 32, 40 and 48 hours post-dose. Samples were collected in heparinized tubes, plasma was separated by centrifugation, and samples were stored at -20°C. Plasma levels of

Table 61 below.

Table 61. Assay for sildenafil and UK-103,320 (Study 148-002).

	Sildenafil		UK-103,320	
		Comment		Comment

C_{max}, AUC_{τ}, k_{el}, AUC, T_{max}, and $t_{1/2}$ were calculated.

Routine safety data were recorded.

A5.5. Results

A5.5.1. Conduct

Twenty-two subjects were randomized and treated. Two subjects withdrew after the first dose. Thus, 20 subjects, age 18 to 39, contributed data. Protocol violations appear to have been minor.

A5.5.2. Pharmacokinetics

Plasma levels of sildenafil and UK-103,320 are shown in Figure 26 below. Pharmacokinetic parameters are shown in Table 62 below.

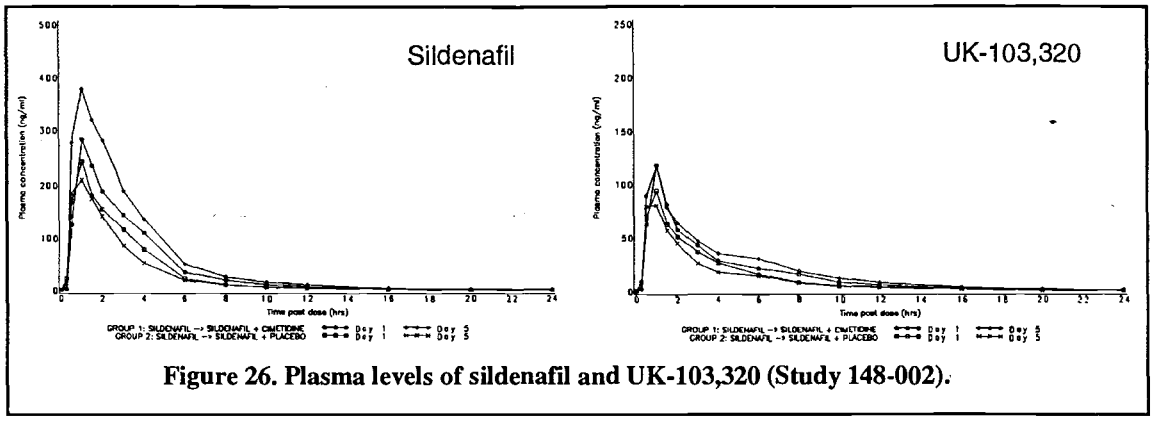


Figure 26. Plasma levels of sildenafil and UK-103,320 (Study 148-002).

Table 62. Pharmacokinetic parameters (Study 148-002).

	Sildenafil				UK-103,320			
	Placebo		Cimetidine		Placebo		Cimetidine	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
AUC (ng.h/mL)	716±260	605±174	889±246	1250±379	270±140	243±105	351±160	414±220
AUC_t (ng.h/mL)	666±271	598±174	883±245	1241±374	261±140	236±104	346±158	407±117
C_{max} (ng/mL)	250±105	220±58	283±69	383±111	98±42	88±30	111±42	115±51
T_{max} (h)	1.1±0.7	1.1±0.5	1.4±0.9	0.9±0.3	1.1±0.7	0.9±0.05	1.2±0.7	1.1±0.7
K_{el} (h^{-1})	0.27±0.09	0.27±0.03	0.23±0.05	0.20±0.05	0.18±0.03	0.22±0.05	0.21±0.06	0.18±0.04
$t_{1/2}$ (h)	2.6	2.6	3.0	3.5	3.9	3.2	3.3	3.9

A5.5.3. Safety

Two subjects withdrew consent (did not like venous puncture). No serious adverse events were reported. One subject had borderline CK elevation at baseline, and developed a substantial increase on day 2, and peaking on day 3 at about 60 times ULN (100% MM band). His CK diminished thereafter and he completed study about 6 weeks later. CK elevation was attributed to exercise.

A5.6. Summary

Cimetidine reduces gastric pH, potentially affecting bioavailability of sildenafil, and it is a non-specific inhibitor of cytochrome P450, potentially affecting the metabolism of sildenafil. The single-dose AUC and C_{max} for sildenafil increased about 50% when co-administered with cimetidine. Its metabolite, UK-103,320, was increased by about 30%. There was no effect of cimetidine on the elimination rate constants for sildenafil or UK-103,320, so the modest observed effect of cimetidine is apt to have been a result of alteration of absorption.

A6. Study 148-003: Phase I open study to assess the effect of concomitant antacid administration on the absorption of sildenafil (UK-92,480) in normal, healthy male subjects.

A6.1. Source documents

Study protocol NDA 20-895, vol 1.41; study report: NDA vol 1.41; electronic document: 46917205.pdf.

A6.2. Investigators

A6.3. Study dates

8 January 1996 to 12 February 1996.

A6.4. Study design

This study description was based upon the final study report, dated 9 June 1997.

The objective was to determine the effect of antacid administration on the absorption of a single dose of sildenafil.

Drug supplies are shown in Table 63 below.

Table 63. Drug supplies (Study 148-003).

	Lot
Sildenafil 25 mg capsules	ED-S-347-994

A total of 12 health male volunteers, age 18 to 45, were to be recruited.

This was an open-label, two-way crossover study in which subjects received single oral doses of sildenafil 50 mg on study days separated by at least 14 days. In random order, on one of the study days, subjects received Maalox® 30 ml just prior to administration of sildenafil. All dosing was done after overnight fasting and fasting continued for 4 hours after dosing.

Blood samples for determination of plasma levels of sildenafil and UK-103,320 were obtained on days 1 and 5 pre-dose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 32, 40 and 48 hours post-dose. Samples were collected in heparinized tubes, plasma was separated by centrifugation, and samples were stored at -20°C. Plasma levels of

Table 64 below.

Table 64. Assay for sildenafil and UK-103,320 (Study 148-003).

	Sildenafil		UK-103,320	
	LLQ	Comment	LLQ	Comment

C_{max}, AUC_{τ}, k_{el}, AUC, T_{max}, and $t_{1/2}$ were calculated.

Routine safety data were recorded.

A6.5. Results

A6.5.1. Conduct

Twelve subjects were randomized and treated. Protocol violations appear to have been minor.

A6.5.2. Pharmacokinetics

Plasma levels of sildenafil and UK-103,320 are shown in Figure 27 below. Pharmacokinetic parameters are shown in Table 65 below.

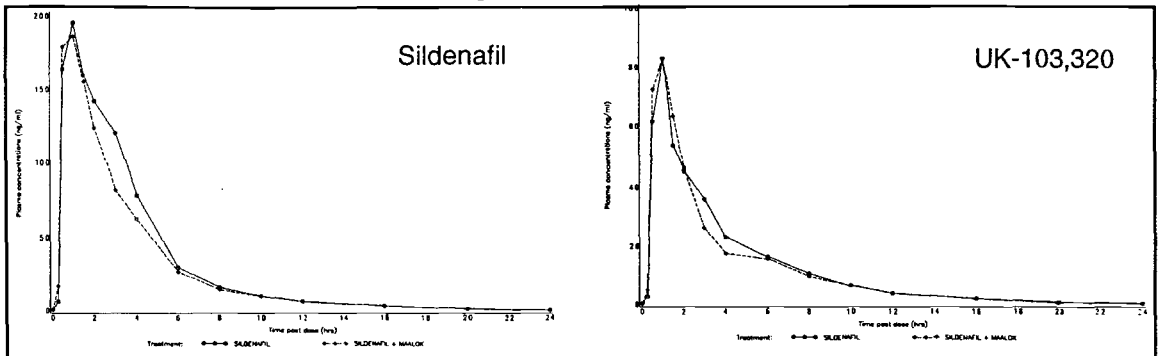


Figure 27. Plasma levels of sildenafil and UK-103,320 (Study 148-003).

Table 65. Pharmacokinetic parameters (Study 148-003).

	Sildenafil		UK-103,320	
	Nothing	Maalox	Nothing	Maalox
AUC (ng.h/mL)	700±198	627±164	272±76	259±75
AUC_t (ng.h/mL)	693±197	621±163	265±75	252±76
C_{max} (ng/mL)	238±86	238±106	96±37	100±31
T_{max} (h)	1.2±0.8	0.8±0.4	1.1±0.7	0.8±0.4
K_{el} (h^{-1})	0.22±0.02	0.23±0.05	0.22±0.05	0.21±0.02
$t_{1/2}$ (h)	3.1	3.0	3.2	3.3

A6.5.3. Safety

There was one case of syncope associated with phlebotomy. There were no serious adverse events. Seven subjects reported penile erections.

A6.6. Summary

Plasma levels of sildenafil and its primary metabolite, UK-103,320, were unaffected by Maalox. The study appears to have been powered adequately to detect a 50% change in AUC or C_{max}.

A7. Study 148-004: Phase I investigator-blind, placebo-controlled, evaluation of safety, toleration, and pharmacokinetics of sildenafil following escalating single oral doses in healthy male volunteers.

A7.1. Source documents Study protocol NDA 20-895, vol 1.42; study report: NDA vol 1.42; electronic document: 46314080.pdf.

A7.2. Investigators Single-center study with 1 investigator in the US.

A7.3. Study dates 9 December 1996 to 9 January 1997.

A7.4. Study design This study description was based upon the final study report, dated 22 July 1997.

Drug supplies are shown in Table 66 below.

Table 66. Drug supplies (Study 148-004).

	Lot		Lot
Placebo	N5275-G1	Sildenafil 100 mg	N6064-G1

A total of 20 health male volunteers, age 40 to 65, were to be recruited.

Two cohorts of 10 subjects were to receive single ascending doses of sildenafil or placebo in a 4:1 randomization. Planned doses were 100, 200, 400, and 800 mg. Because of effects on blood pressure observed at 200 mg, the actual doses used were 100, 200, 300, 400, 600, and 800 mg. Doses were administered with water after overnight fast. Data collected were vital signs, ECGs, and plasma drug levels. Pharmacokinetic sampling was at 0 (pre-dose) and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 32, 40 and 48 hours post-dose. Drug levels were determined by

detection.

Routine safety data were recorded.

A7.5. Results

A7.5.1. Conduct Twenty subjects were randomized and treated. The mean age was 53 years. All but 3 were Caucasian.

Few subjects had standing vital signs measured at doses over 200 mg. Other protocol violations appear to have been minor.

A7.5.2. Pharmacokinetics Mean plasma levels of sildenafil and metabolite UK-103,320 are shown in Figure 28 below. Table 67 below shows all available pharmacokinetic parameters. C_{max} and AUC increased more than proportionally with dose, as shown in Figure 29 below.

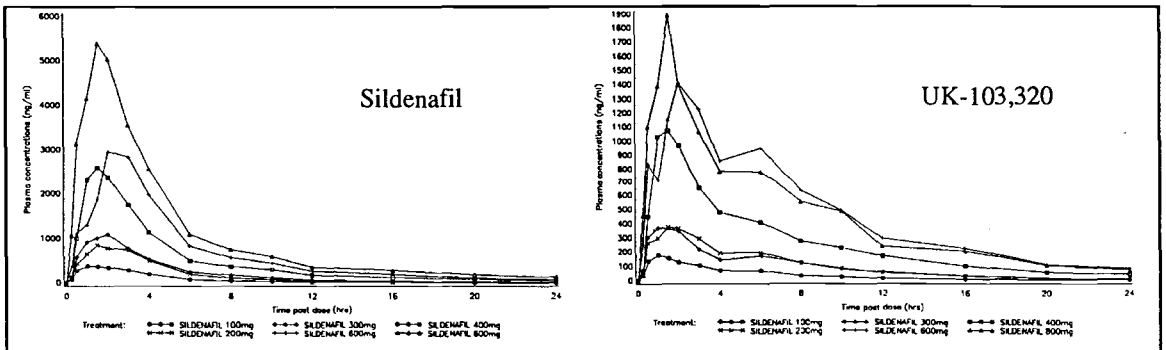


Figure 28. Mean plasma levels of sildenafil and UK-103,320 (Study 148-004).

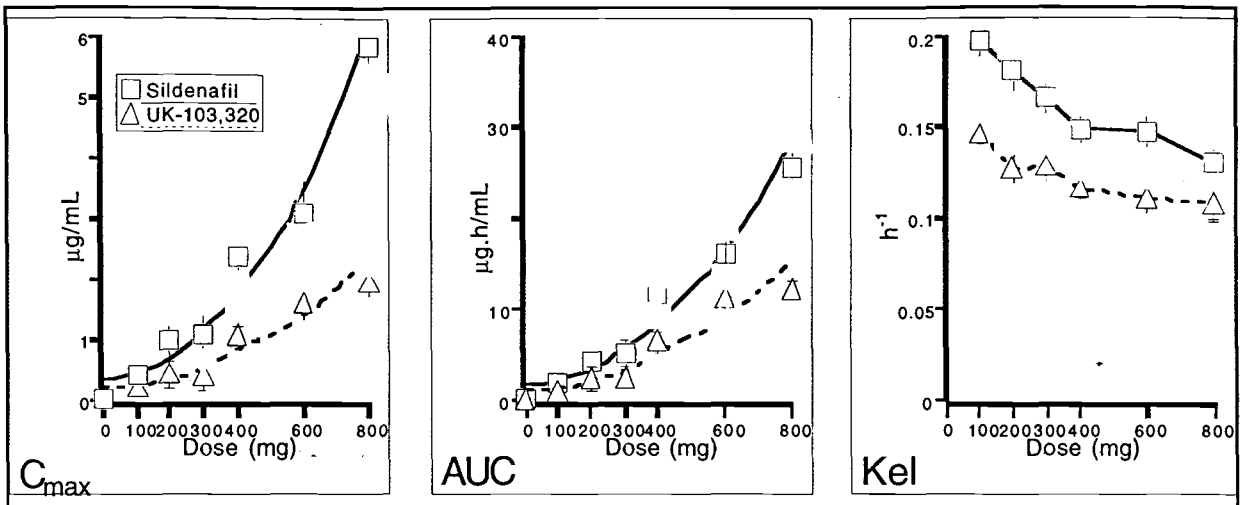


Figure 29. Pharmacokinetic parameters by dose (Study 148-004).

Table 67. Pharmacokinetic parameters (Study 148-004).

	Sildenafil (mg)						UK-103,320 (mg)					
	100 N=8	200 N=8	300 N=4	400 N=4	600 N=4	800 N=4	100 N=8	200 N=8	300 N=4	400 N=4	600 N=4	800 N=4
AUC (ng.h/mL)	1691	4088	5156	11588	16114	25686	834	2392	2351	6531	11274	12095
AUC_τ (ng.h/mL)	1682	4081	5142	11578	16079	25611	826	2378	2342	11569	11213	12029
C_{max} (ng/mL)	411	1001	1081	2367	3084	5834	201	430	391	1066	1590	1939
T_{max} (h)	1.4	2.2	2.1	1.4	2.5	1.8	1.1	1.8	1.4	1.1	2.4	1.5
K_{el} (h^{-1})	0.20	0.18	0.17	0.15	0.15	0.13	0.15	0.13	0.13	0.12	0.11	0.11
$t_{1/2}$ (h)	3.5	3.8	4.2	4.7	4.7	5.3	4.8	5.5	5.4	6.0	6.2	6.5

Effects of the 800-mg dose on sitting vital signs (double differences from placebo and baseline) are shown in Figure 30 below. Somewhat larger and more sustained effects on blood pressure were observed with the 600-mg dose. The study was too small and the intra-subject variability too great to make a hysteresis plot useful.

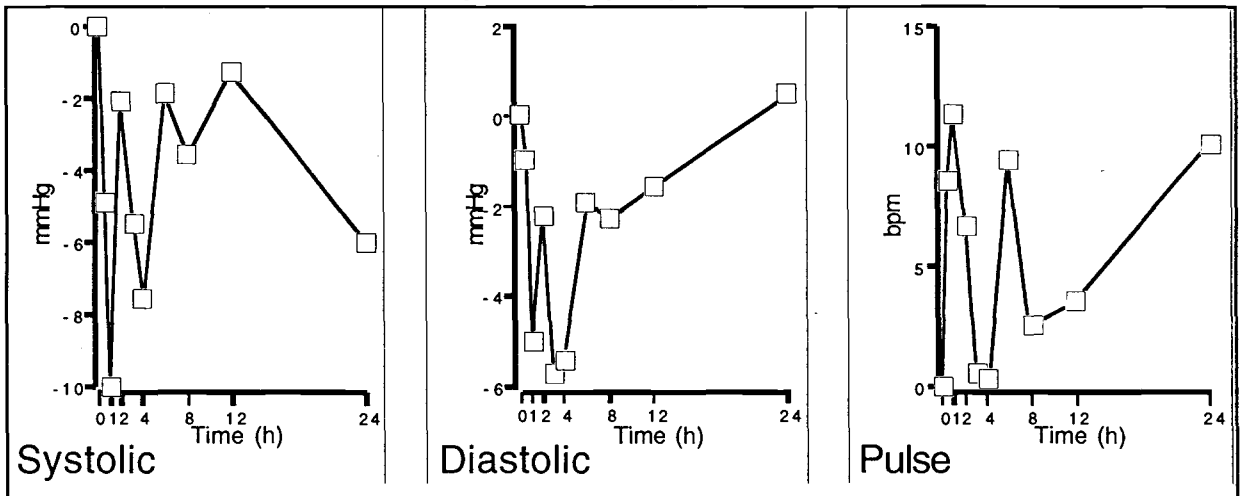


Figure 30. Pharmacodynamic effects at 800 mg, while standiug (Study 148-004).

A7.5.3. Safety

Adverse events were reported by all but one subject at doses >100 mg. There were two cases described as severe dizziness, one of which was associated with syncope.

Visual disturbances were reported by about half of the subjects at doses >100 mg. The frequency of such reports did not trend upwards with dose, but the duration of events increased from 0.25 to 1.4 hours at doses up to 400 mg and 2.5 to 8 hours at higher doses. The effects were described as difficulty seeing in dim light, color aberration, and color tinges. Effects tended to be seen near the expected time of peak plasma concentration.

About half of the subjects at 100 and 200 mg reported erections. The rate was less at higher doses, but one subject on 600 mg reported an erection lasting 5 hours.

A variety of minor laboratory abnormalities were reported. None were serious or had any apparent relationship to treatment.

Inspection of line listings of ECG parameters revealed no treatment-related trends or significant outliers.

A7.6. Summary

Single doses of sildenafil >200 mg were associated with greater-than-proportional increases in C_{max} and AUC for both sildenafil and its major metabolite, consistent with some saturable binding or elimination step. At about the time of peak sildenafil levels in plasma, there are substantial reductions in sitting and standing blood pressure and a compensatory increase in heart rate. The time course was not well characterized here, but it produces symptomatic effects at doses >200 mg and it probably lasts several hours. Effects attributable to inhibition of retinal phosphodiesterase appeared at doses >100 mg.

A8. Study 148-101/101B: A randomised, double-blind, placebo controlled, parallel-group, fixed-dose, multicentre, long-term dose-response study to assess the efficacy and safety of sildenafil (UK-92,480) administered prior to sexual activity to male patients with erectile dysfunction.

A8.1. Source documents

Study protocol NDA 20-895, vol 1.104; study report: NDA vol 1.104; electronic document: 47100356.pdf.

A8.2. Investigators

Multi-center study with 22 investigators in the United States.

A8.3. Study dates

30 June 1995 to 1 May 1996.

A8.4. Study design

This study description was based upon the protocol dated 19 May 1995. There were no amendments.

Drug supplies are shown in Table 68 below.

Table 68. Drug supplies (Study 148-101/101B).

	Lot		Lot
Placebo 5 mg	ED-S-072-295	Sildenafil 5 mg	ED-S-106-395
Placebo 25 mg	ED-S-073-295	Sildenafil 25 mg	4469-005-GI ED-S-074-295 ED-S-239-795 ED-S-240-795 ED-S-343-995

The intent was to randomize 375 male subjects age >18, with erectile dysfunction[1] of >6 months' duration, and in a heterosexual relationship for >6 months. Subjects were excluded for (1) anatomical deformities such as severe penile fibrosis, (2) spinal cord injury, (3) regular use of nitrates, estrogens, anti-androgens, anticoagulants, or psychotropic drugs, (4) history of hematologic, renal, or hepatic disease, (5) stroke or myocardial infarction within 6 months, (6) life-threatening cardiac arrhythmia or coronary artery disease, (7) migraine or cluster headache, (8) history of depression or major psychiatric disorder, (9) history of bleeding disorder or active peptic ulcer disease, (10) suspected sexually transmitted disease, (11) postural hypotension, or blood pressure outside 90/50 to 160/95 mmHg, (12) other experimental drug use within 4 weeks, (13) alcohol or drug dependence, or (14) recent blood donation.

At the end of a 4-week treatment-free run-in period during which baseline sexual performance data were collected, subjects were randomized to placebo or sildenafil 5, 25, 50, or 100 mg and followed for 8 weeks with follow-up at 2-week intervals. Subjects were instructed to take study drug approximately one hour before planned sexual activity, not more than once per day. Subjects completed an event log noting time of study drug administration and subsequent sexual activity. Subjects completed a sexual function questionnaire, and partners completed a separate questionnaire.

Subjects who completed the first 8 weeks of double-blind treatment were eligible to continue double-blind treatment for an additional 16 weeks (protocol 148-101B).

The primary efficacy assessment was at week 8. The primary end point was the ability to achieve an erection during sexual activity, as retrospectively assessed by the response toquestion 1 of the sexual function questionnaire.

Safety assessments included (1) ECGs at screening and week 8, (2) laboratory tests (CBC, SMA20, urinalysis), (3) vital signs, and (4) physical examination. Clinical adverse events and their relationship to the study drug were recorded.

1. 'the inability to attain and/or maintain penile erection sufficient for satisfactory sexual performance'

A8.5. Results

A8.5.1. Conduct

Five hundred and twenty-three subjects were screened, 416 were randomized, and 359 (86%) completed study.

Demographics of the 2 treatment groups are shown in Table 69 below. About 38% of all randomized subjects had received previous drug therapy for erectile dysfunction, and about 11% had used non-drug treatments.

Table 69. Demographics (Study 148-101/101B).

		Placebo N=83	Sildenafil 5 mg N=86	Sildenafil 25 mg N=82	Sildenafil 50 mg N=85	Sildenafil 100 mg N=82
Race (%)	White	90	93	88	89	85
	Black	7.2	5.8	7.3	7.2	13
	Other	2.4	1.2	4.9	3.6	1.2
Age	Mean	58	58	57	56	59
	Range	36-79	26-80	32-79	35-73	37-79
Etiology (%)	Organic	76	80	70	70	74
	Psychogenic	7	5	12	7	10
	Mixed	17	15	18	23	16
Duration (y)	Mean	5.1	5.1	4.2	3.8	4.9
Med hx (%)	Diabetes	14	15	8.5	18	20
	Hypertension	33	31	30	34	38
	IHD	7.2	4.7	6.1	8.4	6.1
	Periph vasc dis	0	0	2.4	1.2	1.2
	Depression	1.2	1.2	1.2	0	0
	Prostatectomy	3.6	7.0	3.7	2.4	4.9

Protocol violations are described in Table 70 below. Not all such subjects were excluded from the sponsor's 'evaluable subjects' analyses.

Table 70. Protocol violations (Study 148-101/101B).

At randomization		On treatment	
	n		n
Concomitant meds	19	>1 dose per day	52
Lab abnormalities	8	Other ED treatment	12
Penile anatomical defect	6	Concomitant meds	7
Confounding medication	5	Blind broken for AE	1
Self-injection during single-blind	4		
Active medical problems	3		
Other	9		
Total[a]	53	Total	52

a. Some subjects had more than one violation.

Nineteen percent of subjects on placebo and 12% of subjects on sildenafil discontinued. One to two subjects per group discontinued for lack of efficacy.

A8.5.2. Effectiveness

Eleven to 22 subjects per group were excluded from evaluation for effectiveness, generally because the week-8 data were not returned. All randomized subjects with a post-randomization assessment were included in the sponsor's ITT analyses. Responses to EF question 1 (ability to attain an erection) were scored as 0 for no

attempts, 1 for never or rarely successful, etc., up to 5 for always or almost always successful. The sponsor's results are summarized in Table 71 below.

Table 71. ITT analyses of EF question 1 (Study 148-101/101B).

		Placebo N=83		Sildenafil 5 mg N=86		25 mg N=82		50 mg N=83		100 mg N=82		*P*
		n	Q	n	Q	n	Q	n	Q	n	Q	
How often were you able to get erection?	Baseline	—	1.8[a]	—	—	—	—	—	—	—	—	
	Week 8	78	2.1	81	2.7	81	3.1	81	3.5	77	3.7	<0.0001
	Week 24	78	2.1	81	2.7	81	2.9	81	3.1	77	3.6	<0.0001

a. This is apparently the pooled baseline value for all subjects.

There were monotonic, dose-related increases in the proportion of subjects who said treatment had improved their erections at week 8 and week 24. With the exception of questions pertaining to sexual desire, there were highly significant treatment effects for all sexual function questionnaire elements. Ninety-four percent of partners responded to the partner questionnaire at weeks 8 and 24, with increasing partner satisfaction with dose at both of these time points. The proportion of successful attempts at intercourse, as determined from the event logs, increased monotonically by dose.

The reviewers performed no analyses of these data.

A8.5.3. Safety

Safety will be reviewed for all placebo-controlled studies together.

A8.6. Summary

The population included subjects with erectile dysfunction of organic etiology, but not spinal cord injury. Although not analyzed extensively by the reviewers, the results of the sponsor's assessments of erectile function and sexual performance were highly internally consistent and also consistent with later studies with sexual performance as a primary end point. The LOCF analyses were not useful for assessing the degree to which treatment effects were sustained for the period of study.

A9. Study 148-101C: An open, non-comparative study to assess the long-term safety of sildenafil in patients with erectile dysfunction.

A9.1. Source documents Study protocol NDA 20-895, vol 1.130; study report: NDA vol 1.130; electronic document: 46916369.pdf.

A9.2. Investigators Multi-center study with 22 investigators in the United States.

A9.3. Study dates 11 January 1996 to 21 January 1997.

A9.4. Study design This study description was based upon the protocol amendment dated 21 June 1996. This amendment increased the duration of open-label study from 28 to 36 weeks.

Drug supplies are shown in Table 72 below.

Table 72. Drug supplies (Study 148-101C).

	Lot
Sildenafil 25 mg	4469-005-G1 4469-006-G1 4469-0008-G1 ED-S-343-995

Subjects were all previous participants in Study 148-101/101B[1]. Subjects must have completed the blinded study without a serious adverse event possibly related to study drug.

Visits were scheduled at 2, 4, 8, 12, 20, and 28 weeks. Subjects could have their doses adjusted between 25 and 100 mg. The primary end point was whether subjects were satisfied with the effect of treatment. Subjects also kept an event log.

Routine safety data were collected.

A9.5. Results

A9.5.1. Conduct Three hundred and thirty-seven subjects (94% of subjects completing the previous study) entered long-term open-label study, and 269 (80%) completed study.

Subject demographics were similar to those in Study 148-101/101B. The mean age was 57. The mean duration of erectile dysfunction was 5 years. Etiology of erectile dysfunction was described as organic in 72%, psychogenic in 8.6%, and mixed in 19%. Eighty-two percent of subjects had received sildenafil for 24 weeks during the previous study.

A variety of protocol violations in Study 148-101/101B should have precluded participation in the open-label study.

Twenty percent of subjects discontinued. Reasons for discontinuation included lack of effectiveness (8.6%, mostly titrated to the highest allowed dose) and adverse events or laboratory abnormalities (5%).

Exposure is characterized in Figure 31 below. The proportion of subjects exposed for different periods of time is shown in the left panel. The proportion of subjects receiving different ranges of number of doses is shown in the center panel. The proportion of subjects receiving each dose level is shown in the right panel.

[1] *Study 148-101/101B: A randomised, double-blind, placebo controlled, parallel-group, fixed-dose, multicentre, long-term dose-response study to assess the efficacy and safety of sildenafil (UK-92,480) administered prior to sexual activity to male patients with erectile dysfunction.* on page 99.

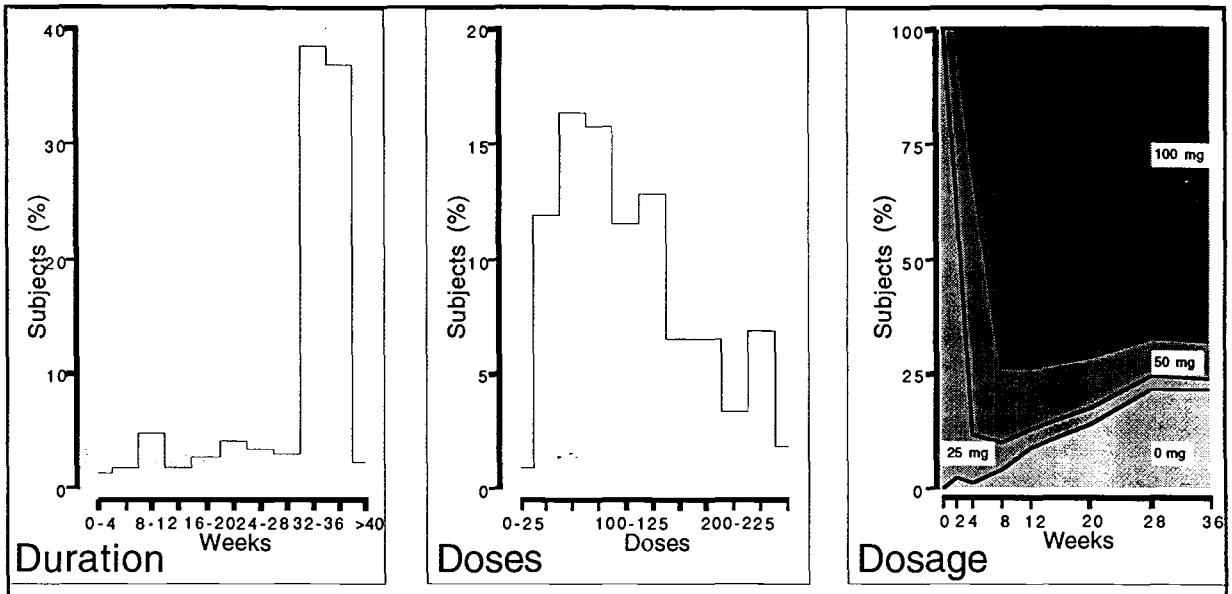


Figure 31. Exposure to study drug (Study 148-101C).

A9.5.2. Effectiveness

By the sponsor's analyses, the number of subjects expressing satisfaction with the treatment rose from 10% at 2 weeks, when essentially all were receiving 25 mg, to 90% at the study end, when most subjects were on 100 mg. The reviewers performed no analyses of these data.

A9.5.3. Safety

Safety will be reviewed for all open-label studies together.

A9.6. Summary

Roughly 80% of subjects had some exposure to sildenafil for 6 months prior to enrollment in this study, so the actual withdrawal rate here is somewhat lower than could be expected in a naive population. Few subjects remained on 25 or 50 mg when given the opportunity to move to a higher dose.

Center for Drug Evaluation and Research

Viagra (Sildenafil)

"Joint Clinical Review" for NDA-20-895

Appendix A10, page 104 through Appendix A13.6, page 125

A10. Study 148-102: A double-blind, randomized, placebo-controlled, parallel group, fixed-dose, multicenter study to assess the efficacy and safety of UK-92,480 administered over six months to male patients with erectile dysfunction.

A10.1. Source documents

Study protocol IND vol 15.1; study report: NDA vol 1.91-1.94; electronic document: 47099527.pdf; SAS datasets.

A10.2. Investigators

Multi-center study with 23 investigators in the United States.

A10.3. Study dates

30 November 1995 to 30 October 1996.

A10.4. Study design

This study description was based upon the amended protocol dated 4 April 1996. On 25 January 1996, the primary end point was changed, upon advice by FDA, to be sexual performance-related (questionnaire) rather than erectile function (questionnaire). The same amendment called for the primary analysis to be based upon an "ITT" population with at least one efficacy assessment post-baseline.

Drug supplies are shown in Table 73 below.

Table 73. Drug supplies (Study 148-102).

	Lot		Lot
Placebo 25 mg	ED-S-362-995 ED-S-363-995	Sildenafil 25 mg	ED-S-355-995
Placebo 50 mg	ED-S-350-995 ED-S-351-995 ED-S-352-995 ED-S-353-995	Sildenafil 50 mg	ED-S-356-995 ED-S-358-995

The intent was to randomize 500 male subjects age >18, with erectile dysfunction[1] of >6 months' duration, and in a heterosexual relationship for >6 months. Subjects were excluded for (1) anatomical deformities such as severe penile fibrosis, (2) other sexual disorders such as hypoactive sexual desire, (3) elevated prolactin (3x ULN) or low free testosterone (20% below LLN), (4) major, uncontrolled psychiatric disorders, (5) history of alcohol or drug abuse, (6) history of major hematologic, renal, or hepatic disorder, (7) erectile dysfunction following spinal cord injury, (8) uncontrolled diabetes or diabetic retinopathy, (9) stroke or myocardial infarction within 6 months, (10) cardiac failure, unstable angina, ECG ischemia, or life-threatening arrhythmia within 6 months, (11) blood pressure outside 90/50 to 170/100 mmHg, (12) active peptic ulcer disease or bleeding disorder, (13) any clinically significant baseline laboratory abnormality, (14) need for anticoagulants, nitrates, androgens, or trazodone, (15) need for aspirin or NSAIDs and a history of peptic ulcer disease, (16) unwillingness to cease use of vacuum devices, intracavernosal injection, or other therapy for erectile dysfunction, (17) other experimental drug use within 3 months, or (18) history of retinitis pigmentosa.

At the end of a 4-week treatment-free run-in period during which baseline sexual performance data were collected, subjects were randomized to placebo or sildenafil 25, 50, or 100 mg and followed for 24 weeks. A 2:1 placebo:active randomization was implemented to compensate for the expected differences in the rate of withdrawal for lack of efficacy. Subjects were instructed to take study drug approximately one hour before planned sexual activity, not more than once per day. Alcohol use during this hour was discouraged. Prior to clinic visits at the end of weeks 4, 8, 12, 16, and 24, subjects also took study drug. Subjects completed an event log noting time of study drug administration and subsequent sexual activity. Subjects completing study without an adverse event were eligible for participation in an open-label follow-on study.

[1] 'the inability to attain and/or maintain penile erection sufficient for satisfactory sexual performance'

The primary efficacy assessment was at week 12. At this visit, subjects completed a global assessment question, sexual function questionnaire (containing the primary efficacy questions), and a quality of life questionnaire. Optionally, partners filled out another questionnaire.

Plasma samples were drawn for determination of parent compound and metabolite UK-103,320 at weeks 2, 4, 8, 12, and 24, a random, but recorded, time after the last dose.

The study was originally sized to achieve 80% power at α=0.05 to detect a 70% improvement in erections on study drug compared with a 50% improvement on placebo. The study was not resized when the end point was changed. Randomization was not stratified.

The primary end point was the answer, at 12 weeks, to two questions on the sexual function questionnaire:

> *[3] Over the past 4 weeks, when you attempted sexual intercourse, how often were you able to penetrate (enter) your partner?*
>
> *[4] Over the past 4 weeks, during sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?*

Both questions had the same set of possible responses, either "did not attempt intercourse" or a 5-level semi-quantitative response. Analysis was to be by ANCOVA, based on table scores, where the "no attempt" response was lumped with the worst frequency category. Each question was to be analyzed separately with $p<0.05$ on both necessary for demonstrating efficacy. The model was to include terms for center, baseline, and "other covariates deemed to be appropriate". The primary test was a single-degree-of-freedom test for a linear trend by dose. Any interim analyses were not to affect the ongoing trial.

The primary analysis was described as ITT with last observation carried forward. However, the sponsor's description of the ITT population includes only subjects with at least one observation post-randomization.

Secondary end points were (1) response to the global assessment question (originally the primary end point):

> *Has the treatment you have been taking over the past 4 weeks improved your erections? [yes] [no]*

(2) the responses to other sexual function questions (there were 13 in addition to the primary efficacy questions), (3) proportion of successful attempts at intercourse, determined from the event log, (4) responses on the optional partner questionnaire, (5) responses on the quality of life assessment, and (6) time to discontinuation for lack of efficacy.

Pharmacokinetic data were to be analyzed by nonlinear mixed-effect modeling (NONMEM) utilizing a large selection of baseline attributes as covariates.

Safety assessments included (1) ECGs at screening and week 12, (2) laboratory tests (CBC, SMA20, urinalysis), (3) vital signs, and (4) physical examination. Clinical adverse events and their relationship to the study drug were recorded.

A10.5. Results

A10.5.1. Conduct

Six hundred and four subjects were screened, 532 were randomized, and 465 (87%) completed study. Individual sites enrolled 3 to 40 subjects.

Demographics of the 4 treatment groups are shown in Table 74 below. About half of all randomized subjects had received previous drug therapy for erectile dysfunction, and about 9% had used non-drug treatments.

Table 74. Demographics (Study 148-102).

		Placebo N=216	Sildenafil 25 mg N=102	Sildenafil 50 mg N=107	Sildenafil 100 mg N=107
Race (%)	White	85	88	91	91
	Black	9.7	5.9	7.5	6.5
	Other	5.1	7.8	1.9	2.8
Age	Mean	57	58	57	59
	Range	20-79	24-79	29-81	25-87
Etiology (%)	Organic	77	76	80	77
	Psychogenic	10	7	8	11
	Mixed	13	17	11	12
Duration (y)	Mean	3.2	2.9	3.2	3.5
	Range	0.4-20	0.5-11	0.5-14	0.1-16
Med hx (%)	Hypertension	31	35	34	30
	Diabetes	16	19	12	9
	Prostatectomy	15	18	20	17
	Depression	6.5	6.9	5.6	8.4
	IHD	9.7	6.9	13	13

Protocol violations are described in Table 75 below. Not all such subjects were excluded from the sponsor's 'evaluable subjects' analyses.

Table 75. Protocol violations (Study 148-102).

At randomization	n	On treatment	n
Prohibited meds	30	>1 dose/day	56
Baseline lab abn	25	Blind broken for AE	9
Peyronie's disease or anatomic defect	23		
Psychiatric disorder	19		
Ethanol or drug abuse	13		
Confounding condition/treatment	9		
Poorly controlled hypertension	4		
Use of other experimental drug	1		
Sexually transmitted disease	1		
Erectile dysfunction <6 months	1		
Total[a]	114	Total	64

a. Some subjects had more than one violation.

The disposition of subjects in the trial is shown in Figure 32 below, which shows the placebo group in the left panel and all active treatment groups combined in the right panel. Most subjects remained in study for more than 24 weeks, but some "completed" several weeks early. As the sponsor predicted, fewer subjects on active treatment withdrew for lack of efficacy (or withdrew consent).

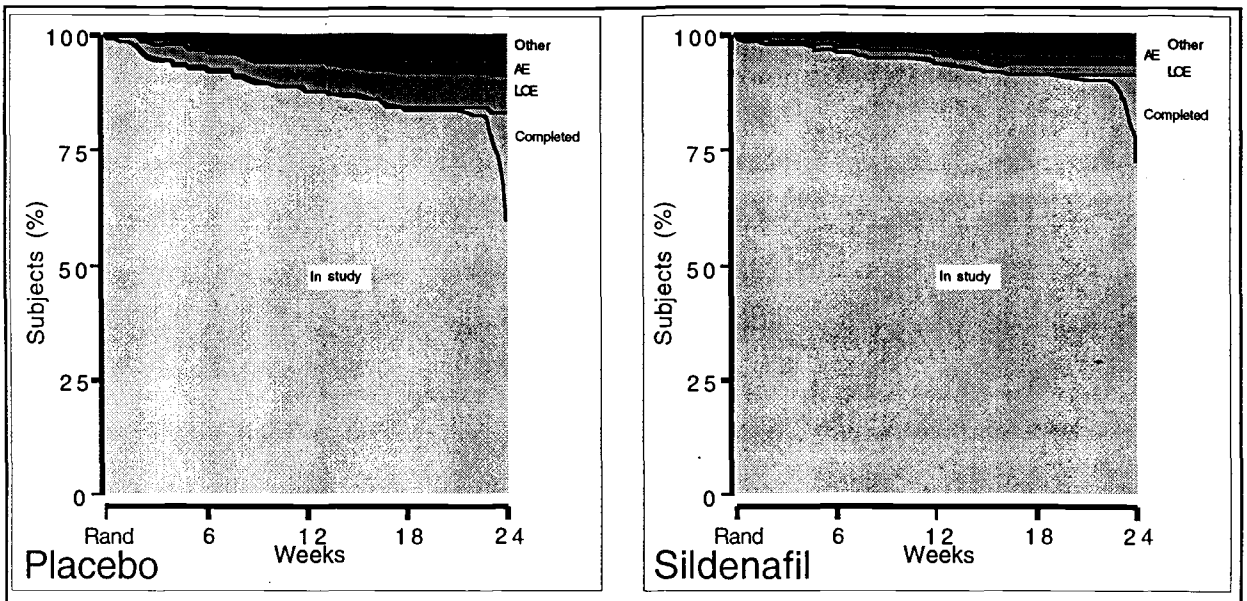


Figure 32. Disposition of subjects (Study 148-102).

The reviewers counted all subjects as "in study" until they reach a state in an all-inclusive set of mutually exclusive final states. In this particular case, the band labeled "LOE" (lack of efficacy) includes subjects who withdrew consent, the band labeled "AE" (adverse event) includes subjects withdrawn for laboratory abnormalities, and the "Other" band includes subjects withdrawn for protocol violations and subjects lost to follow-up.

A10.5.2. Effectiveness

All randomized subjects with a post-randomization assessment were included in the sponsor's ITT analyses. Responses to IIEF questions 3 and 4 were scored as 0 for no attempts[2], 1 for never or rarely successful, etc., up to 5 for always or almost always successful. The sponsor's analyses were LOCF, which tends to make placebo, which had a higher withdrawal rate, better than it otherwise would be. Results are summarized in Table 76 below.

Table 76. ITT analyses of IIEF questions 3 and 4 (Study 148-102).

		Placebo N=216		Sildenafil 25 mg N=102		Sildenafil 50 mg N=107		Sildenafil 100 mg N=107		P-value[a]
		n	Q[b]	n	Q	n	Q	n	Q	
How often were you able to penetrate your partner?	Baseline	—	2.0[c]	—	—	—	—	—	—	
	Week 12	190	2.3	95	3.3	100	3.7	96	4.0	<0.0001
	Week 24	199	2.2	96	3.2	105	3.5	100	4.0	<0.0001
How often were you able to maintain your erection after penetration?	Baseline	—	1.6	—	—	—	—	—	—	
	Week 12	189	2.2	95	3.2	100	3.5	96	3.9	<0.0001
	Week 24	199	2.1	96	3.1	105	3.5	101	3.9	<0.0001

a. P-value for non-zero slope to dose-response.
b. Mean score.
c. Pooled baseline value for all subjects.

Secondary end points from the other IIEF questions are described in Table 77 below (sponsor's analyses only). All treatment effects were highly statistically significant,

[2] Although this is not strictly as specified for this protocol, it is reasonable and in accordance with other phase III protocols and analyses.

except for those pertaining to sexual desire, for which there appeared to be no treatment effect at week 12. Generally similar findings were obtained at week 24, but a small treatment effect on sexual desire was also nominally statistically significant at week 24.

Table 77. ITT analyses of non-primary IIEF questions at week 12 (Study 148-102)[a].

Domain	Question	Baseline	Placebo N=216		Sildenafil 25 mg N=102		Sildenafil 50 mg N=107		Sildenafil 100 mg N=107		p^b
			n	Q	n	Q	n	Q	n	Q	
Erectile function	Able to get erection	2.5	189	2.9	95	3.8	100	4.0	97	4.4	<0.0001
	Erections hard enough	2.1	190	2.1	95	3.3	99	3.8	97	4.0	<0.0001
	Erection maintained to completion	1.5	190	2.1	95	3.2	100	3.6	97	3.9	<0.0001
	Confidence in erection	1.6	190	2.1	95	2.7	98	3.3	96	3.4	<0.0001
Intercourse satisfaction	Attempted intercourse	1.9	191	2.7	95	3.1	100	3.0	97	3.6	<0.0001
	Satisfaction of intercourse	1.8	191	2.3	94	3.4	100	3.7	97	3.9	<0.0001
	Enjoyment of intercourse	1.8	191	2.3	94	3.0	100	3.6	97	3.8	<0.0001
Orgasmic function	Frequency of ejaculation	3.1	189	3.2	93	4.0	97	4.2	97	4.3	<0.0001
	Frequency of orgasm	3.0	190	3.2	94	3.5	100	4.2	97	4.1	<0.0001
Sexual desire	Frequency of desire	3.5	190	3.3	95	3.3	100	3.5	97	3.6	0.2
	Rating of desire	3.2	190	3.2	95	3.3	100	3.4	97	3.3	0.2
Overall satisfaction	Satisfaction with sex life	1.9	190	2.4	95	3.1	100	3.4	97	3.6	<0.0001
	Satisfaction with relationship	2.7	187	3.1	94	3.7	100	3.8	95	4.1	<0.0001

a. Sponsor's analyses.
b. P-value for non-zero slope to dose-response.

About 25% of placebo group partners and 33% of active group partners responded on the partner questionnaire, which is perhaps as telling as the observed statistically significant treatment effects, at 12 and 24 weeks, on questions to rate the partner's erections and satisfaction of sexual intercourse.

The global assessment by subjects whether treatment improved their erections, the original primary end point, was answered in the affirmative at week 12 by 27% on placebo, 58% on 25 mg, 74% on 50 mg, and 81% on 100 mg. Results were similar at 24 weeks.

The sponsor's analysis of the event logs focussed on the proportion of successful attempts at intercourse, but did not describe the number of such attempts by treatment group, or the success rate for subjects. Table 78 below therefore combines results of the sponsor's and the reviewers' analyses.

The only quality of life component (out of 11) with a statistically significant treatment effect (by the sponsor's analyses), at both week 12 and week 24, was impact of erectile dysfunction on quality of life.

The reviewers analyzed the proportion of subjects improving, staying the same, or worsening, on the primary effectivenes questions, by treatment group, at week 24, as shown in Table 79 below.

The reviewers also carried out an analysis of the primary end point on sub-groups defined by etiology of erectile dysfunction, duration of erectile dysfunction, history of nocturnal erections, history of prior treatment for erectile dysfunction, and history of

Table 78. Successful intercourse by event logs (Study 148-102).

	Sponsor (12 weeks)				Reviewers (24 weeks)			
	Placebo	Sildenafil			Placebo	Sildenafil		
	N=216	25 mg N=102	50 mg N=107	100 mg N=107	N=216	25 mg N=102	50 mg N=107	100 mg N=107
Attempts								
Total	—	—	—	—	14004	7023	7795	7055
Per subject mean	—	—	—	—	65	69	73	66
Successes								
Total	—	—	—	—	3388	2701	3994	3627
Per subject mean	—	—	—	—	16	26	37	34
Success by attempts (%)	22	39	54	56	24	38	51	51
Success by subjects (%)								
During run-in	—	—	—	—	43	33	47	48
During DB treatment	—	—	—	—	69	86	89	92

Table 79. ITT shift analyses of IIEF questions 3 and 4 at week 24 (Study 148-102).

		Placebo	Sildenafil		
		N=198	25 mg N=96	50 mg N=107	100 mg N=107
How often were you able to penetrate your partner?	Decr (%)	28	15	8	8
	Same (%)	40	40	35	22
	Incr (%)	31	46	57	71
How often were you able to maintain your erection after penetration?	Decr (%)	22	14	7	9
	Same (%)	45	31	27	21
	Incr (%)	33	55	66	72

diabetes mellitus. The results of comparisons of the slope of the dose-response curves (change in score per g) are summarized in Table 80 below. The results are consistent with there being similar treatment effects regardless of classification of etiology, presence or absence of nocturnal erections, previous use of drugs or devices for treatment of erectile dysfunction, or duration of erectile dysfunction. Of the factors evaluated, only subjects with a history of diabetes mellitus appeared to have a reduced treatment effect, as indicated by smaller estimates of the slope in subjects with diabetes, statistical significant treatment*diabetes interaction, and the lack of nominal statistical significance for the slope.

Table 80. Sub-group analyses of IIEF questions 3 and 4[a] (Study 148-102).

	N	How often were you able to penetrate your partner?				How often were you able to maintain your erection after penetration?			
		Factors[b]	Intcpt	Slope	*P*[c]	Factors	Intcpt	Slope	*P*
Etiology		Baseline				Baseline			
Organic	411	Age	0.2±0.1	16±2	0.0001		0.4±0.1	15±2	0.0001
Psychogenic	50	Etiology	0.2±0.2	23±5	0.0001		0.1±0.2	28±5	0.0001
Mixed	70		0.7±0.3	19±6	0.003		1.1±0.3	16±5	0.005
Nocturnal erections		Baseline				Baseline			
Yes	308	Noct	0.5±0.1	18±2	0.0001	Noct	0.7±0.1	18±2	0.0001
No	175		0.0±0.1	16±3	0.0001		0.1±0.2	15±3	0.0001
Unknown	48		0.2±0.4	17±6	0.01		0.7±0.4	12±7	0.09
Duration		Baseline				Baseline			
<3 years	325		0.1±0.1	19±2	0.0001	Age	0.5±0.1	17±2	0.0001
>3 years	206		0.5±0.1	13±3	0.0001		0.5±0.1	17±3	0.0001
Previous treatment		Baseline				Baseline			
Yes	284		0.4±0.1	16±3	0.0001	Age	0.6±0.1	15±3	0.0001
No	247		0.1±0.1	17±2	0.0001		0.3±0.1	18±3	0.0001
Diabetes mellitus		Baseline				Baseline			
Yes	72	Tx*diabetes	0.4±0.2	5±5	0.29	Age	0.5±0.2	8±5	0.11
No	459		0.3±0.1	18±2	0.0001	Tx*diabetes	0.5±0.1	17±2	0.0001

a. Reviewers' LOCF analyses; slope of dose-response (change in score per g)
b. Statistically significant effects ($P<0.05$) by ANCOVA from among baseline score, age classified as <55 or >55, sub-grouping (etiology, etc.), treatment by age (Tx*age) interaction, or treatment by sub-grouping.
c. P-value for non-zero slope to dose-response analysis of treatment alone.

A10.5.3. Safety

Safety will be reviewed for all placebo-controlled experience together.

A10.5.4. Long-term

Four hundred and two subjects entered the 24-week, long-term, open-label extension to Study 148-102. As of the cut-off date of 3 February 1997, 20 subjects had completed, and 23 subjects had withdrawn (15 for lack of effectiveness, 4 for withdrawal of consent, and 1 each for laboratory abnormality[3], protocol violation, and loss to follow-up). Twelve subjects reported vision abnormalities, all but 2 being described as moderate, and none leading to withdrawal. Three additional subjects were listed as discontinuations for serious adverse events, 2 hospitalized for coronary artery disease and one for myocardial infarction. Common adverse events were headache (11%), vasodilation/flushing (10%), and dyspepsia (5%).

A10.6. Summary

These subjects had erectile dysfunction of organic, but otherwise ill characterized, etiology. One-third to one-half of these subjects were able to achieve erections sufficient for sexual intercourse during a 4-week run-in period. In this population of moderately disabled men, whether analyzed by sexual function questionnaire or event log, there were highly statistically significant, internally consistent, and dose-related treatment effects. Treatment effects were consistent across classes of etiology, presence or absence of nocturnal erections, duration of erectile dysfunction, and history of previous treatment for erectile dysfunction, but the data are indicative of a reduced effect in subjects with diabetes mellitus.

3. Elevated PSA.

A11. Study 148-103: A double-blind, randomized, placebo-controlled, parallel group, multicenter, flexible dose escalation study to assess the efficacy and safety of sildenafil administered as required to male patients with erectile dysfunction.

A11.1. Source documents

Study protocol INL vol 15.1; study report: NDA vol 1.95-1.97; electronic document: 46289525.pdf; SAS datasets.

A11.2. Investigators

Multi-center study with 20 investigators in the United States.

A11.3. Study dates

24 April 1996 to 18 November 1996.

A11.4. Study design

This study description was based upon the protocol dated 28 February 1996. There were no amendments

Drug supplies are shown in Table 81 below.

Table 81. Drug supplies (Study 148-103).

	Lot		Lot
Placebo 25 mg	4469-101A-G1	Sildenafil 25 mg	4469-120A-G1
Placebo 50 mg	4469-104-G1	Sildenafil 50 mg	4469-121A-G1
Placebo 100 mg	4469-084-G1	Sildenafil 100 mg	4469-119A-G1

The intent was to randomize 230 male subjects age >18, with erectile dysfunction[1] of >6 months' duration, and in a heterosexual relationship for >6 months. Subjects were excluded for (1) anatomical deformities such as severe penile fibrosis, (2) other sexual disorders such as hypoactive sexual desire, (3) elevated prolactin (3x ULN) or low free testosterone (20% below LLN), (4) major, uncontrolled psychiatric disorders, (5) history of alcohol or drug abuse, (6) history of major hematologic, renal, or hepatic disorder, (7) erectile dysfunction following spinal cord injury, (8) uncontrolled diabetes or diabetic retinopathy, (9) stroke or myocardial infarction within 6 months, (10) cardiac failure, unstable angina, ECG ischemia, or life-threatening arrhythmia within 6 months, (11) blood pressure outside 90/50 to 170/100 mmHg, (12) active peptic ulcer disease or bleeding disorder, (13) any clinically significant baseline laboratory abnormality, (14) need for anticoagulants, nitrates, androgens, or trazodone, (15) need for aspirin or NSAIDs and a history of peptic ulcer disease, (16) unwillingness to cease use of vacuum devices, intracavernosal injection, or other therapy for erectile dysfunction, other experimental drug use within 3 months, or (17) history of retinitis pigmentosa.

At the end of a 4-week treatment-free run-in period during which baseline sexual performance data were collected, subjects were randomized to placebo or sildenafil 50 mg and followed for 12 weeks. A 1:1 placebo:active randomization was implemented, although there were expected differences in the rate of withdrawal for lack of efficacy. Subjects were instructed to take study drug approximately one hour before planned sexual activity, not more than once per day. Alcohol use during this hour was discouraged. Prior to clinic visits at the end of weeks 2, 4, 8, and 12, subjects also took study drug. Subjects completed an event log noting time of study drug administration and subsequent sexual activity. At any visit, subjects who were intolerant of the starting dose could have the dose halved and tolerant subjects with inadequate efficacy could have the dose doubled. Subjects completing study without an adverse event were eligible for participation in an open-label follow-on study.

The primary efficacy assessment was at week 12. At this visit, subjects completed a global assessment question, sexual function questionnaire (containing the primary

[1] 'the inability to attain and/or maintain penile erection sufficient for satisfactory sexual performance'

efficacy questions), and a quality of life questionnaire. Optionally, partners filled out another questionnaire.

Plasma samples were drawn for determination of parent compound and metabolite UK-103,320 at weeks 2, 4, 8, and 12, a random, but recorded, time after the last dose.

The study was sized to achieve 90% power at α=0.05 to detect a treatment effect the same size as seen in a previous study. Randomization was not stratified.

The primary end point was the answer, at 12 weeks, to two questions on the sexual function questionnaire:

> *[3] Over the past 4 weeks, when you attempted sexual intercourse, how often were you able to penetrate (enter) your partner?*
>
> *[4] Over the past 4 weeks, during sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?*

Both questions had the same set of possible responses, either "did not attempt intercourse" or a 5-level semi-quantitative response. Analysis was to be by ANCOVA, based on table scores, where the "no attempt" response was not lumped with the worst frequency category. Each question was to be analyzed separately with $p<0.05$ on both necessary for demonstrating efficacy. The model was to include terms for center, baseline, and "other covariates deemed to be appropriate". Any interim analyses were not to affect the ongoing trial.

The primary analysis was described as ITT with last observation carried forward. However, the sponsor's description of the ITT population includes only subjects with at least one observation post-randomization.

Secondary end points were (1) response to the global assessment question:

> *Has the treatment you have been taking over the past 4 weeks improved your erections? [yes] [no]*

(2) the responses to other sexual function questions (there were 13 in addition to the primary efficacy questions), (3) proportion of successful attempts at intercourse, determined from the event log, (4) responses on the optional partner questionnaire, (5) responses on the quality of life assessment, and (6) time to discontinuation for lack of efficacy.

Pharmacokinetic data were to be analyzed by nonlinear mixed-effect modeling (NONMEM) utilizing a large selection of baseline attributes as covariates.

Safety assessments included (1) ECGs at screening and week 12,(2) laboratory tests (CBC, SMA20, urinalysis), (3) vital signs, and (4) physical examination. Clinical adverse events and their relationship to the study drug were recorded.

A11.5. Results

A11.5.1. Conduct

Three hundred and sixty-eight subjects were screened, 329 were randomized, and 307 (93%) completed study. Individual sites enrolled 9 to 23 subjects.

Demographics of the 2 treatment groups are shown in Table 82 below. About 56% of all randomized subjects had received previous drug therapy for erectile dysfunction, and about 11% had used non-drug treatments.

Protocol violations are described in Table 83 below. Not all such subjects were excluded from the sponsor's 'evaluable subjects' analyses.

Table 82. Demographics (Study 148-103).

		Placebo N=166	Sildenafil N=163			Placebo N=166	Sildenafil N=163
Race (%)	White	93	95	Duration (y)	Mean	4.7	5.0
	Black	5.4	2.5		Range	0.6-26	0.5-26
	Other	1.8	2.5				
Age	Mean	59	60	Med hx (%)	Hypertension	31	28
	Range	31-81	26-79		Diabetes	11	8.0
Etiology (%)	Organic	63	55		Prostatectomy	20	18
	Psychogenic	16	14		Depression	5.4	7.4
	Mixed	22	31		IHD	11	21

Table 83. Protocol violations (Study 148-103).

At randomization	n	On treatment	n
Prohibited meds	33	>1 dose/day	34
Baseline lab abn	4	Blind broken for AE	8
Peyronie's disease or anatomic defect	7		
Active medical problem	5		
Ethanol or drug abuse	6		
Confounding condition/treatment	6		
Poorly controlled hypertension	5		
Total[a]	55	Total	38

a. Some subjects had more than one violation.

The disposition of subjects in the trial is shown in Figure 33 below, which shows the placebo group in the left panel and the active treatment group in the right panel. Most subjects remained in study for more than 12 weeks, but some "completed" several weeks early. As the sponsor predicted, fewer subjects on active treatment withdrew for lack of efficacy (or withdrew consent).

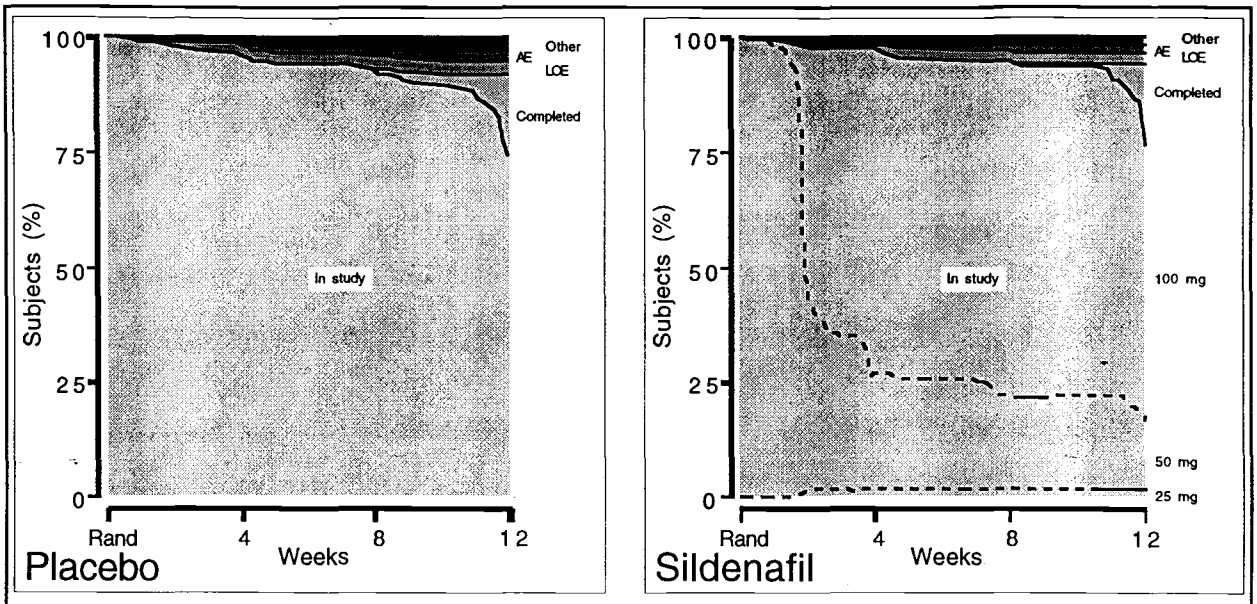


Figure 33. Disposition of subjects (Study 148-103).

The reviewers counted all subjects as "in study" until they reach a state in an all-inclusive set of mutually exclusive final states. In this particular case, the band labeled "LOE" (lack of efficacy) includes subjects who withdrew consent, the band labeled "AE" (adverse event) includes subjects withdrawn for laboratory abnormalities, and the "Other" band includes subjects withdrawn for protocol violations and subjects lost to follow-up. The dashed lines through the "in study" area of the active treatment group show the proportion of subjects on each dose.

A11.5.2. Effectiveness

All randomized subjects with a post-randomization assessment were included in the sponsor's ITT analyses. Responses to IIEF questions 3 and 4 were scored as 0 for no attempts, 1 for never or rarely successful, etc., up to 5 for always or almost always successful. The sponsor's analyses were LOCF, which tends to make placebo, which had a higher withdrawal rate, better than it otherwise would be. Results are summarized in Table 84 below.

Table 84. ITT analyses of IIEF questions 3 and 4 (Study 148-103).

		Placebo N=166		Sildenafil N=163		*P*
		n	Q	n	Q	
How often were you able to penetrate your partner?	Baseline	—	2.0[a]	—	—	
	Week 12	138	2.3	138	3.9	<0.0001
How often were you able to maintain your erection after penetration?	Baseline	—	1.5	—	—	
	Week 12	138	1.8	137	3.6	<0.0001

a. Pooled baseline value for all subjects.

Secondary end points from the other IIEF questions are described in Table 85 below (sponsor's analyses only). All treatment effects were highly statistically significant, except for one pertaining to sexual desire, for which there appeared to be no treatment effect.

About 22% of placebo and active group partners responded on the partner questionnaire. There were statistically significant treatment effects, at 12 weeks, on questions to rate the partner's erections and satisfaction of sexual intercourse.

Table 85. ITT analyses of non-primary IIEF questions at week 12 (Study 148-103)[a].

Domain	Question	Baseline	Placebo N=166		Sildenafil N=163		P
			n	Q	n	Q	
Erectile function	Able to get erection	2.4	138	2.4	138	3.9	<0.0001
	Erections hard enough	2.0	138	2.1	138	3.8	<0.0001
	Difficulty maintaining erection	1.6	138	1.9	138	3.7	<0.0001
	Confidence in erection	1.6	137	1.9	136	3.3	<0.0001
Intercourse satisfaction	Attempted intercourse	2.2	139	2.9	138	3.5	<0.0001
	Satisfaction of intercourse	1.8	139	2.0	138	3.7	<0.0001
	Enjoyment of intercourse	1.9	139	2.2	138	3.6	0.0001
Orgasmic function	Frequency of ejaculation	2.8	139	2.8	134	3.9	<0.0001
	Frequency of orgasm	2.7	139	2.9	138	3.8	<0.0001
Sexual desire	Frequency of desire	3.6	138	3.5	138	3.5	0.7
	Rating of desire	3.3	139	3.3	138	2.5	0.006
Overall satisfaction	Satisfaction with sex life	1.8	138	2.0	138	3.7	<0.0001
	Satisfaction with relationship	2.6	138	2.8	137	4.0	<0.0001

a. Sponsor's analyses.

The global assessment by subjects whether treatment improved their erections, the original primary end point, was answered in the affirmative at week 12 by 16% on placebo and 74% on sildenafil, a highly statistically significant difference.

The sponsor's analysis of the event logs focussed on the proportion of successful attempts at intercourse, but did not describe the number of such attempts by treatment group, or the success rate for subjects. Table 86 below shows the reviewers' analyses.

Table 86. Successful intercourse by event logs (Study 148-103).

	Placebo N=166	Sildenafil N=163
Attempts		
Total	5645	5971
Per subject mean	34	37
Successes		
Total	732	2792
Per subject mean	4.4	17.1
Success by attempts (%)	13	47
Success by subjects (%)		
During run-in	37	32
During DB treatment	55	87

Several quality of life questions demonstrated a nominally highly statistically significant treatment effect—health compared to a year ago, satisfaction with relationship, impact of erectile problems—but the treatment effect size was, in each case, small.

The reviewers analyzed the proportion of subjects improving, staying the same, or worsening, on the primary effectivenes questions, by treatment group, at week 24, as shown in Table 87 below.

Table 87. ITT shift analyses of IIEF questions 3 and 4 at week 24 (Study 148-103).

		Placebo N=161	Sildenafil N=158
How often were you able to penetrate your partner?	Decr (%)	25	3
	Same (%)	41	22
	Incr (%)	34	75
How often were you able to maintain your erection after penetration?	Decr (%)	20	6
	Same (%)	48	15
	Incr (%)	32	79

The reviewers also carried out an analysis of the primary end point on sub-groups defined by etiology of erectile dysfunction, duration of erectile dysfunction, history of nocturnal erections, history of prior treatment for erectile dysfunction, and history of diabetes mellitus. The results of ANCOVA analyses of the sildenafil-placebo difference in score, after adjustment for baseline and age, are summarized in Table 88 below. The results are consistent with there being similar treatment effects regardless of classification of etiology, presence or absence of nocturnal erections, previous use of drugs or devices for treatment of erectile dysfunction, duration of erectile dysfunction, or history of diabetes.

Table 88. Sub-group analyses of IIEF questions 3 and 4[a] (Study 148-103).

	N	How often were you able to penetrate your partner?				How often were you able to maintain your erection after penetration?			
		Factors[b]	Pcbo	Sil	*P*	Factors	Pcbo	Sil	*P*
Etiology		Baseline				Baseline			
Organic	193	Etiology	0.1	1.6	0.0001	Etiology	0.2	1.9	0.0001
Psychogenic	49		0.2	2.3	0.0001		0.3	2.4	0.0001
Mixed	87		0.4	1.9	0.0001		0.4	2.2	0.0001
Nocturnal erections		Baseline				Baseline			
Yes	202	Noct	0.2	1.9	0.0001	Noct	0.3	2.2	0.0001
No	102		0.6	2.0	0.0001		0.4	2.0	0.0001
Unknown	25		0.0	0.9	0.19		0.0	1.5	0.04
Duration		Baseline				Baseline			
<3 years	132	Age	0.4	1.7	0.0001	Age	0.3	2.1	0.0001
>3 years	197		0.1	1.9	0.0001		0.2	2.1	0.0001
Previous treatment		Baseline				Baseline			
Yes	230	Age	0.1	1.9	0.0001		0.2	2.1	0.0001
No	99		0.3	1.5	0.0001		0.3	1.9	0.0001
Diabetes mellitus		Baseline				Baseline			
Yes	31	Age	0.6	1.6	0.07		0.3	1.6	0.02
No	298		0.2	1.8	0.0001		0.3	2.1	0.0001

a. Reviewers' LOCF analyses; sildenafil-placebo difference in score, after adjustment for baseline and age, classified as <55 or >55.

b. Statistically significant effects ($P<0.05$) by ANCOVA from among baseline score, age classified as <55 or >55, sub-grouping (etiology, etc.), treatment by age (Tx*age) interaction, or treatment by sub-grouping.

A11.5.3. Safety

Safety will be reviewed for all placebo-controlled experience together.

A11.5.4. Long-term

Two hundred and twenty-five subjects entered the 36-week, long-term, open-label extension to Study 148-103. As of the cut-off date of 3 February 1997, 0 subjects had completed, and 18 subjects had withdrawn (8 for lack of effectiveness, 2 for headaches, 1 for headache and abdominal pain, 1 for blurred vison and facial flushing,

1 for prostate cancer, 3 for withdrawal of consent, and 1 each for laboratory abnormality[2] and protocol violation). Eleven subjects reported vision abnormalities, generally described as moderate, with one contributing to withdrawal. Two additional subjects are listed as discontinuations for serious adverse events, one subject hospitalized for CHF and one for dyspnea; the latter died 3 months after discontinuation. Common adverse events were headache (12%), vasodilation/flushing (10%), and dyspepsia (5%).

A11.6. Summary

One-third of subjects were able to attain and maintain an erection sufficient for sexual intercourse during a 4-week baseline period. In this population of moderately disabled men, with largely organic, but otherwise ill-characterized, erectile dysfunction, whether analyzed by sexual function questionnaire or event log, there were highly statistically significant and internally consistent treatment effects. There was a strong tendency to migrate to the highest available dose.

[2] Elevated alkaline phosphatase.

A12. Study 148-104: A double-blind, randomized, placebo-controlled, parallel group, multicenter, flexible dose escalation study to assess the efficacy and safety of sildenafil administered as required to male diabetic patients with erectile dysfunction.

A12.1. Source documents

Study protocol IND vol 15.1; study report: NDA vol 1.108-1.110; electronic document: 46132596.pdf; SAS datasets.

A12.2. Investigators

Multi-center study with 19 investigators in the United States.

A12.3. Study dates

2 May 1996 to 14 November 1996.

A12.4. Study design

This study description was based upon the protocol dated 28 February 1996. There were no amendments

Drug supplies are shown in Table 89 below.

Table 89. Drug supplies (Study 148-104).

	Lot		Lot
Placebo 25 mg	4469-101A-G1	Sildenafil 25 mg	4469-120A-G1
Placebo 50 mg	4469-104-G1	Sildenafil 50 mg	4469-121A-G1
Placebo 100 mg	4469-084-G1	Sildenafil 100 mg	4469-119A-G1 4469-119B-G1

The intent was to randomize 230 male subjects age >18, with erectile dysfunction[1] of >6 months' duration, diabetes mellitus type I for >5 years or type II for >2 years, and in a heterosexual relationship for >6 months. Diabetes was to be stable for at least 3 months, with glycated hemoglobin <12%, and screening fasting glucose <300 mg/dL. Subjects were excluded for (1) anatomical deformities such as severe penile fibrosis, (2) other sexual disorders such as hypoactive sexual desire, (3) elevated prolactin (3x ULN) or low free testosterone (20% below LLN), (4) major, uncontrolled psychiatric disorders, (5) history of alcohol or drug abuse, (6) history of major hematologic, renal, or hepatic disorder, (7) erectile dysfunction following spinal cord injury, (8) uncontrolled diabetes, active diabetic retinopathy, history of serious hypoglycemia within 6 months, severe autonomic neuropathy, ketoacidosis within 3 years, or diabetes secondary to pancreatic damage, Cushing's disease, or acromegaly, (9) stroke or myocardial infarction within 6 months, (10) cardiac failure, unstable angina, ECG ischemia, or life-threatening arrhythmia within 6 months, (11) blood pressure outside 90/50 to 170/100 mmHg, (12) active peptic ulcer disease or bleeding disorder, (13) any clinically significant baseline laboratory abnormality, (14) need for anticoagulants, nitrates, androgens, or trazodone, (15) need for aspirin or NSAIDs and a history of peptic ulcer disease, (16) unwillingness to cease use of vacuum devices, intracavernosal injection, or other therapy for erectile dysfunction, other experimental drug use within 3 months, or (17) history of retinitis pigmentosa.

At the end of a 4-week treatment-free run-in period during which baseline sexual performance data were collected, subjects were randomized to placebo or sildenafil 50 mg and followed for 12 weeks. A 1:1 placebo:active randomization was implemented, although there were expected differences in the rate of withdrawal for lack of efficacy. Subjects were instructed to take study drug approximately one hour before planned sexual activity, not more than once per day. Alcohol use during this hour was discouraged. Prior to clinic visits at the end of weeks 2, 4, 8, and 12, subjects also took study drug. Subjects completed an event log noting time of study drug administration and subsequent sexual activity. Each question was to be analyzed separately with $p<0.05$ on both necessary for demonstrating efficacy. Subjects

[1] 'the inability to attain and/or maintain penile erection sufficient for satisfactory sexual performance'

completing study without an adverse event were eligible for participation in an open-label follow-on study.

The primary efficacy assessment was at week 12. At this visit, subjects completed a global assessment question, sexual function questionnaire (containing the primary efficacy questions), and a quality of life questionnaire. Optionally, partners filled out another questionnaire.

Plasma samples were drawn for determination of parent compound and metabolite UK-103,320 at weeks 2, 4, 8, and 12, a random, but recorded, time after the last dose.

The study was sized to achieve 90% power at α=0.05 to detect a treatment effect the same size as seen in a previous study. Randomization was not stratified.

The primary end point was the answer, at 12 weeks, to two questions on the sexual function questionnaire:

> *[3] Over the past 4 weeks, when you attempted sexual intercourse, how often were you able to penetrate (enter) your partner?*
>
> *[4] Over the past 4 weeks, during sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?*

Both questions had the same set of possible responses, either "did not attempt intercourse" or a 5-level semi-quantitative response. Analysis was to be by ANCOVA, based on table scores, where the "no attempt" response was not lumped with the worst frequency category. Each question was to be analyzed separately with $p<0.05$ on both necessary for demonstrating efficacy. The model was to include terms for center, baseline, and "other covariates deemed to be appropriate". Any interim analyses were not to affect the ongoing trial.

The primary analysis was described as ITT with last observation carried forward. However, the sponsor's description of the ITT population includes only subjects with at least one observation post-randomization.

Secondary end points were (1) response to the global assessment question:

> *Has the treatment you have been taking over the past 4 weeks improved your erections? [yes] [no]*

(2) the responses to other sexual function questions (there were 13 in addition to the primary efficacy questions), (3) proportion of successful attempts at intercourse, determined from the event log, (4) responses on the optional partner questionnaire, (5) responses on the quality of life assessment, and (6) time to discontinuation for lack of efficacy.

Pharmacokinetic data were to be analyzed by nonlinear mixed-effect modeling (NONMEM) utilizing a large selection of baseline attributes as covariates.

Safety assessments included (1) ECGs at screening and week 12, (2) laboratory tests (CBC, SMA20, urinalysis), (3) vital signs, and (4) physical examination. Clinical adverse events and their relationship to the study drug were recorded.

A12.5. Results

A12.5.1. Conduct

Three hundred and fifty-five subjects were screened, 268 were randomized, and 252 (94%) completed study. Individual sites randomized 6 to 31 subjects.

Demographics of the 2 treatment groups are shown in Table 90 below. About 38% of all randomized subjects had received previous drug therapy for erectile dysfunction, and about 11% had used non-drug treatments.

Table 90. Demographics (Study 148-104).

		Placebo N=132	Sildenafil N=136			Placebo N=132	Sildenafil N=136
Race (%)	White	76	81	Duration (y)	Mean	5.8	5.0
	Black	14	12		Range	0.6-22	0.5-26
	Other	9.8	7.4				
Age	Mean	57	57	Med hx (%)	Diabetes I	21	16
	Range	27-79	33-76		Diabetes II	79	84
Etiology (%)	Organic	96	95		Hypertension	51	53
	Psychogenic	0	0		IHD	25	27
	Mixed	3.8	5.1		Prostatectomy	7.6	4.4
					Periph vasc dis	7.6	3.7

Protocol violations are described in Table 91 below. Not all such subjects were excluded from the sponsor's 'evaluable subjects' analyses.

Table 91. Protocol violations (Study 148-104).

At randomization	n	On treatment	n
Prohibited meds	16	>1 dose/day	19
Baseline lab abn	11	Blind broken for AE	2
Diabetes diagnosis < 2 years	1	Mis-dosed	2
Active medical problem	25		
Ethanol or drug abuse	11		
Confounding condition/treatment	9		
Poorly controlled hypertension	4		
Total[a]	75	Total	22

a. Some subjects had more than one violation.

The disposition of subjects in the trial is shown in Figure 34 below, which shows the placebo group in the left panel and the active treatment group in the right panel. Most subjects remained in study for more than 12 weeks, but some "completed" several weeks early. As the sponsor predicted, fewer subjects on active treatment withdrew for lack of efficacy (or withdrew consent).

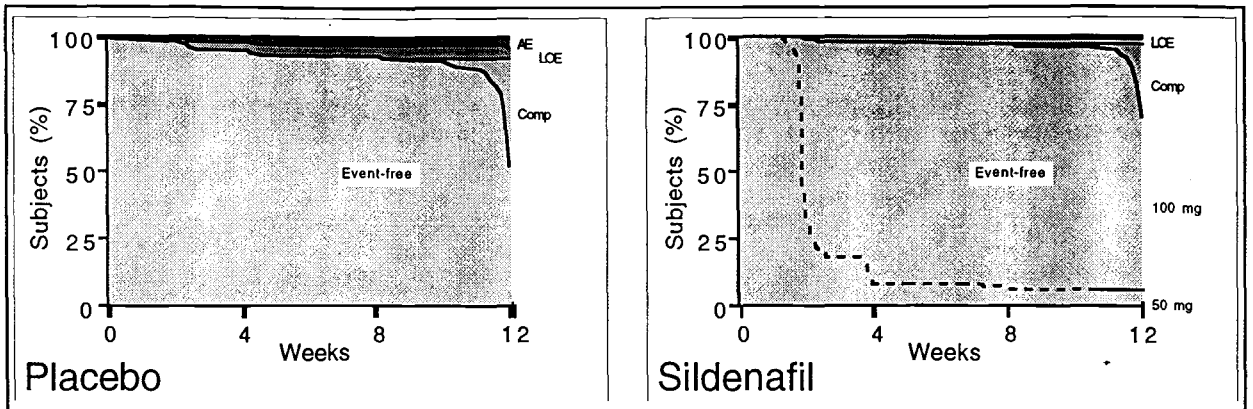


Figure 34. Disposition of subjects (Study 148-104).

The reviewers counted all subjects as "in study" until they reach a state in an all-inclusive set of mutually exclusive final states. In this particular case, the band labeled "LOE" (lack of efficacy) includes subjects who withdrew consent, the band labeled "AE" (adverse event) includes subjects withdrawn for laboratory abnormalities, and the "Other" band includes subjects withdrawn for protocol violations and subjects lost to follow-up. The dashed line through the "in study" area of the active treatment group shows the proportion of subjects on each dose.

A12.5.2. Effectiveness

All randomized subjects with a post-randomization assessment were included in the sponsor's ITT analyses. Responses to IIEF questions 3 and 4 were scored as 0 for no attempts, 1 for never or rarely successful, etc., up to 5 for always or almost always successful. The reviewers' ITT analyses included all randomized subjects, assigning a worst rank to subjects with no assessment post-randomization[2]. Both the sponsor's and the reviewers' analyses were LOCF, which tends to make placebo, which had a higher withdrawal rate, better than it otherwise would be. Results are summarized in Table 92 below.

Table 92. ITT analyses of IIEF questions 3 and 4 (Study 148-104).

		Placebo N=132		Sildenafil N=136		p
		n	Q	n	Q	
How often were you able to penetrate your partner?	Baseline	—	1.7[a]	—	—	
	Week 12	126	2.0	131	3.2	<0.0001
How often were you able to maintain your erection after penetration?	Baseline	—	1.4	—	—	
	Week 12	125	1.6	131	2.9	<0.0001

a. Pooled baseline value for all subjects.

Secondary end points from the other IIEF questions are described in Table 93 below (sponsor's analyses only). All treatment effects were highly statistically significant, except for one pertaining to sexual desire, for which there appeared to be no treatment effect.

About 20% of placebo and active group partners responded on the partner questionnaire. There were no statistically significant treatment effects, at 12 weeks, on questions to rate the partner's erections and satisfaction of sexual intercourse, although the trend is in favor of active treatment.

The global assessment by subjects whether treatment improved their erections, the original primary end point, was answered in the affirmative at week 12 by 10% on placebo and 57% on sildenafil, a highly statistically significant difference.

2. Worst rank for all withdrawals?

Table 93. ITT analyses of non-primary IIEF questions at week 12 (Study 148-104)[a].

Domain	Question	Baseline	Placebo N=166		Sildenafil N=163		P
			n	Q	n	Q	
Erectile function	Able to get erection	2.0	126	1.8	131	3.1	<0.0001
	Erections hard enough	1.6	126	1.8	131	3.1	<0.0001
	Difficulty maintaining erection	1.3	127	1.6	131	2.7	<0.0001
	Confidence in erection	1.5	127	1.6	131	2.5	<0.0001
Intercourse satisfaction	Attempted intercourse	2.0	126	2.7	131	3.4	<0.0001
	Satisfaction of intercourse	1.5	127	1.7	131	2.7	<0.0001
	Enjoyment of intercourse	1.7	126	1.8	131	2.8	<0.0001
Orgasmic function	Frequency of ejaculation	2.9	127	3.3	131	3.9	0.0006
	Frequency of orgasm	2.9	127	3.3	131	3.7	0.02
Sexual desire	Frequency of desire	3.6	127	3.7	131	3.7	0.7
	Rating of desire	3.3	127	3.4	131	3.5	0.2
Overall satisfaction	Satisfaction with sex life	1.8	127	2.1	131	2.9	<0.0001
	Satisfaction with relationship	2.5	127	2.8	130	3.3	0.001

a. Sponsor's analyses.

The sponsor's analysis of the event logs focussed on the proportion of successful attempts at intercourse, but did not describe the number of such attempts by treatment group, or the success rate for subjects. Table 94 below shows the reviewers' analyses.

Table 94. Successful intercourse by event logs (Study 148-104).

	Placebo N=132	Sildenafil N=136
Attempts		
Total	3763	4746
Per subject mean	29	35
Successes		
Total	270	1439
Per subject mean	2.0	11
Success by attempts (%)	7.2	30
Success by subjects (%)		
During run-in	17	22
During DB treatment	32	72

Several quality of life questions demonstrated a nominally highly statistically significant treatment effect—mental health, impact of erectile problems—but the treatment effect size was, in each case, small.

A12.5.3. Safety

Safety will be reviewed for all placebo-controlled studies together.

A12.5.4. Long-term

One hundred and eighty-five subjects entered the 36-week, long-term, open-label extension to Study 148-104. As of the cut-off date of 3 February 1997, 0 subjects had completed, and 22 subjects had withdrawn (16 for lack of effectiveness, 1 each for leg pain, bloodshot eyes and heartburn, and dizziness and hypertension, and 1 each for protocol violation, leaving country, and withdrawal of consent). Three subjects reported vision abnormalities, none contributing to withdrawal. Three other subjects are listed as discontinuing for serious adverse events (3 for coronary artery disease, one

of whom died, and 1 for transient ischemic attack). Common adverse events were headache (6%) and vasodilation/flushing (8%).

A12.6. Summary

One-fifth of these subjects with well-controlled diabetes mellitus had erections sufficient for sexual intercourse during a 4-week baseline assessment period. Whether analyzed by sexual function questionnaire or event log, there were highly statistically significant and internally consistent treatment effects. There was a strong tendency to migrate to the highest available dose.

A13. Study 148-105: A double-blind, randomised, placebo controlled, four-way crossover study to investigate the efficacy, safety and toleration of single oral dose of sildenafil (25, 50, and 100 mg) in patients with male erectile dysfunction.

A13.1. Source documents

Study protocol NDA 20-895, vol 1.111; study report: NDA vol 1.111; electronic document: 46004687.pdf.

A13.2. Investigators

Multi-center study with 2 investigators in the United States.

A13.3. Study dates

13 August 1996 to 27 November 1996.

A13.4. Study design

This study description was based upon the protocol dated 12 June 1996. There were no amendments

Drug supplies are shown in Table 95 below.

Table 95. Drug supplies (Study 148-105).

	Lot		Lot
Placebo 25 mg	4469-101A-G1	Sildenafil 25 mg	4469-144-G1
Placebo 50 mg	4469-104-G1	Sildenafil 50 mg	4469-142B-G1
Placebo 100 mg	4469-084-G1	Sildenafil 100 mg	4469-119C-G1

The intent was to randomize 48 male subjects age >18, with erectile dysfunction[1] of >6 months' duration, and in a heterosexual relationship for >6 months. Subjects were excluded for (1) anatomical deformities such as severe penile fibrosis, (2) hypoactive sexual drive, (3) elevated prolactin or low testosterone, (4) major psychiatric illness, (5) history of alcohol or drug abuse, (6) major hematologic, renal, or hepatic disease, (7) spinal cord injury, (8) poorly controlled diabetes, (9) stroke or myocardial infarction within 6 months, (10) cardiac failure, unstable angina, or life-threatening arrhythmia within 6 months, (11) blood pressure outside 90/50 to 170/100 mmHg, (12) active peptic ulcer disease, (13) bleeding disorder, (14) baseline lab abnormality, (15) recent changes in medication associated with erectile dysfunction, (16) regular use of nitrates, androgens, or trazodone, (17) other medical or social problems limiting participation, (18) other treatment of erectile dysfunction, (19) experimental drug use within 3 months, (20) blood donation within 1 month, and (21) retinitis pigmentosa.

Subjects had routine safety evaluations carried out at a screening visit. They then received single doses of placebo or study drug 25, 50, or 100 mg in random order on clinic visits separated by at least 7 days. Penile plethysmography was performed in the setting of a 20-minute videotape of sexual activity and for the following hour. Plasma samples were obtained at baseline and at 90 minutes.

The primary end point was the log-transformed duration of 60% rigidity[2]. The log-transformed duration of 80% rigidity was a secondary end point.

Safety assessments included (1) laboratory tests (CBC, SMA20, urinalysis), (2) vital signs, and (3) physical examination. Clinical adverse events and their relationship to the study drug were recorded.

A13.5. Results

A13.5.1. Conduct

Fifty-seven subjects were screened, 54 were randomized, and 53 (98%) completed study.

1. 'the inability to attain and/or maintain penile erection sufficient for satisfactory sexual performance'
2. The sponsor cites studies to show that 60% rigidity is thought adequate for penetration.

Demographics of the 4 treatment groups were similar: mean ages ranged from 51 to 55 years. All but 3 subjects were Caucasian. Etiology of erectile dysfunction was organic in 48%, psychogenic in 20%, and mixed in 31%.

Protocol violations included anatomical defects (1), prohibited medication (1), history of drug abuse (5). None of these subjects were excluded from the sponsor's 'evaluable subjects' analyses.

One subject discontinued after the 25-mg dose, for treatment-related facial flushing and vertigo.

A13.5.2. Effectiveness

The sponsor's results of ITT analysis of the primary end point are summarized in Table 96 below. No statistically significant effect was seen in the duration of 80% rigidity.

Table 96. ITT analyses of Rigiscan data (Study 148-105).

	Placebo N=54	Sildenafil 25 mg N=54	Sildenafil 50 mg N=53	Sildenafil 100 mg N=53	P
Duration of 60% rigidity (min)	0.06	0.53	0.39	0.95	0.0002

A13.5.3. Pharmacokinetics

Approximately dose-proportional plasma levels of sildenafil were seen at 60 and 90 minutes after dosing, as shown in Table 97 below. Plasma levels of the principal metabolite, UK-103,320, were about 40% as high as for the parent drug, and were also approximately dose-proportional.

Table 97. Plasma levels (±SD) of sildenafil (Study 148-105).

	25 mg	50 mg	100 mg
60 min	96±43	194±128	426±219
90 min	81±39	200±129	370±167

The reviewers performed no analyses of these data.

A13.5.4. Safety

Safety will be reviewed for all placebo-controlled studies together.

A13.6. Summary

The study population appeared to similar to that studied for effects on sexual performance. Dose-related effects were found on duration of erections, but the durations attained in the clinic were small.

Center for Drug Evaluation and Research

Viagra (Sildenafil)

"Joint Clinical Review" for NDA-20-895

Appendix A14, page 126 through Appendix A22.6, page 149

A14. Study 148-106: A double-blind, randomised, placebo controlled, parallel group, multicentre, fixed-dose study to assess the efficacy and safety of sildenafil administered as required to male subjects with erectile dysfunction.

A14.1. Source documents

Study protocol NDA 20-895, vol 1.112; study report: NDA vol 1.112; electronic document: 47090249.pdf.

A14.2. Investigators

Multi-center study with 27 investigators in Canada.

A14.3. Study dates

16 July 1996 to 6 January 1997.

A14.4. Study design

This study description was based upon the amended protocol dated 21 March 1996. There were no amendments.

Drug supplies are shown in Table 98 below.

Table 98. Drug supplies (Study 148-106).

	Lot		Lot
Placebo 50 mg	N5274-G1	Sildenafil 50 mg	4469-142B-G1
Placebo 100 mg	N5275-G1	Sildenafil 100 mg	N5277-G1

The intent was to randomize 460 male subjects age >18, with erectile dysfunction[1] of >6 months' duration, and in a heterosexual relationship for >6 months. Subjects were excluded for (1) anatomical deformities such as severe penile fibrosis, (2) other sexual disorders such as hypoactive sexual desire, (3) elevated prolactin (3x ULN) or low free testosterone (20% below LLN), (4) major, uncontrolled psychiatric disorders, (5) history of alcohol or drug abuse, (6) history of major hematologic, renal, or hepatic disorder, (7) erectile dysfunction following spinal cord injury, (8) uncontrolled diabetes or diabetic retinopathy, (9) stroke or myocardial infarction within 6 months, (10) cardiac failure, unstable angina, ECG ischemia, or life-threatening arrhythmia within 6 months, (11) blood pressure outside 90/50 to 170/100 mmHg, (12) active peptic ulcer disease or bleeding disorder, (13) any clinically significant baseline laboratory abnormality, (14) need for anticoagulants, nitrates, androgens, or trazodone, (15) need for aspirin or NSAIDs and a history of peptic ulcer disease, (16) unwillingness to cease use of vacuum devices, intracavernosal injection, or other therapy for erectile dysfunction, (17) other experimental drug use within 3 months, or (18) history of retinitis pigmentosa.

At the end of a 4-week treatment-free run-in period during which baseline sexual performance data were collected, subjects were randomized equally to placebo or sildenafil 50, 100, or 200 mg and followed for 12 weeks. Subjects were instructed to take study drug approximately one hour before planned sexual activity, not more than once per day. Alcohol use during this hour was discouraged. Prior to clinic visits at the end of weeks 2, 4, 8, and 12, subjects also took study drug. Subjects completed an event log noting time of study drug administration and subsequent sexual activity. Subjects completing study without an adverse event were eligible for participation in an open-label follow-on study.

The primary efficacy assessment was at week 12. At this visit, subjects completed a global assessment question, sexual function questionnaire (containing the primary efficacy questions), and a quality of life questionnaire. Optionally, partners filled out another questionnaire.

[1] 'the inability to attain and/or maintain penile erection sufficient for satisfactory sexual performance'

Plasma samples were drawn for determination of parent compound and metabolite UK-103,320 at weeks 2, 4, 8, and 12, a random, but targeted and recorded, time after the last dose.

The study was originally sized to achieve 90% power at α=0.05 to detect a 75% improvement in erections on study drug compared with placebo. Randomization was not stratified.

The primary end point was the answer, at 12 weeks, to two questions on the sexual function questionnaire:

> *[3] Over the past 4 weeks, when you attempted sexual intercourse, how often were you able to penetrate (enter) your partner?*
>
> *[4] Over the past 4 weeks, during sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?*

Both questions had the same set of possible responses, either "did not attempt intercourse" or a 5-level semi-quantitative response. Analysis was to be by ANCOVA, based on table scores, where the "no attempt" response was lumped with the worst frequency category. Each question was to be analyzed separately with $p<0.05$ on both necessary for demonstrating efficacy. The model was to include terms for center, baseline, and "other covariates deemed to be appropriate". The primary test was a single-degree-of-freedom test for a linear trend by dose. Any interim analyses were not to affect the ongoing trial.

The primary analysis was described as ITT with last observation carried forward. However, the sponsor's description of the ITT population includes only subjects with at least one observation post-randomization.

Secondary end points were (1) response to the global assessment question (originally the primary end point):

> *Has the treatment you have been taking over the past 4 weeks improved your erections? [yes] [no]*

(2) the responses to other sexual function questions (there were 13 in addition to the primary efficacy questions), (3) proportion of successful attempts at intercourse, determined from the event log, (4) responses on the optional partner questionnaire, (5) responses on the quality of life assessment, and (6) time to discontinuation for lack of efficacy.

Pharmacokinetic data were to be analyzed by nonlinear mixed-effect modeling (NONMEM) utilizing a large selection of baseline attributes as covariates.

Safety assessments included (1) ECGs at screening and week 12,(2) laboratory tests (CBC, SMA20, urinalysis), (3) vital signs, and (4) physical examination. Clinical adverse events and their relationship to the study drug were recorded.

A14.5. Results

A14.5.1. Conduct

Five hundred and eighty-two subjects were screened, 497 were randomized, and 436 (88%) completed study.

Demographics of the 4 treatment groups are shown in Table 99 below. About half of all randomized subjects had received previous drug therapy for erectile dysfunction, and about 9% had used non-drug treatments.

Protocol violations are described in Table 100 below. Not all such subjects were excluded from the sponsor's 'evaluable subjects' analyses.

Table 99. Demographics (Study 148-106).

		Placebo N=122	Sildenafil 50 mg N=127	Sildenafil 100 mg N=124	Sildenafil 200 mg N=124
Race (%)	White	95	95	91	86
	Black	4.1	1.7	4.0	4.8
	Other	4.9	0.8	5.2	7.2
Age	Mean	57	60	58	58
	Range	25-79	39-80	24-80	21-79
Etiology (%)	Organic	59	62	58	53
	Psychogenic	14	18	16	18
	Mixed	27	19	26	29
Duration (y)	Mean	5.2	5.5	5.6	5.2
	Range	0.5-26	0.5-34	0.5-37	0.5-23
Med hx (%)	Hypertension	27	22	30	21
	Diabetes	18	22	12	17
	Peripheral vascular disease	4.9	3.9	3.2	0.8
	Depression	7.4	5.5	4.9	4.8
	IHD	17	15	16	15

Table 100. Protocol violations (Study 148-106).

At randomization	n	On treatment	n
Prohibited meds	3	>1 dose/day	36
Baseline lab abn	21	Blind broken for AE	5
Peyronie's disease or anatomic defect	24		
Active medical condition	8		
Ethanol or drug abuse	20		
Confounding condition/treatment	28		
Poorly controlled hypertension	2		
Hypogonadism	1		
Total[a]	130	Total	41

a. Some subjects had more than one violation.

Discontinuation rates were 11 to 15% in the 4 treatment groups, but most of the withdrawals from placebo were for lack of efficacy and most of the withdrawals from the 200-mg group were for treatment-related adverse events.

A14.5.2. Effectiveness

All randomized subjects with a post-randomization assessment were included in the sponsor's ITT analyses. Responses to IIEF questions 3 and 4 were scored as 0 for no attempts[2], 1 for never or rarely successful, etc., up to 5 for always or almost always successful. The sponsor's analyses was LOCF, which tends to make placebo, which had a higher withdrawal rate, better than it otherwise would be. Results are summarized in Table 101 below.

Secondary end points from the other IIEF questions are described in Table 102 below (sponsor's analyses only). All treatment effects were highly statistically significant,

2. Although this is not strictly as specified for this protocol, it is reasonable and in accordance with other phase III protocols and analyses.

Table 101. ITT analyses of IIEF questions 3 and 4 (Study 148-106).

		Placebo N=122		Sildenafil 50 mg N=127		100 mg N=124		200 mg N=124		*P*
		n	Q	n	Q	n	Q	n	Q	
How often were you able to penetrate your partner?	Baseline	—	1.8[a]	—	—	—	—	—	—	
	Week 12	109	2.2	116	3.5	112	3.7	112	3.5	<0.0001
How often were you able to maintain your erection after penetration?	Baseline	—	1.5	—	—	—	—	—	—	
	Week 12	109	1.7	115	3.2	112	3.6	112	3.4	<0.0001

a. This is apparently the pooled baseline value for all subjects.

except for one pertaining to sexual desire, for which there appeared to be no treatment effect.

Table 102. ITT analyses of non-primary IIEF questions at week 12 (Study 148-106)[a].

Domain	Question	Base-line	Placebo N=216		Sildenafil 25 mg N=102		50 mg N=107		100 mg N=107		*P*
			n	Q	n	Q	n	Q	n	Q	
Erectile function	Able to get erection	2.3	108	2.3	116	3.3	112	3.7	112	3.7	<0.0001
	Erections hard enough	1.8	110	2.0	116	3.3	112	3.6	112	3.4	<0.0001
	Erection maintained to completion	1.5	110	1.7	116	3.4	113	3.6	112	3.4	<0.0001
	Confidence in erection	1.6	108	1.8	113	3.0	111	3.2	110	3.1	<0.0001
Intercourse satisfaction	Attempted intercourse	2.0	110	2.7	116	3.3	113	3.3	112	3.2	0.001
	Satisfaction of intercourse	1.6	110	1.9	116	3.2	112	3.5	112	3.6	<0.0001
	Enjoyment of intercourse	1.8	110	1.9	115	3.1	112	3.2	112	3.2	<0.0001
Orgasmic function	Frequency of ejaculation	2.7	108	2.9	116	3.6	110	3.7	111	3.6	0.0002
	Frequency of orgasm	2.7	109	2.9	116	3.6	113	3.7	112	3.5	0.0002
Sexual desire	Frequency of desire	3.3	109	3.3	115	3.5	112	3.5	111	3.5	0.4
	Rating of desire	3.1	110	3.1	116	3.3	112	3.3	111	3.4	0.008
Overall satisfaction	Satisfaction with sex life	1.9	109	2.1	116	3.2	112	3.4	111	3.5	<0.0001
	Satisfaction with relationship	2.5	108	2.6	116	3.6	111	3.8	110	3.8	<0.0001

a. Sponsor's analyses.

About 11% of partners responded on the partner questionnaire; no treatment effect was demonstrated.

The global assessment by subjects whether treatment improved their erections, the original primary end point, was answered in the affirmative at week 12 by 25% on placebo, 70% on 50 mg, 82% on 100 mg, and 80% on 200 mg.

The sponsor's analysis of the event logs focussed on the proportion of successful attempts at intercourse, but did not describe the number of such attempts by treatment group, or the success rate for subjects. The success rates were 15%, 45%, 49%, and 49% on placebo, 50 mg, 100 mg, and 200 mg.

The only quality of life component (out of 11) with a statistically significant treatment effect (by the sponsor's analyses), at week 12, was impact of erectile dysfunction on quality of life.

A14.5.3. Safety

Safety will be reviewed for all placebo-controlled experience together.

A14.6. Summary

SAS datasets were not made available for this study, so all analyses are the sponsor's. The study population appears to have been similar to that in other major studies. There were, as well, comparably robust and dose-related treatment effects, as assessed by erectile and sexual function questionnaires and by event log.

A15. Study 148-203: A single blind, four way crossover study to investigate the pharmacokinetics of and assess the safety and tolerance of UK-92480 after administration of escalating intravenous doses in the fasted state.

A15.1. Source documents

Study protocol NDA 20-895, vol 1.46; study report: NDA vol 1.46-1.47; electronic document: 47159495.pdf.

A15.2. Investigators

A15.3. Study dates

8 November 1991 to 31 December 1991.

A15.4. Study design

This study description was based upon the final study report, dated 16 October 1996.

A15.4.1. Objectives

The objectives were

- To assess the safety and toleration of intravenously administered sildenafil.
- To investigate the pharmacokinetics of sildenafil after IV administration.
- To assess the effect of sildenafil on plasma and/or platelet-rich plasma cGMP after IV administration.

A15.4.2. Formulation

Sildenafil was supplied as an injection solution 1 mg/ml (lot 975-19). Placebo was a 5% mannitol solution (lot 975-31).

A15.4.3. Population

Eight healthy male subjects between 45 and 60 years inclusive participated in this study.

A15.4.4. Procedures

The study was a single-blind four-way crossover dose escalation study of three single IV doses of sildenafil (20, 40, and 80 mg) plus a randomly inserted dose of placebo. Each dose was separated by a washout period of at least 7 days. The drug solutions was to be diluted with 5% mannitol solution. For each dose, 80 ml of solution was administered at 2 ml/min over 40 minutes. For the first study period, plasma samples were collected at 0, 20, 30, 40, and 50 minutes and at 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 18, 24, 32 and 48 hours post-dose. For the second, third and fourth study periods, additional samples were taken at 72 and 96 hours.

A15.4.5. Assay

A15.4.6. Analysis

Pharmacokinetic parameters were calculated using standard non-compartmental techniques. The linear relationship between log-dose and the log-transformed C_{max} or AUC was statistically evaluated. Pharmacokinetic parameters were deemed dose-proportional if the linear model was a good fit and the 95% confidence interval for the proportionality term included 1.

A15.4.7. Safety

Routine safety data were recorded.

A15.5. Results

A15.5.1. Pharmacokinetics

Mean plasma concentrations vs. time profiles for sildenafil and its main metabolite are shown as a function of dose in Figure 35 below with the corresponding pharmacokinetic parameters summarized in Table 103 below.

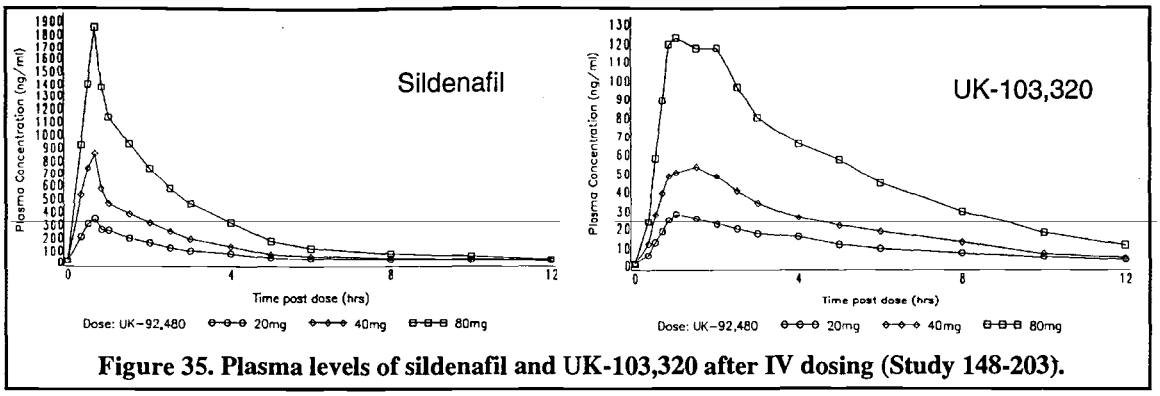


Figure 35. Plasma levels of sildenafil and UK-103,320 after IV dosing (Study 148-203).

Table 103. Pharmacokinetic parameters after IV dosing (Study 148-203).

	Sildenafil			UK-103,320		
	20 mg	40 mg	80 mg	20 mg	40 mg	80 mg
C_{max} (ng/ml)	331	833	1822	27	50	124
AUC_{τ} (ng.h/ml)	714	1554	3711	119	220	584
T_{max} (h)	0.67	0.58	0.69	1.17	1.17	1.33

The sponsor's analysis showed that both AUC and C_{max} for sildenafil, shown in Figure 36 below, were slightly more than dose proportional. The proportionality factor for C_{max} was 1.23 with a 95% CI of 1.1 to 1.36. For AUC the proportionality factor was 1.19 with a 95% CI of 1.09 to 1.29.

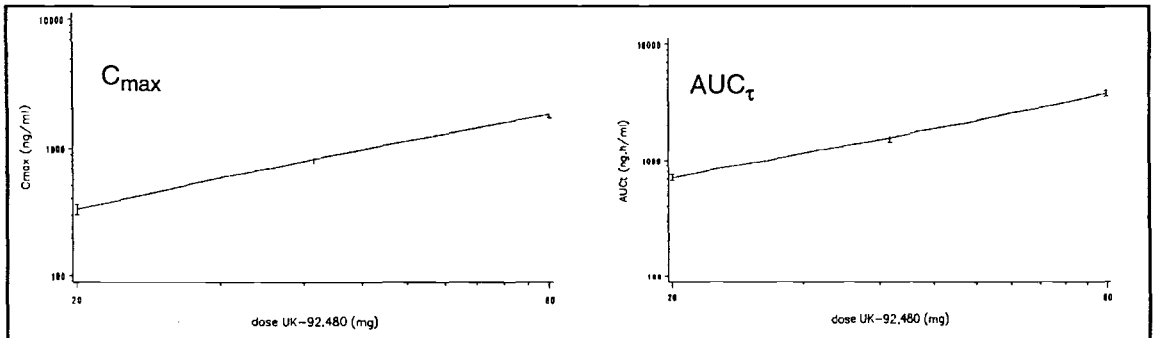


Figure 36. Log-log plots of C_{max} and AUC for sildenafil after IV dosing (Study 148-203).

For metabolite UK 103,320, both C_{max} and AUC increased in a dose-proportional manner. The proportionality factor for C_{max} was 1.09 with a 95% CI of 0.93-1.25 and for AUC it was 1.15 with a 95% CI of 0.96-1.34.

A15.6. Summary

The results of the study showed that after IV infusion of sildenafil 20 to 80 mg over 40 minutes, AUC and C_{max} for sildenafil exhibited a slight nonlinearity with dose. AUC and C_{max} for UK-103,320 appeared to be similar functions of dose, but wider confidence limits prevent one from excluding dose-proportionality for the metabolite.

A16. Study 148-204: An open study in normal volunteers to investigate the effects of an escalating brachial artery infusion of UK-92,480 on forearm blood flow and forearm venous compliance.

A16.1. Source documents	Study protocol NDA 20-895, vol 1.48; study report: NDA vol 1.48; electronic document: 47059260.pdf.
A16.2. Investigators	Single-center study with 1 investigator in the UK.
A16.3. Study dates	10 January 1992 to 23 April 1992.
A16.4. Study design	This study description was based upon the final study report, dated 26 March 1997. A total of 12 health male volunteers, age 18 to 45, were to be recruited. Subjects received open-label 10-minute brachial artery infusions of dextrose 5%, dextrose 5%, mannitol 5%, sildenafil 0.3 to 100 µg/min (later changed to 3 to 300 µg/min), dextrose, and dextrose. All but the first dextrose infusions were accompanied by noradrenaline 0.5 to 2 µg/min. Some subjects returned for a second mannitol infusion on day 2. Forearm blood flow and venous compliance were measured during the latter half of each infusion, using standard techniques. Routine safety data were recorded.
A16.5. Results	
A16.5.1. Conduct	Thirteen subjects were recruited, but only 12 were dosed. All but 1 subject were Caucasian.
A16.5.2. Pharmacokinetics	Although plasma drug levels were recorded, they were not reported a fashion amenable to comparison with pharmacodynamic results.
A16.5.3. Pharmacodynamics	Forearm blood flow increased by 16% at 3 µg/min, and by 50% at 300 µg/min. Forearm volume increased by about 15% at the highest dose.
A16.5.4. Safety	No adverse events and no significant laboratory abnormalities were reported.
A16.6. Summary	Forearm vessel tone was set by noradrenaline infusion and antagonized by sildenafil in a dose-related manner.

A17. Study 148-206: A single blind, two way crossover, placebo controlled pilot study to investigate the effects of UK-92,480 (sildenafil) on platelet function in normal male volunteers.

A17.1. Source documents

Study protocol NDA 20-895, vol 1.50; study report: NDA vol 1.50; electronic document: 47164938.pdf.

A17.2. Investigators

Single-center study with 1 investigator in the UK.

A17.3. Study dates

24 March 1992 to 20 May 1992.

A17.4. Study design

This study description was based upon the final study report, dated 29 July 1997.

A total of 8 health male volunteers, age 18 to 45, were to be recruited.

This was a crossover study. During the two phases, subjects received 2 doses of either placebo or sildenafil 50 mg, 16 hours apart. The two phases were at least 10 days apart. After each dose, subjects had blood drawn at 2, 8, and 12 hours for measurement of sildenafil and metabolite and for assessment of platelet aggregability.

Platelet aggregation in plasma was assessed by a dose-response relationship for the IC_{50} for sodium nitroprusside inhibition of aggregation by ADP, with and without superoxide dismutase (to prolong the lifetime of nitric oxide). The aggregation of platelets in whole blood was assessed as the response to ADP.

Routine safety data were recorded.

A17.5. Results

A17.5.1. Conduct

Eight subjects were recruited and dosed.

A17.5.2. Pharmacokinetics

Although plasma drug levels were recorded, the study is too small to allow comparison with pharmacodynamic results.

A17.5.3. Pharmacodynamics

ADP produces platelet aggregation. Sodium nitroprusside antagonizes aggregation caused by ADP. Greater antagonism was observed in the presence of sildenafil. At about the time of the peak sildenafil concentration, the effect was about a 10-fold decrease in the IC_{50} for sodium nitroprusside. No effect on platelets was observed in whole blood.

Sildenafil had no effect on bleeding time.

A17.5.4. Safety

No serious adverse events or discontinuations and no significant laboratory abnormalities were reported.

A17.6. Summary

These results are consistent with sildenafil having no direct effect on platelet aggregation. However, in the presence of sodium nitroprusside, a donor of nitric oxide, platelet aggregation was enhanced.

A18. Study 148-207: A double blind, placebo controlled, single dose study followed by a double blind, placebo controlled 10-day multiple dose study to investigate the pharmacokinetics, platelet effects, safety and toleration of UK-92,480 (sildenafil) in healthy male volunteers.

A18.1. Source documents

Study protocol NDA 20-895, vol 1.51; study report: NDA vol 1.51; electronic document: 47053693.pdf.

A18.2. Investigators

A18.3. Study dates

25 February 1992 to 22 May 1992.

A18.4. Study design

This study description was based upon the final study report, dated 23 July 1997.

A18.4.1. Objectives

The objectives were

- To assess the pharmacokinetics of sildenafil following single and multiple capsule doses.
- To assess the safety and toleration of multiple doses of sildenafil.
- To assess the effects of single and multiple doses of sildenafil on platelet aggregability

A18.4.2. Formulation

Drug supplies were 25-mg capsules, lot 979-12 and matching placebo capsules, lot 748-43.

A18.4.3. Population

A total of 36 health male volunteers, age 18 to 45, were to be recruited.

A18.4.4. Procedures

Subjects were equally randomized to placebo or sildenafil 25, 50, or 75 mg and received a single morning dose of study drug after overnight fast. At least 14 days later, they began thrice-daily dosing for 8 days and a single morning dose on day 9. Pharmacokinetic data were collected during the single-dose phase (0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 32, 48, 72, and 96 hours after dosing) and on days 0, 4, (0.5, 1, 1.5, 2, 3, 4, 6, and 8 hours after dosing) and 9 (sampling as in single-dose phase) of the multiple-dose phase.

Platelet aggregation in plasma was assessed by a dose-response relationship for the IC_{50} for sodium nitroprusside inhibition of aggregation by ADP.

A18.4.5. Assay

A18.4.6. Analysis

Pharmacokinetic parameters were calculated using standard non-compartmental techniques. The predicted accumulation was calculated as AUC_{SD} / $AUC_{0\text{-}8h,SD}$. The observed accumulation was calculated as $AUC_{0\text{-}8h}$ (day 4 or day 9) / $AUC_{0\text{-}8h,SD}$. A repeated measure analysis of variance model was fitted to each pharmacokinetic parameter in turn, allowing for the effects of dose, subject within dose, day, and dose

by day interaction. Each of the following effects were tested: dose against subject within dose, and day and dose by day interaction against the residual. Each comparison of the pharmacokinetic parameters of interest was made using a two-sample t-test with the estimate of variability taken from the ANOVA. For the comparisons of AUC_{0-8h}, AUC, and C_{max}, the mean difference and 90% confidence limits of the mean difference were shown on a log-scale together with the transformed mean difference ratio (ratio of geometric means expressed as a percentage) and back-transformed 90% confidence limits of the ratio.

A18.4.7. Safety

Routine safety data were recorded. In addition, visual impairment was assessed with tests for visual acuity, color perception, and pupil response to light.

A18.5. Results

A18.5.1. Conduct

Thirty-eight subjects were recruited and dosed (9 to 10 per treatment group).

A18.5.2. Pharmacokinetics

Mean plasma concentration time profiles for sildenafil for both the single and multiple dose periods for the 25-, 50-, and 75-mg doses are shown in Figure 37 below. The corresponding pharmacokinetic parameters are summarized in Table 104 below. Figure 38 below shows the relationship between AUC_{0-8h} and C_{max} for sildenafil and the sildenafil dose. Figure 39 below shows the mean plasma concentration profiles for metabolite UK-103,320 for the single and multiple dose periods at a dose of 75 mg.

The results of the study suggest some nonlinearity in C_{max} and AUC appearing by the 75-mg dose level. Thrice-daily administration of sildenafil produced slight accumulation at all of the studied doses. The accumulation ratios between day 9 and single dose administration, based on AUC_{0-8h}, for the 25-, 50-,

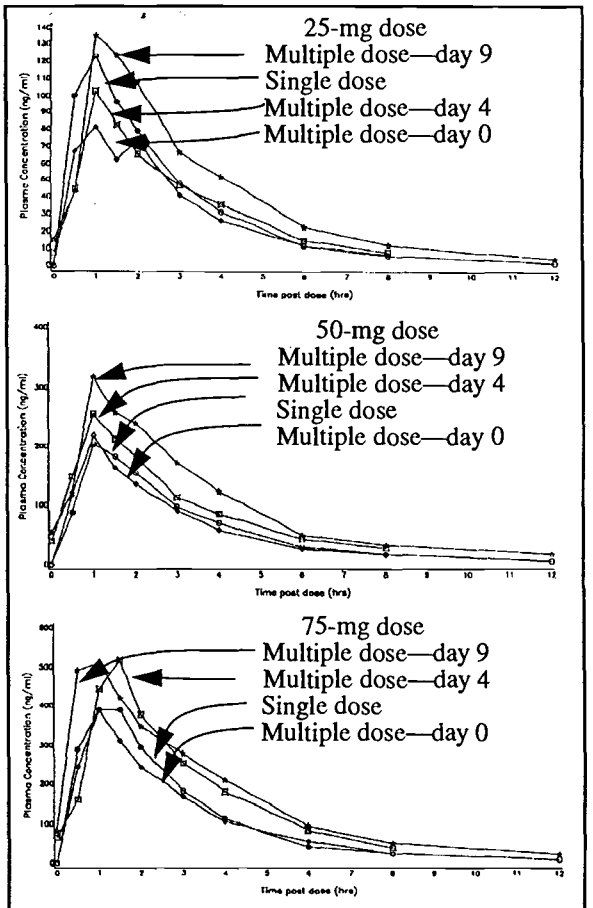


Figure 37. Plasma sildenafil levels after single and multiple dosing (Study 148-207).

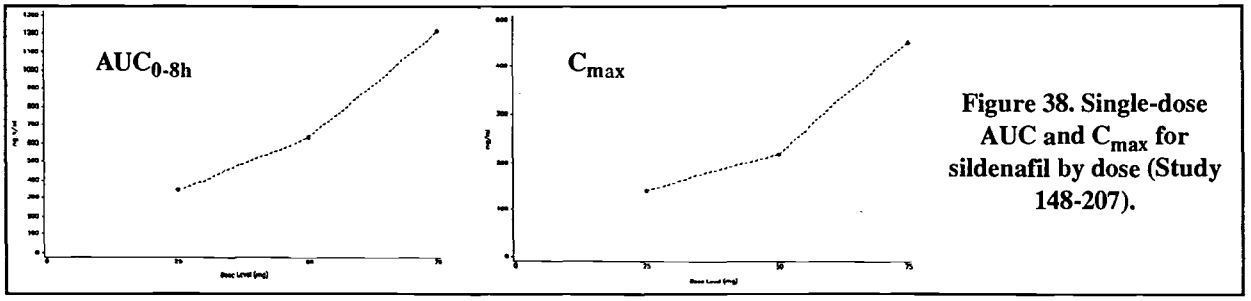


Figure 38. Single-dose AUC and C_{max} for sildenafil by dose (Study 148-207).

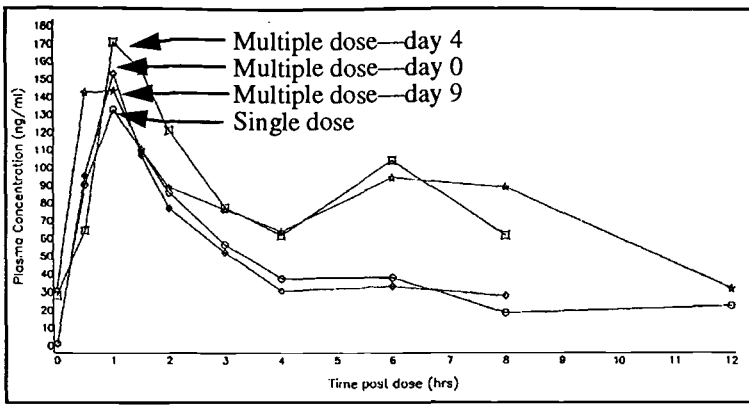


Figure 39. Plasma levels of UK-103,320 after single and multiple doses of sildenafil 75 mg (Study 148-207).

Table 104. Pharmacokinetic parameters after single and multiple dosing (Study 148-207).

	Day	Sildenafil dose				Day	Sildenafil dose		
		25 mg	50 mg	75 mg			25 mg	50 mg	75 mg
C_{max} (ng/mL)	SD	133	200	437	AUC_{0-8h} (ng.h/mL)	SD	320	583	1168
	MD0	99	211	421		MD0	254	575	1060
	MD4	104	229	519		MD4	292	701	1517
	MD9	141	310	494		MD9	403	929	1707
T_{max} (h)	SD	0.95	1.22	1.11	k_{el} (h^{-1})	SD	0.17	0.14	—
	MD0	1.05	0.94	1.06		MD0	—	—	—
	MD4	1.44	1.17	1.36		MD4	—	—	—
	MD9	1.28	1.39	1.25		MD9	0.16	0.15	—
AUC (ng.h/mL)	SD	346	662	1212	$t_{1/2}$ (h)	SD	4.0	4.8	—
	MD0	—				MD0	—	—	—
	MD4	—				MD4	—	—	—
	MD9	461	1107	1950		MD9	4.4	4.8	—

and 75-mg doses were 1.26, 1.59, and 1.32 respectively. The accumulation ratios, based on C_{max}, were 1.07, 1.55, and 1.09, respectively.

Plasma concentrations of the metabolite UK-103,320 were analyzed for the 75-mg dose. The results of this study show that its pharmacokinetics follow those of the parent drug, with about the same degree of accumulation with multiple dosing.

No attempt was made to compare plasma drug levels with pharmacodynamic results.

A18.5.3. Pharmacodynamics

ADP produces platelet aggregation. Sodium nitroprusside antagonizes aggregation caused by ADP. Measurements of the mean IC_{50} for sodium nitroprusside varied over 5-fold at various time points in the placebo group. Various measurements in the active treatment groups varied over essentially the same range, so that, although there appeared to be no treatment effect, there are quite wide confidence limits about this conclusion.

A18.5.4. Safety

No serious adverse events were reported. Apparently treatment-related adverse events included back pain, myalgia, headache, and penile erection. No visual disturbance adverse events were reported.

No subject showed a reduction in visual acuity. No statistically significant effect was found in pupillary response or color discrimination.

A18.6. Summary

Thrice-daily dosing from 25 to 75 mg resulted in a small degree of accumulation of sildenafil and metabolite UK-103,320. The AUC and C_{max} for sildenafil showed a slightly greater than linear dependence on dose. Sildenafil had no detectable effect on

platelet aggregation, although the trial could easily have missed a clinically significant effect. No effects of sildenafil were found on assessments of visual acuity, color discrimation, or pupillary response to light, at doses up to 75 mg/day.

A19. Study 148-208: An open randomised, two way crossover study to investigate the pharmacokinetics of UK-92480 after oral administration and IV administration in the fasted state.

A19.1. Source documents

Study protocol NDA 20-895, vol 1.53; study report: NDA vol 1.53; electronic document: 47154232.pdf.

A19.2. Investigators

A19.3. Study dates

7 September 1992 to 27 October 1992.

A19.4. Study design

This study description was based upon the final study report, dated 16 October 1996.

A19.4.1. Objectives

The objectives were

- To investigate the pharmacokinetics of sildenafil administered orally and intravenously and to determine its absolute oral bioavailability.
- To assess the safety and toleration of sildenafil 50 mg administered orally and intravenously.

A19.4.2. Formulation

Sildenafil 1 mg/ml injection solution was lot 975-30. Sildenafil 25 mg capsules were lot 979-12.

A19.4.3. Population

Twelve healthy male subjects between the ages 18 and 45 years participated in this study.

A19.4.4. Procedures

The study was an open, randomized, two-way crossover of two single doses of sildenafil 50 mg (oral and IV). On 2 study days separated by at least 10 days, subjects received, in random order, sildenafil 2x25 mg capsules or a 50 ml of 1 mg/ml solution infused at 1 ml/minute. After the IV dose of the drug, plasma samples were collected pre-dose and at 20, 30, 40, 50, 60, 75, and 90 minutes and at 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 18, 24, 32, 48, 72, 96 and 120 hours post dose. After the oral dose plasma samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 18, 24, 32, 48, 72, 96 and 120 hours post-dose.

A19.4.5. Assay

A19.4.6. Analysis

Pharmacokinetic parameters were calculated using standard non-compartmental techniques.

A19.4.7. Safety

Routine safety data were recorded.

A19.5. Results

A19.5.1. Pharmacokinetics

Mean plasma concetrations time profile for sildenafil for both routes of administration are shown in Figure 40 below with the corresponding parameters summarized in Table 105 below.

Table 105. Pharmacokinetic parameters for sildenafil after IV and oral administration (Study 148-208).

	IV	PO		IV	PO
AUC (ng.h/mL)	1291	530	$t_{1/2}$ (h)	3.9	4.1
AUC_τ (ng.h/mL)	1289	528	CL (L/h)	41	—
C_{max} (ng/mL)	531	160	V (L)	234	—
T_{max} (h)	0.7	1.5	V_{ss} (L)	105	—
K_{el} (h^{-1})	0.18	0.17			

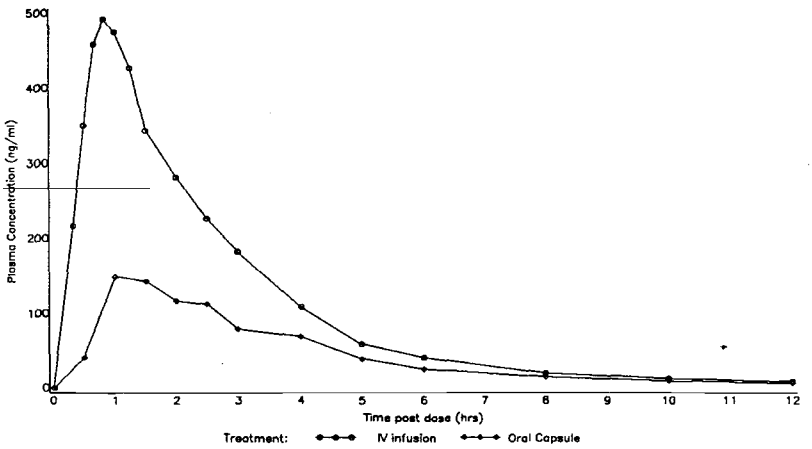


Figure 40. Mean plasma sildenafil after IV and oral dosing (Study 148-208).

The results of the study also seem to indicate that both V_{ss} and clearance are correlated to body weight as can be seen in Figure 41 below.

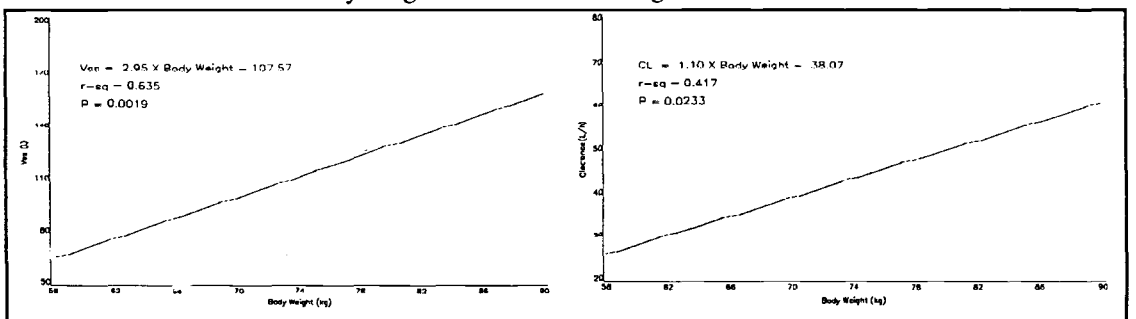


Figure 41. V_{ss} and clearance by body weight (Study 148-208).

Intravenous administration showed less inter-subject variability in K_{el} (10% CV) compared to the oral administration (21% CV).

A19.6. Summary

The results of the study showed that after a 50-mg dose, the absolute bioavailability of sildenafil was estimated to be 41%. The results are in good agreement with the ^{14}C study[1] in which the absolute bioavailability was estimated to be 38%.

[1] *Study 148-215: An open, parallel group study to investigate the absorption, metabolism and excretion of a single oral and a single intravenous dose of radiolabeled [14C]-UK-92,480.* on page 145.

A20. Study 148-209: A double blind, randomised, placebo controlled, two-way crossover study to examine the effects of 25mg tid UK-92,480, administered as capsules, on the haemodynamic responses to glyceryl trinitrate in normal volunteers.

A20.1. Source documents

Study protocol NDA 20-895, vol 1.54; study report: NDA vol 1.54; electronic document: 47053170.pdf.

A20.2. Investigators

Single-center study with 1 investigator in the UK.

A20.3. Study dates

8 July 1992 to 13 September 1992.

A20.4. Study design

This study description was based upon the final study report, dated 19 March 1997.

A total of 12 health male volunteers, age 18 to 45, were to be recruited.

In a crossover design, subjects received, in random order and separated by at least 10 days, placebo or sildenafil 25 mg tid for 4 days. On day 4, subjects received an intravenous infusion of glyceryl trinitrate stepped from 2.5 to 40 mg/min over 25 minutes while tilted at 70° (head up), and had vital signs monitored. The infusion was stopped when a subject's systolic pressure fell by 25 mmHg. This was repeated with a sublingual dose of 500 µg on day 5. Subjects spit out the tablet when they experienced symptomatic hypotension or systolic pressure fell 25 mmHg.

Blood samples were drawn for study drug levels once at the time of hemodynamic assessment.

Routine safety data were recorded.

A20.5. Results

A20.5.1. Conduct

Twelve subjects were randomized and completed both study phases. Four subjects did not have hemodynamic data returned for at least one session because of technical problems.

A20.5.2. Pharmacokinetics

At the time of hemodynamic assessment on days 4 and 5, mean plasma levels of sildenafil were about 160 ng/mL.

A20.5.3. Pharmacodynamics

Two of 12 subjects completed glyceryl trinitrate infusion on placebo, while none did so on sildenafil. The median time of infusion was 9 minutes on sildenafil vs. 13 minutes on placebo.

Similarly, with sublingual nitroglycerin, the median time was 4.5 minutes on sildenafil, but only 4 subjects on placebo stopped early. Blood pressure was reduced to a greater extent on sildenafil than on placebo (difference from baseline and placebo of about -15/-5 mmHg). Heart rate changes were not different on placebo and sildenafil.

A20.5.4. Safety

Adverse events other than hypotension with apparent relationship to study drug included headache, back pain, and myalgia. There were no serious or severe adverse events or laboratory abnormalities.

A20.6. Summary

Sildenafil augmented the hypotensive effect of glyceryl trinitrate, but had no discerible effect on blood pressure alone.

A21. Study 148-214: An open, parallel group study to determine the effects of impaired renal function on the pharmacokinetics, safety and toleration of sildenafil administered as a single 50 mg capsule dose.

A21.1. Source documents

Study protocol NDA 20-895, vol 1.59; study report: NDA vol 1.59; electronic document: 47053558.pdf.

A21.2. Investigators

A21.3. Study dates

7 November 1994 to 25 February 1995.

A21.4. Study design

This study description was based upon the final study report, dated 12 May 1997.

A21.4.1. Objectives

The objectives were

- To assess the plasma pharmacokinetics of sildenafil and the metabolite UK-103,320 after oral administration of a 50-mg capsule in the fasted state to subjects with varying degrees of renal function.
- To assess the safety and toleration of a single 50-mg dose of sildenafil in subjects with varying degrees of renal function.

A21.4.2. Formulation

Sildenafil 50-mg capsules were from lot 979-12.

A21.4.3. Population

A total of 24 male subjects between 18 and 70 years inclusive were to recruited into 4 treatment groups on the basis of baseline renal function: (a) healthy subjects with normal renal function (Cl_{cr} >80 mL/min), (b) subjects with mild renal impairment (Cl_{cr} between 50 and 80 mL/min), (c) subjects with moderate renal impairment (Cl_{cr} between 30 and 49 mL/min), and (d) subjects with severe renal impairment (Cl_{cr} <30 mL/min).

A21.4.4. Procedures

Subjects attended the clinical unit on the evening before dosing. On the morning of dosing (day 1), blood samples were collected immediately prior to dosing and at 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 18, 24, 36, and 48 hours post-dosing. An additional blood sample was collected immediately before dosing for protein binding determination and urine was collected from 0 to 24 hours post-dosing for the determination of creatinine clearance.

A21.4.5. Assay

A21.4.6. Analysis

Pharmacokinetic parameters were calculated using standard non-compartmental techniques. Oral clearance for sildenafil was analyzed using regression techniques to investigate its relationship with creatinine clearance and age. In the first analysis, creatinine clearance was fitted first to examine whether it alone accounted for a significant amount of variability in Cl/f. Age was then fitted to determine whether it significantly explained significantly more of the variability. In the second analysis, age was fitted first followed by creatinine clearance. The choice of final model was based on the significance levels found in the previous two analyses. The same types of analyses were also done for AUC and C_{max}.

A21.4.7. Safety

Routine safety data were recorded.

A21.5. Results

A21.5.1. Pharmacokinetics

Mean plasma concentration vs. time profiles for sildenafil and its metabolite for the 4 groups of subjects with varying degrees of renal impairment are shown in Figure 42 below while the corresponding parameters are shown in Table 106 below. Figure 43 below shows the relationship between oral clearance of sildenafil and creatinine

clearance and between C_{max} for sildenafil and creatinine clearance. Figure 44 below shows the relationships between the oral clearance and age and between C_{max} and age.

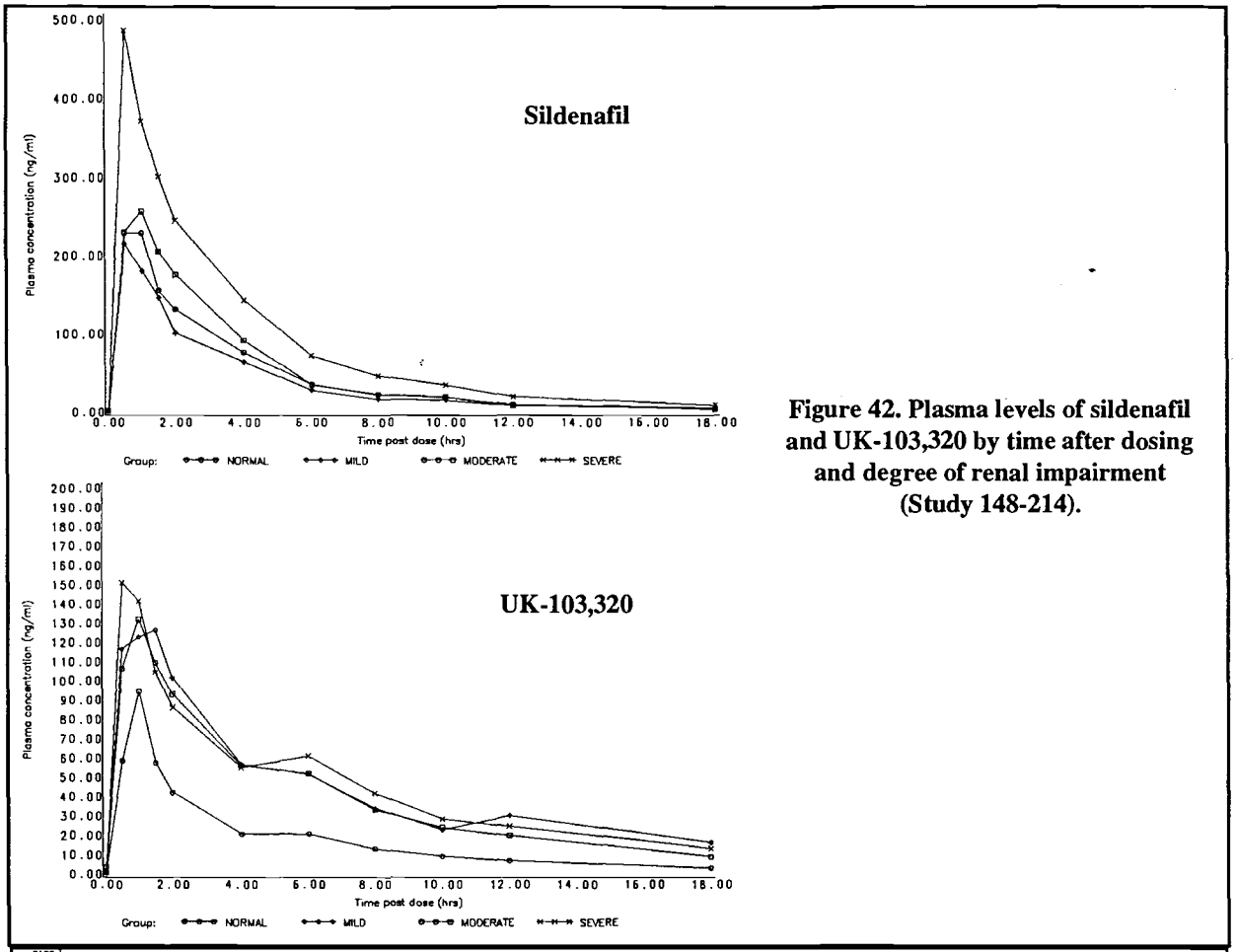


Figure 42. Plasma levels of sildenafil and UK-103,320 by time after dosing and degree of renal impairment (Study 148-214).

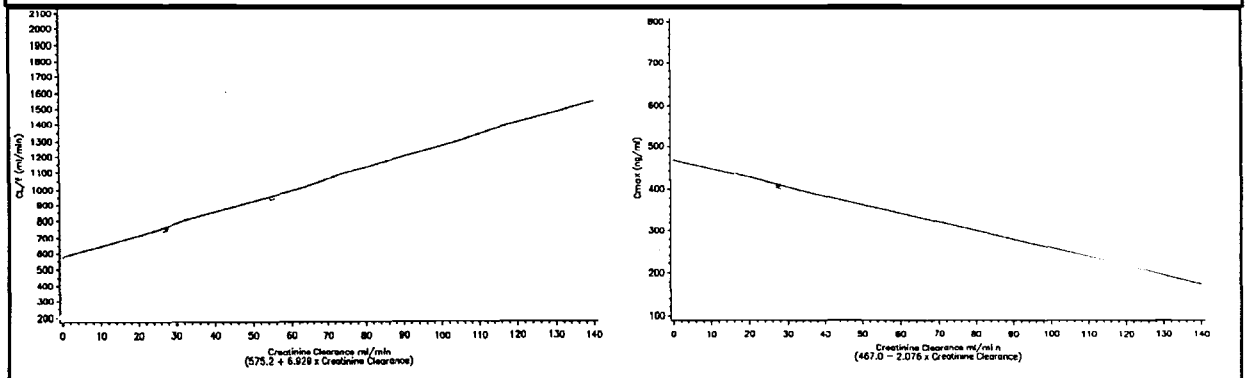


Figure 43. Clearance and C_{max} for sildenafil as a function of creatinine clearance (Study 148-214).

C_{max} in the severely impaired subjects was almost twice that in the normal group; the ratio of the geometric means was 1.88 with a 95% confidence interval of 1.24 to 2.87. The same trend was observed with AUC; severely impaired subjects had AUCs about twice as high as the group with normal renal function. Regression analysis of the C_{max} data revealed that creatinine clearance explained a significant amount (26%) of the variability (p=0.014) while age alone did not (p=0.191). Inclusion of age as an additional parameter in the regression model did not account for a significant amount of the variability above what was explained by creatinine clearance. Thus the model that best fits the C_{max} data is $C_{max} = 467 - 2.08 \times Cl_{cr}$.

Table 106. Pharmacokinetic parameters (Study 148-214).

	CL_{cr} (mL/min)							
	Sildenafil				UK-103,320			
	>80 N=8	50-80 N=5	30-49 N=4	<30 N=7	>80 N=8	50-80 N=5	30-49 N=4	<30 N=7
Clearance/f (mL/min)	1102	1220	945	549	—	—	—	—
AUC (ng.h/mL)	756	683	882	1519	302	684	525	907
C_{max} (ng/mL)	246	256	288	464	87	151	103	156
T_{max} (h)	0.8	0.8	1.0	0.5	1.0	0.9	1.1	0.8
k_{el} (h^{-1})	0.20	0.16	0.23	0.18	0.13	0.09	0.12	0.09
$t_{1/2}$ (h)	3.4	4.2	3.0	3.9	5.4	7.7	5.9	7.7
PPB (% free)	2.7	2.4	2.0	2.2	3.4	2.9	2.5	2.6

Regression analyses of the oral clearance data revealed that creatinine clearance explained a significant amount of the variability (32%; p=0.05) while age alone did not (p=0.115). Thus, the best model to describe the relationship between oral clearance and creatinine clearance is $Cl/f = 575 + 6.93 \times Cl_{cr}$.

Analysis of the protein binding data revealed no significant difference between the groups in free fraction with mean values of 2.7, 2.4, 2 and 2.2% for the normal, mild, moderate, and severe groups respectively.

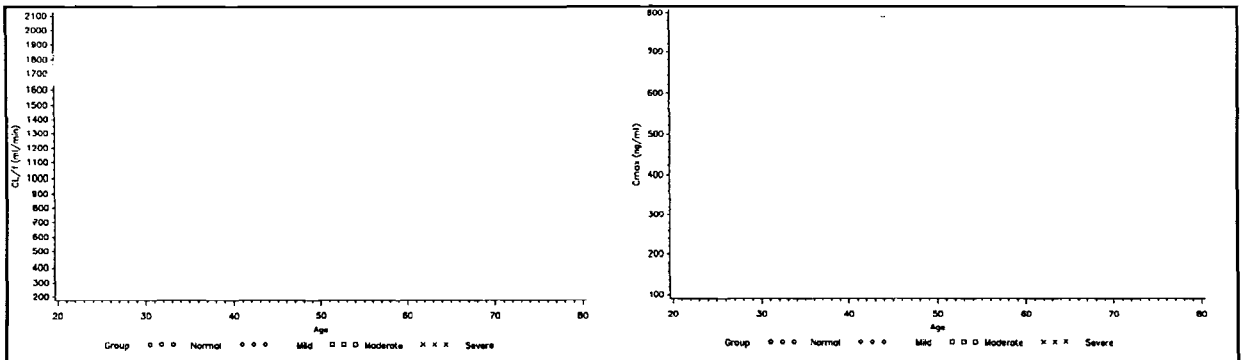


Figure 44. Clearance and C_{max} for sildenafil as a function of age (Study 148-214).

A21.5.2. Safety

There were no treatment-emergent adverse events reported.

A21.6. Summary

The results of the study showed that in patients the plasma levels of sildenafil and its metabolite UK-103,320 were almost doubled in subjects with severe renal impairment (creatinine clearance <30 mL/min) compared to subjects with normal renal function. This doubling of plasma concentrations might warrant the starting of certain subjects on lower dose of sildenafil—25 mg instead of 50 mg.

A22. Study 148-215: An open, parallel group study to investigate the absorption, metabolism and excretion of a single oral and a single intravenous dose of radiolabeled [^{14}C]-UK-92,480.

A22.1. Source documents

Study protocol NDA 20-895, vol 1.60; study report: NDA vol 1.60; electronic document: 47081663.pdf.

A22.2. Investigators

A22.3. Study dates

8 April 1995 to 8 May 1995.

A22.4. Study design

This study description was based upon the final study report, dated 6 August 1997.

A22.4.1. Objectives

The objectives were

- To measure the cumulative amount of drug related, radiolabeled material excreted in the urine and faces following a single dose of either oral (50mg) or IV (25 mg) [^{14}C]-sildenafil (nominally 50 μCi each).
- To characterize urinary and fecal radioactivity as unchanged sildenafil or its metabolites and, where possible, identify metabolites.
- To quantify blood and plasma total radioactivity and unchanged drug concentrations and, where possible, the major circulating metabolites following both oral and IV administration.

A22.4.2. Formulation

Sildenafil was to be supplied as powder for oral solution with sachets of sterile water (lot #3043-108) for reconstitution and as a 1 mg/ml solution for IV infusion (lot #3043-107). Each dose was to contain 50 μCi of radioactivity.

A22.4.3. Population

Six healthy male subjects between 45 and 60 years inclusive participated in this study.

A22.4.4. Procedures

The trial was an open, parallel group study of single oral and IV doses of ^{14}C-sildenafil. The oral dose was 50 mg and the IV dose was 25 mg, chosen to provide similar plasma concentrations. Three subjects received the oral solution and three subjects received the IV infusion. Subjects were to take 100 ml of oral solution. The bottle containing the oral solution was to be rinsed with 140 ml of potable water which was to be taken also. The 1-mg/ml IV infusion was to be given at 1 ml/min over 25 minutes.

After oral dosing, blood samples were collected at the following times: 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48 and 72 hours post-dose. After IV infusion, samples were taken at 5, 10, and 25 minutes and 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, and 72 hours after the start of the infusion. Subsequent samples were to be taken at 24-hour intervals until the subject left the unit.

A urine sample was collected prior to dosing. After dosing all urine samples were collected for approximately 5 days post dose. The collection periods were as follows: 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-72, and 72-96 hours post-dose and subsequently at 24-hour intervals until less than 3 times baseline radioactivity level was reached.

All feces were collected into pre-weighed plastic containers in the 24 hours prior to dosing and at 24-hour intervals after dosing until approximately 5 days after dosing or until less than 3 times baseline radioactivity level was reached.

A22.4.5. Assay

A22.4.6. Analysis

Pharmacokinetic parameters were calculated using standard non-compartmental techniques. Oral absorption was estimated from the recovery ratios using the following formula:

$$\frac{\text{mean(urinary recovery/urinary+fecal recovery)}_{oral}}{\text{mean(urinary recovery/urinary+fecal recovery)}_{IV}}$$

A22.4.7. Safety

Routine safety data were recorded.

A22.5. Results

A22.5.1. Pharmacokinetics

Total radioactivity-time profiles in plasma and whole blood are shown in Figure 45 below for both routes of administration while the corresponding parameters are shown in Table 107 below.

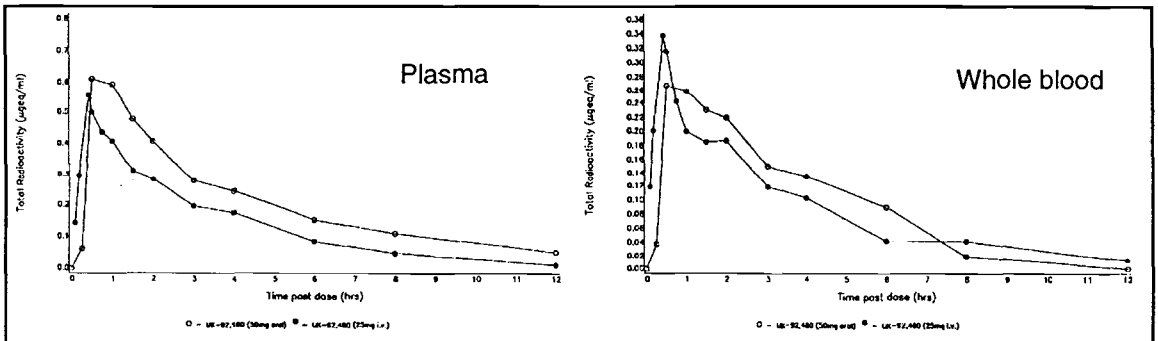


Figure 45. Plasma and whole-blood levels of total radioactivity after IV and oral dosing (Study 148-

Table 107. Pharmacokinetic parameters for total radioactivity after IV and oral dosing (Study 148-215).

	Sildenafil 25 mg IV		Sildenafil 50 mg oral	
	Plasma	Blood	Plasma	Blood
C_{max} (ng-eq/mL)	560	342	610	288
T_{max} (h)	0.53	0.45	1.1	0.97
AUC_{τ} (ng-eq.h/mL)	1603	1012	2258	960

Values are means and ranges. The sponsor notes that estimates of C_{max} and T_{max} will have been affected by mis-sampling at early time points in orally-dosed subjects.

After IV administration the ratio of total radioactivity in plasma to whole blood based on geometric mean C_{max} and AUC was 1.64 and 1.58. The ratios after oral administration were 2.12 and 2.35, respectively. The difference between IV and oral administration was attributed to the presence of higher levels of metabolites which are more polar and have less tendency to partition into the red cells.

Mean plasma concentration profiles for sildenafil after IV and oral administration are shown in Figure 46 below with the corresponding pharmacokinetic parameters summarized in Table 108 below. The absolute bioavailability of sildenafil based on the ratio of AUC after oral and IV administration was calculated to be 38%.

Figure 47 below show the plasma concentration profiles for both UK-103,320 and UK-150,564, two of the main sildenafil metabolites, with the corresponding parameters summarized in Table 109 below.

The total amount of radioactivity recovered after IV infusion was 88.5% with 75.5% of radioactivity in the feces and 13.1% in the urine. Similar recoveries were observed after oral administration (91.3% of the dose with 79% in feces and 12.3% in urine).

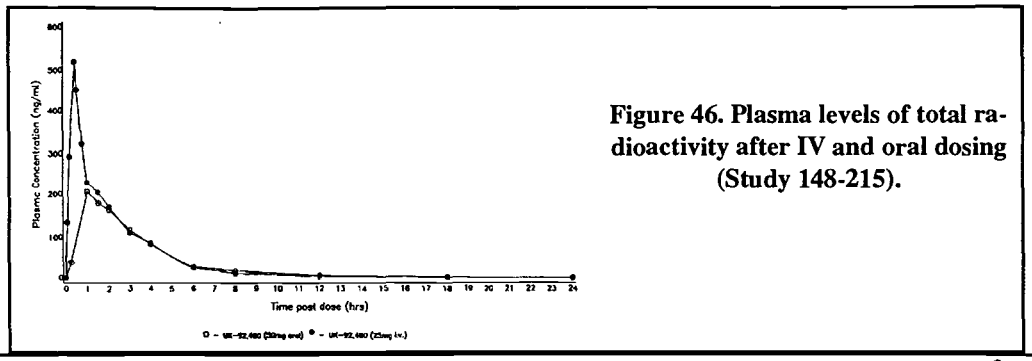


Figure 46. Plasma levels of total radioactivity after IV and oral dosing (Study 148-215).

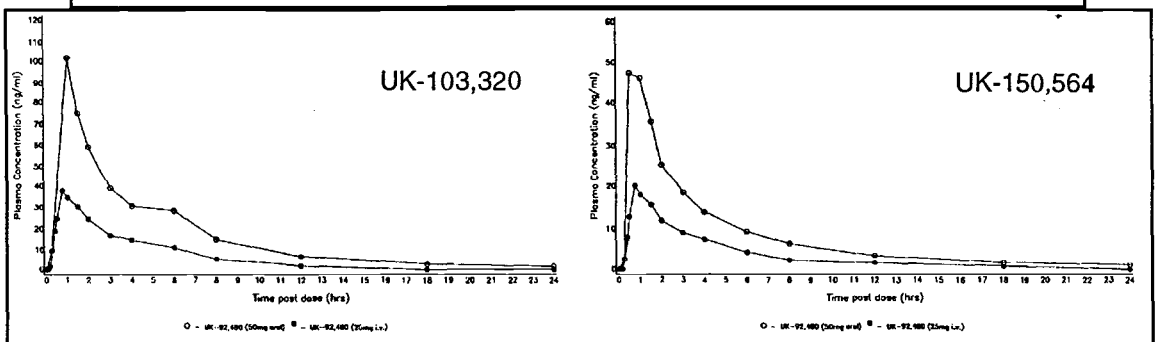


Figure 47. Plasma levels of sildenafil metabolites after IV and oral dosing (Study 148-215).

Table 108. Pharmacokinetic parameters for plasma sildenafil after IV and oral dosing (Study 148-215).

	25 mg IV	50 mg oral		25 mg IV	50 mg oral
C_{max} (ng/mL)	518	207	$t_{1/2}$ (h)	2.2	3.2
T_{max} (h)	0.42	1.17	CL_p (L/h)	26	—
AUC_τ (ng.h/mL)	964	721	V_d (L)	88	—
AUC (ng.h/mL)	971	729	V_{ss} (L)	57	—
k_{el} (h^{-1})	0.32	0.22			

Values are means and ranges. The sponsor notes that estimates of C_{max} and T_{max} will have been affected by mis-sampling at early time points in orally-dosed subjects.

Table 109. Pharmacokinetic parameters for sildenafil metabolites after IV and oral dosing (Study 148-215).

	UK-103,320		UK-150,564	
	25 mg IV	50 mg oral	25 mg IV	50 mg oral
C_{max} (ng-eq/mL)	38	101	19	49
T_{max} (h)	0.83	1.0	0.83	0.98
AUC_τ (ng-eq.h/mL)	139	389	76	179
AUC (ng.h/mL)	147	400	86	197
k_{el} (h^{-1})	0.30	0.13	0.13	0.10
$t_{1/2}$ (h)	2.3	5.5	5.4	7.0

Values are means and ranges. The sponsor notes that estimates of C_{max} and T_{max} will have been affected by mis-sampling at early time points in orally-dosed subjects.

Oral absorption as estimated from the above formula was 92%. Therefore, the low absolute bioavailability (38%) was due to first-pass metabolism and not due to incomplete absorption.

Sixty-eight percent of the plasma radioactivity after IV administration was accounted for by sildenafil while the parent drug only accounted for 47% of the plasma radioactivity after oral dosing.

Figure 48 below summarizes the metabolic pathways for sildenafil with the percentage of each metabolite in the urine and feces. There was no unchanged drug recovered in either urine or feces, indicating that the major clearance mechanism for sildenafil is metabolism. The major urinary metabolite was the aliphatic hydroxylated metabolite accounting for 41% of the urinary radioactivity (5.2% of the total dose). Urine contained a further 8 metabolites, accounting each for less than 1.5% of the dose. The major metabolite in feces was UK-150,564 and it accounted for 28% of radioactivity in the feces (22% of the dose). A further 16 metabolites were identified in feces, accounting each for less than 5% of the dose.

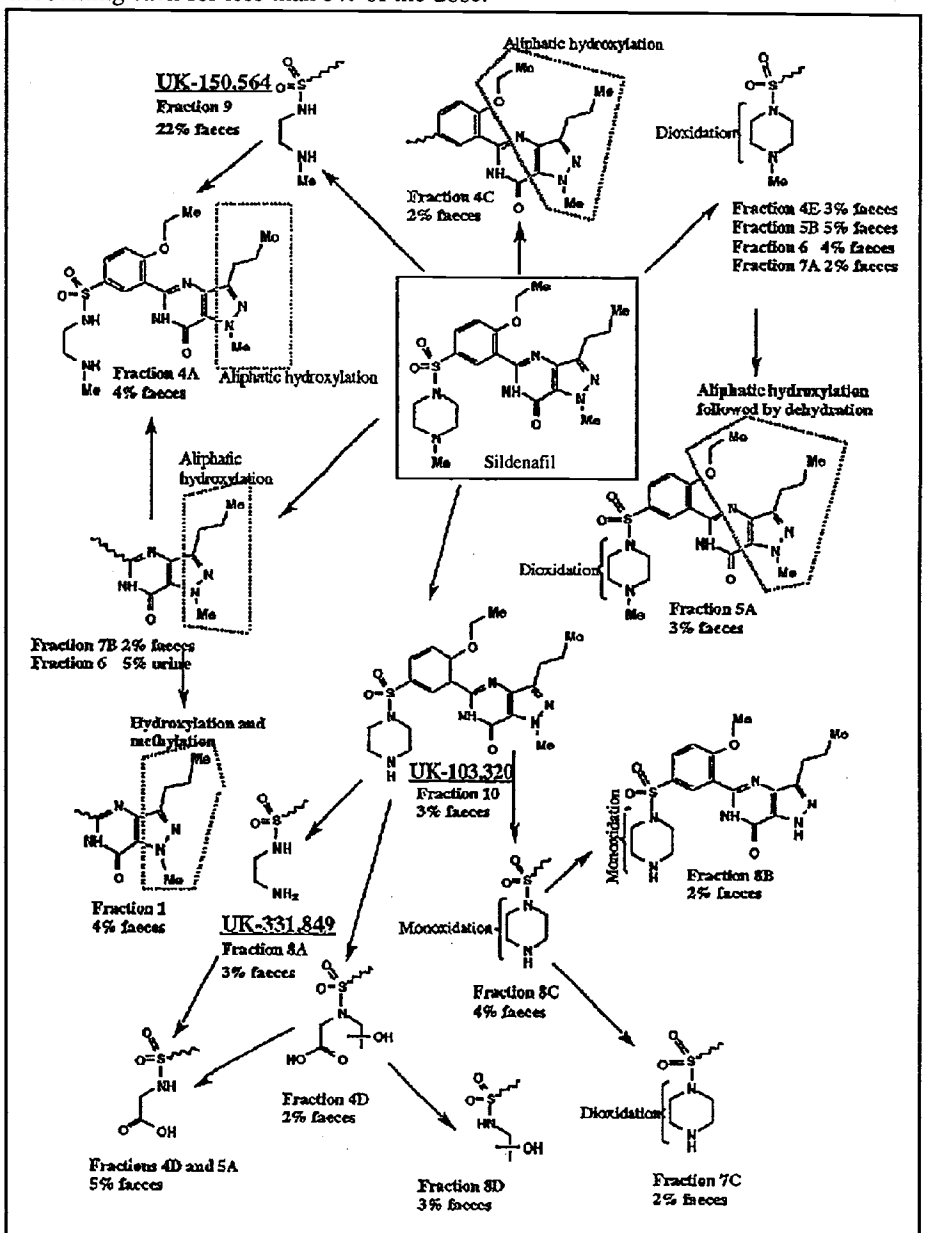


Figure 48. Proposed routes of metabolism for sildenafil (Study 148-215).

A22.6. Summary

The principle routes of metabolism of sildenafil were N-demethylation at the N-methyl piperazine and N-methyl pyrazole moieties, multiple oxidation and loss of a 2-carbon fragment from the piperazine ring, and aliphatic hydroxylation.

Center for Drug Evaluation and Research

Viagra (Sildenafil)

"Joint Clinical Review" for NDA-20-895

Appendix A23, page 150 through Appendix A33.6, page 169

A23. Study 148-216: An open study to investigate the effects of a single dose of UK-92,480 (50mg) on bleeding time, followed by a double-blind, placebo-controlled, two-way crossover study to investigate the effects of a single dose of UK-92,480 (50mg) on aspirin-induced prolongation of bleeding time in healthy male volunteers.

A23.1. Source documents

Study protocol NDA 20-895, vol 1.61; study report: NDA vol 1.61; electronic document: 47153405.pdf.

A23.2. Investigators

Single-center study with 1 investigator in the UK.

A23.3. Study dates

17 October 1994 to 20 December 1994.

A23.4. Study design

This study description was based upon the final study report, dated 20 September 1996.

A total of 18 health male volunteers, age 18 to 45, were to be recruited.

There were two study phases. The first phase was an open study in which subjects received a single dose of sildenafil 50 mg. The second phase was a double-blind crossover study in which subjects received aspirin 150 mg qd for 7 days and, in random order, placebo and sildenafil on days 4 and 7. Bleeding time was assessed by the 'simplate technique' prior to dosing with study drug and then at 1 and 4 hours after dosing.

Routine safety data were recorded.

A23.5. Results

A23.5.1. Conduct

Eighteen subjects were randomized and completed both study phases. There were minor protocol deviations, but no subject was excluded from analyses.

A23.5.2. Pharmacodynamics

Bleeding time data are shown in Table 110 below, as analyzed by the sponsor. The 4-hour data were not analyzed. The 95% confidence limits on the ratio of bleeding time on aspirin plus sildenafil to bleeding time on aspirin plus placebo were 89 to 128%.

Table 110. Bleeding time (Study 148-216).

	Baseline	1 hour
Sildenafil alone (min±SD)	8.2±3.9	7.2±2.9
Aspirin + placebo	11.0±3.9	12.9±5.6
Aspirin + sildenafil	10.7±4.1	14.7±8.8

Although mean effects were not statistically significant, 3 subjects had bleeding times doubled one hour after aspirin + sildenafil compared with aspirin + placebo.

A23.5.3. Safety

There were no serious or treatment-related severe adverse reactions.

A23.6. Summary

Despite the lack of a statistically significant treatment effect, sildenafil probably does produce prolonged bleeding times in some individuals receiving aspirin.

A24. Study 148-217: A double blind, randomised, placebo controlled, three way crossover study to investigate the haemodynamic and pharmacokinetic interactions of sildenafil and alcohol in healthy male volunteers.

A24.1. Source documents

Study protocol NDA 20-895, vol 1.62; study report: NDA vol 1.62; electronic document: 47151059.pdf.

A24.2. Investigators

Dr. MD Eve, Pfizer Clinical Research Unit, Kent and Canterbury Hospital, Ethelbert Road, Canterbury CT1 3NG England.

A24.3. Study dates

2 November 1994 to 21 December 1994.

A24.4. Study design

This study description was based upon the final study report, dated 22 August 1997.

A24.4.1. Objectives

The objectives were

- To study the hemodynamic effects of sildenafil when taken with alcohol.
- To characterize any pharmacokinetic interaction between sildenafil and alcohol.
- To examine the safety and toleration of a single dose of sildenafil when taken acutely with alcohol.

A24.4.2. Formulation

Sildenafil was to be supplied 25-mg capsules (lot #979-12). Matching placebo capsules were from lot #748-43. Absolute ethanol was from lot #L-435402. Placebo alcohol was orange juice and two drops of absolute ethanol.

A24.4.3. Population

Twelve healthy male subjects between 18 and 45 years were to be recruited.

A24.4.4. Procedures

The study was a double blind, randomised, placebo controlled, 3-way crossover study in which subjects received, in random order, sildenafil 50 mg alone, 0.5 g/kg of alcohol alone, and sildenafil plus alcohol together. There was a minimum of a 7 day washout period between treatments. The alcohol was diluted to 200 ml with orange juice. The contents were drunk in 2 minutes or less. For sildenafil and metabolite measurement, plasma samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose. For alcohol determination, plasma samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hours post-dose.

A24.4.5. Assay

A24.4.6. Analysis

Pharmacokinetic parameters were calculated using standard non-compartmental techniques. Pair-wise comparisons were made between the sildenafil plus alcohol group and the sildenafil group using a t-test, where the estimate of variability was obtained from ANOVA. Ninety-five percent confidence intervals were also constructed for the parameters of interest.

A24.4.7. Safety

Routine safety data were recorded.

A24.5. Results

A24.5.1. Pharmacokinetics

Mean plasma concentration time profiles for sildenafil and its metabolite with and without alcohol are shown in Figure 49 below, while the corresponding parameters are

summarized in Table 111 below, along with 95% confidence limits for the comparison of sildenafil alone to sildenafil plus ethanol. Figure 50 below shows the mean ethanol plasma concentration vs. time profile with and without sildenafil.

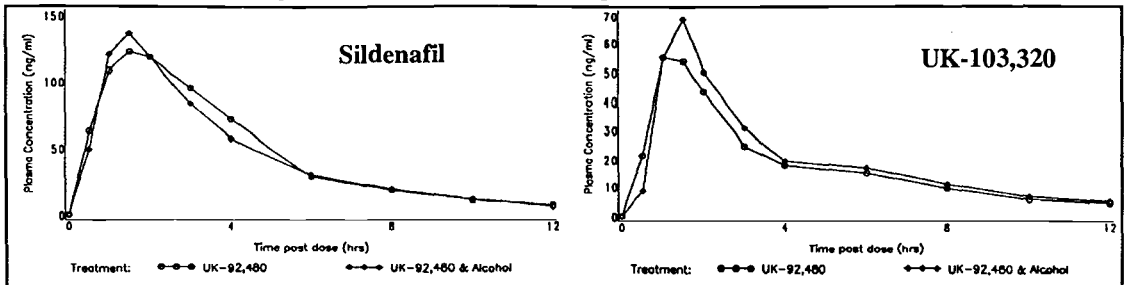


Figure 49. Plasma levels of sildenafil and UK-103,320 after sildenafil ± ethanol (Study 148-217).

Table 111. Pharmacokinetic parameters for sildenafil, UK-103,320, and ethanol (Study 148-217).

	Sildenafil			UK-103,320			Ethanol[a]		
	Sil only	Sil+EtOH	Ratio or Δ[b] (95% CI)	Sil only	Sil+EtOH	Ratio or Δ (95% CI)	Sil only	Sil+EtOH	Ratio or Δ (95% CI)
AUC (ng.h/mL)	552	542	0.77 - 1.26	224	542	0.96 - 1.34	195	542	0.98 - 1.30
C_{max} (ng/mL)	145	157	0.75 - 1.57	58	157	0.96 - 1.71	80	157	0.97 - 1.35
T_{max} (h)	1.6	1.4	-0.68 - 0.35	1.5	1.4	-0.53 - 0.37	0.7	1.4	-0.40 - 0.06
k_{el} (h^{-1})	0.23	0.23	-0.03 - 0.04	0.18	0.23	-0.05 - 0.05	—	0.23	—
$t_{1/2}$ (h)	3.0	3.0	—	3.9	3.0	—	—	3.0	—

a. For ethanol, AUC is in mg.h/dL and C_{max} is mg/dL.
b. (Sildenafil+EtOH)/(Sildenafil) for AUC and C_{max}; (Sildenafil+EtOH)-(Sildenafil) for T_{max} and k_{el}.

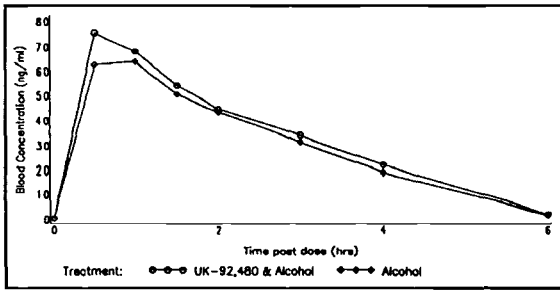


Figure 50. Plasma levels of ethanol after sildenafil ± ethanol (Study 148-217).

The results of the study show that there in general there was a slight increase in the metabolite concentrations with the co-administration of ethanol. AUC increased by 13% and C_{max} by 28%. However, the increase in these pharmacokinetic parameters did not achieve statistical significance. A statistically insignificant increase in the ethanol plasma concentrations was observed with the co-administration of sildenafil; C_{max} and AUC increased by 13 and 15%, respectively.

A24.5.2. Safety

There were no serious or severe adverse events. Common events—headache, penile erection, and vasodilation—had a similar incidence on sildenafil alone and sildenafil plus ethanol.

A24.6. Summary

The results of the study showed that co-administration sildenafil with ethanol did not result in any clinically significant alterations of the pharmacokinetics of either sildenafil or ethanol.

A25. Study 148-218: A double blind, randomised, placebo controlled, two-way crossover study to investigate any pharmacokinetic or pharmacodynamic interaction between orally administered UK-92,480 and tolbutamide in healthy male volunteers.

A25.1. Source documents

Study protocol NDA 20-895, vol 1.63; study report: NDA vol 1.63; electronic document: 47150192.pdf.

A25.2. Investigators

Dr M D Eve, Pfizer Clinical Research Unit, Kent and Canterbury Hospital, Ethelbert Road, Canterbury, Kent, United Kingdom.

A25.3. Study dates

18 January 1995 to 6 April 1995.

A25.4. Study design

This study description was based upon the final study report, dated 2 December 1996.

A25.4.1. Objectives

The objectives were

- To characterize any pharmacokinetic interaction between oral doses of sildenafil and tolbutamide.
- To characterize any pharmacodynamic interaction between oral doses of sildenafil and tolbutamide by measuring plasma glucose concentrations.
- To assess the safety and toleration of a single dose of sildenafil when taken concomitantly with tolbutamide.

A25.4.2. Formulation

Drug supplies are shown in Table 112 below.

Table 112. Drug supplies (Study 148-218).

	Lot		Lot
Tolbutamide 500 mg	3818-033A 3818-033B	Placebo for sildenafil	748-43
		Sildenafil 25 mg capsules	979-12

A25.4.3. Population

A total of 12 health male volunteers, age 18 to 45, were to be recruited.

A25.4.4. Procedures

In random order and separated by 7 days, subjects received single oral doses of tolbutamide 250 mg accompanied by placebo or sildenafil 50 mg.

Blood glucose levels were to be assessed pre-dose and 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hours after dosing.

Blood samples for determination of plasma levels of tolbutamide were obtained pre-dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, 24, 30, and 36 hours post-dose. Samples were collected in heparinized tubes, plasma was separated by centrifugation, and samples were stored at -20°C.

Urine was collected for assay of hydroxytolbutamide and carboxytolbutamide in intervals 0-12 and 12-24 hours after dosing.

A25.4.5. Assay

A25.4.6. Analysis

C_{max}, AUC_{τ}, k_{el}, AUC, T_{max}, and $t_{1/2}$ were calculated. In addition, the ratio of urinary metabolites was assessed.

A25.4.7. Safety

Routine safety data were recorded.

A25.5. Results

A25.5.1. Conduct

Twelve subjects were randomized and treated. Eleven of 12 subjects broke fast for treatment of hypoglycemia, so blood glucose data were not analyzed. Other protocol violations appear to have been minor.

A25.5.2. Pharmacokinetics

Plasma levels of sildenafil and UK-103,320 are shown in Figure 51 below. Pharmacokinetic parameters are shown in Table 113 below.

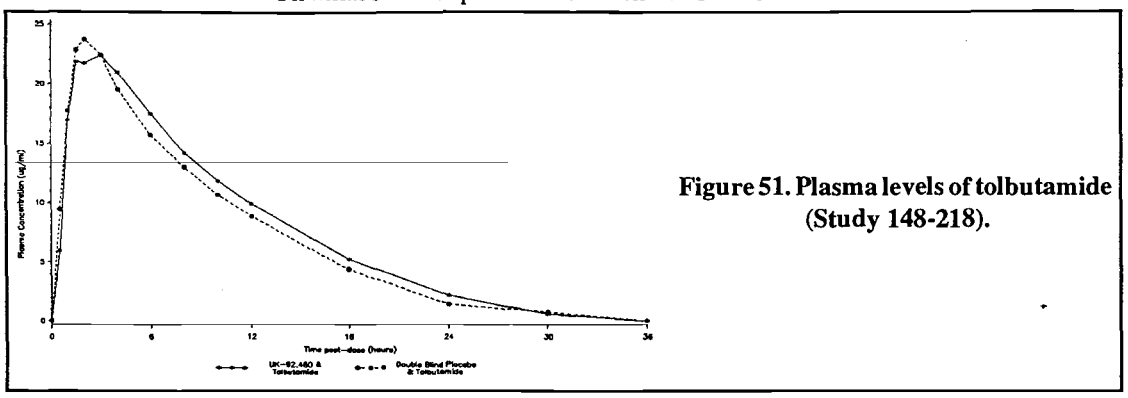


Figure 51. Plasma levels of tolbutamide (Study 148-218).

Table 113. Pharmacokinetic parameters for tolbutamide (Study 148-218).

	Placebo	Sildenafil		Placebo	Sildenafil
AUC_t (µg.h/mL)	231±65	247±71	T_{max} (h)	1.8±0.7	2.2±1.0
AUC (µg.h/mL)	266±69	285±74	K_{el} (h^{-1})	0.11±0.02	0.10±0.02
C_{max} (µg/mL)	24.7±2.7	24.8±4.0	$t_{1/2}$ (h)	6.6	6.8

Excretion of metabolites of tolbutamide is characterized in Table 114 below.

Table 114. Urinary excretion of tolbutamide metabolites (Study 148-218).

	Carboxytolbutamide		Hydroxytolbutamide		Carboxy:hydroxy	
	Placebo	Sildenafil	Placebo	Sildenafil	Placebo	Sildenafil
0-12 hours	105±23	115±35	20±6.3	22±6.6	5.8±3.1	5.4±2.3
12-24 hours	43±5.5	44±12	7.0±1.7	7.2±2.2	6.7±3.0	6.1±0.9

A25.5.3. Safety

Other than hypoglycemia, the only common adverse event was headache. Two subjects reported erections. One subject reported visual disturbances.

A25.6. Summary

Sildenafil inhibits CYP2C9 activity in vitro. CYP2C9 is involved in metabolism of tolbutamide, an oral hypoglycemic agent, so a potentially important drug interaction was possible. However, plasma levels of tolbutamide and urinary excretion of its metabolites were unaffected by single oral doses of sildenafil 50 mg. The study appears to have been powered adequately to detect a 30% change in AUC or C_{max}, but single doses of sildenafil below the maximum recommended dose was not the optimum study design.

A26. Study 148-219: A double-blind, randomised, placebo-controlled, two-way crossover study to assess the potential interaction between orally administered UK-92,480 (sildenafil) and warfarin in healthy male volunteers.

A26.1. Source documents Study protocol NDA 20-895, vol 1.64; study report: NDA vol 1.64; electronic document: 47152646.pdf.

A26.2. Investigators Dr D Kleinermans, Hôpital Erasme, Pfizer Clinical Research Unit, 808 Route de Lennik, 1070 Bruxelles, Belgium.

A26.3. Study dates 26 January to 3 March 1995.

A26.4. Study design This study description was based upon the final study report, dated 10 December 1996.

A26.4.1. Objectives The objective was to examine the safety, toleration and hemostatic effects (bleeding time and prothrombin time) of oral sildenafil taken concomitantly with warfarin.

A26.4.2. Formulation Drug supplies are shown in Table 115 below.

Table 115. Drug supplies (Study 148-219).

	Lot		Lot
Warfarin 40 mg	—	Placebo for sildenafil	748-43
		Sildenafil 25 mg capsules	3039-134

A26.4.3. Population A total of 12 health male volunteers, age 18 to 45, were to be recruited.

A26.4.4. Procedures In random order and separated by 4 days, subjects received oral doses of placebo or sildenafil 50 mg in the evenings for 6 days. Subjects took an additional dose of placebo or sildenafil on the morning of day 2 accompanied by a single oral dose of warfarin 40 mg.

Blood samples for prothrombin time were obtained prior to the first dose of placebo or sildenafil, prior to administration of warfarin, and then at 4, 8, 12, 24, 36, 48, 60, 72, 96, 120, and 144 hours post-dosing. Bleeding time was assessed prior to the first dose of placebo or sildenafil and then 38 hours after administration of warfarin.

A26.4.5. Assay Prothrombin time was assessed by standard methods.

A26.4.6. Safety Routine safety data were recorded.

A26.5. Results

A26.5.1. Conduct Twelve subjects were randomized and treated. Protocol violations appear to have been minor.

A26.5.2. Pharmacodynamics Individual prothrombin time profiles are shown in Figure 52 below. Mean bleeding times and prothrombin times are shown in Table 116 below. The sponsor's analysis of AUEC[1] for prothrombin time showed a statistically significant difference between treatment groups.

[1] Area under the effect curve.

Table 116. Bleeding time and prothrombin time (Study 148-219).

Bleeding time (sec±SD)			Prothrombin time		
	Placebo	Sildenafil		Placebo	Sildenafil
Baseline	343±114	332±86	Baseline (sec±SD)	13.5±0.8	13.6±0.8
On treatment	367±85	378±88	AUEC (sec.h±SD)	3006±667	2895±460
			MaxΔ (sec±SD)	15.6±9.2	14.4±7.3

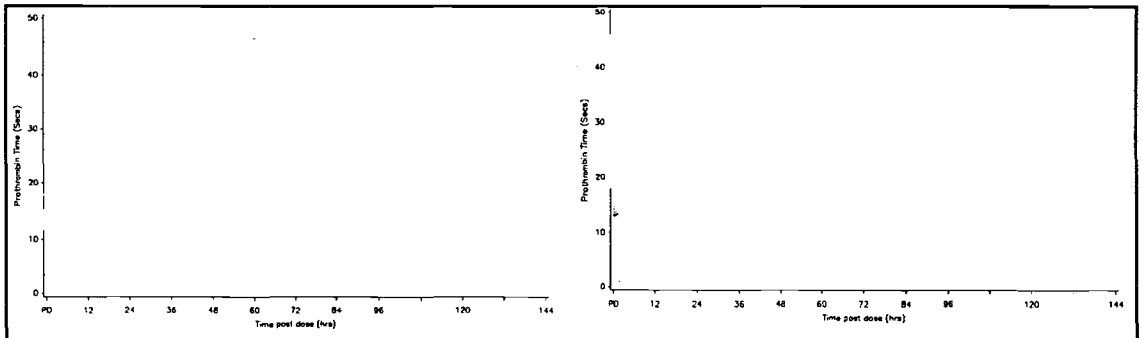


Figure 52. Individual prothrombin times on placebo and sildenafil (Study 148-219)

A26.6. Summary

Oxidation by CYP2C9 is thought to be the principal path of elimination for warfarin. Sildenafil inhibits CYP2C9 activity in vitro and sildenafil inhibits platelet aggregation. Both effects of sildenafil could result in increased bleeding times in patients receiving both drugs. The study design was sub-optimum in utilizing a dose of sildenafil (50 mg) less than the sponsor's recommended maximum. A reduction in the effect of warfarin was evidenced by a statistically significant but clinically meaningless decrease in AUEC; this unexpected result is most likely spurious.

A27. Study 148-221: An open, single dose study to compare the pharmacokinetics, safety and toleration of a single oral dose of sildenafil in patients with chronic stable hepatic cirrhosis to healthy subjects with normal hepatic function.

A27.1. Source documents

Study protocol NDA 20-895, vol 1.66; study report: NDA vol 1.66; electronic document: 47053746.pdf.

A27.2. Investigators

A27.3. Study dates

20 December 1995 to 27 February 1996.

A27.4. Study design

This study description was based upon the final study report, dated 14 May 1997.

A27.4.1. Objectives

The objectives were

- To determine the pharmacokinetics of sildenafil and the metabolite UK-103,320 following single oral doses in subjects with chronic stable hepatic cirrhosis and to compare them with those for age and weight matched normal subjects.
- To assess the safety and toleration of a single 50-mg dose of sildenafil in subjects with chronic stable hepatic cirrhosis.

A27.4.2. Formulation

Sildenafil was supplied as 50-mg capsules (lot 3039-135).

A27.4.3. Population

Twelve healthy male subjects between 18 and 70 years were to be recruited to match age (±5 years) and weight (±10 kg) of 12 subjects with chronic stable hepatic cirrhosis. The diagnosis of cirrhosis was to include previous liver biopsy with laboratory and clinical findings supporting cirrhosis. Six (±2) of the subjects with cirrhosis were to conform to the Child-Pugh classification A and 6±2 to the Child-Pugh classification B.

A27.4.4. Procedures

The study was an open, single oral dose, parallel group study. Subjects were given a standard light breakfast on the morning of dosing. Breakfast was completed at least 2 hours before dosing. Blood samples were collected at 0, 0.25, 0.5, 1, 1.5, 2, 6, 10, 18, 24, 36, and 48 hours post-dosing.

A27.4.5. Assay

A27.4.6. Analysis

Pharmacokinetic parameters were calculated using standard non-compartmental techniques. Paired tests were used (equivalent to a two-way analysis of variance). AUC and C_{max} were log-transformed before analysis. Mean differences and 95% confidence intervals were calculated on the log scale and then back-transformed to give the ratio and confidence intervals as percentages.

A27.4.7. Safety

Routine safety data were recorded.

A27.5. Results

A27.5.1. Pharmacokinetics

Mean plasma concentration time profiles for sildenafil and its metabolite for the normal and cirrhotic subjects are shown in Figure 53 below while the corresponding parameters are summarized in Table 117 below. Figure 54 below shows the ln(AUC) as a function of the Child-Pugh score.

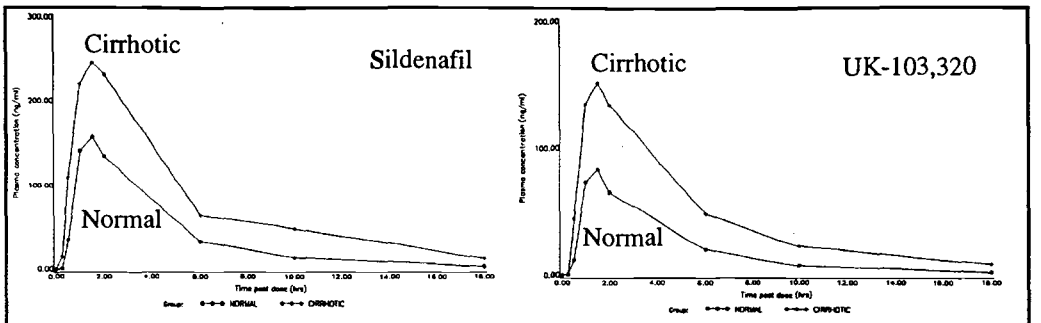


Figure 53. Plasma levels of sildenafil and UK-103,320 (Study 148-221).

Table 117. Pharmacokinetic parameters (Study 148-221).

	Sildenafil		UK-103,320			Sildenafil		UK-103,320	
	Normal	Cirrhotic	Normal	Cirrhotic		Normal	Cirrhotic	Normal	Cirrhotic
C_{max} (ng/ml)	155	228	83	155	$t_{1/2}$ (h)	3.2	4.3	3.1	5.8
AUC_{τ} (ng.h/ml)	664	1225	343	873	CL/f (mL/min)	1255	680	—	—
T_{max} (h)	1.4	1.6	1.3	1.5	Protein binding (% free)	3.5	3.7	4.9	5.6
k_{el} (h^{-1})	0.22	0.16	0.22	0.12					

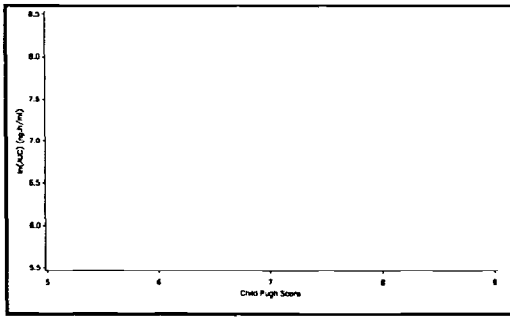


Figure 54. Ln(AUC) as a function of Child-Pugh score (Study 148-221).

The results of the study show that there was almost doubling of the sildenafil plasma concentrations in the liver impaired subjects. The ratio of geometric AUC means was 185% with a 95% confidence interval of 111 to 307%. The ratio of geometric C_{max} means was 145% with a 95% confidence interval of 97 to 222%. This difference in plasma concentrations can be partially explained by the differences in oral clearance. The cirrhotic patients' oral clearance was 46% lower compared to the healthy volunteers (680 mL/min vs. 1255 mL/min). Analysis of the protein binding revealed no significant difference in free fraction between the two groups, with mean values of 3.5 and 3.7%, respectively. There was no relationship between AUC and the Child-Pugh score.

In view of the above results, one would expect that the concentrations of UK 103,320 would be reduced. However, the results of this study show that plasma levels of the metabolite were doubled in the cirrhotic subjects. The results suggest that the metabolite concentrations are dependent upon the elimination rate and that the intrinsic clearance of the metabolite is affected to a greater extent than is that of the parent drug.

A27.6. Summary

The results of the study showed that in subjects with liver cirrhosis, the plasma levels of sildenafil and its metabolite UK-103,320 were almost double the levels in age- and weight-matched normal controls. This doubling of plasma concentrations might warrant starting such patients on a lower dose of sildenafil.

A28. Study 148-222: Single blind, placebo controlled, parallel group study to investigate the effects of a single oral dose of sildenafil (UK-92,480) (100mg) and isosorbide dinitrate (20mg) on aspirin-induced prolongation of bleeding time in healthy male volunteers.

A28.1. Source documents

Study protocol NDA 20-895, vol 1.67; study report: NDA vol 1.67; electronic document: 47147979.pdf.

A28.2. Investigators

Single-center study with 1 investigator in the UK.

A28.3. Study dates

25 August 1995 to 3 November 1995.

A28.4. Study design

This study description was based upon the final study report, dated 21 February 1997.

A total of 36 healthy male volunteers, age 18 to 45, were to be recruited.

This was an investigator-blind, parallel study. Subjects received aspirin 100 mg qd for 4 days and then a single dose of randomized treatment—placebo, ISDN 20 mg, or sildenafil 100 mg. One hour after dosing, blood samples were drawn for plasma levels of sildenafil and UK-103,320. Bleeding times were measured on day 1 and 1 hour after dosing on day 4.

Routine safety data were recorded.

A28.5. Results

A28.5.1. Conduct

Forty-five subjects were randomized and completed both study phases. There were minor protocol deviations, but only one subject was excluded from analyses because of use of aspirin at baseline.

A28.5.2. Pharmacokinetics

One hour after dosing, plasma levels of sildenafil were 124 to 487 ng/mL (mean 298 ng/mL). Plasma levels of UK-103,320 were 43 to 236 ng/mL (mean of 132 ng/mL).

A28.5.3. Pharmacodynamics

Bleeding time data are shown in Table 118 below, as analyzed by the sponsor.

Table 118. Bleeding time (Study 148-222).

	Placebo	ISDN	Sildenafil
Day 1 pre-dose (min±SD)	4.0±0.9	5.5±2.1	4.9±1.2
Day 4 pre-dose	7.6±2.9	11.0±7.5	6.6±1.5
Day 4 hour 1	8.0±2.6	10.5±4.7	8.1±2.1

There were no evident outliers in the sildenafil group.

A28.5.4. Safety

There were no serious or treatment-related severe adverse reactions. The incidence of headache was greater on sildenafil than on placebo, and greater on ISDN than on sildenafil.

A28.6. Summary

Aspirin increased bleeding time, but neither ISDN 20 mg nor sildenafil 100 mg produced further increases in bleeding time 1 hour after dosing.

A29. Study 148-223: A double-blind, randomised, placebo controlled, four period crossover study to assess the effect of orally administered sildenafil (50, 100 and 200mg) on visual function in healthy male volunteers.

A29.1. Source documents

Study protocol NDA 20-895, vol 1.68; study report: NDA vol 1.68; electronic document: 47111394.pdf.

A29.2. Investigators

Single-center study with 1 investigator in Belgium.

A29.3. Study dates

18 March 1996 to 6 May 1996.

A29.4. Study design

This study description was based upon the final study report, dated 5 August 1997.

A total of 16 health male volunteers, age 40 to 60, were to be recruited.

This was a double-blind, 4-period crossover study in which subjects received single oral doses of placebo and sildenafil 50, 100, and 200 mg in random order on study days at least 7 days apart. Visual effects were assessed by Snellen charts (visual acuity), Pelli-Robson charts (contrast sensitivity), Farnsworth-Munsell 100-hue tests (color discrimination), and pupillometry performed pre-dose, and 1, 2, 4, 6, and 24 to 36 hours after dosing. Supine vital signs were monitored for 4 hours after dosing.

Blood samples for assay of plasma levels of sildenafil and UK-103,320 were taken pre-dose, and 0.5, 1, 2, 3, 4, 6, 7, and 24 to 36 hours after dosing.

Routine safety data were recorded.

A29.5. Results

A29.5.1. Conduct

Sixteen subjects were randomized and completed study. There were minor protocol deviations, but no subject was excluded from analyses.

A29.5.2. Pharmacokinetics

C_{max} for sildenafil was 271 ng/mL after a 50-mg dose, increasing dose-proportionally to 1081 ng/mL after a 200-mg dose. The time of the maximum concentration was about 1 hour at all doses. Metabolite UK-103,320 levels were about 40% as high as those for sildenafil. Sildenafil and its metabolite were each about 95% protein-bound.

A29.5.3. Pharmacodynamics

Results of the color discrimination test 1 hour after dosing are shown in Figure 55 below. Pane A shows the change from baseline in the total color discrimination errors as a function of dose. Pane B shows the color distribution of errors in the 200-mg dose group. Treatment effects were not apparent after 4 hours.

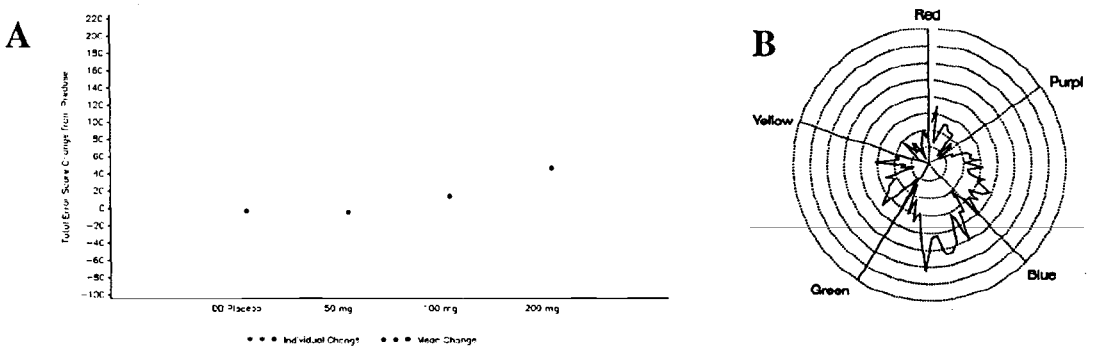


Figure 55. Color discrimination scores (A) by dose and (B) by color at 200 mg (Study 148-223).

In contrast, no apparent treatment effects were observed for tests of contrast sensitivity, visual acuity, or pupillometry.

The main study report makes reference to a PK/PD report, not in evidence. The description of the findings does not indicate whether plasma levels of study drug or metabolite correlate better with color discrimination scores than does dose.

A29.5.4. Safety

There were no serious or treatment-related severe adverse reactions. There was a dose-related increase in the incidence of total adverse events, the most common of which were vasodilation and headache. Other adverse events included visual disturbance

A29.6. Summary

At doses up to 200 mg, the only demonstrated visual effect was impairment of color discrimination. The effect lasted at most a few hours.

A30. Study 148-225: A double-blind, placebo controlled, two way crossover study to investigate the effects of a single dose of sildenafil (100 mg) on blood pressure in subjects with essential hypertension being treated with amlodipine.

A30.1. Source documents

Study protocol NDA 20-895, vol 1.70; study report: NDA vol 1.70; electronic document: 47053931.pdf.

A30.2. Investigators

Single-center study with 1 investigator in the United Kingdom.

A30.3. Study dates

7 August 1996 to 24 January 1997.

A30.4. Study design

This study description was based upon the final study report, dated 29 July 1997.

A total of 16 subjects with uncomplicated hypertension treated with a stable dose of amlodipine only, age 18 to 75, were to be recruited.

On each of two clinic days separated by 7 days, subjects received a single oral dose of placebo or sildenafil 100 mg after overnight fast and 2 hours after the usual amlodipine dose. Vital signs and blood samples for assay of plasma levels of amlodipine were taken over the succeeding 8 hours.

Routine safety data were recorded.

A30.5. Results

A30.5.1. Conduct

Sixteen subjects were randomized and completed study. There were minor protocol deviations, but no subject was excluded from analyses.

A30.5.2. Pharmacokinetics

Pharmacokinetic parameters for amlodipine, AUC and C_{max}, were unaffected by sildenafil (with 95% confidence limits of about ±20%). T_{max} for amlodipine did not appear to have been affected either, but the confidence limits there are much wider.

A30.5.3. Pharmacodynamics

The two treatment periods had comparable vital signs at baseline. Effects on vital signs are summarized in Table 119 below. By the sponsor's analyses, most of the treatment group differences were nominally highly statistically significant.

Table 119. Effects on vital signs (Study 148-225).

		Systolic		Diastolic		Heart rate	
		Placebo	Sildenafil	Placebo	Sildenafil	Placebo	Sildenafil
MaxΔ (±SD)	Supine	-8.7±5.4	-17.0±9.7	-2.1±5.4	-7.9±5.2	-0.7±5.1	1.2±4.7
AUC (mmHg.h)		-6.1	-30.0	7.1	-5.5	-19.9	-18.8
MaxΔ (±SD)	Standing	-9.6±7.7	-20.1±13.3	-3.0±4.6	-11.6±11.2	-3.7±4.1	3.7±6.8
AUC (mmHg.h)		-11.2	-35.1	6.8	-16.0	-28.4	-14.5

A30.5.4. Safety

There were no serious or treatment-related severe adverse reactions. Adverse events overall were more common on sildenafil, headache, diarrhea, and penile erections all occurring only on sildenafil. There was one case of postural hypotension (BP fall from 136/76 mmHg supine to 68/43 mmHg standing), but a concomitant fall in pulse suggests this was vaso-vagal in nature.

A30.6. Summary

Placebo-subtracted effects on supine and standing blood pressure averaged -8/-6 mmHg and -11/-9 mmHg, respectively. Little change in heart rate accompanied changes in blood pressure, but that may have been related to the background antihypertensive agent used. Blood pressure effects had onset within half an hour and persisted for several hours. Although substantial, subjects were not symptomatic, at least under the controlled clinical conditions.

A31. Study 148-226: An open, randomised, single oral dose, three way crossover bioequivalence study to determine the pharmacokinetics of sildenafil in healthy male volunteers following administration of 100mg as capsules and tablets in the fasted state.

A31.1. Source documents

Study protocol NDA 20-895, vol 1.71; study report: NDA vol 1.71; electronic document: 47081254.pdf.

A31.2. Investigators

Dr. D. Kleinermans, Pfizer Clinical Research Unit, Hopital Erasme ,Route de Lennik 808, 1070 Bruxelles, Belgium.

A31.3. Study dates

12 June 1996 to 29 July 1996.

A31.4. Study design

This study description was based upon the final study report, dated 4 July 1997.

A31.4.1. Objectives

The objectives were

- To compare the pharmacokinetics of sildenafil and the metabolite UK-103,320 following single oral doses of research capsules (4x25 mg), research tablet (1x100 mg) and commercial tablet (1x100 mg) in the fasted state and determine bioequivalence between formulations.
- To assess the safety and toleration of single 100-mg doses of sildenafil in healthy male subjects.

A31.4.2. Formulation

Drug supplies were 25-mg capsules (lot 3509-051, size 35,000), 100-mg tablets (lot 4469-119, size 61,358) and the to-be-marketed 100-mg tablet formulation (lot N6060, size 216,721).

A31.4.3. Population

A total of 36 healthy male volunteers, age 18 to 45, were recruited.

A31.4.4. Procedures

This was an open, randomised, single-dose, three-way crossover bioequivalence study. After an overnight fast, dosing took place between 0700 and 0900 on each dosing day. There was a minimum of a 6-day washout period between treatments. Plasma samples were collected before each dose and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose.

A31.4.5. Assay

A31.4.6. Analysis

Pharmacokinetic parameters were calculated using standard non-compartmental techniques. C_{max} and AUC were log-transformed. The Schuirman two one-sided test was applied using a bioequivalence interval of 80 to 125%.

A31.4.7. Safety

Routine safety data were recorded.

A31.5. Results

A31.5.1. Conduct

Thirty-seven subjects were recruited and all but one completed all study phases. Protocol violations appear to have been minor.

A31.5.2. Pharmacokinetics

Mean plasma concentration time profiles for sildenafil and its metabolite for the three treatments are shown in Figure 56 below and the corresponding parameters along with the relevant 90% confidence intervals are summarized in Table 1.

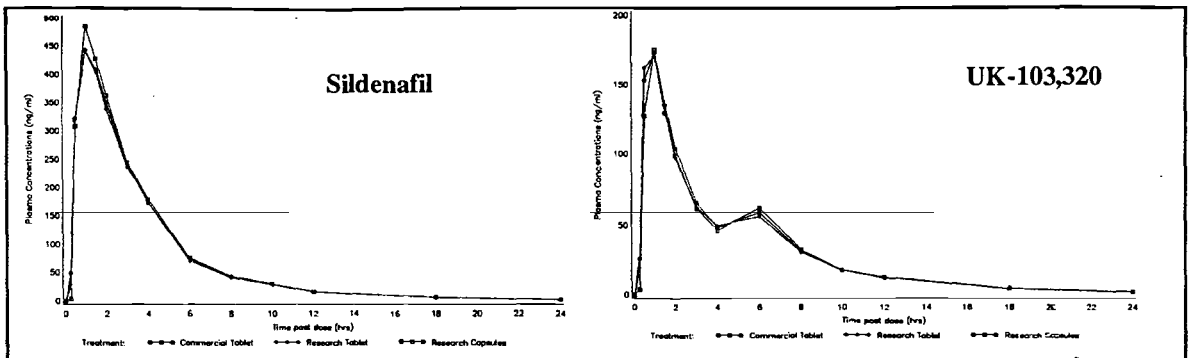


Figure 56. Plasma levels of sildenafil and UK-103,320 (Study 148-226).

Table 120. Pharmacokinetic parameters (Study 148-226).

	Research formulation		Commercial	Ratios (90% CI)		
	25-mg capsule	100-mg tablet	100-mg tablet	Commercial: research tab	Commercial: research cap	Research tab: research cap
C_{max} (ng/mL)	478	446	438	0.99 (0.92-1.07)	0.97 (0.90-1.05)	0.98 (0.91-1.06)
T_{max} (h)	1.1	1.1	1.1	—	—	—
AUC (ng.h/mL)	1645	1540	1609	1.05 (0.99-1.11)	1.01 (0.96-1.07)	0.96 (0.91-1.02)
AUC_{τ} (ng.h/mL)	1629	1526	1593	—	—	—
k_{el} (h^{-1})	0.22	0.21	0.23	—	—	—
$t_{1/2}$ (h)	3.2	3.3	3.1	—	—	—

A31.5.3. Safety

There were no serious or severe adverse events reported. Headache, vasodilation, abnormal vision, and penile erections were reported with all treatments.

A31.6. Summary

The results of the study show that the research tablet, the commercial tablet and the research capsule are all bioequivalent to each other.

A32. Study 148-227: An open randomised, single oral dose, two way crossover study to determine the pharmacokinetics of sildenafil in healthy male volunteers following administration of 100 mg as commercial tablets in the fed and fasted state.

A32.1. Source documents

Study protocol NDA 20-895, vol 1.72; study report: NDA vol 1.72; electronic document: 46660186.pdf.

A32.2. Investigators

A32.3. Study dates

3 June 1996 to 4 August 1996.

A32.4. Study design

This study description was based upon the final study report, dated 21 July 1997.

A32.4.1. Objectives

The objectives were

- To compare the pharmacokinetics of sildenafil and the metabolite (UK-103,320) following single oral doses of commercial tablets (1x100 mg) in the fed and fasted states.
- To assess the safety and toleration of sildenafil 100 mg in healthy male subjects.

A32.4.2. Formulation

Drug supplies were the to-be-marketed 100-mg tablet formulation, lot 6060.

A32.4.3. Population

A total of 34 healthy male volunteers, age 18 to 45, were recruited.

A32.4.4. Procedures

The study was an open, randomized, two-way crossover single-dose study of sildenafil commercial 100-mg tablets under fed and fasted states. There was a washout period of at least 7 days between treatments. After an overnight fast, subjects in the fed arm of the study received a high-fat breakfast consisting of: 2 fried eggs in vegetable oil, 2 bacon rashers grilled, 1 slice of white bread toasted with butter, 90 gm of hash brown potatoes, 240 ml full fat milk. A standard lunch and dinner were served after the 4-hour and 10-hour post-dosing blood samples.

During each treatment period, 7-ml blood samples were collected at the following times: 0, 0.25, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18 and 24-hours post-dose.

A32.4.5. Assay

A32.4.6. Analysis

Pharmacokinetic parameters were calculated using standard non-compartmental techniques. Ninety percent confidence intervals were calculated on the ratios of the log-transformed C_{max} and AUC in the fasted and fed states.

A32.4.7. Safety

Routine safety data were recorded.

A32.5. Results

A32.5.1. Conduct

All 34 subjects completed both study phases. Protocol violations appear to have been minor.

A32.5.2. Pharmacokinetics

Mean plasma concentrations vs. time profiles for sildenafil and its metabolite in both the fed and fasted states are shown in Figure 57 below. The corresponding pharmacokinetic parameters are summarized in Table 121 below. The results of the study seem to indicate that food slowed the rate of absorption of sildenafil since C_{max} decreased by 29% and T_{max} was prolonged by 1 hour. The relative bioavailability fed/fasted was 89%.

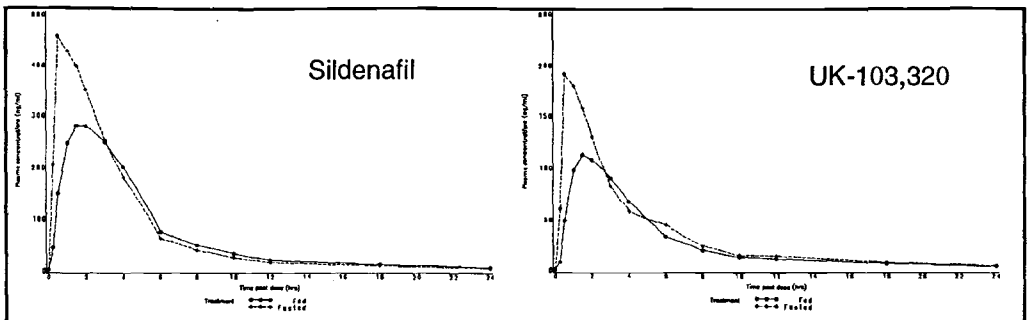


Figure 57. Plasma sildenafil and UK-103,320 levels (Study 148-227).

Table 121. Pharmacokinetic parameters for sildenafil and UK-103,320 (Study 148-227).

	Sildenafil		UK-103,320	
	Fed	Fasted	Fed	Fasted
C_{max} (ng/mL)	364	514	137	215
T_{max} (h)	2.0	1.0	1.9	0.9
AUC (ng.h/mL)	1489	1651	571	729
AUC_{τ} (ng.h/mL)	1465	1651	547	700
k_{el} (h-1)	0.16	0.17	0.11	0.12
$t_{1/2}$ (h)	4.3	4.0	6.1	5.9

A32.5.3. Safety

There were no serious or severe adverse events reported. Headache and visual defects were the most common adverse events. Penile erections were reported by 1 subject in the fasted state and 4 subjects in the fed state.

A32.6. Summary

The results of the study showed that coadministration of a high-fast breakfast with sildenafil slightly decreased its rate of absorption. However, this decrease in the rate of absorption is not expected to have any clinical consequences.

A33. Study 148-228: An open, randomised, single oral dose, four way crossover study to determine the dose proportionality of the pharmacokinetics of sildenafil in healthy male volunteers over the dose range 25mg to 200mg.

A33.1. Source documents

Study protocol NDA 20-895, vol 1.73; study report: NDA vol 1.73; electronic document: 47111104.pdf.

A33.2. Investigators

A33.3. Study dates

10 June 1996 to 10 September 1996.

A33.4. Study design

This study description was based upon the final study report, dated 8 August 1997.

A33.4.1. Objectives

The objectives were

- To determine the dose proportionality of the pharmacokinetics of sildenafil following single oral doses of the commercial tablet over the dose range 25 to 200 mg.
- To assess the safety and toleration of single doses (25 to 200 mg) of sildenafil in healthy male subjects.

A33.4.2. Formulation

Drug supplies were to the to-be-marketed tablet formulation shown in Table 122 below.

Table 122. Drug supplies (Study 148-228).

	Lot		Lot		Lot
25 mg tablet	N6056	50 mg tablet	N6058	100 mg tablet	N6060

A33.4.3. Population

A total of 32 health male volunteers, age 18 to 45, were to be recruited.

A33.4.4. Procedures

In random order and separated by 7 days, subjects received oral doses of sildenafil 1x25 mg, 1x50 mg, 1x100 mg, and 2x100 mg in the morning after overnight fast. Subjects continued to fast for 4 hours after dosing.

Blood samples for plasma levels of sildenafil and UK-103,320 were obtained pre-dose, and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose.

A33.4.5. Assay

A33.4.6. Analysis

The following pharmacokinetic parameters were calculated: C_{max}, T_{max}, AUC_{τ}, AUC, k_{el}, and $t_{1/2}$.

A33.4.7. Safety

Routine safety data were recorded.

A33.5. Results

A33.5.1. Conduct

Thirty-three subjects were randomized and treated. One was withdrawn after the first study period because of protocol violations. Other protocol violations appear to have been minor.

A33.5.2. Pharmacokinetics

Mean plasma levels of sildenafil and UK-103,320 are shown as a function of dose in Figure 58 below. Pharmacodynamic parameters are shown in Table 123 below.

[1] However, the same assay was used in other trials for which adequate documentation was provided.

Table 123. Pharmacokinetic parameters for sildenafil and UK-103,320 (Study 148-228).

	Sildenafil				UK-103,320			
	25	50	100	200	25	50	100	200
C_{max} (ng/mL)	127	271	560	1150	54	126	254	526
T_{max} (h)	1.02	0.79	0.83	0.94	0.97	0.78	0.77	0.88
AUC (ng.h/mL)	361	738	1685	3755	147	328	776	1822
AUC_τ (ng.h/mL)	334	727	1667	3702	134	318	756	1772
k_{el} (h-1)	0.27	0.23	0.19	0.18	0.24	0.20	0.15	0.14
$t_{1/2}$ (h)	2.6	3.0	3.7	3.8	2.9	3.5	4.6	4.9

The sponsor's analyses of dose-normalized ratios of geometric means for AUC and C_{max} for sildenafil increase with the ratio of doses, as shown in Table 124 below. The AUC and C_{max} for sildenafil increase more than dose-proportionally; a doubling of dose increases AUC by 2.23 and C_{max} by 2.08.

Table 124. Test of dose-proportionality for AUC and C_{max} of sildenafil (Study 148-228).

	AUC		C_{max}	
	Ratio	90% CI	Ratio	90% CI
50/25	1.08	1.01-1.15	1.07	0.96-1.19
100/50	1.14	1.07-1.22	1.04	0.93-1.15
200/100	1.11	1.05-1.19	1.03	0.92-1.14
100/25	1.23	1.16-1.32	1.11	1.00-1.23
200/25	1.38	1.29-1.47	1.14	1.02-1.27

T_{max} for sildenafil did not appear to be a function of dose. The elimination rate constant, k_{el}, and $t_{1/2}$ were functions of dose.

Although not formally analyzed by the sponsor, the dose-relatedness of AUC, C_{max}, T_{max}, k_{el}, and $t_{1/2}$ for UK-103,320 appear similar to sildenafil.

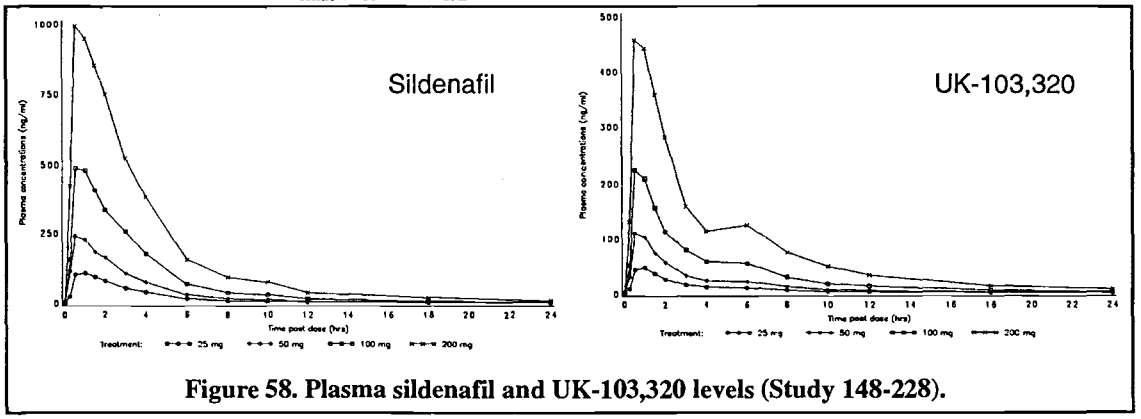


Figure 58. Plasma sildenafil and UK-103,320 levels (Study 148-228).

A33.5.3. Safety

There were no serious or severe adverse events reported. Adverse events, total and treatment-related, increased with dose, with headache and visual defects being most common.

A33.6. Summary

When sildenafil is administered as single doses using the to-be-marketed tablet formulation, AUC and C_{max} for sildenafil and metabolite UK-103,320 increase more

than proportionally with dose over the range of sildenafil 25 to 200 mg. This was true even for the 100- to 200-mg doses, both of which utilized the 100-mg tablets, suggesting that this effect is related to the drug substance rather than the formulation. The increase in AUC and C_{max} were associated with increased $t_{1/2}$ and reduced k_{el}, suggestive of a saturable elimination process.

Center for Drug Evaluation and Research

Viagra (Sildenafil)

"Joint Clinical Review" for NDA-20-895

Appendix A34, page 170 through Appendix A43.6, page 190

A34. Study 148-229: A double-blind, randomised, single oral dose, four period, two-way crossover pilot study to investigate the acute effects of sildenafil on sperm motility.

A34.1. Source documents

Study protocol NDA 20-895, vol 1.74; study report: NDA vol 1.74; electronic document: 47111402.pdf.

A34.2. Investigators

Single-center study with 1 investigator in Norway.

A34.3. Study dates

20 August 1996 to 14 October 1996.

A34.4. Study design

This study description was based upon the final study report, dated 7 August 1997.

A34.4.1. Objectives

- To determine the acute effects of sildenafil (100 mg) on sperm motility (percentage motile, static, rapid, progressive, progressive motility and mean lateral head displacement) in healthy male subjects.
- To determine the acute effects of sildenafil on sperm count, sperm density, sperm morphology, and vitality, ejaculate volume and viscosity in healthy male subjects.
- To determine sildenafil and UK-103,320 concentrations in ejaculate and to compare these with plasma concentrations.
- To assess the safety and toleration of a single dose of sildenafil (100 mg) in healthy male subjects.

A34.4.2. Formulation

Sildenafil 100 mg tablets were from lot 4469-115. Matching placebo tablets were from lot 4469-084.

A34.4.3. Population

A total of 16 normal volunteers, age 18 to 45, were to be recruited.

A34.4.4. Procedures

On each of four clinic days separated by 7 days, subjects received single oral doses of placebo or sildenafil 100 mg at least 2 hours after the last meal. Semen samples were collected at 1.5 and 4 hours after dosing.

Semen samples were assessed for sperm motility, count, density, morphology, and vitality. Ejaculate volume and viscosity were also assessed.

Blood samples for assay of plasma levels of sildenafil and UK-103,320 were taken at baseline, 0.25, 0.5, 1, 2, 3, 4, and 6 hours after dosing.

Routine safety data were recorded.

A34.4.5. Assay

A34.4.6. Analysis

Pharmacokinetic parameters were calculated using standard non-compartmental techniques. The relationships between the total amounts of sildenafil or UK 103,320 in the semen and the corresponding total and free plasma concentrations were investigated using linear regression analyses.

A34.5. Results

A34.5.1. Conduct

Seventeen subjects were randomized and 16 completed study. One subject discontinued for back pain and urinary retention, and was replaced[1]. There were minor protocol deviations, but no subject was excluded from analyses.

A34.5.2. Pharmacokinetics

Mean plasma concentration-time profiles for sildenafil and UK-103,320 are shown in Figure 59 below and the corresponding parameters are summarized in Table 125 below.

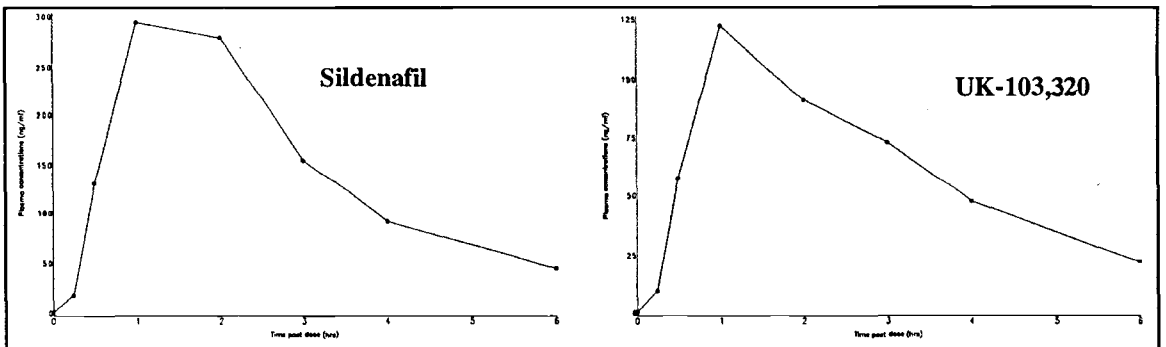


Figure 59. Concentrations of sildenafil and UK-103,320 in plasma (Study 148-229).

Table 125. Pharmacokinetic parameters (Study 148-229).

	Sildenafil	UK-103,320		Sildenafil		UK-103,320	
				1-2 h	4 h	1-2 h	4 h
Plasma AUC_6 (ng.h/mL)	841	341	Plasma binding (%free)	5.7	4.7	6.9	5.5
Plasma C_{max} (ng/mL)	331	125	Total plasma conc (ng/mL)	286	91	106	47
Plasma T_{max} (h)	1.4	1.4	Free plasma conc (ng/mL)	15	4.2	7.0	2.5
Total in semen (ng)	188	18	Semen conc (ng/mL)	51	16	5.1	7.1

Figure 60 below shows the mean semen concentration-time profiles for both sildenafil and its metabolite with the corresponding parameters summarized in Table 125. The mean semen sildenafil concentrations were approximatley 18% of the plasma concentrations at the same time points. However, the same trend was not observed for UK 103,320 . The mean semen concentrations were approximately 5 and 15% of the plasma concentrations. Figure 61 below shows the relationship between the concentration or amount in the semen and the total plasma concentrations for both sildenafil and UK 103,320.

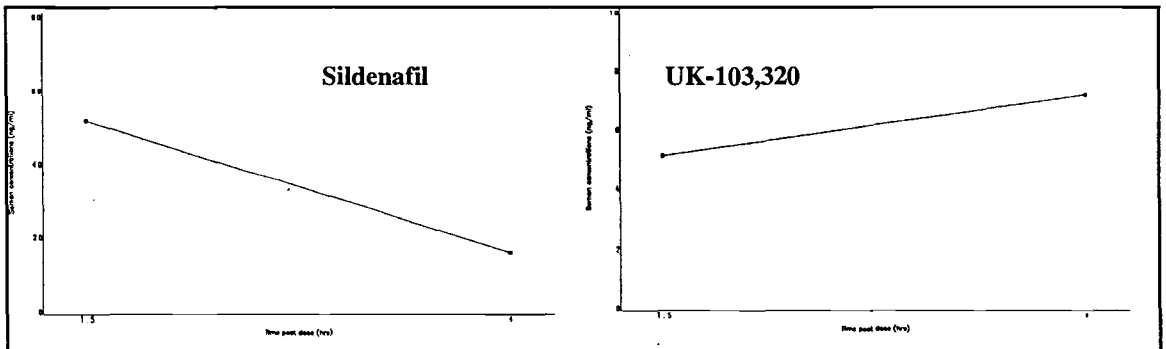


Figure 60. Concentrations of sildenafil and UK-103,320 in semen (Study 148-229).

[1] Adverse event noted after first study period. The subject had received placebo.

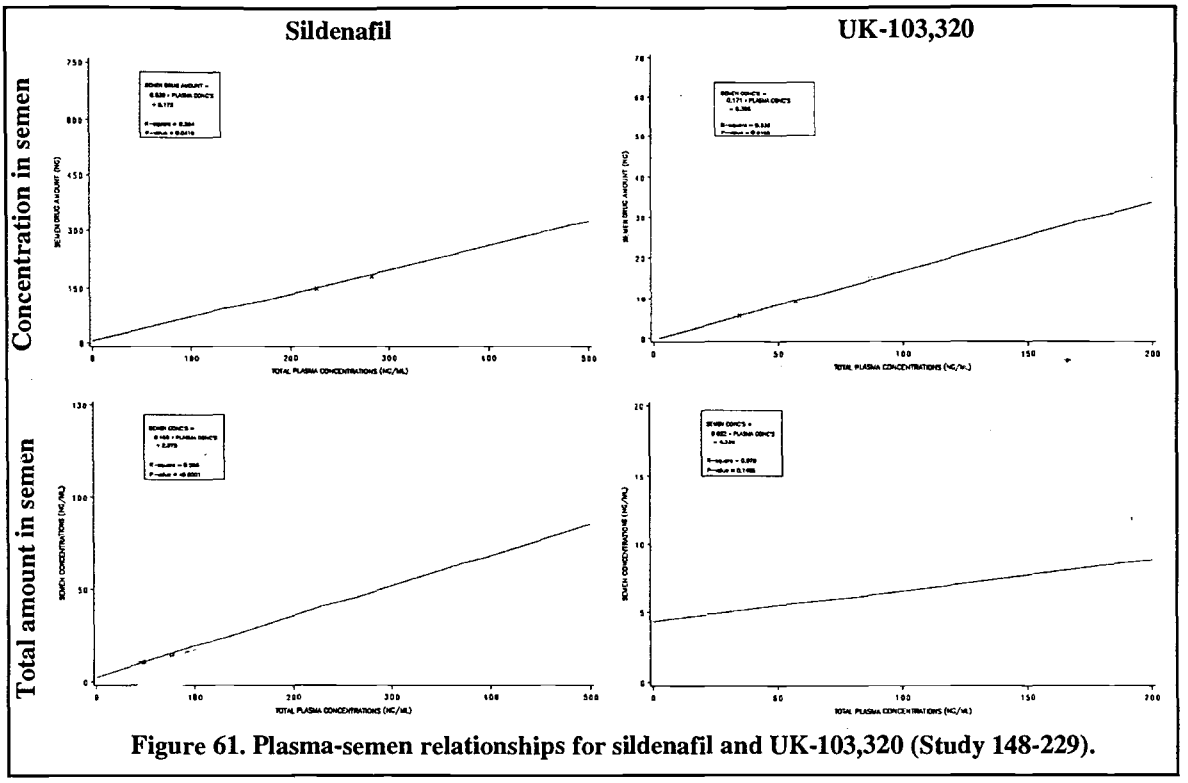


Figure 61. Plasma-semen relationships for sildenafil and UK-103,320 (Study 148-229).

The relationship between the amount of sildenafil and metabolite and the correponding total concentrations was statistically significant ($p<0.05$). Moreover, there was a highly statistically significant relationship betwen sildenafil and metabolite semen concentrations and either free or total plasma concentrations of either sildenafil or metabolite ($p<0.001$).

A34.5.3. Pharmacodynamics

Sperm motility parameters remained within the normal range of the laboratory and showed no mean differences between placebo and sildenafil periods. Ninety-five percent confidence limits on the difference was typically <10%. Similar results with similar confidence limits were obtained for sperm count, density, morphology, and vitality, and for ejaculate volume and viscosity.

A34.5.4. Safety

There were no serious or treatment-related severe adverse reactions. Adverse events overall were more common on sildenafil, with headache, vasodilation, and penile erection most notable on sildenafil. Three subjects reported color-related visual disturbances, all shortly after exposure to sildenafil.

A34.6. Summary

The study was adequately powered to detect small effects on sperm motility, count, etc., probably below levels of clinical significance. No such effects were found in response to single doses of sildenafil 100 mg. The sildenafil concentration in semen was 18% of the total plasma concentration at 1.5 and 4 hours post-dose, while the concentration of UK-103,320 in semen was 5% (1.5 hours)and 15% (4 hours) of the corresponding plasma levels. The effects of recurrent exposure were not assessed.

A35. Study 148-230: A double blind, placebo controlled, randomised, two way crossover study to investigate the effects of a single dose of sildenafil (50mg) in patients with stable angina taking isosorbide mononitrate oral therapy.

A35.1. Source documents

Study protocol NDA 20-895, vol 4.1; study report: NDA vol 4.1; electronic document: 46505263.pdf.

A35.2. Investigators

Single-center study with 1 investigator in the UK.

A35.3. Study dates

16 December 1996 to 16 April 1997.

A35.4. Study design

This study description was based upon the final study report, dated 21 November 1997.

The objective was to determine the effect of a single 50-mg dose of sildenafil on vital signs in subjects receiving chronic nitrates.

A total of 16 subjects age 18 to 75, with chronic stable angina, on nitrate therapy, were to be recruited. Exclusions were made for (1) myocardial infarction, (2) exercise-induced angina, (3) blood pressure outside 100/60 to 170/100 mmHg, (4) orthostatic hypotension (>10 mmHg decrease or symptoms), (5) other significant baseline abnormalities, and (6) excessive alcohol consumption.

Subjects switched their baseline nitrate to isosorbide mononitrate 20 mg 5 to 7 days prior to the first study day. In randomized order on study days at least 7 days apart, subjects received isosorbide mononitrate 20 mg plus placebo or sildenafil 50 mg.

Vital sign assessments were made sitting and standing -0.25, 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 3, 4, 5, and 6 hours after dosing.

Routine safety data were recorded.

A35.5. Results

A35.5.1. Conduct

Eighteen subjects age 45 to 78 years were randomized and all but one completed both study phases. There were minor protocol deviations.

A35.5.2. Pharmacodynamics

Vital sign data are shown in Table 126 below, as analyzed by the sponsor. Differences in blood pressures between treatment groups were all highly statistically significant.

Table 126. Vital signs (Study 148-230).

	MaxΔ Systolic		MaxΔ Diastolic		MaxΔ Pulse	
	Placebo N=17	Sildenafil N=18	Placebo N=17	Sildenafil N=18	Placebo N=17	Sildenafil N=18
Sitting	-22	-41	-13	-26	14	16
Standing	-25	-52	-15	-29	17	19

The time courses of group-mean standing blood pressure changes from baseline are shown in Figure 62 below. Changes in sitting blood pressure were similar. There was a 5-bpm greater increase in pulse rate on sildenafil sustained for the first 3 hours after dosing, as shown in Figure 63 below.

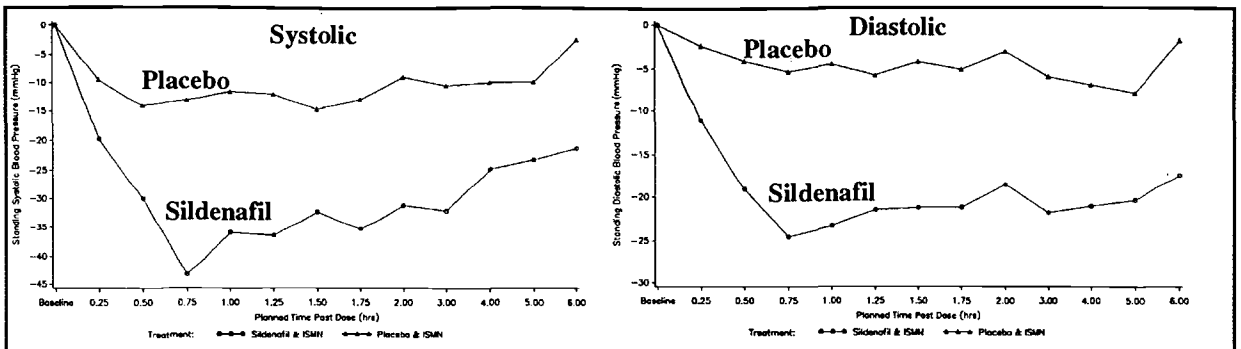


Figure 62. Changes in standing blood pressure (Study 148-230).

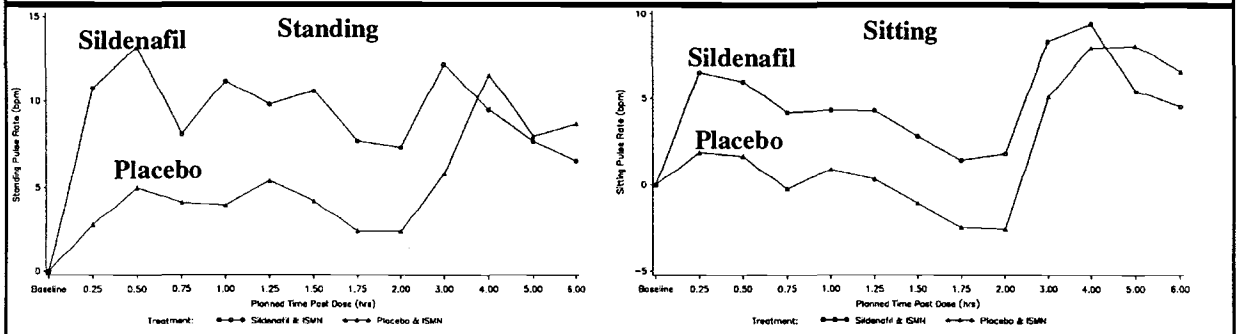


Figure 63. Changes in heart rate (Study 148-230).

A35.5.3. Safety

There were no serious adverse events and no discontinuations for adverse events. Five subjects on sildenafil and 2 subjects on placebo dizziness that probably represented symptomatic hypotension.

A35.6. Summary

Sildenafil substantially decreased blood pressure for more than 6 hours and increased heart rate for about 3 hours when co-administered with isosorbide mononitrate, often to the point of being symptomatic.

A36. Study 148-231: A double blind, placebo controlled, randomised, two way crossover study to investigate the effects of a single dose of sildenafil (50mg) in patients with stable angina taking sublingual glyceryl trinitrate (GTN) therapy.

A36.1. Source documents

Study protocol NDA 20-895, vol 4.2; study report: NDA vol 4.2; electronic document: 44785859.pdf.

A36.2. Investigators

Multi-center study with 2 investigators in the UK.

A36.3. Study dates

10 March 1997 to 3 July 1997.

A36.4. Study design

This study description was based upon the final study report, dated 21 November 1997.

The objective was to determine the effect of a single 50-mg dose of sildenafil on vital signs in subjects receiving glyceryl trinitrate.

A total of 16 subjects age 18 to 75, with chronic stable angina, on prn nitroglycerin therapy, were to be recruited. Exclusions were made for (1) myocardial infarction, (2) exercise-induced angina, (3) blood pressure outside 100/60 to 170/100 mmHg, (4) orthostatic hypotension (>10 mmHg decrease or symptoms), (5) other significant baseline abnormalities, and (6) excessive alcohol consumption.

Subjects were to have taken no nitrates other than prn sublingual nitroglycerin for 14 days prior to the first study day. In randomized order on study days at least 7 days apart, subjects received glyceryl trinitrate 0.5 mg plus placebo or sildenafil 50 mg.

Vital sign assessments were made sitting and standing -0.25, 0, 0.25, 0.5, 0.75, and 1 hour, then every 3 minutes out to 2 hours, and 2.25, 2.5, 2.75, 3, 4, 5, and 6 hours after dosing.

Routine safety data were recorded.

A36.5. Results

A36.5.1. Conduct

Sixteen subjects age 47 to 77 years were randomized and all but one completed both study phases. There were minor protocol deviations.

A36.5.2. Pharmacodynamics

Vital sign data are shown in Table 127 below, as analyzed by the sponsor. Differences in blood pressures between treatment groups were all highly statistically significant.

Table 127. Vital signs (Study 148-231).

	MaxΔ Systolic		MaxΔ Diastolic		MaxΔ Pulse	
	Placebo N=15	Sildenafil N=16	Placebo N=15	Sildenafil N=16	Placebo N=15	Sildenafil N=16
Sitting	-26	-36	-11	-21	10	16

The time courses of group-mean sitting vital sign changes from baseline are shown in Figure 64 below.

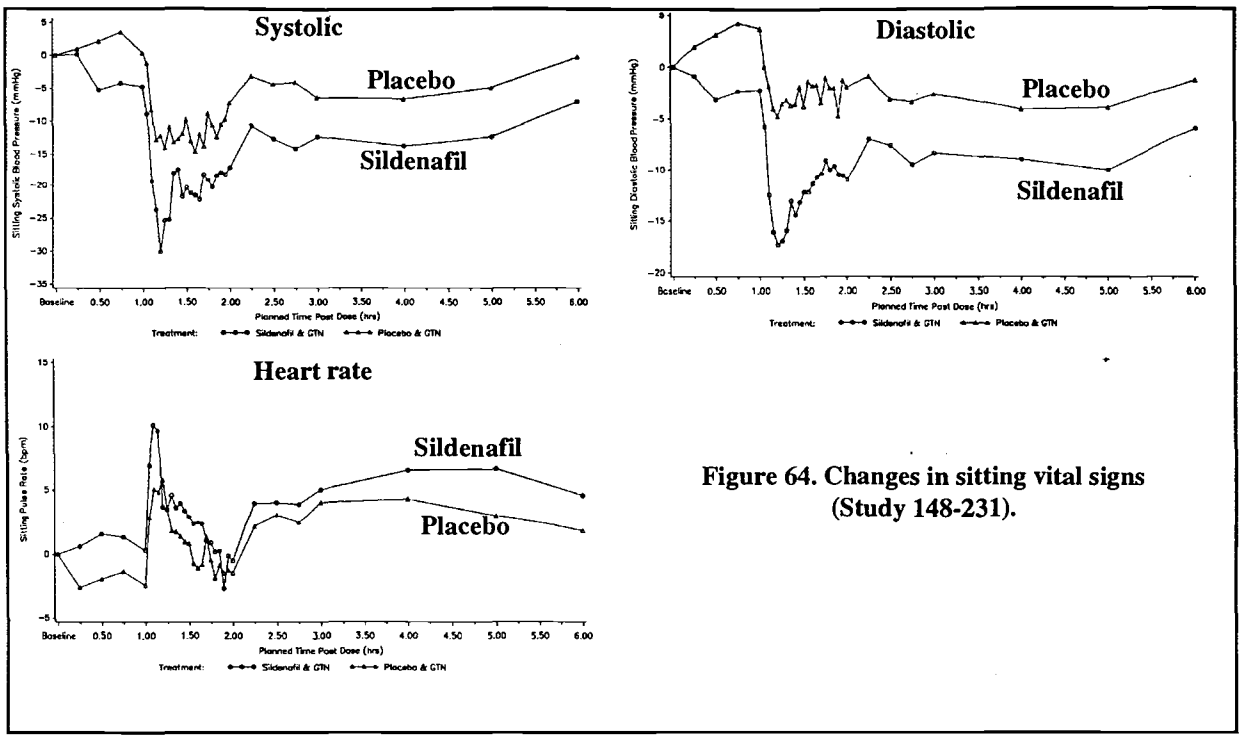


Figure 64. Changes in sitting vital signs (Study 148-231).

A36.5.3. Safety

There were no serious adverse events. One subject (age 77) discontinued with symptomatic hypotension after first receiving sildenafil. Three subjects on sildenafil and 1 subject on placebo dizziness that probably represented symptomatic hypotension.

A36.6. Summary

Sildenafil substantially decreased blood pressure for more than 6 hours and increased heart rate for about 2 hours when co-administered with glyceryl trinitrate, often to the point of being symptomatic.

A37. Study 148-232: A randomised, double-blind, placebo-controlled, crossover pilot study to investigate the effects of a single oral tablet dose of sildenafil (200mg) on visual function (electroretinogram, photostress, visual field and colour discrimination tests) in healthy male volunteers and patients with diabetic retinopathy.

A37.1. Source documents

Study protocol NDA 20-895, vol 1.75; study report: NDA vol 1.75 and 4.4; electronic document: 45252576.pdf.

A37.2. Investigators

Single-center study with 1 investigator in France.

A37.3. Study dates

12 November 1996 to 14 January 1997.

A37.4. Study design

This study description was based upon the final study report, dated 22 August 1997.

A total of 8 normal volunteers and 8 subjects with diabetic retinopathy, age 40 to 65, were to be recruited.

Both normal volunteers and diabetic subjects participated in a 2-period, 2-arm crossover study. During the 2 periods 7 days apart, subjects received a single oral dose of placebo or sildenafil 200 mg in random order and underwent a battery of visual function tests consisting of visual acuity, photostress test, Farnsworth-Munsell 100-hue color discrimination test, and Amsler grid (tests for visual field defects in the central 10°). These assessments were made at baseline, and then 1, 2, 5, and 8 hours wafter dosing. Normal volunteers participated in a further 2 periods with the same double-blind treatments and underwent electroretinogram, Humphrey 30-2 visual field test, and assessment of intraocular pressure, at 1.25 and 5 hours after dosing.

Blood samples for assay of plasma levels of sildenafil and UK-103,320 were taken at baseline, 0.5, 1, 2, 3, 4, 5, and 8 hours after dosing.

Routine safety data were recorded.

A37.5. Results

The study report only deals with results for normal volunteers.

A37.5.1. Conduct

Eight normal subjects and 7 diabetic subjects were randomized and completed study. Diabetic subjects did not undergo ERG. All normal subjects received topical eye treatment following the ERG study. There were minor protocol deviations, but no subject was excluded from analyses.

A37.5.2. Pharmacokinetics

Pharmacokinetic parameters following administration of sildenafil 200 mg are summarized in Table 128 below. Normal subjects and subjects with diabetes had similar findings. Times to peak were somewhat longer than in other studies.

Table 128. Pharmacokinetic parameters (Study 148-232).

	Sildenafil		UK-103,320	
	Normal	Diabetes	Normal	Diabetes
AUC (ng.h/mL)	2178	2155	1274	1176
C_{max} (ng/mL)	615	586	356	337
T_{max} (h)	2.2	3.4	2.3	3.6

A37.5.3. Pharmacodynamics

Color discrimination error scores for normal subjects are shown in Table 129 below as a function of time after dosing. Figure 65 below shows the color distribution of errors in the 200-mg dose group at the time of peak plasma levels and highest error rate.

Only 3 normal subjects reported abnormalities on the Amsler grid visual field test, and 2 of those were on placebo. The photostress test showed no differences between treatment groups, but it could only have detected a 30% change. No subject on placebo

Table 129. Color discrimination error scores for normals (Study 148-232).

	Placebo			Sildenafil		
	1 h	2 h	5 h	1 h	2 h	5 h
Errors	78	77	89	100	129	97
Change from baseline	8	7	19	26	55	22

or sildenafil showed a change in visual acuity. There were no effects detected on intraocular pressure or in the Humphrey visual field test.

The photopic electroretinogram showed a treatment effect—50% reduction in the amplitude of the response to blue light statistically significant or nearly so at both 1.25 and 5 hours after dosing. Various components of the electroretinogram were delayed as well, typically by a few percent. The scotopic electroretinogram showed about a 30% reduction in amplitude at the 1.25- and 5-hour measurements of the response to orange light.

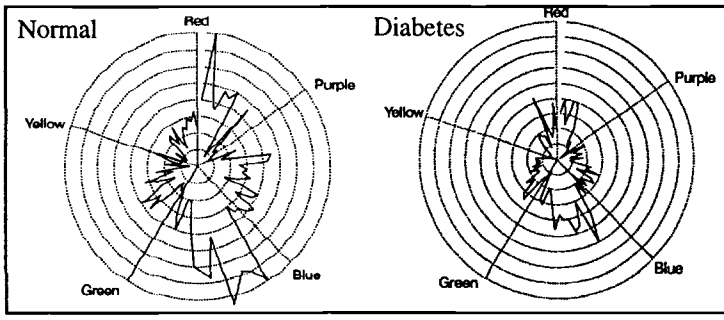


Figure 65. Color distribution of color discrimination errors on sildenafil at 2 hours (Study 148-232).

A37.5.4. Safety

There were no serious or treatment-related severe adverse reactions. Adverse events overall were more common on sildenafil, with headache and visual disturbances most notable on sildenafil among normal and diabetic subjects. Three normal and 4 diabetic subjects reported color-related visual disturbances, all shortly after exposure to sildenafil.

A37.6. Summary

Sildenafil was associated with aberrations in color vision manifest most clearly in the blue-green axis on color discrimination tests and also subjectively. Effects were also detectable by amplitude reduction and delays in the electroretinogram waveform in both the light- and dark-adapted states. Visual disturbances appear to have roughly tracked the time courses of plasma levels of sildenafil or its metabolite. None of the effects on vision appear to have been incapacitating. Subjects with diabetic retinopathy fared no worse than did normal volunteers.

A38. Study 148-234: An open, randomised, placebo controlled, parallel group study to investigate the effects of multiple doses of erythromycin on the pharmacokinetics of a single 100mg dose of sildenafil.

A38.1. Source documents

Study protocol NDA 20-895, vol 4.3; study report: NDA vol 4.3; electronic document: 46504995.pdf.

A38.2. Investigators

A38.3. Study dates

24 February 1997 to 20 May 1997.

A38.4. Study design

This study description was based upon the final study report, dated 19 November 1997.

A38.4.1. Objectives

The objectives were

- To investigate the effects of multiple doses of erythromycin (500 mg bid) on the pharmacokinetics of a single 100-mg dose of sildenafil.
- To investigate the safety and toleration of sildenafil co-administered with erythromycin.

A38.4.2. Formulation

Drug supplies were 250-mg erythromycin tablets, lot 87033VA, placebo lot 3039-124A, and 100-mg sildeanfil tablets, lot 4469-115.

A38.4.3. Population

A total of 24 healthy male volunteers, age 18 to 45, were recruited.

A38.4.4. Procedures

This was an open, randomized, placebo controlled, parallel controlled study. On day 1, all subjects received a single dose of sildenafil 100 mg. Subjects were then allocated to either one of 2 treatment groups. On days 2 to 6, one group of 12 subjects received erythromycin 500 mg twice daily and the other group of 12 subjects was to receive placebo twice daily. On day 6 all subjects received a single dose of sildenafil 100 mg one hour after dosing with erythromycin. On days 1 and 6, blood samples for the measurement of sildenafil and its metabolite were collected at the following time points: 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose.

A38.4.5. Assay

A38.4.6. Analysis

Pharmacokinetic parameters were calculated using standard non-compartmental techniques. The difference between the mean values on day 1 and day 6 was estimated for each treatment group, together with the associated standard error and 95% confidence interval for the difference. For comparisons of AUC and CMAX, the linearized ratio and confidence intervals were also presented.

A38.4.7. Safety

Routine safety data were recorded.

A38.5. Results

A38.5.1. Conduct

Twenty-six subjects were randomized and all but one in each group completed all study phases. Protocol violations appear to have been minor.

A38.5.2. Pharmacokinetics

Mean plasma concentration time profiles for sildenafil and its metabolite for day 1 and 6 for both the placebo and erythromycin treated groups are shown in Figure 66 below and the corresponding parameters are summarized in Table 130 below.

The results show that for sildenafil, coadministration with erythromycin both increased the AUC and C_{max} of sildenafil by 2.6- and 2.1-fold, respectively. This

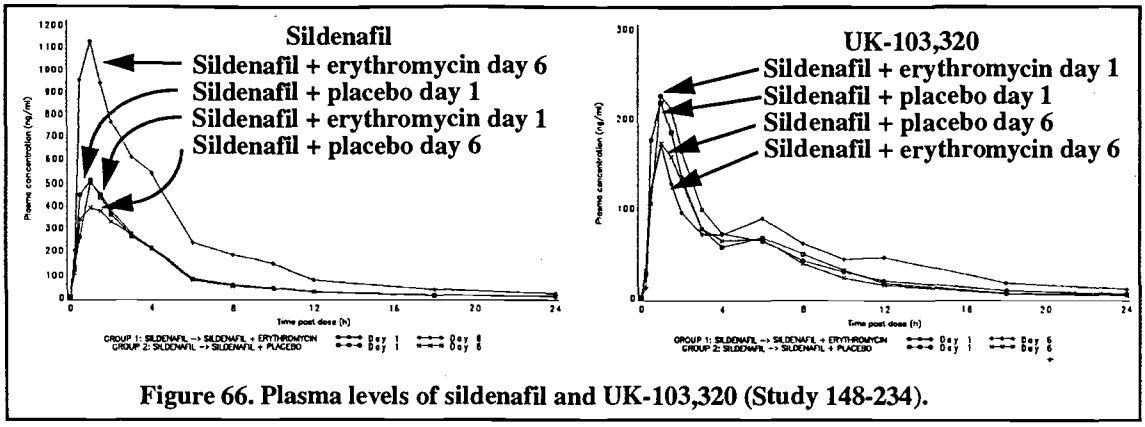


Figure 66. Plasma levels of sildenafil and UK-103,320 (Study 148-234).

Table 130. Pharmacokinetic parameters (Study 148-234).

	Sildenafil				UK-103,320			
	Placebo		Erythromycin		Placebo		Erythromycin	
	Day 1[a]	Day 6	Day 1	Day 6	Day 1	Day 6	Day 1	Day 6
AUC (ng.h/mL)	1893	1732	1904	4911	867	769	905	1099
AUC_τ (ng.h/mL)	1879	1718	1889	4832	853	757	884	1031
C_{max} (ng/mL)	639	512	596	1245	277	227	270	169
T_{max} (h)	1.0	1.2	1.1	0.8	0.9	1.2	1.1	0.9
k_{el} (h^{-1})	0.21	0.20	0.20	0.17	0.18	0.19	0.16	0.13
$t_{1/2}$ (h)	3.3	3.5	3.5	4.1	3.9	3.6	4.2	5.3

a. Both groups received sildenafil alone on day 1, and then sildenafil plus erythromycin or placebo on day 6.

increase in exposure most probably did not result from inhibiting the elimination pathway for sildenafil, since the elimination rate constant was not affected by erythromycin.

As for the effect of erythromycin on the levels of the metabolite UK-103,320, the ratio of geometric AUC means was 1.2 while the ratio of geometric C_{max} was 0.6. The results also show that multiple dosing of erythromycin increased the half-life of the metabolite by 1 hour (from 4.2 to 5.3 hours). This increase in $t_{1/2}$ was found to be statistically significant.

A38.5.3. Safety

There were no serious or severe adverse events reported. Headache, vasodilation, abnormal vision, and penile erections were reported with both treatments, at roughly the same rate.

A38.6. Summary

These findings strongly suggest that erythromycin inhibits CYP3A4 found in the gastrointestinal tract, thereby affecting pre-systemic metabolism of sildenafil. The magnitude of increase in plasma levels of sildenafil suggests that patients who are on known inhibitors of CYP3A4 should be started on the lowest possible dose—25 mg—and titrated up as needed, although the safety results of this study do not indicate this is a major safety concern.

A39. Study 148-301: An open single intravenous dose study of the haemodynamic effects of UK-92,480 (sildenafil) in patients with stable ischaemic heart disease.

A39.1. Source documents

Study protocol NDA 20-895, vol 1.77; study report: NDA vol 1.77; electronic document: 46822388.pdf.

A39.2. Investigators

Single-center study with 1 investigator in the United Kingdom.

A39.3. Study dates

6 April 1993 to 7 September 1993.

A39.4. Study design

This study description was based upon the final study report, dated 24 March 1997.

A total of 10 subjects with stable, uncomplicated ischemic heart disease, age 18 to 70, were to be recruited.

Subjects were to have vasoactive drugs withheld 48 hours prior to right heart catheterization. Subjects underwent a baseline 4-minute exercise test and then had hemodynamic measurements made beginning after 20 or more minutes of rest. Sildenafil was administered by intravenous infusion to provide 10 mg over the first 30 minutes, 10 mg over the next 15 minutes, and then 20 mg over the final 15 minutes (total of 40 mg over 1 hour). Hemodynamic measurements were made in the 3 minutes prior to the end of four 15-minute sampling periods. Assessments to be made included systemic arterial pressure, pulmonary artery wedge pressure, pulmonary artery pressure, right atrial pressure, heart rate, cardiac volume, stroke volume, systemic vascular resistance, pulmonary vascular resistance, and left and right ventricular work indices.

Blood samples for assay of plasma levels of sildenafil were taken at baseline and at the end of each 15-minute period of infusion.

Routine safety data were recorded.

A39.5. Results

A39.5.1. Conduct

Eight subjects were randomized and completed study. Some of the derived parameters were not reported.

A39.5.2. Pharmacokinetics

Mean plasma levels by 15-minute period were 153 to 174, 217 to 278, 432 to 708, and 950 to 2023 ng/mL, respectively.

A39.5.3. Pharmacodynamics

Selected hemodynamic data (mean±SD) are shown in Table 131 below. In general, pressures were somewhat lower following sildenafil than after placebo.

Table 131. Hemodynamic data at highest dose (Study 148-301).

	At rest		After exercise	
	Baseline	Sildenafil	Baseline	Sildenafil
PAWP (mmHg)	11±4.8	7.8±4.7	36±13.7	28±15.3
Mean PAP (mmHg)	18±3.9	13±4.1	39±12.9	32±13.2
Mean RAP (mmHg)	5.7±3.7	4.1±3.7	—	—
SBP (mmHg)	155±17	143±17	200±37	188±30
DBP (mmHg)	74±8.3	63±15	85±9.7	80±9.4
CO (L/min)	6.6±1.6	5.2±0.4	12±2.4	10±3.5
Heart rate (bpm)	64±9.8	70±14	102±12	99±20

A39.5.4. Safety

One subject died after emergency CABG on day 12 after dosing. There were no other noteworthy findings.

A39.6. Summary

At these doses, intravenous sildenafil produced modest changes in pulmonary and systemic blood pressures. The dose-response relationship and the time course of these effects were not well-characterized by the study.

A40. Study 148-350: A double blind, randomised, placebo controlled, two way crossover pilot study to investigate the efficacy and safety of UK-92,480 (sildenafil, 25mg tid for 7 days) in patients with impotence.

A40.1. Source documents

Study protocol NDA 20-895, vol 1.78; study report: NDA vol 1.787; electronic document: 47081406.pdf.

A40.2. Investigators

Single-center study with 1 investigator in the United Kingdom.

A40.3. Study dates

28 July 1993 to 15 November 1993.

A40.4. Study design

This study description was based upon the final study report, dated 8 August 1997.

A total of 16 subjects with a 6-month history of erectile dysfunction of no known neurological or vascular cause, age 18 to 70, were to be recruited.

For two study periods 7 days apart, subjects received in random order placebo or sildenafil 25 mg tid for 7 days. The last dose on the evening of 7th day was administered in the clinic where penile plethysmography was performed for 10 hours in the setting of visual sexual stimulation.

During at-home dosing, subjects maintained a diary of erections and erections associated with sexual stimulation.

Routine safety data were recorded.

A40.5. Results

A40.5.1. Conduct

Sixteen subjects were randomized and 15 completed both phases of the study. Minor protocol violations were described, but the only subject excluded from the plethysmography assessment was one who did not participate in the second crossover phase.

A40.5.2. Pharmacodynamics

Penile plethysmography data (Rigiscan) obtained during presentation of sexual stimulation are shown in Table 132 below. There was no treatment effect during the succeeding 8 hours of monitoring.

Table 132. Penile plethysmography (Study 148-350).

	Placebo	Sildenafil	*P*
Duration >60% (base; min)	12	49	0.005
Duration >60% (tip; min)	7.4	36	0.002
Duration >80% (base; min)	3.5	15	0.002
Duration >80% (tip; min)	1.2	10	0.0006

Diary data showed a nominally statistically significant increase in erections on sildenafil, but interpretation is complicated by apparent treatment period effects.

A40.5.3. Safety

One subject discontinued from dosing on active treatment because of dyspepsia, considered treatment-related. Adverse events were more commonly reported on sildenafil, the most common of which were headaches and myalgia involving the back and legs.

A40.6. Summary

The Rigiscan and diary data are consistent with a clinically significant improvement of erectile function in subjects receiving sildenafil 25 mg tid. The trial design does not permit any assessment of the time course of effects after a dose or with repeated dosing.

A41. Study 148-351: A double blind, randomised, placebo controlled, four way crossover study followed by a double blind, randomised, placebo controlled, two way crossover study to investigate the efficacy of single doses of UK-92,480 (sildenafil) in patients with erectile dysfunction with no established organic cause.

A41.1. Source documents

Study protocol NDA 20-895, vol 1.79; study report: NDA vol 1.79; electronic document: 47081294.pdf.

A41.2. Investigators

Single-center study with 1 investigator in the United Kingdom.

A41.3. Study dates

24 February 1994 to 30 May 1994.

A41.4. Study design

This study description was based upon the final study report, dated 15 July 1997.

A total of 12 subjects with a 6-month history of erectile dysfunction of no known neurological[1] cause, age 18 to 70, were to be recruited.

The study consisted of two parts. In the first part, subjects received, in random order, single doses of placebo and sildenafil 10, 25, and 50 mg on separate study days 3 days apart. These subjects underwent penile plethysmography accompanied by visual sexual stimulation. Blood samples for plasma sildenafil and UK-103,320 were obtained at the end of a 2.5-hour Rigiscan evaluation. In the second part, subjects received, in random order, placebo and sildenafil 25 mg per day for 7 days in phases separated by 7 days. Subjects maintained a diary during these two 1-week periods.

Routine safety data were recorded.

A41.5. Results

A41.5.1. Conduct

Twelve subjects were randomized and all completed both parts of the study. All subjects reported spontaneous erections. Minor protocol violations were described, but no subject was excluded from analyses.

A41.5.2. Pharmacokinetics

Mean plasma levels at 2.5 hours after dosing were 26 ng/mL after 10 mg, 62 ng/mL after 25 mg, and 122 ng/mL after 50 mg. Corresponding mean levels of UK-103,320 were about 40% of the parent compound.

A41.5.3. Pharmacodynamics

Penile plethysmography data (Rigiscan) obtained during presentation of sexual stimulation are shown in Table 133 below. Plasma levels of sildenafil or its metabolite correlated strongly with Rigiscan data, but accounted for a small fraction of the observed variance. In all subjects with plasma concentrations >100 ng/mL, the duration of >60% rigidity exceeded 30 minutes.

Table 133. Penile plethysmography (Study 148-351).

	Placebo	Sildenafil		
		10 mg	25 mg	50 mg
Duration >60% (base; min)	3.2	26	24	32
Duration >60% (tip; min)	2.9	19	26	27
Duration >80% (base; min)	1.4	3.5	7.7	11
Duration >80% (tip; min)	1.1	4.6	6.7	7.4

Diary data showed a statistically significant increase in erections on sildenafil.

A41.5.4. Safety

Minor adverse events were reported with no clear relationship to treatment.

[1] The intent was clearly to enroll subjects with psychogenic erectile dysfunction, but the exclusion criteria do not appear to encompass erectile dysfunction of vascular etiology. All subjects enrolled had erectile dysfunction attributed to psychogenic etiology.

A41.6. Summary

This was an early demonstration of effects of sildenafil on erectile function in subjects with no established organic cause.

A42. Study 148-353: A randomised, double-blind, placebo controlled, parallel-group, multicentre, dose-response study to assess the efficacy and safety of sildenafil (UK-92,480) administered once daily for 28 days to patients with erectile dysfunction.

A42.1. Source documents

Study protocol NDA 20-895, vol 1.116; study report: NDA vol 1.116; electronic document: 47098656.pdf.

A42.2. Investigators

Multi-center study with 36 investigators in France, Sweden, and United Kingdom.

A42.3. Study dates

5 September 1994 to 25 July 1995.

A42.4. Study design

This study description was based upon the amended protocol dated 17 June 1994. There is no mention of amendments.

Drug supplies are shown in Table 134 below.

Table 134. Drug supplies (Study 148-353).

	Lot		Lot
Placebo	3039-100	Sildenafil 5 mg	3039-131 3039-050
		Sildenafil 25 mg	3039-133 3039-135

The intent was to randomize 300 male subjects age >18, with erectile dysfunction of >3 months' duration, and in a heterosexual relationship. Subjects were excluded for (1) advanced vascular or neurological erectile dysfunction, (2) regular use of nitrates, anticoagulants, major tranquilizers, estrogens, or antiandrogens, (3) elevated prolactin or low testosterone, (4) major hematologic, renal, or hepatic disease, (5) history of stroke, bleeding disorder, or active peptic ulcer disease, (6) postural hypotension or blood pressure outside 90/50 to 170/110 mmHg, (7) experimental drug use within 3 months, (8) alcohol abuse, (9) blood donation within 1 month, (10) HBsAg positivity, (11) significant abnormalities at screening, and (12) inadequate compliance during screening.

At the end of a 2-week treatment-free run-in period during which baseline sexual performance data were collected, subjects were randomized to placebo or sildenafil 10, 25, or 50 mg and followed for 4 weeks. Subjects were instructed to take study drug once per day. Subjects completed an event log noting time of study drug administration and subsequent sexual activity. Subjects completing study without serious adverse events were eligible to participate in a 52-week open-label study.

The primary efficacy assessment was at week 4. The primary end points were (1) the proportion of subjects reporting an improvement in erections, (2) the proportion of subjects interested in continuing treatment, and (3) weekly erection count.

Routine safety data were recorded.

A42.5. Results

A42.5.1. Conduct

Four hundred and four subjects were screened, 351 were randomized, and 317 (90%) completed study.

Demographics of the 4 treatment groups are shown in Table 135 below.

Protocol violations are described in Table 136 below. Not all such subjects were excluded from the sponsor's 'evaluable subjects' analyses.

Table 135. Demographics (Study 148-353).

		Placebo N=95	Sildenafil 10 mg N=90	Sildenafil 25 mg N=85	Sildenafil 50 mg N=81
Race (%)	White	91	90	94	94
	Black	6.3	5.6	3.5	1.2
	Other	3.2	4.4	2.4	4.9
Age	Mean	53	52	53	52
	Range	26-70	28-70	24-70	26-69
Etiology (%)	Psychogenic	54	59	61	59
	Mixed	46	41	39	41
Duration (y)	Mean	4.3	4.7	4.5	4.5
	Range	0.3-40	0.4-30	0.3-30	0.3-23
Med hx (%)	Hypertension	14	8.9	18	12
	Diabetes	1.1	3.3	3.5	1.2
	Prostatectomy	2.1	3.3	2.4	2.5

Table 136. Protocol violations (Study 148-353).

At randomization	n	On treatment	n
Screen phase compliance low	24	Compliance	24
Appropriate consent lacking	7	No efficacy data	12
No erections during screening	3		
Organic erectile dysfunction	3		
Concomitant meds	1		
Total[a]	38	Total	36

a. Some subjects had more than one violation.

A42.5.2. Effectiveness

Affirmative responses to global questions are summarized in Table 137 below. The mean number of erections per week per subject varied monotonically from 1.8 in placebo (same as overall mean baseline rate) to 3.8 on 50 mg.

Table 137. ITT analyses of global effectiveness data (Study 148-353).

	Placebo N=95		Sildenafil 10 mg N=90		Sildenafil 25 mg N=85		Sildenafil 50 mg N=81		P
	n	%	n	%	n	%	n	%	
Treatment has improved erections	91	39	84	64	82	79	76	88	<0.0001
Would use this treatment again	87	51	82	78	78	84	75	91	<0.0001

Secondary end points from the other Sexual Function Questionnaire (SFQ) questions are described in Table 138 below (sponsor's analyses only). There is a fairly consistent pattern, with significant treatment effects generally confined to effects pertaining to erectile function and then less compelling and less consistent effects in areas like satisfaction with intercourse or satisfaction more generally.

Table 138. ITT analyses of non-primary SFQ questions at week 4 (Study 148-353)[a].

Domain	Question	Base-line	Placebo N=95		Sildenafil 25 mg N=90		50 mg N=85		100 mg N=81		*P*
			n	Q	n	Q	n	Q	n	Q	
Erectile function	Waking erections	2.7	94	2.7	90	2.9	85	3.0	78	3.2	0.007
	Frequency of stimulated erections	2.4	94	2.7	90	2.9	85	3.1	78	3.4	0.003
	Firmness of erections	2.0	91	2.4	90	2.9	80	3.0	74	3.3	<0.0001
	Duration of erections	1.5	91	2.1	90	2.6	80	2.8	75	3.2	<0.0001
Intercourse satisfaction	Attempted intercourse	2.9	83	3.1	72	3.6	69	3.8	63	4.3	0.08
	Satisfaction of intercourse	1.3	69	2.1	66	2.5	60	3.0	49	4.0	0.004
	Enjoyment of intercourse	3.4	94	2.1	88	2.8	85	3.0	76	3.2	<0.0001
Orgasmic function	Frequency of ejaculation	3.5	94	3.6	89	4.0	85	4.0	78	4.5	0.0009
Sexual desire	Frequency of desire	3.0	94	3.2	90	3.2	85	3.4	78	3.6	0.03
	Rating of desire	2.8	94	3.0	90	3.1	85	3.1	78	3.3	0.2
Overall satisfaction	Satisfaction with sex life	2.2	93	2.4	88	2.9	85	3.1	77	3.5	<0.0001
	Self confidence	2.9	76	3.0	67	3.2	70	3.1	60	3.4	0.09
	Satisfaction with relationship	3.2	76	3.2	66	3.7	70	3.6	60	3.6	0.05
	Enjoyment of life	3.5	76	3.5	67	3.6	70	3.7	60	3.8	0.07

a. Sponsor's analyses.

About 66% of partners responded on the partner questionnaire. There strongly dose-related improvements in partners' assessments of quality of erections and their own sex lives.

A42.5.3. Safety

Safety will be reviewed for all placebo-controlled experience together.

A42.6. Summary

All subjects had erectile dysfunction wholly or partly of psychogenic origin. The placebo response rate (as assessed by subjects' assertion that treatment had improved erections) was predictably high. Nonetheless, substantial dose-related effects were demonstrated, by sexual function questionnaire and erections per week.

A43. Study 148-354A: An open, non-comparative study to assess the efficacy and safety of UK-92,480 (sildenafil) taken over a 52-week period by patients with erectile dysfunction.

A43.1. Source documents Study protocol NDA 20-895, vol 1.132; study report: NDA vol 1.132; electronic document: 46006894.pdf.

A43.2. Investigators Multi-center study with 36 investigators in United Kingdom, France, and Sweden.

A43.3. Study dates 20 December 1994 to 3 September 1996.

A43.4. Study design This study description was based upon the amended protocol dated 7 April 1995. There were 2 minor amendments.

Drug supplies are shown in Table 139 below.

Table 139. Drug supplies (Study 148-354A).

	Lot		Lot
Sildenafil 10 mg	3039-132 3039-132A 3509-042	Sildenafil 25 mg	3039-134A 3509-044 3509-051 3509-078 3509-080 3509-081

Subjects were all previous participants in studies 148-350[1], 148-351[2], 148-353[3], or 148-355[4]. Subjects must have completed the blinded study without a serious adverse event possibly related to study drug.

Visits were scheduled at 4, 8, 16, 24, 36, and 52 weeks. Subjects began on 25 mg, but could have their doses adjusted between 10 and 100 mg. The primary end point was whether subjects were interested in continuing treatment, subjects' assessments of erections, and the proportion of responders by dose. Subjects also kept an event log and completed a sexual function questionnaire.

Routine safety data were collected.

A43.5. Results

A43.5.1. Conduct Three hundred and eight subjects entered long-term open-label study, and 269 (87%) completed study.

The mean age was 54 years. Ninety-three percent were Caucasian. The mean duration of erectile dysfunction was 4 years. Etiology of erectile dysfunction was organic in 0.7%, psychogenic in 57%, and mixed in 42%.

---

1. A double-blind, randomized, placebo-controlled two-way crossover pilot study to investigate the efficacy and safety of UK-92,480 (25 mg tid for 7 days) in patients with impotence.
2. A double-blind, randomized, placebo-controlled, 4-way crossover study followed by a double-blind, randomized, placebo-controlled, two-way crossover study to investigate the effect of single doses of UK-92,480 in patients with erectile dysfunction with no established organic cause.
3. *Study 148-353: A randomised, double-blind, placebo controlled, parallel-group, multicentre, dose-response study to assess the efficacy and safety of sildenafil (UK-92,480) administered once daily for 28 days to patients with erectile dysfunction.* on page 186.
4. *Study 148-355: A double blind, randomised, placebo controlled, two way crossover study to investigate the efficacy of single doses of sildenafil (UK-92,480) (taken when required over a 28 day period) in patients with erectile dysfunction with no established organic cause.* on page 191.

A variety of protocol violations included more than one dose per day (38), participation for more than 52 weeks (24), and use of forbidden concomitant medications (32).

Thirteen percent of subjects discontinued. Reasons for discontinuation included lack of effectiveness (3%, mostly titrated to the highest allowed dose) and adverse events or laboratory abnormalities.

Exposure is characterized in Figure 67 below. The proportion of subjects exposed for different periods of time is shown in the left panel. The proportion of subjects receiving different ranges of number of doses is shown in the center panel. The proportion of subjects receiving each dose level is shown in the right panel.

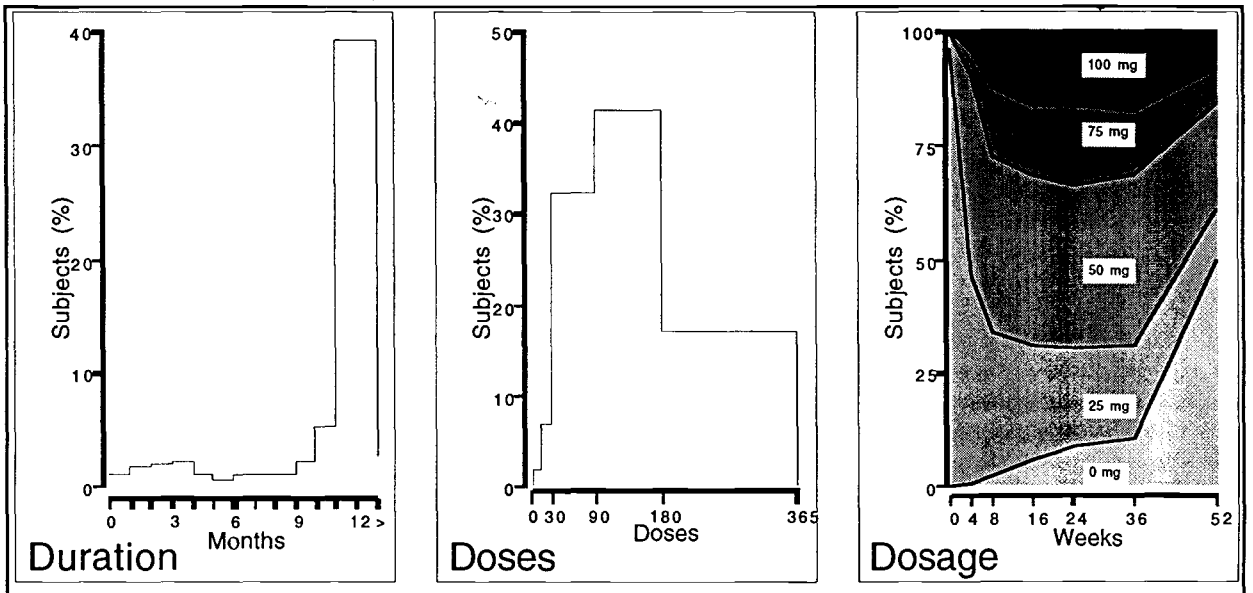


Figure 67. Exposure to study drug (Study 148-354A).

A43.5.2. Effectiveness

By the sponsor's analyses, the proportion of subjects indicating improvement in erections was 88% and the proportion indicating an interest in continuing treatment was 90%. There were significant improvements from baseline for each of 7 items in the sexual function questionnaire. The reviewers performed no analyses of these data.

A43.5.3. Safety

Safety will be reviewed for all open-label studies together.

A43.5.4. Long-term

Documentation is incomplete. One hundred and thirty-two subjects entered the 52-week, long-term, open-label extension toStudy 148-354A. As of the cut-off date of 3 February 1997, 0 subjects had completed, and 0 subjects had withdrawn. One subject on β-blocker reported a syncopal episode after a hot bath. Two subjects reported visual disturbances. Common adverse events were headache (5%), vasodilation/flushing (7%), and dyspepsia (5%).

A43.6. Summary

The study population's erectile dysfunction was wholly or partly psychogenic in etiology. The absence of a control group makes it difficult to assess effectiveness. Most subjects gravitated to the 50-mg dose, not the highest dose available.

Center for Drug Evaluation and Research

Viagra (Sildenafil)

"Joint Clinical Review" for NDA-20-895

Appendix A44, page 191 through Appendix A51.6, page 211

A44. Study 148-355: A double blind, randomised, placebo controlled, two way crossover study to investigate the efficacy of single doses of sildenafil (UK-92,480) (taken when required over a 28 day period) in patients with erectile dysfunction with no established organic cause.

A44.1. Source documents

Study protocol NDA 20-895, vol 1.118; study report: NDA vol 1.118; electronic document: 46870169.pdf.

A44.2. Investigators

Multi-center study with 4 investigators in United Kingdom.

A44.3. Study dates

18 October 1994 to 23 May 1995.

A44.4. Study design

This study description was based upon the protocol dated 30 June 1994. There is no mention of amendments.

Drug supplies are shown in Table 140 below.

Table 140. Drug supplies (Study 148-355).

	Lot		Lot
Placebo	3039-100	Sildenafil 25 mg	3039-135 3039-134 3039-133

The intent was to randomize 36 male subjects age >18, with erectile dysfunction of no established organic cause, of >6 months' duration, but able to attain an erection under some circumstances during a 3-week run-in period, and in a heterosexual relationship. Subjects were excluded for (1) advanced neurological or vascular causes for impotence, (2) history of alcohol abuse, (3) regular use of nitrates, anticoagulants, or aspirin, (4) need for antidepressants or major tranquilizers, (5) history of asthma, eczema, or drug hypersensitivity, (6) family history of bleeding disorder, active peptic ulcer disease, or migraines, (7) significant abnormality on screening lab or physical exam, (8) experimental drug use within 4 months, (9) recent or planned blood donation, or (10) HBsAg positivity.

At the end of a 3-week treatment-free run-in period during which baseline sexual performance data were collected, subjects were randomized to placebo or sildenafil 25 to 75 mg and followed for 4 weeks. Subjects were then switched to the other treatment and again followed for 4 weeks. Subjects were instructed to take study drug once per day. Subjects completed an event log noting time of study drug administration and subsequent sexual activity. Subjects completing study without serious adverse events were eligible to participate in a 52-week open-label study.

The primary efficacy assessment was at week 4. The primary end points were (1) median and minimum time between doses, (2) the number of doses, and (3) the frequency of erections adequate for penetration.

Routine safety data were recorded.

A44.5. Results

A44.5.1. Conduct

Forty-seven subjects were screened, 44 were randomized, and 43 (98%) completed both study phases.

Subjects had a mean age of 53 and all but 4 were Caucasian. The mean duration of impotence was about 3 years. Six subjects had received previous drug treatments for impotence.

Protocol violations included compliance outside targeted range (8) and drug abuse (1). Not all such subjects were excluded from the sponsor's 'evaluable subjects' analyses.

A44.5.2. Effectiveness

The average number of erections adequate for penetration was 1.4 per week on placebo and 4.2 per week on sildenafil. The number of such erections sexually stimulated was 0.8 per week on placebo and 2.4 per week on sildenafil ($p<0.0001$). The average number of doses per week showed a lesser treatment effect—2.6 on placebo and 3.4 on sildenafil ($p=0.001$). After adjustment for a period effect, based on the event log data, the sponsor estimated the odds of successful intercourse on sildenafil to be 12-times higher than on placebo.

Responders were much more likely to say that sildenafil improved erections than to say that placebo did so.

A44.5.3. Safety

Safety will be reviewed for all placebo-controlled experience together.

A44.5.4. Long-term

Documentation is incomplete. Thirty-two subjects entered the 52-week, long-term, open-label extension to Studies 148-355, 148-357, 148-358, 148-359, 148-360, and 166-301 (not reviewed). As of the cut-off date of 3 February 1997, 11 subjects had completed, and 21 subjects had withdrawn (6 for lack of effectiveness, 8 for loss to follow-up, 4 for withdrawal of consent, 1 for headache, and 2 for elevated hepatic transaminases). One subject reported vision abnormalities, not contributing to withdrawal. Common adverse events were headache (n=7), vasodilation/flushing (n=4), and dyspepsia (n=8).

A44.6. Summary

This was a pilot crossover study in subjects with erectile dysfunction of psychogenic etiology. Results were consistent with treatment effects observed in later and larger studies.

A45. Study 148-356: A multi-centre study consisting of a 16-week open, dose-escalation phase followed by an 8-week randomised, double-blind, placebo controlled phase to assess the efficacy and safety of oral doses of UK-92,480 (sildenafil) taken as required by patients with erectile dysfunction.

A45.1. Source documents

Study protocol NDA 20-895, vol 1.124; study report: NDA vol 1.124; electronic document: 46640637.pdf.

A45.2. Investigators

Multi-center study with 15 investigators in United Kingdom, France, and Norway.

A45.3. Study dates

24 November 1994 to 21 November 1995.

A45.4. Study design

This study description was based upon the amended protocol dated 31 August 1994. There were only minor amendments.

Drug supplies are shown in Table 141 below.

Table 141. Drug supplies (Study 148-356).

	Lot		Lot
Placebo	3039-100A	Sildenafil 10 mg	3039-132
		Sildenafil 25 mg	3039-133A 3039-134A 3039-135A

The intent was to randomize 200 male subjects age 18 to 70, with erectile dysfunction of >3 months' duration, but must have had at least one spontaneous or medically-induced erection within 4 weeks of screening. Subjects were excluded for (1) known vascular, neurological, endocrine or anatomical causes of erectile dysfunction, (2) regular use of nitrates, anticoagulants, major tranquilizers, estrogens, antiandrogens, or other drugs possibly contributing to erectile dysfunction, (3) elevated prolactin or low free testosterone, (4) history of major hematologic, renal, or hepatic disease, (5) history of stroke, subarachnoid hemorrhage, bleeding disorder, or peptic ulcer disease, (6) postural hypotension or blood pressure outside 90/50 to 170/110 mmHg, (7) diabetes if poorly controlled or possibly contributory to erectile dysfunction, (8) experimental drug use within 3 months, (9) alcohol abuse, (10) recent or planned blood donation, (11) clinical depression, or (12) other factors which might affect completion of study.

The study consisted of 3 phases. The first phase was a 2-week single-blind run-in period. The second phase was a 16-week open-label, dose titration phase. During this phase, subjects began treatment on sildenafil 10 mg and had opportunities to escalate or reduce the dose as indicated by effectiveness and tolerability step-wise to a maximum of 100 mg. The third and final phase was an 8-week, randomized, double-blind, parallel-group study in which subjects completing phase 2 were randomized to placebo or their optimum dose, as determined in the open-label phase. There was one clinic visit at 4 weeks prior to the final assessment at 8 weeks.

The primary efficacy assessment was at week 8. The primary end point was the proportion of subjects willing to continue to use their randomized treatment, were it available. Other effectiveness data were obtained in an event log and a sexual function questionnaire.

Routine safety data were recorded.

A45.5. Results

A45.5.1. Conduct

Two hundred and ninety-two subjects were screened, 233 were randomized and received open-label treatment, 205 entered double-blind treatment, and 202 (99%) completed study.

The mean age was 54, and all but 2 subjects were Caucasian. The mean duration of erectile dysfunction was 4.9 years. Erectile dysfunction was of psychogenic origin in 40% and of mixed psychogenic and organic origin in 60%. About half had received pervious treatment for erectile dysfunction. The two treatment groups were similar with respect to these attributes.

Eight subjects were discontinued from open-label study because of protocol violations. Other protocol violations appear to have been minor.

Of 31 subjects who withdrew during open-label or double-blind treatment, there was one death, 7 were for lack of effectiveness, and 7 were for adverse events.

A45.5.2. Effectiveness

At the end of the open-label phase 93% of subjects thought study drug had improved erections and 96% thought they would continue its use if it were available. The distribution of subjects on each dose was 1% on 10 mg, 11% on 25 mg, 29% on 50 mg, and 58% on 100 mg.

At the end of double-blind treatment, 40% of subjects on placebo (N=102) said they would continue using that treatment if it were available, compared with 85% randomized to sildenafil (N=93). Twenty-six percent of placebo subjects and 82% of sildenafil subjects said they thought the treatment improved their erections.

From the event logs, placebo subjects experienced a mean of 0.5 erections per week (sufficient for intercourse) compared with 1.4 per week on sildenafil.

The sexual function questionnaire tended to show highly statistically significant effects on questions pertaining to erectile function, but not on effects pertaining to sexual intercourse, although the trend there was in favor of study drug.

A45.5.3. Safety

Safety will be reviewed for all placebo-controlled experience together.

A45.5.4. Long-term

Documentation is incomplete. One hundred and forty-eight subjects entered the 52-week, long-term, open-label extension to Study 148-356. As of the cut-off date of 3 February 1997, 129 subjects had completed, and 19 subjects had withdrawn (2 for lack of effectiveness, 5 for loss to follow-up, and 1 each for left ventricular failure, drowsiness and headache, nasal congestion and pain, testicular seminoma, prostatic carcinoma, and myocardial infarction). Seven subjects reported vision abnormalities, generally described as moderate, with none contributing to withdrawal. Common adverse events were headache (10%), vasodilation/flushing (10%), and dyspepsia (15%).

A45.6. Summary

Subjects had erectile dysfunction of wholly or substantially psychogenic etiology. During the initial open-label phase, most subject migrated to the 100-mg dose. On this responder population, the placebo response rate (interest in continuing randomized treatment) was 40% (26% for belief that treatment improved erections). By all indications, the response rate for sildenafil was much higher.

A46. Study 148-357: A multi-centre, double blind, randomised, placebo controlled, three way crossover study to investigate the efficacy of single oral doses of sildenafil (UK-92,480) in diabetic patients with penile erectile dysfunction.

A46.1. Source documents

Study protocol NDA 20-895, vol 1.119; study report: NDA vol 1.119; electronic document: 46811438.pdf.

A46.2. Investigators

Multi-center study with 2 investigators in United Kingdom.

A46.3. Study dates

2 November 1994 to 11 April 1995.

A46.4. Study design

This study description was based upon the protocol dated 2 August 1994. There is no mention of amendments.

Drug supplies are shown in Table 142 below.

Table 142. Drug supplies (Study 148-357).

	Lot		Lot
Placebo	3039-100	Sildenafil 25 mg	979-12

The intent was to randomize 18 male subjects age >18, with a 5-year history of diabetes, and erectile dysfunction of >6 months' duration. Subjects were excluded for (1) poor diabetic control, (2) significant arterial disease, (3) regular use of nitrates, anticoagulants, or aspirin, (4) need for antidepressants or major tranquilizers, (5) history of asthma, eczema, or drug hypersensitivity, (6) family history of bleeding disorder, active peptic ulcer disease, or migraines, (7) alcohol abuse, or (8) severe, untreated diabetic retinopathy.

In a 3-phase crossover design, subjects received placebo, sildenafil 25 mg, and sildenafil 50 mg in random order. The first dose was administered in the clinic where subjects underwent penile plethysmography (Rigiscan) from 15 minutes prior to dosing to 2 hours post-dosing. Visual sexually stimulating materials were provided. Subjects were then discharged home on that dose and instructed to take study drug once daily, a half hour prior to usual sexual activity, for 10 days, maintaining a diary.

Plasma drug levels were measured during the in-clinic phases.

Routine safety data were recorded. In addition, subjects had assessments of heart rate variation during deep breathing, in response to Valsalva, and in response to standing.

A46.5. Results

A46.5.1. Conduct

Twenty-one subjects were randomized, and 20 (95%) completed all study phases.

Subjects had a mean age of 51 and all but 1 were Caucasian.

Protocol violations included diabetes diagnosed <5 years (4), poor diabetic control (1), and use of a different mode of Rigisan (4).

A46.5.2. Effectiveness

Rigiscan and diary data are summarized in Table 143 below. A greater proportion of subjects on sildenafil than on placebo reported improvement of erections (50%, similar for both doses, vs. 11%).

Sildenafil levels in plasma were dose-proportional at 2 hours. Metabolite UK-103,320 levels were about half as high as those of the parent drug at 2 hours, and also proportional to dose. Higher plasma levels of sildenafil were weakly positively correlated with longer duration and more frequent erections.

Table 143. Effectiveness data (Study 148-357).

	Plcbo	Sildenafil	
		25 mg	50 mg
Rigidity >60% (minutes)			
Base of penis	1.9	3.8	8.4
Tip of penis	1.3	2.7	4.3
Rigidity >80% (minutes)		0.4	1.0
Base of penis	0.4	0.6	0.9
Tip of penis	0.4		
Erections/week	0.6	0.8	1.6

A46.5.3. Safety

Safety will be reviewed for all placebo-controlled experience together.

A46.5.4. Long-term

Documentation is incomplete. Thirty-two subjects entered the 52-week, long-term, open-label extension to Studies 148-355, 148-357, 148-358, 148-359, 148-360, and 166-301 (not reviewed). As of the cut-off date of 3 February 1997, 11 subjects had completed, and 21 subjects had withdrawn (6 for lack of effectiveness, 8 for loss to follow-up, 4 for withdrawal of consent, 1 for headache, and 2 for elevated hepatic transaminases). One subject reported vision abnormalities, not contributing to withdrawal. Common adverse events were headache (n=7), vasodilation/flushing (n=4), and dyspepsia (n=8).

A46.6. Summary

This was a small pilot crossover study in subjects with diabetes mellitus as the likely etiology of their erectile dysfunction. Erectile function was improved in a dose-related manner.

A47. Study 148-358: A two stage, double blind, placebo-controlled study to assess the efficacy and safety of oral doses of sildenafil (UK-92,480) in spinal cord injury patients with erectile dysfunction.

A47.1. Source documents

Study protocol NDA 20-895, vol 1.120; study report: NDA vol 1.120; electronic document: 47062844.pdf.

A47.2. Investigators

Multi-center study with 3 investigators in United Kingdom.

A47.3. Study dates

13 June 1995 to 29 May 1996.

A47.4. Study design

This study description was based upon the protocol dated 13 January 1995. There is no mention of amendments.

Drug supplies are shown in Table 144 below.

Table 144. Drug supplies (Study 148-358).

	Lot		Lot
Placebo	3039-100	Sildenafil 25 mg	3039-135

The intent was to randomize 88 male subjects age 21 to 49, with a 6-month history of spinal cord injury, but having the ability to achieve an erection in response to a vibrator. Subjects were excluded for (1) anatomical defect or vascular or endocrine etiology to erectile dysfunction, (2) drugs associated with erectile dysfunction, (3) major hematologic, renal or hepatic dysfunction, (4) diabetes, (5) history of stroke, subarachnoid hemorrhage, bleeding disorder, or peptic ulcer disease, (6) spinal cord injury above T5 or T6 (because of risk of reflex hypertension in response to vibrator), (7) postural hypotension or blood pressure <90/50 mmHg, (8) regular use of nitrates or anticoagulants, (9) experimental drug use within 3 months, (10) alcohol abuse, (11) other serious medical, psychological, or social conditions apt to interfere with participation, or (12) recent or planned blood donation.

The study consisted of two phases. During the first phase, subjects participated in a double-blind, randomized, single-dose, 2-way cross-over study comparing placebo and sildenafil 50 mg. This study was conducted in the clinic. Subjects received up to 4-minutes of vibratory stimulation 0.5, 1, and 1.5 hours after study drug administration, with erectile function assessed by penile plethysmography (Rigiscan). Plasma drug levels were measured at 2 hours. The 2 study days were separated by at least 3 days. The second phase was a double-blind, randomized, placebo-controlled parallel-group study of home administration over 4 weeks. Subjects kept a diary and subjects and partners completed a sexual function questionnaire. The primary end point was whether subjects were interested in continued use of the drug at the end of 4 weeks.

Routine safety data were recorded.

A47.5. Results

A47.5.1. Conduct

Thirty-four subjects were screened, 27 were randomized, and 24 (89%) completed all study phases.

Subjects had a mean age of 33, a mean of a 6-year history of erectile dysfunction, and all were Caucasian. Spinal cord injury was described as complete (motor and sensory) in 14 subjects. Half of the subjects had previously received drug treatment for erectile dysfunction.

Only minor protocol violations were reported.

A47.5.2. Effectiveness

By questionnaire, treatment improved erections in 7% of subjects on placebo and 75% of subjects on sildenafil. Fifteen percent of subjects on placebo said they would continue to use randomized treatment, compared with 67% on sildenafil. The proportion of successful intercourse attempts was 38% on placebo and 67% on sildenafil. The number of attempts on the two treatments were not described. Subjects on placebo reported a mean of 0.4 erections per week; those on sildenafil reported 1.5 erections per week. The study was too small to yield useful results on the sexual function questionnaire, but the results were not inconsistent with larger studies. During the in-clinic phase, 4% of placebo and 46% of sildenafil subjects achieved erections with >60% rigidity.

A47.5.3. Safety

Safety will be reviewed for all placebo-controlled experience together.

A47.5.4. Long-term

Documentation is incomplete. Thirty-two subjects entered the 52-week, long-term, open-label extension to Studies 148-355, 148-357, 148-358, 148-359, 148-360, and 166-301 (not reviewed). As of the cut-off date of 3 February 1997, 11 subjects had completed, and 21 subjects had withdrawn (6 for lack of effectiveness, 8 for loss to follow-up, 4 for withdrawal of consent, 1 for headache, and 2 for elevated hepatic transaminases). One subject reported vision abnormalities, not contributing to withdrawal. Common adverse events were headache (n=7), vasodilation/flushing (n=4), and dyspepsia (n=8).

A47.6. Summary

This was a small pilot study in subjects with erectile dysfunction resulting from spinal cord injury, but still evidencing reflex activity. By questionnaire and event log, subjects showed treatment related improvements.

A48. Study 148-359: A 12 week, double blind, placebo controlled, parallel group, multicentre study to evaluate a new sexual function questionnaire in the assessment of the efficacy of sildenafil (UK-92,480) in patients with erectile dysfunction.

A48.1. Source documents

Study protocol NDA 20-895, vol 1.126; study report: NDA vol 1.126; electronic document: 47081108.pdf.

A48.2. Investigators

Multi-center study with 7 investigators in United Kingdom.

A48.3. Study dates

14 July 1995 to 13 June 1996.

A48.4. Study design

This study description was based upon an undated protocol. An undated amendment permitted titration of sildenafil to 100 mg.

Drug supplies are shown in Table 145 below.

Table 145. Drug supplies (Study 148-359).

	Lot		Lot
Placebo	3509-057	Sildenafil 25 mg	3509-079

The intent was to randomize 100 male subjects age >18, with a 6-month history of erectile dysfunction, erectile dysfunction evidenced by success <2/3 times during run-in period, and in stable heterosexual relationship for >6 months. Subjects were excluded for (1) advanced vascular, neurological, endocrine, or anatomical causes for erectile dysfunction, (2) regular use of nitrates or anticoagulants, (3) history of major hematologic, renal, or hepatic disease, (4) history of stroke, subarachnoid hemorrhage, bleeding disorder, or peptic ulcer disease, (5) experimental drug use within 3 months, (6) alcohol or drug dependence, (7) recent or planned blood donation, (8) significant abnormalities on screening labs or physical exam, or (9) other factors which might impact on ability to complete study.

After a 2- to 4-week treatment-free run-in period, subjects were randomized to placebo or sildenafil 25 mg and followed for 12 weeks, with clinic visits at the end of weeks 2, 4, and 8. At these visits, the dose could be increased to 50 mg if response was considered inadequate, and it could be dropped back to 25 mg if there were intolerable side effects. Effectiveness data included event logs and sexual function questionnaires. The primary end point was the proportion of subjects reporting an improvement in their erections at 12 weeks. Assessment of the sensitivity and specificity of the sexual function questionnaire was a secondary end point.

Routine safety data were recorded.

A48.5. Results

A48.5.1. Conduct

One hundred and twenty-seven subjects were screened, 111 were randomized, and 97 (87%) completed all study phases.

Subjects had a mean age of 56, a mean of a 4.5-year history of erectile dysfunction, and 93% were Caucasian. Erectile dysfunction was described as organic in 21%, psychogenic in 40%, and mixed in 39%. About 2/3 of subjects had pervious treatment for erectile dysfunction, mostly intracavernosal injection.

Protocol violations at baseline included advanced erectile dysfunction (3), >2/3 successes during run-in (1), use of intracavernosal injection during run-in (1). Violations after randomization included >1 dose per day (27), randomized period > 14 weeks (8), and issue of study drug at screening visit (5).

A48.5.2. Effectiveness

By questionnaire, treatment improved erections in 18% of subjects on placebo and 81% of subjects on sildenafil.

Results pertaining to the validation of the IIEF are discussed in *Development and validation of the primary efficacy instrument (International Index of Erectile Function; IIEF).* on page 87.

A48.5.3. Safety

Safety will be reviewed for all placebo-controlled experience together.

A48.5.4. Long-term

Documentation is incomplete. Thirty-two subjects entered the 52-week, long-term, open-label extension to Studies 148-355, 148-357, 148-358, 148-359, 148-360, and 166-301 (not reviewed). As of the cut-off date of 3 February 1997, 11 subjects had completed, and 21 subjects had withdrawn (6 for lack of effectiveness, 8 for loss to follow-up, 4 for withdrawal of consent, 1 for headache, and 2 for elevated hepatic transaminases). One subject reported vision abnormalities, not contributing to withdrawal. Common adverse events were headache (n=7), vasodilation/flushing (n=4), and dyspepsia (n=8).

A48.6. Summary

The sponsor has used this study for its (secondary end point) assessment of the validity of the sexual function questionnaire used in other major trials, but it is also entirely consistent in its demonstration of treatment effects assessed with that questionnaire, in subjects with psychogenic and organic erectile dysfunction.

A49. Study 148-360: A double-blind, randomised, placebo controlled, two-way crossover study to investigate the onset of action of single oral doses of UK-92,480 (sildenafil) 50mg in patients with penile erectile dysfunction without an established organic cause.

A49.1. Source documents

Study protocol NDA 20-895, vol 1.80; study report: NDA vol 1.80; electronic document: 46063109.pdf.

A49.2. Investigators

Single-center study with 1 investigator in the United Kingdom.

A49.3. Study dates

5 September 1995 to 13 February 1996.

A49.4. Study design

This study description was based upon the final study report, dated 15 July 1997.

A total of 18 subjects with a 6-month history of erectile dysfunction of no known organic cause, age 18 to 70, were to be recruited.

Subjects received, in random order, single doses of placebo or sildenafil 50 mg on study days 7 days apart. Subjects underwent a 1-hour penile plethysmography study (base only) accompanied by visual sexual stimulation, beginning 10 minutes after dosing. Blood samples were obtained for assessment of plasma sildenafil and UK-103,320 at the end of plethysmography.

Routine safety data were recorded.

A49.5. Results

A49.5.1. Conduct

Seventeen subjects were randomized and 16 completed both crossover phases of the study. Fifteen subjects reported spontaneous erections. Minor protocol violations and technical problems were described, but no subject was excluded from analyses.

A49.5.2. Pharmacokinetics

Mean plasma levels at 70 minutes after dosing were 255 ng/mL for sildenafil and 94 ng/mL for UK-103,320.

A49.5.3. Pharmacodynamics

Penile plethysmography data (Rigiscan) obtained during presentation of sexual stimulation showed that 14 subjects on sildenafil and 9 subjects on placebo had erections with >60% rigidity. Erections on sildenafil lasted 3.7-fold longer, on average. However, neither the response rate nor the duration of rigidity results represented statistically significant treatment effects.

As shown in Figure 68 below, there was a poor correlation between plasma levels of sildenafil at 70 minutes and the time to or duration of 60% rigidity.

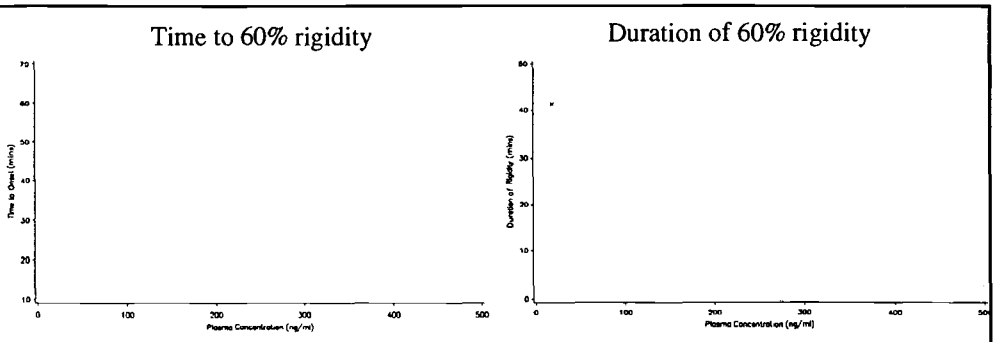


Figure 68. Dependence of erectile response on plasma sildenafil (Study 148-360).

Diary data showed a statistically significant increase in erections on sildenafil.

A49.5.4. Safety

One subject was hospitalized for paranoid psychosis 9 days after receiving sildenafil. Minor adverse events were reported with no clear relationship to treatment.

A49.5.5. Long-term

Documentation is incomplete. Thirty-two subjects entered the 52-week, long-term, open-label extension to Studies 148-355, 148-357, 148-358, 148-359, 148-360, and 166-301 (not reviewed). As of the cut-off date of 3 February 1997, 11 subjects had completed, and 21 subjects had withdrawn (6 for lack of effectiveness, 8 for loss to follow-up, 4 for withdrawal of consent, 1 for headache, and 2 for elevated hepatic transaminases). One subject reported vision abnormalities, not contributing to withdrawal. Common adverse events were headache (n=7), vasodilation/flushing (n=4), and dyspepsia (n=8).

A49.6. Summary

This was an early demonstration of effects of sildenafil on erectile function in subjects with no established organic cause.

A50. Study 148-361: A 12-week, double-blind, placebo controlled, parallel group, multi-centre study followed by a 40 week open label extension to evaluate the efficacy and safety of UK-92,480 (sildenafil) in patients with erectile dysfunction.

A50.1. Source documents

Study protocol NDA 20-895, vol 1.128; study report: NDA vol 1.128; electronic document: 47062943.pdf.

A50.2. Investigators

Multi-center study with 4 investigators in Australia.

A50.3. Study dates

9 January 1996 to 15 November 1996.

A50.4. Study design

This study description was based upon an undated protocol. There was one minor amendment.

Drug supplies are shown in Table 146 below.

Table 146. Drug supplies (Study 148-361).

	Lot		Lot
Placebo 25 mg	3509-060 3509-057	Sildenafil 25 mg	3509-115 3509-117 3509-080 3509-082 3509-090 3509-135 3509-184 3509-185 3509-136

The intent was to randomize 200 male subjects age >18, with erectile dysfunction of >6 months' duration, and in a heterosexual relationship for >6 months. Subjects were excluded for (1) anatomical deformities such as severe penile fibrosis, (2) other sexual disorders such as hypoactive sexual desire, (3) spinal cord injury, (4) poorly controlled diabetes or diabetic retinopathy, (5) major hematologic, renal, or hepatic dysfunction, (6) history of stroke or myocardial infarction within 6 months, (7) major psychiatric disorder or alcohol or drug abuse, (8) history of bleeding disorder or peptic ulcer disease, (9) cardiac failure, unstable angina, or life-threatening arrhythmia, (10) postural hypotension or blood pressure outside 90/50 to 170/110 mmHg, (11) clinically significant abnormalities on screening, (12) concomitant drugs associated with erectile dysfunction, (13) regular use of nitrates or anticoagulants, (14) other medical, psychological, or social impediments to study, (15) use of other treatments of erectile dysfunction, (16) experimental drug use within 3 months, and (17) recent or planned blood donation.

At the end of a 2-week treatment-free run-in period during which baseline sexual performance data were collected, subjects were randomized to placebo or sildenafil 50, 100, or 200 mg and followed for 12 weeks. Interim visits were scheduled at 2, 4, and 8 weeks. Subjects kept an event log and completed a sexual function questionnaire. Subjects completing study without an adverse event were eligible for a 40-week open-label follow-on study.

The primary end point was the response to IIEF question 1 (*How often were you able to get an erection during sexual activity?*), at 12 weeks.

Routine safety data were recorded.

A50.5. Results

A50.5.1. Conduct

Two hundred and seventy-seven subjects were screened, 254 were randomized, and 241 (95%) completed study.

Demographics of the 4 treatment groups are shown in Table 147 below. Fifty-six percent of subjects had received prior treatment for erectile dysfunction.

Table 147. Demographics (Study 148-361).

		Placebo N=59	Sildenafil 50 mg N=62	Sildenafil 100 mg N=66	Sildenafil 200 mg N=67
Race (%)	White	98	98	97	95
Age	Mean	60	57	57	58
	Range	44-74	32-74	34-77	38-75
Etiology (%)	Organic	49	48	52	46
	Psychogenic	8	5	5	10
	Mixed	41	47	44	43
Duration (y)	Mean	4.9	5.8	5.1	5.1

Twenty-five subjects were randomized in spite of various exclusion criteria, the most common of which were vision abnormalities (10).

A50.5.2. Effectiveness

All randomized subjects with a post-randomization assessment were included in the sponsor's ITT analyses. Responses to IIEF question 1 were scored as 0 for no attempts, 1 for never or rarely successful, etc., up to 5 for always or almost always successful. The reviewers' ITT analyses included all randomized subjects, assigning a worst rank to subjects with no assessment post-randomization[1]. Both the sponsor's and the reviewers' analyses were LOCF, which tends to make placebo, which had a higher withdrawal rate, better than it otherwise would be. Results are summarized in Table 148 below.

Table 148. ITT analyses of IIEF question1 (Study 148-361).

		Placebo N=59		Sildenafil 50 mg N=62		Sildenafil 100 mg N=66		Sildenafil 200 mg N=67		*P*
		n	Q	n	Q	n	Q	n	Q	
How often were you able to get an erection during sexual activity?	Baseline	—	2.1[a]	—	—	—	—	—	—	
	Week 12	58	2.1	61	3.8	64	3.8	66	3.9	<0.0001

a. This is apparently the pooled baseline value for all subjects.

Secondary end points from the other IIEF questions are described in Table 149 below (sponsor's analyses only). All treatment effects were highly statistically significant.

The global assessment by subjects whether treatment improved their erections, the original primary end point, was answered in the affirmative at week 12 by 13% on placebo, and 76 to 78% on sildenafil.

Event log analyses showed a mean of 0.6 erections per week on placebo and 1.4 erections per week on sildenafil (very similar results in the 3 active treatment groups).

1. Worst rank for all discontinuations?

Table 149. ITT analyses of non-primary IIEF questions at week 12 (Study 148-361)[a].

Domain	Question	Base-line	Placebo N=216		Sildenafil 25 mg N=102		50 mg N=107		100 mg N=107		*P*
			n	Q	n	Q	n	Q	n	Q	
Erectile function	Erections hard enough	1.9	58	2.0	61	3.5	64	3.8	66	3.8	<0.0001
	Frequency of penetration	1.9	58	1.9	61	3.4	64	3.7	66	3.7	<0.0001
	Erection maintained to completion	1.7	58	1.9	61	3.3	64	3.7	65	3.7	<0.0001
	Difficulty in maintaining erection	1.6	58	1.7	61	3.2	64	3.5	66	3.6	<0.0001
	Confidence in erection	1.7	57	1.9	61	3.2	64	3.4	65	3.4	<0.0001
Intercourse satisfaction	Attempted intercourse	1.5	58	1.8	61	2.6	64	2.8	66	2.9	<0.0001
	Satisfaction of intercourse	1.8	58	1.9	61	3.4	64	3.7	66	3.6	<0.0001
	Enjoyment of intercourse	1.8	58	1.8	61	2.8	64	3.1	66	3.3	<0.0001
Orgasmic function	Frequency of ejaculation	2.8	55	2.8	61	3.8	63	4.2	65	4.0	<0.0001
	Frequency of orgasm	2.8	55	2.7	61	3.7	63	4.1	65	3.9	<0.0001
Sexual desire	Frequency of desire	3.0	55	3.0	61	3.3	63	3.6	65	3.7	0.0005
	Rating of desire	2.8	56	2.8	61	3.0	63	3.4	66	3.5	0.0004
Overall satisfaction	Satisfaction with sex life	1.9	56	2.0	61	3.2	63	3.4	66	3.5	<0.0001
	Satisfaction with relationship	2.5	56	2.7	61	3.7	63	3.9	66	4.0	<0.0001

a. Sponsor's analyses.

A50.5.3. Safety

Safety will be reviewed for all placebo-controlled experience together.

A50.5.4. Long-term

Documentation is incomplete. Two hundred and twenty-seven subjects entered the 52-week, long-term, open-label extension to Study 148-361. As of the cut-off date of 3 February 1997, 0 subjects had completed, and 7 subjects had withdrawn (4 for lack of effectiveness, 2 for withdrawal of consent, and 1 for sinusitis and hot flushes). Four subjects reported visual disturbances, none of which led to withdrawal. Common adverse events were headache (1%), vasodilation/flushing (4%), and dyspepsia (2%).

A50.6. Summary

Subjects had erectile dysfunction of wholly or substantially organic etiology, excluding spinal cord injury. The primary end point was related to erectile function, but it and the usual secondary end points—other aspects of the sexual function questionnaire and sexual performance by event log—were highly statistically significant, internally consistent, and dose-related. Long-term data are not available.

A51. Study 148-363: A double-blind, randomised, placebo-controlled, parallel group, multi-centre, flexible dose escalation study to assess the efficacy and safety of UK-92,480 administered over six months to male patients with erectile dysfunction.

A51.1. Source documents

Study protocol IND vol 15.2; study report: NDA vol 1.98-1.100; electronic document: 47101479.pdf; SAS datasets.

A51.2. Investigators

Multi-center study with 25 investigators in Europe.

A51.3. Study dates

12 October 1995 to 22 September 1996.

A51.4. Study design

This study description was based upon the protocol dated 10 July 1995. There were two amendments; apparently the changes were indicated as italicized text in the submitted protocol.

Drug supplies are shown in Table 150 below.

Table 150. Drug supplies (Study 148-363).

	Lot		Lot
Placebo	3827-157 3827-158	Sildenafil 25 mg	3827-166 3827-167

The intent was to randomize 300 male subjects age >18, with erectile dysfunction[1] of >6 months' duration, and in a heterosexual relationship for >6 months. Subjects were excluded for (1) anatomical deformities such as severe penile fibrosis, (2) other sexual disorders such as hypoactive sexual desire, (3) elevated prolactin (3x ULN) or low free testosterone (20% below LLN), (4) major, uncontrolled psychiatric disorders, (5) history of alcohol or drug abuse, (6) history of major hematologic, renal, or hepatic disorder, (7) erectile dysfunction following spinal cord injury, (8) uncontrolled diabetes or diabetic retinopathy, (9) stroke or myocardial infarction within 6 months, (10) cardiac failure, unstable angina, ECG ischemia, or life-threatening arrhythmia within 6 months, (11) blood pressure outside 90/50 to 170/100 mmHg, (12) active peptic ulcer disease or bleeding disorder, (13) any clinically significant baseline laboratory abnormality, (14) need for anticoagulants, nitrates, androgens, or trazodone, (15) need for aspirin or NSAIDs and a history of peptic ulcer disease, (16) unwillingness to cease use of vacuum devices, intracavernosal injection, or other therapy for erectile dysfunction, other experimental drug use within 3 months, or (17) history of retinitis pigmentosa.

At the end of a 4-week treatment-free run-in period during which baseline sexual performance data were collected, subjects were randomized to placebo or sildenafil 25 mg and followed for 26 weeks. A 1:1 placebo:active randomization was implemented, although there were expected differences in the rate of withdrawal for lack of efficacy. Subjects were instructed to take study drug approximately one hour before planned sexual activity, not more than once per day. Alcohol use during this hour was discouraged. Subjects completed an event log noting time of study drug administration and subsequent sexual activity. At any visit, subjects who were intolerant of the starting dose were to be discontinued, and tolerant subjects with inadequate efficacy could have the dose doubled to 50 or 100 mg. Subjects completing study without an adverse event were eligible for participation in an open-label follow-on study.

The primary efficacy assessment was at week 12. At this visit, subjects completed a global assessment question, sexual function questionnaire (containing the primary

[1] 'the inability to attain and/or maintain penile erection sufficient for satisfactory sexual performance'

efficacy questions), and a quality of life questionnaire. Optionally, partners filled out another questionnaire.

No pharmacokinetic data were collected.

The study was sized to achieve 80% power at α=0.05 to detect a difference between a 70% improvement rate on the global assessment on active treatment against a 50% improvement rate on placebo. Randomization was not stratified.

The primary end point was the answer, at 12 weeks, to two questions on the sexual function questionnaire:

> *[3] Over the past 4 weeks, when you attempted sexual intercourse, how often were you able to penetrate (enter) your partner?*
>
> *[4] Over the past 4 weeks, during sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?*

Both questions had the same set of possible responses, either "did not attempt intercourse" or a 5-level semi-quantitative response. Analysis was to be by ANCOVA, based on table scores, where the "no attempt" response was lumped with the worst frequency category. Each question was to be analyzed separately with $p<0.05$ on both necessary for demonstrating efficacy. The model was to include terms for center, baseline, and "other covariates deemed to be appropriate". Any interim analyses were not to affect the ongoing trial.

The primary analysis was described as ITT with last observation carried forward. However, the sponsor's description of the ITT population includes only subjects with at least one observation post-randomization.

Secondary end points were (1) response to the global assessment question:

> *Has the treatment you have been taking over the past 4 weeks improved your erections? [yes] [no]*

(2) the responses to other sexual function questions (there were 13 in addition to the primary efficacy questions), (3) proportion of successful attempts at intercourse, determined from the event log, (4) responses on the optional partner questionnaire, (5) responses on the quality of life assessment, and (6) time to discontinuation for lack of efficacy.

Pharmacokinetic data were to be analyzed by nonlinear mixed-effect modeling (NONMEM) utilizing a large selection of baseline attributes as covariates.

Safety assessments included (1) ECGs at screening and week 12,(2) laboratory tests (CBC, SMA20, urinalysis), (3) vital signs, and (4) physical examination. Clinical adverse events and their relationship to the study drug were recorded.

A51.5. Results

A51.5.1. Conduct

Three hundred and seventy-eight subjects were screened, 315 were randomized, and 307 (64%) completed study. Individual sites enrolled 1 to 25 subjects.

Demographics of the 2 treatment groups are shown in Table 151 below. About 71% of all randomized subjects had received some therapy for erectile dysfunction.

Protocol violations are described in Table 152 below. Not all such subjects were excluded from the sponsor's 'evaluable subjects' analyses.

Table 151. Demographics (Study 148-363).

		Placebo N=156	Sildenafil N=159			Placebo N=156	Sildenafil N=159
Race (%)	White	95	97	Duration (y)	Mean	5.1	4.8
	Black	1.9	1.9		Range	1-27	1-35
	Other	3.2	0.6				
Age	Mean	54	55	Med hx (%)	Hypertension	19	21
	Range	23-82	24-77		Diabetes	15	16
Etiology (%)	Organic	30	29		Prostatectomy	12	10
	Psychogenic	32	31		Depression	4.5	8.2
	Mixed	35	38		IHD	6.4	13
	Other	3.8	1.9				

Table 152. Protocol violations (Study 148-363).

At randomization	n	On treatment	n
Missing baseline event log	36	>1 dose/day	51
Ethanol or drug abuse	15	Baseline ECG abn	6
Prohibited drug use	4	Prohibited drug use	8
Poorly controlled hypertension	2	Poorly controlled hypertension	3
		Mis-dosed	1
Total[a]	55	Total	63

a. Some subjects had more than one violation.

The disposition of subjects in the trial is shown in Figure 69 below, which shows the placebo group in the left panel and the active treatment group in the right panel. Most subjects remained in study for more than 24 weeks, but some "completed" several weeks early. As the sponsor predicted, fewer subjects on active treatment withdrew for lack of efficacy (or withdrew consent).

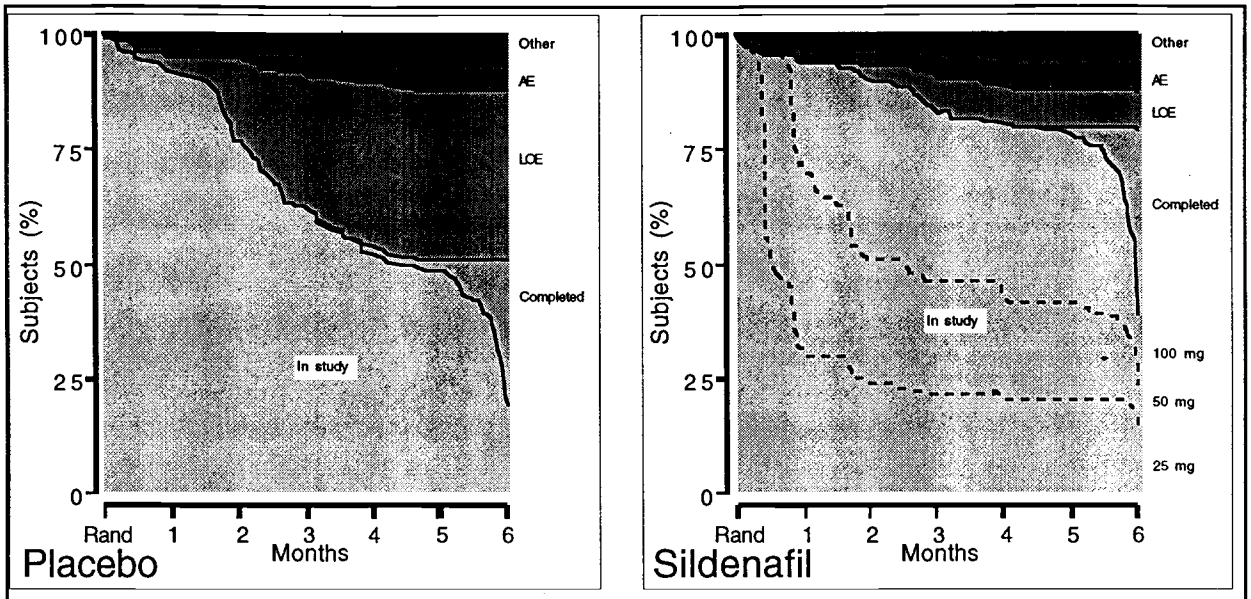


Figure 69. Disposition of subjects (Study 148-363).

The reviewers counted all subjects as "in study" until they reach a state in an all-inclusive set of mutually exclusive final states. In this particular case, the band labeled "LOE" (lack of efficacy) includes subjects who withdrew consent, the band labeled "AE" (adverse event) includes subjects withdrawn for laboratory abnormalities, and the "Other" band includes subjects withdrawn for protocol violations and subjects lost to follow-up. The dashed lines through the "in study" area of the active treatment group show the proportion of subjects on each dose.

A51.5.2. Effectiveness

All randomized subjects with a post-randomization assessment were included in the sponsor's ITT analyses. Responses to IIEF questions 3 and 4 were scored as 0 for no attempts, 1 for never or rarely successful, etc., up to 5 for always or almost always successful. The sponsor's analyses were LOCF, which tends to make placebo, which had a higher withdrawal rate, better than it otherwise would be. Results for week 12 are summarized in Table 153 below.

Table 153. ITT analyses of IIEF questions 3 and 4 (Study 148-363).

		Placebo N=156		Sildenafil N=159		*P*
		n	Q	n	Q	
How often were you able to penetrate your partner?	Baseline	—	1.9[a]	—	—	
	Week 12	101	2.2	124	3.5	<0.0001
	Week 24	130	2.2	144	3.7	<0.0001
How often were you able to maintain your erection after penetration?	Baseline	—	1.6	—	—	
	Week 12	116	2.1	136	3.5	<0.0001
	Week 24	128	2.1	144	3.6	<0.0001

a. Pooled baseline value for all subjects.

Secondary end points from the other IIEF questions are described in Table 154 below (sponsor's analyses only). All treatment effects were highly statistically significant, except for those pertaining to sexual interest, for which there was at least a trend in favor of increased interest on sildenafil.

About 25% of placebo and active group partners responded on the partner questionnaire at 12 and 26 weeks. There were statistically significant treatment effects, at 12 and 26 weeks, on questions to rate the partner's erections and satisfaction of sexual intercourse.

Table 154. ITT analyses of non-primary IIEF questions at week 12 (Study 148-363)[a].

Domain	Question	Base-line	Week 12					Week 26				
			Placebo N=156		Sildenafil N=159		*P*	Placebo N=156		Sildenafil N=159		*P*
			n	Q	n	Q		n	Q	n	Q	
Erectile function	Able to get erection	2.2	120	2.4	139	3.8	<0.0001	131	2.5	147	3.9	<0.0001
	Erections hard enough	1.9	117	2.1	138	3.6	<0.0001	128	2.2	145	3.7	<0.0001
	Difficulty maintaining erection	1.7	113	2.3	134	3.6	<0.0001	124	2.1	143	3.6	<0.0001
	Confidence in erection	2.0	120	2.2	137	3.4	<0.0001	130	2.2	146	3.4	<0.0001
Intercourse satisfaction	Attempted intercourse	2.1	121	2.7	139	2.9	0.4	131	2.7	146	3.1	0.01
	Satisfaction of intercourse	1.7	121	1.9	136	3.4	<0.0001	131	2.0	143	3.5	<0.0001
	Enjoyment of intercourse	1.9	121	2.2	138	3.0	<0.0001	131	2.1	145	3.1	<0.0001
Orgasmic function	Frequency of ejaculation	2.9	120	2.9	138	3.8	<0.0001	131	3.1	146	3.8	<0.0001
	Frequency of orgasm	2.6	119	2.7	137	3.7	<0.0001	130	2.9	145	3.7	<0.0001
Sexual desire	Frequency of desire	3.2	120	3.4	136	3.6	0.02	131	3.3	145	3.6	0.02
	Rating of desire	3.0	120	3.2	135	3.4	0.08	131	3.2	144	3.4	0.3
Overall satisfaction	Satisfaction with sex life	1.9	120	2.4	138	3.6	<0.0001	130	2.4	147	3.6	<0.0001
	Satisfaction with relationship	2.4	117	2.9	137	3.7	<0.0001	127	2.9	145	3.8	<0.0001

a. Sponsor's analyses.

The global assessment by subjects whether treatment improved their erections, the original primary end point, was answered in the affirmative at week 12 by 22% on placebo and 82% on sildenafil and at week 26 by 23% on placebo and 80% on sildenafil, both highly statistically significant differences.

The sponsor's analysis of the event logs focussed on the proportion of successful attempts at intercourse, but did not describe the number of such attempts by treatment group, or the success rate for subjects. Table 155 below shows the reviewers' analyses.

Table 155. Successful intercourse by event logs (Study 148-363).

	Placebo N=156	Sildenafil N=159
Attempts		
Total	6984	8978
Per subject mean	45	56
Successes		
Total	1780	5284
Per subject mean	11	33
Success by attempts (%)	25	59
Success by subjects (%)		
During run-in	33	38
During DB treatment	63	89

Among quality of life components, impact of erectile problems was highly statistically significantly on sildenafil at 12 and 26 weeks.

The reviewers also carried out an analysis of the primary end point on sub-groups defined by etiology of erectile dysfunction, duration of erectile dysfunction, history of nocturnal erections, history of prior treatment for erectile dysfunction, and history of diabetes mellitus. The results of ANCOVA analyses of the sildenafil-placebo difference

in score, after adjustment for baseline and age, are summarized in Table 156 below. The results are consistent with there being similar treatment effects regardless of classification of etiology, presence or absence of nocturnal erections, previous use of drugs or devices for treatment of erectile dysfunction, duration of erectile dysfunction, or history of diabetes.

Table 156. Sub-group analyses of IIEF questions 3 and 4[a] (Study 148-363).

	N	How often were you able to penetrate your partner?				How often were you able to maintain your erection after penetration?			
		Factors[b]	Pcbo	Sil	*P*	Factors	Pcbo	Sil	*P*
Etiology		Baseline				Baseline			
Organic	91	Age	0.7	1.5	0.0001	Age	0.3	2.0	0.0001
Psychogenic	100		0.3	1.5	0.0001		0.3	1.7	0.0001
Mixed	114		0.7	1.7	0.0001		0.2	1.8	0.0001
Other	9		-0.3	3.0	0.03		0.2	1.5	0.36
Nocturnal erections		Baseline				Baseline			
Yes	181	Age	0.3	1.7	0.0001	Age	0.3	1.8	0.0001
No	112		0.1	1.7	0.0001		0.3	1.9	0.0001
Unknown	21		0.5	1.3	0.27		0.3	1.5	0.14
Duration		Baseline				Baseline			
<3 years	134	Age	0.1	1.9	0.0001	Age	0.5	1.6	0.0001
>3 years	180	Tx*duration	0.3	1.4	0.0001	Tx*duration	0.1	1.9	0.0001
Previous treatment		Baseline				Baseline			
Yes	255	Age	0.2	1.7	0.0001	Age	0.2	1.8	0.0001
No	59		0.3	1.5	0.002		0.4	1.6	0.02
Diabetes mellitus		Baseline				Baseline			
Yes	43	Age	0.1	2.1	0.0001	Age	0.0	1.8	0.0001
No	269		0.2	1.6	0.0001		0.3	1.8	0.0001

a. Reviewers' LOCF analyses; sildenafil-placebo difference in score, after adjustment for baseline and age, classified as <55 or >55.
b. Statistically significant effects ($P<0.05$) by ANCOVA from among baseline score, age classified as <55 or >55, sub-grouping (etiology, etc.), treatment by age (Tx*age) interaction, or treatment by sub-grouping.

A51.5.3. Safety

Safety will be reviewed for all placebo-controlled experience together.

A51.5.4. Long-term

Documentation is incomplete. Two hundred and three subjects entered the 26-week, long-term, open-label extension toStudy 148-363. As of the cut-off date of 3 February 1997, 0 subjects had completed, and 9 subjects had withdrawn (7 for lack of effectiveness, 1 for increased transaminases[2], and 1 for loss to follow-up). One subject reported vision abnormalities, described as severe, but not leading to withdrawal. Common adverse events were headache (6%), vasodilation/flushing (3%), and dyspepsia (2%).

A51.6. Summary

Erectile dysfunction was equally attributable to organic, psychogenic, and mixed etiologies. About one-third of subjects had successful sexual intercourse in the run-in period, and two-thirds of placebo group subjects had some success post-randomization, but one-third of placebo subjects withdrew for lack of effectiveness by 26 weeks. Treatment effects were highly statistically significant and internally consistent in favor of sildenafil at 12 and 26 weeks. Subjects on active treatment distributed approximately equally among the 25-, 50-, and 100-mg doses.

2. Transaminase levels were acutely 3-fold above ULN andreturned to normal range over 2 months.

Center for Drug Evaluation and Research

Viagra (Sildenafil)

"Joint Clinical Review" for NDA-20-895

Appendix A52, page 212 through Appendix A56, page 224

A52. Study 148-364: A double-blind, randomised, placebo-controlled, parallel group, multi-centre study to assess the efficacy and safety of fixed doses of sildenafil administered for three months to male patients with erectile dysfunction.

A52.1. Source documents

Study protocol IND vol 15.2; study report: NDA vol 1.101-1.103; electronic document: 46161691.pdf; SAS datasets.

A52.2. Investigators

Multi-center study with 32 investigators in Europe.

A52.3. Study dates

2 May 1996 to 19 November 1996.

A52.4. Study design

This study description was based upon the protocol dated 2 February 1995. There was one amendment; apparently the changes were indicated as italicized text in the submitted protocol.

Drug supplies are shown in Table 157 below.

Table 157. Drug supplies (Study 148-364).

	Lot		Lot
Placebo 25 mg	4469-101	Sildenafil 25 mg	4469-120
Placebo 50 mg	4469-066 4469-067	Sildenafil 50 mg	4469-121
Placebo 100 mg	4469-091	Sildenafil 100 mg	4469-122

The intent was to randomize 460 male subjects age >18, with erectile dysfunction[1] of >6 months' duration, and in a heterosexual relationship for >6 months. Subjects were excluded for (1) anatomical deformities such as severe penile fibrosis, (2) other sexual disorders such as hypoactive sexual desire, (3) elevated prolactin (3x ULN) or low free testosterone (20% below LLN), (4) major, uncontrolled psychiatric disorders, (5) history of alcohol or drug abuse, (6) history of major hematologic, renal, or hepatic disorder, (7) erectile dysfunction following spinal cord injury, (8) uncontrolled diabetes or diabetic retinopathy, (9) stroke or myocardial infarction within 6 months, (10) cardiac failure, unstable angina, ECG ischemia, or life-threatening arrhythmia within 6 months, (11) blood pressure outside 90/50 to 170/100 mmHg, (12) active peptic ulcer disease or bleeding disorder, (13) any clinically significant baseline laboratory abnormality, (14) need for anticoagulants, nitrates, androgens, or trazodone, (15) need for aspirin or NSAIDs and a history of peptic ulcer disease, (16) unwillingness to cease use of vacuum devices, intracavernosal injection, or other therapy for erectile dysfunction, other experimental drug use within 3 months, or (17) history of retinitis pigmentosa.

At the end of a 4-week treatment-free run-in period during which baseline sexual performance data were collected, subjects were randomized to placebo or sildenafil 25, 50, or 100 mg and followed for 12 weeks. A 1:1:1:1 randomization was implemented, although there were expected differences in the rate of withdrawal for lack of efficacy. Subjects were instructed to take study drug approximately one hour before planned sexual activity, not more than once per day. Alcohol use during this hour was discouraged. Subjects completed an event log noting time of study drug administration and subsequent sexual activity. Subjects completing study without an adverse event were eligible for participation in an open-label follow-on study.

The primary efficacy assessment was at week 12. At this visit, subjects completed a global assessment question, sexual function questionnaire (containing the primary

[1] 'the inability to attain and/or maintain penile erection sufficient for satisfactory sexual performance'

efficacy questions), and a quality of life questionnaire. Optionally, partners filled out another questionnaire.

Pharmacokinetic data (plasma samples) were collected at weeks 4, 8, and 12.

The study was sized to achieve 90% power at α=0.05 to detect a difference of 0.75 on both primary end point questions. Randomization was not stratified.

The primary end point was the answer, at 12 weeks, to two questions on the sexual function questionnaire:

> *[3] Over the past 4 weeks, when you attempted sexual intercourse, how often were you able to penetrate (enter) your partner?*
>
> *[4] Over the past 4 weeks, during sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?*

Both questions had the same set of possible responses, either "did not attempt intercourse" or a 5-level semi-quantitative response. Analysis was to be by ANCOVA, based on table scores, where the "no attempt" response was lumped with the worst frequency category. Each question was to be analyzed separately with $p<0.05$ on both necessary for demonstrating efficacy. The model was to include terms for center, baseline, and "other covariates deemed to be appropriate". The primary test was a single-degree-of-freedom test for a linear trend by dose. Any interim analyses were not to affect the ongoing trial.

The primary analysis was described as ITT with last observation carried forward. However, the sponsor's description of the ITT population includes only subjects with at least one observation post-randomization.

Secondary end points were (1) response to the global assessment question:

> *Has the treatment you have been taking over the past 4 weeks improved your erections? [yes] [no]*

(2) the responses to other sexual function questions (there were 13 in addition to the primary efficacy questions), (3) proportion of successful attempts at intercourse, determined from the event log, (4) responses on the optional partner questionnaire, (5) responses on the quality of life assessment, and (6) time to discontinuation for lack of efficacy.

Pharmacokinetic data were to be analyzed by nonlinear mixed-effect modeling (NONMEM) utilizing a large selection of baseline attributes as covariates.

Safety assessments included (1) ECGs at screening and week 12, (2) laboratory tests (CBC, SMA20, urinalysis), (3) vital signs, and (4) physical examination. Clinical adverse events and their relationship to the study drug were recorded.

A52.5. Results

A52.5.1. Conduct

Five hundred and sixty-four subjects were screened, 514 were randomized, and 484 (94%) completed study. Individual sites enrolled 4 to 42 subjects.

Demographics of the 2 treatment groups are shown in Table 158 below. About half of all randomized subjects had received some therapy for erectile dysfunction.

Protocol violations are described in Table 159 below. Not all such subjects were excluded from the sponsor's 'evaluable subjects' analyses.

Table 158. Demographics (Study 148-364).

		Placebo	Sildenafil					Placebo	Sildenafil		
		N=127	25 mg N=128	50 mg N=132	100 mg N=127			N=127	25 mg N=128	50 mg N=132	100 mg N=127
Race (%)	White	98	98	99	97	Duration (y)	Mean	5.0	4.5	4.6	5.0
	Black	0	0.8	1.8	0.8		Range	0.6-30	0.5-30	0.5-40	0.5-30
	Other	1.6	1.6	0	2.4						
Age	Mean	55	55	57	56	Med hx (%)	Hypertension	14	17	11	15
	Range	20-77	19-74	30-76	25-79		Diabetes	10	7.8	9.8	7.1
Etiology (%)	Organic	46	44	41	39		Prostatectomy	19	14	14	12
	Psychogenic	29	28	36	35		Depression	0.8	1.6	2.3	1.6
	Mixed	24	28	23	25		IHD	2.4	1.6	1.6	0.8

Table 159. Protocol violations (Study 148-364).

At randomization		On treatment	
	n		n
Missing baseline evaluations	20	>1 dose/day	46
Ethanol or drug abuse	1	Baseline ECG abn	6
Lack of consent	4	Prohibited drug use	9
Poorly controlled hypertension	6	Poorly controlled hypertension	2
Low testosterone	1	Mis-dosed	2
		Blind broken for AE	2
		Mis-diagnosed or other	4
Total[a]	33	Total	62

a. Some subjects had more than one violation.

The disposition of subjects in the trial is shown in Figure 70 below, which shows the placebo group in the left panel and all active treatment groups combined in the right panel. Most subjects remained in study for more than 24 weeks, but some "completed" several weeks early. As the sponsor predicted, fewer subjects on active treatment withdrew for lack of efficacy (or withdrew consent).

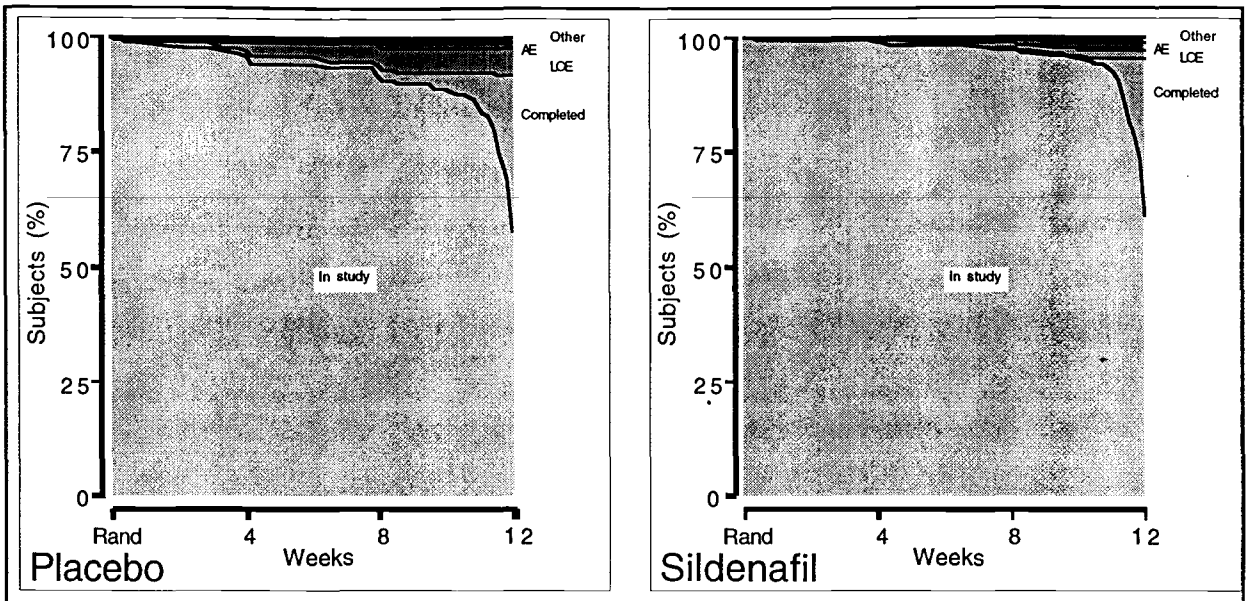


Figure 70. Disposition of subjects (Study 148-364).

The reviewers counted all subjects as "in study" until they reach a state in an all-inclusive set of mutually exclusive final states. In this particular case, the band labeled "LOE" (lack of efficacy) includes subjects who withdrew consent, the band labeled "AE" (adverse event) includes subjects withdrawn for laboratory abnormalities, and the "Other" band includes subjects withdrawn for protocol violations and subjects lost to follow-up.

A52.5.2. Effectiveness

All randomized subjects with a post-randomization assessment were included in the sponsor's ITT analyses. Responses to IIEF questions 3 and 4 were scored as 0 for no attempts, 1 for never or rarely successful, etc., up to 5 for always or almost always successful. The sponsor's analyses were LOCF, which tends to make placebo, which had a higher withdrawal rate, better than it otherwise would be. Results for week 12 are summarized in Table 160 below.

Table 160. ITT analyses of IIEF questions 3 and 4 (Study 148-364).

		Placebo N=127		Sildenafil 25 mg N=128		Sildenafil 50 mg N=132		Sildenafil 100 mg N=127		P
		n	Q	n	Q	n	Q	n	Q	
How often were you able to penetrate your partner?	Baseline	—	2.2[a]	—	—	—	—	—	—	
	Week 12	117	2.2	121	3.2	123	3.7	120	3.8	<0.0001
How often were you able to maintain your erection after penetration?	Baseline	—	1.8	—	—	—	—	—	—	
	Week 12	115	2.0	119	3.0	122	3.4	118	3.6	<0.0001

a. Pooled baseline value for all subjects.

Secondary end points from the other IIEF questions are described in Table 161 below (sponsor's analyses only). All treatment effects were highly statistically significant.

About 1/3 of placebo and active group partners responded on the partner questionnaire at 12 weeks. There were statistically significant treatment effects on questions to rate the partner's erections and satisfaction of sexual intercourse.

The global assessment by subjects whether treatment improved their erections, the original primary end point, was answered in the affirmative at week 12 by 24% on

Table 161. ITT analyses of non-primary IIEF questions at week 12 (Study 148-364)[a].

Domain	Question	Base-line	Placebo N=127		Sildenafil 25 mg N=128		50 mg N=132		100 mg N=127		*P*
			n	Q	n	Q	n	Q	n	Q	
Erectile function	Able to get erection	2.5	118	2.4	123	3.4	125	3.7	120	3.9	<0.0001
	Erections hard enough	2.2	118	2.2	123	3.3	125	3.6	117	3.9	<0.0001
	Erection maintained to completion	1.7	114	1.9	118	3.1	124	3.6	118	3.6	<0.0001
	Confidence in erection	2.0	117	2.3	120	3.0	123	3.2	117	3.5	<0.0001
Intercourse satisfaction	Attempted intercourse	2.0	114	2.4	120	3.0	123	3.1	117	3.3	<0.0001
	Satisfaction of intercourse	1.9	114	2.1	118	3.1	122	3.5	116	3.8	<0.0001
	Enjoyment of intercourse	2.0	113	2.2	118	2.9	123	3.4	117	3.4	<0.0001
Orgasmic function	Frequency of ejaculation	3.0	118	3.2	118	3.5	121	3.9	120	4.0	<0.0001
	Frequency of orgasm	2.8	118	2.8	117	3.4	121	3.6	119	3.8	<0.0001
Sexual desire	Frequency of desire	3.3	116	3.2	120	3.2	123	3.5	119	3.6	0.001
	Rating of desire	3.1	116	3.1	118	3.2	123	3.3	119	3.4	0.01
Overall satisfaction	Satisfaction with sex life	2.1	118	2.3	118	3.1	123	3.4	117	3.6	<0.0001
	Satisfaction with relationship	2.6	118	2.9	116	3.3	122	3.7	116	3.8	<0.0001

a. Sponsor's analyses.

placebo, 67% on sildenafil 25 mg, 78% on 50 mg, and 86% on 100 mg, a highly statistically significant difference.

The sponsor's analysis of the event logs focussed on the proportion of successful attempts at intercourse, but did not describe the number of such attempts by treatment group, or the success rate for subjects. Table 162 below shows the reviewers' analyses.

Table 162. Successful intercourse by event logs (Study 148-364).

	Placebo N=127	Sildenafil 25 mg N=128	50 mg N=132	100 mg N=127
Attempts				
Total	3705	4313	4192	4062
Per subject mean	29	34	32	32
Successes				
Total	481	1635	1808	1879
Per subject mean	3.8	13	14	15
Success by attempts (%)	13	38	43	46
Success by subjects (%)				
During run-in	32	42	33	31
During DB treatment	53	79	91	82

Among quality of life components, general health and well-being showed small but statistically significant effects, and impact of erectile problems was highly statistically significantly better on sildenafil at 12 weeks.

The reviewers also carried out an analysis of the primary end point on sub-groups defined by etiology of erectile dysfunction, duration of erectile dysfunction, history of nocturnal erections, history of prior treatment for erectile dysfunction, and history of

diabetes mellitus. The results of comparisons of the slope of the dose-response curves (change in score per g) are summarized in Table 163 below. The results are consistent with there being similar treatment effects regardless of classification of etiology, presence or absence of nocturnal erections, previous use of drugs or devices for treatment of erectile dysfunction, or duration of erectile dysfunction. Of the factors evaluated, only subjects with a history of diabetes mellitus appeared to have a reduced treatment effect, as indicated by smaller estimates of the slope in subjects with diabetes and the lack of nominal statistical significance for the slope.

Table 163. Sub-group analyses of IIEF questions 3 and 4[a] (Study 148-364).

	N	How often were you able to penetrate your partner?				How often were you able to maintain your erection after penetration?			
		Factors[b]	Intcpt	Slope	P[c]	Factors	Intcpt	Slope	P
Etiology		Baseline				Baseline			
Organic	165		0.1±0.2	13±3	0.0001		0.4±0.2	12±3	0.003
Psychogenic	129		0.3±0.2	15±3	0.0001		0.6±0.2	15±3	0.0001
Mixed	219		0.5±0.2	11±3	0.001		0.6±0.2	12±3	0.003
Nocturnal erections		Baseline				Baseline			
Yes	335	Noct	0.2±0.1	16±2	0.0001	Tx*noct	0.4±0.1	17±2	0.0001
No	151	Tx*noct	0.4±0.2	11±3	0.002		0.7±0.2	8±3	0.02
Unknown	26		1.6±0.5	−11±7	0.15		1.7±0.5	−10±7	0.19
Duration		Baseline				Baseline			
<3 years	177	Duration	0.6±0.2	8±3	0.008	Duration	0.7±0.2	11±3	0.0007
>3 years	336		0.2±0.1	15±2	0.002		0.5±0.1	13±2	0.002
Previous treatment		Baseline				Baseline			
Yes	375		0.4±0.1	12±2	0.0001		0.6±0.1	12±2	0.0001
No	138		0.3±0.2	15±4	0.0001		0.5±0.2	14±3	0.0001
Diabetes mellitus		Baseline				Baseline			
Yes	44	Diabetes	0.3±0.3	2±5	0.72		0.4±0.2	5±4	0.26
No	469		0.4±0.1	14±2	0.0001		0.6±0.1	13±2	0.0001

a. Reviewers' LOCF analyses; slope of dose-response (change in score per g)
b. Statistically significant effects ($P<0.05$) by ANCOVA from among baseline score, age classified as <55 or >55, sub-grouping (etiology, etc.), treatment by age (Tx*age) interaction, or treatment by sub-grouping.
c. P-value for non-zero slope to dose-response analysis of treatment alone.

A52.5.3. Safety

Safety will be reviewed for all placebo-controlled experience together.

A52.6. Summary

Erectile dysfunctions were evenly distributed among organic, psychogenic, and mixed etiologies. One-third had erections sufficient for sexual intercourse during the run-in period. Assessed by sexual function questionnaire or event log, there was a highly statistically significant, internally consistent, and dose-related improvement in erectile function at 12 weeks.

A53. Study 148-367: A double-blind, randomised, placebo-controlled, two way cross-over, flexible dose study to assess the efficacy and safety of oral doses of sildenafil in patients with erectile dysfunction caused by traumatic injuries to the spinal cord.

A53.1. Source documents

Study protocol INT vol 15.2; study report: NDA vol 1.121-1.123; electronic document: 46005090.pdf; SAS datasets.

A53.2. Investigators

Multi-center study with 19 investigators in Europe and Australia.

A53.3. Study dates

3 June 1996 to 24 January 1997.

A53.4. Study design

This study description was based upon the protocol dated 4 March 1996. There were no amendments.

Drug supplies are shown in Table 164 below.

Table 164. Drug supplies (Study 148-367).

	Lot		Lot
Placebo	4469-101	Sildenafil 25 mg	4469-144

The intent was to randomize 150 male subjects age >18, with erectile dysfunction[1] of >6 months' duration, and in a heterosexual relationship for >6 months. Subjects were excluded for (1) anatomical deformities such as severe penile fibrosis, (2) other sexual disorders such as hypoactive sexual desire, (3) elevated prolactin (3x ULN) or low free testosterone (20% below LLN), (4) major, uncontrolled psychiatric disorders, (5) history of alcohol or drug abuse, (6) history of major hematologic, renal, or hepatic disorder, (8) uncontrolled diabetes or diabetic retinopathy, (9) stroke or myocardial infarction within 6 months, (10) cardiac failure, unstable angina, ECG ischemia, or life-threatening arrhythmia within 6 months, (11) blood pressure outside 90/50 to 170/100 mmHg, (12) active peptic ulcer disease or bleeding disorder, (13) any clinically significant baseline laboratory abnormality, (14) need for anticoagulants, nitrates, androgens, or trazodone, (15) need for aspirin or NSAIDs and a history of peptic ulcer disease, (16) unwillingness to cease use of vacuum devices, intracavernosal injection, or other therapy for erectile dysfunction, other experimental drug use within 3 months, or (17) history of retinitis pigmentosa.

At the end of a 4-week treatment-free run-in period during which baseline sexual performance data were collected, subjects were randomized to order of receiving placebo or sildenafil 50 mg, with the two 6-week treatment periods separated by a 2-week wash-out period. Subjects were instructed to take study drug approximately one hour before planned sexual activity, not more than once per day. Alcohol use during this hour was discouraged. Subjects completed an event log noting time of study drug administration and subsequent sexual activity. At any visit, subjects who were intolerant of the starting dose were to be discontinued, and tolerant subjects with inadequate efficacy could have the dose doubled to 50 or 100 mg. Subjects completing study without an adverse event were eligible for participation in an open-label follow-on study.

The primary efficacy assessment was at week 6. At this visit, subjects completed a global assessment question, sexual function questionnaire (containing the primary efficacy questions), and a quality of life questionnaire. Optionally, partners filled out another questionnaire.

No pharmacokinetic data were collected.

[1] 'the inability to attain and/or maintain penile erection sufficient for satisfactory sexual performance'

The study was sized to achieve 90% power at α=0.05 to detect a difference in preference for study drug of 70% vs. 30% for placebo. Randomization was not stratified.

The primary end point was the answer at study end to the question of which treatment was preferred and who indicated that the treatment improved their erections, for subjects with some psychogenic or reflexogenic function. Analysis was to be by logistic regression for binary data. Any interim analyses were not to affect the ongoing trial.

Secondary end points for subjects with some psychogenic or reflexogenic function were (1) responses to the sexual function questionnaire, (2) event log responses, (3) partner responses, and (4) quality of life questionnaire. Secondary end points for all subjects included (1) the preferred treatment, and (2) responses to the sexual function questionnaire.

Pharmacokinetic data were not collected.

Safety assessments included (1) ECGs at screening and week 12,(2) laboratory tests (CBC, SMA20, urinalysis), (3) vital signs, and (4) physical examination. Clinical adverse events and their relationship to the study drug were recorded.

A53.5. Results

A53.5.1. Conduct

One hundred and eighty-three subjects were screened, 178 were randomized, and 168 (94%) completed both crossover phases of the study. Individual sites enrolled 1 to 22 subjects.

Demographics of the 2 treatment groups are shown in Table 165 below. About 55% of all randomized subjects had received previous drug therapy for erectile dysfunction, and about 12% had used non-drug treatments.

Table 165. Demographics (Study 148-367).

		First treatment	
		Placebo N=89	Sildenafil N=89
Race (%)	White	98	97
	Black	1.1	1.1
	Other	1.1	2.2
Age	Mean	38	38
	Range	19-63	21-61
Etiology (%)	Central cord	39	
	Brown-Sequard	2.2	
	Anterior cord	2.2	
	Conus medularis	4.5	
	Cauda equina	8.4	
	Unknown or other	43	

		First treatment	
		Placebo N=89	Sildenafil N=89
Duration (y)	Mean	10.3±8.5	11.7±9.0
	Range	0.7-35	0.7-38
Med hx (%)	Urinary tract infection	12	15
	Depression	5.6	3.4
	Hypertension	4.5	3.4
	Peripheral vascular disease	2.2	2.2
	Ischemic heart disease	2.2	1.1
	TURP	1.1	1.1
	Diabetes type II	0	1.1

Protocol violations are described in Table 166 below. No such subjects were excluded from the sponsor's 'evaluable subjects' analyses.

Table 166. Protocol violations (Study 148-367).

At randomization	n	On treatment	n
Prohibited meds	52	>1 dose/day	16
Baseline lab abn	1	Other dosing viol	64
Ethanol or drug abuse	2		
Hypotension	2		
Poorly controlled hypertension	1		
Total[a]	57	Total	80

a. Some subjects had more than one violation.

A53.5.2. Effectiveness

The primary end point was the subject's preference for a treatment, analyzed for subjects with some residual erectile function. Of 145 such subjects who received both treatments, 118 expressed a preference, of whom 111 subjects preferred sildenafil.

Preference for treatment among all subjects was a secondary end point. Of 171 subjects who received both treatments, 134 subjects expressed a preference, and, for 127 subjects, the preference was for sildenafil.

Other secondary end points included the IIEF questions, as described in Table 167 below (sponsor's analyses only). All treatment effects were highly statistically significant.

Table 167. ITT analyses of IIEF questions at weeks 6 and 12 (Study 148-367)[a].

Domain	Question	Subjects with residual erectile function						All subjects					
		Baseline	Placebo		Sildenafil		*P*	Baseline	Placebo		Sildenafil		*P*
			n	Q	n	Q			n	Q	n	Q	
Erectile function	Able to get erection	2.6	132	2.6	130	4.1	<0.0001	2.4	156	2.4	153	4.0	<0.0001
	Erections hard enough	2.6	130	2.4	130	4.0	<0.0001	2.3	155	2.2	154	3.7	<0.0001
	Able to penetrate	2.2	133	2.4	131	4.1	<0.0001	2.0	158	2.2	155	3.8	<0.0001
	Erection after penetration	1.7	133	1.8	131	3.8	<0.0001	1.5	158	1.7	155	3.6	<0.0001
	Erection to completion	1.5	133	1.7	131	3.7	<0.0001	1.4	157	1.6	155	3.5	<0.0001
	Confidence in erection	1.9	132	2.0	131	3.6	<0.0001	1.9	156	1.9	155	3.5	<0.0001
Intercourse satisfaction	Attempted intercourse	1.8	133	2.7	131	3.3	<0.0001	1.6	158	2.6	155	3.2	<0.0001
	Satisfaction of intercourse	1.8	133	2.0	131	3.6	<0.0001	1.6	158	1.9	155	3.5	<0.0001
	Enjoyment of intercourse	1.9	133	2.2	131	3.3	<0.0001	1.8	158	2.1	155	3.2	<0.0001
Orgasmic function	Frequency of ejaculation	1.9	132	1.8	130	2.2	0.002	1.9	155	1.8	152	2.1	0.001
	Frequency of orgasm	1.8	131	1.8	129	2.5	<0.0001	1.8	155	1.8	152	2.5	<0.0001
Sexual desire	Frequency of desire	3.7	133	3.3	131	3.6	<0.0001	3.7	158	3.3	155	3.7	<0.0001
	Rating of desire	3.7	133	3.2	131	3.6	<0.0001	3.7	158	3.3	155	3.6	<0.0001
Overall satisfaction	Satisfaction with sex life	2.7	132	2.5	131	3.9	<0.0001	2.6	157	2.5	155	3.8	<0.0001
	Satisfaction with relationship	3.0	132	3.0	131	4.0	<0.0001	2.9	157	2.9	155	3.9	<0.0001

a. Sponsor's analyses.

About 50% of partners responded on the partner questionnaire. There were statistically significant treatment effects on questions to rate the partner's erections and satisfaction of sexual intercourse.

The only aspect of the quality of life assessment nominally showing a treatment effect pertained to the impact of erectile problems.

The sponsor's analysis of the event logs focussed on the proportion of successful attempts at intercourse, but did not describe the number of such attempts by treatment group, or the success rate for subjects. The reviewers did not analyze event log data for this study, either, because of difficulty determining when subjects moved from one crossover period to the other.

A53.5.3. Safety

Safety will be reviewed for all placebo-controlled experience together.

A53.6. Summary

Subjects all had spinal cord injury as the etiology of erectile dysfunction, but with intact spinal cord-mediated reflexes. Other risk factors for erectile dysfunction were rare. The study showed highly statistically significant and internally consistent treatment-related effects on erectile function and sexual performance, as assessed by a sexual function questionnaire and preference for treatment.

A54. Study 148-369: A double blind, randomised, placebo controlled, sequential design, two way crossover study to investigate the duration of action of a single oral dose of sildenafil (100 mg) on penile erectile activity during visual sexual stimulation in patients with male erectile dysfunction without an established organic cause.

A54.1. Source documents

Study protocol NDA 20-895, vol 1.81; study report: NDA vol 1.81; electronic document: 47061829.pdf.

A54.2. Investigators

Single-center study with 3 investigators in the United Kingdom.

A54.3. Study dates

23 July 1996 to 19 December 1996.

A54.4. Study design

This study description was based upon the final study report, dated 6 August 1997.

A total of 16 subjects with a 6-month history of erectile dysfunction, but without diabetes or significant vascular disease, age 18 to 65, were to be recruited.

Subjects underwent two 2-period crossover studies. During each crossover study, subjects received, in random order, single oral doses of placebo or sildenafil 100 mg on study days 7 days apart. In the first study, penile plethysmography accompanied by visual sexual stimulation was undertaken 4 hours after dosing. In the second crossover study, such evaluations were performed 2 hours after dosing, the change being made on the basis of interim findings from the first study. Blood samples for assay of plasma levels of sildenafil and UK-103,320 were obtained before and after plethysmography.

Routine safety data were recorded.

A54.5. Results

A54.5.1. Conduct

Sixteen subjects were randomized and 15 completed both crossover studies. Fifteen subjects reported spontaneous erections. Minor protocol violations and technical problems were described, but no subject was excluded from analyses.

A54.5.2. Pharmacokinetics

Mean plasma levels of sildenafil were 123 ng/mL in study 1 and 255 ng/mL in study 2. Corresponding levels of UK-103,320 were 61 and 84 ng/mL, respectively.

A54.5.3. Pharmacodynamics

Penile plethysmography data (Rigiscan) obtained during presentation of sexual stimulation showed erections with >60% rigidity lasted about twice as long (part I) or 3-times as long (part II) on sildenafil. Thirteen of 16 subjects had grade3 or 4 erections on sildenafil and 5 subjects had erections on placebo.

A54.5.4. Safety

Minor adverse events were reported with only headache bearing apparent relationship to active treatment.

A54.6. Summary

The data are consistent with there being effects of sildenafil on erectile function in subjects with no established organic cause, and that these effects last at least 4 hours, correlating poorly with plasma levels of sildenafil or metabolite.

A55. Study 148-401: Statistical report a psychometric validation of the international index of erectile function (IIEF) in male patients with erectile dysfunction and age-matched controls.

A55.1. Source documents

Study protocol NDA 20-895, vol 1.115; study report: NDA vol 1.115; electronic document: 46690347.pdf.

A55.2. Investigators

Single-center study with 1 investigator in the United States.

A55.3. Study dates

15 February 1996 to 22 May 1996.

A55.4. Study design

This study description was based upon the protocol dated 30 November 1995. There were no amendments.

This study was undertaken to validate the IIEF. Subjects with erectile dysfunction and age-matched controls were administered the IIEF on two clinic visits, 4 weeks apart. No subject received drug treatment.

A55.5. Results

A55.5.1. Conduct

Sixty subjects were screened, and 58 (27 controls and 37 subjects with erectile dysfunction) completed study.

The mean age was higher in the control group, but there was a greater proportion of subjects over age 65 in the erectile dysfunction group. About 90% were Caucasian. Erectile dysfunction was similar in etiology and duration to other clinical studies.

A55.5.2. Validation

The results of this study are discussed in the context of the overall validation program, reviewed in *Development and validation of the primary efficacy instrument (International Index of Erectile Function; IIEF).* on page 87.

A56. Study 148-451: A study to generate sexual function and quality of life data in male subjects who do not have a diagnosis of erectile dysfunction.

A56.1. Source documents Study protocol NDA 20-895, vol 1.126; study report: NDA vol 1.126; electronic document: 47081108.pdf.

A56.2. Investigators Multi-center study with 3 investigators in United Kingdom.

A56.3. Study dates 26 February 1996 to 23 July 1996.

A56.4. Study design This study description was based upon a protocol dated 4 December 1995. There is no mention of amendments.

There was no drug treatment, and no randomization.

The intent was to obtain sexual function questionnaire data from 100 normal male subjects, age-matched to subjects in Study 148-359[1], in a stable heterosexual relationship for >6 months.

The sexual function questionnaire (draft IIEF) was administered once in a single clinic visit.

No safety data were recorded.

A56.5. Results

A56.5.1. Effectiveness Results pertaining to the validation of the IIEF are discussed in *Development and validation of the primary efficacy instrument (International Index of Erectile Function; IIEF).* on page 87.

A56.5.2. Safety No safety data were collected.

[1] *Study 148-359: A 12 week, double blind, placebo controlled, parallel group, multicentre study to evaluate a new sexual function questionnaire in the assessment of the efficacy of sildenafil (UK-92,480) in patients with erectile dysfunction.* on page 199.

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

CHEMISTRY REVIEW(S)

NOV 18 1997

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20,895 CHEM.REVIEW #: 1 REVIEW DATE: 10/15/97

SUBMISSION	TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
ORIGINAL	1P	29-Sep-1997	30-Sep-1997	01-Oct-1997

NAME & ADDRESS OF APPLICANT: **Pfizer, Pharmaceutical Production Corp.**
Ringaskiddy
County Cork, Ireland

U.S. Agent Pfizer, Inc
Eastern Point Road
Groton, CT 06340

DRUG PRODUCT NAME:

Proprietary:	Viagra
Nonproprietary/USAN/BAN:	Sildenafil Citrate
I.N.N.:	Sildenafil
Code Name/#:	UK-92,480-10
CAS #	171,599-83-0
Chem.Type/Ther.Class:	1 P

PATENT STATUS: 5,250,534, expires June 18, 2011

PHARMACOL.CATEGORY/INDICATION: Erectile Dysfunction

DOSAGE FORM: Tablets

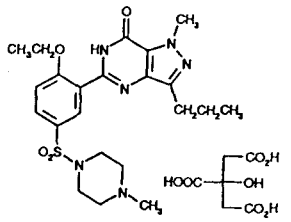
STRENGTHS: 25, 50, 100 mg

ROUTE OF ADMINISTRATION: Oral

DISPENSED: __x__ Rx _____ OTC

STRUCTURAL FORMULA, CHEMICAL NAME, MOLECULAR FORMULA, MOLECULAR WEIGHT:

1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1 H-pyrazolo[4,3-d]pyrimidin-5-yl)phenylsulphonyl]-4-methylpiperazine citrate



Sildenafil Citrate

Molecular Formula: $C_{22}H_{30}N_6O_4S \cdot C_6H_8O_7$ Molecular Weight Citrate: 666.7

Melting range 191-202°C Molecular Weight of Sildenafil: 474.6

SUPPORTING DOCUMENTS:

RELATED DOCUMENTS (if applicable): IND

CONSULTS: None

REMARKS/COMMENTS:

Viagra is a selective inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type 5 (PDE5) and is indicated for the treatment of erectile dysfunction. The physiological mechanism responsible for erection of the penis involves the release of the nitric oxide (NO) in the corpus cavernosum in response to sexual stimulation. Nitric oxide activates the enzyme guanylate cyclase, which results in locally increased levels of cGMP, thereby producing smooth muscle relaxation. By inhibiting PDE5, VIAGRA enhances the normal physiological action of nitric oxide/cGMP, thereby allowing patients to obtain erection adequate for sexual intercourse.

EER is requested on 10/08/97.
Expiration date - 24 months in HDPE containers and blister packs.
Method validation will be requested to be performed by district laboratory.

CONCLUSIONS & RECOMMENDATIONS:
The firm has addressed to our recommendations that were made at the time of pre NDA meeting of 9/16/97. These were regarding strict controls for starting materials, identity tests, particle size specifications, impurity specifications particuarly toxicitv data for
present in the drug substance at data on I have highlighted the firm's actions in these regards in this review.
The information on following items will be obtained by communicating with the firm by telephone.
1) Partition coefficient data on drug substance
2) Data on IND product used in the clinical trials to support 24 months expiration date
3) Information on markers.

cc:
Orig. NDA
HFD-110/Division File
HFD-110/JAdvani/11/17/97
HFD-110/CSO
District
HFD-810/CHoiberg

R/D Init by: RWolters/

J.V. Advani, Review Chemist
filename: N20-895

11/18/97

1

G. Buehler

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

JAN 30 1998

NDA #: 20-895 **CHEM.REVIEW #: 2** **REVIEW DATE: 01/09/98**

SUBMISSION	TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
ORIGINAL	1P	29-Sep-1997	30-Sep-1997	01-Oct-1997
Amendment	**(BC)**	**03-Dec-1997**	**04-Dec-1997**	**05-Dec-1997**
Amendment	**(BC)**	**19-Dec-1997**	**22-Dec-1997**	**29-Dec-1997**

NAME & ADDRESS OF APPLICANT: **Pfizer, Pharmaceutical Production Corp.**
Ringaskiddy
County Cork, Ireland

U.S. Agent Pfizer, Inc
Eastern Point Road
Groton, CT 06340

DRUG PRODUCT NAME:

Proprietary:	Viagra
Nonproprietary/USAN/BAN:	Sildenafil Citrate
I.N.N.:	Sildenafil
Code Name/#:	UK-92,480-10
CAS #	171,599-83-0
Chem.Type/Ther.Class:	1 P

PATENT STATUS: 5,250,534, expires June 18, 2011

PHARMACOL.CATEGORY/INDICATION: Erectile Dysfunction

DOSAGE FORM: Tablets

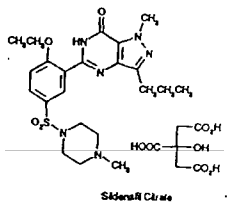
STRENGTHS: 25, 50, 100 mg

ROUTE OF ADMINISTRATION: Oral

DISPENSED: __x__ Rx _____ OTC

STRUCTURAL FORMULA, CHEMICAL NAME, MOLECULAR FORMULA, MOLECULAR WEIGHT:

1-[4-Ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)phenylsulphonyl]-4-methylpiperazine citrate



Molecular Formula: $C_{22}H_{30}N_6O_4S \cdot C_6H_8O_7$ Molecular Weight Citrate: 666.7

Melting range 191-202°C Molecular Weight of Sildenafil: 474.6

SUPPORTING DOCUMENTS:

IND #

Drug Master Files:

RELATED DOCUMENTS (if applicable): None

CONSULTS: None

REMARKS/COMMENTS:
Viagra is a selective inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type 5 (PDE5) and is indicated for the treatment of erectile dysfunction. The physiological mechanism responsible for erection of the penis involves the release of nitric oxide (NO) in the corpus cavernosum in response to sexual stimulation. Nitric oxide activates the enzyme guanylate cyclase, which results in locally increased levels of cGMP, thereby producing smooth muscle relaxation. By inhibiting PDE5, VIAGRA enhances the normal physiological action of nitric oxide/cGMP, thereby allowing patients to obtain erection adequate for sexual intercourse.

EER was requested on 10/08/97. All establishments are acceptable.
Expiration date - 24 months in HDPE containers and blister packs.
A request for methods validation was sent to PHI-District lab. on 11/20/97.

Labeling and nomenclature committee as of 10/3/96, has provided provisional acceptance of the proprietary name pending final acceptance of the USAN name. The Sildenafil has been approved as the USAN name on 8/96.

The amendments of 12/3/97 and 12/22/97 provide responses to telephone requests for information related to Chemist's Review #1.

CONCLUSIONS & RECOMMENDATIONS:

The firm has responded to our recommendations that were made at the time of pre NDA meeting of 9/16/97. These were regarding strict controls for starting materials, identity tests, particle size specifications, impurity specifications particularly toxicity data for the present in the drug substance , data on This information was included in the original submission, and has been reviewed.

The information on the following items that was requested following review of the original submission, has now been obtained in these two amendments.

1) Partition coefficient data on drug substance
2) Data on IND product used in the clinical trials to support 24 months expiration date
3) Information on markers and amended dosage form monographs (DFMs) for sildenafil citrate tablets
4) Updated 6 months stability studies

Information supplied is reviewed, and found satisfactory.

Approvable

cc:
Orig. NDA
HFD-110/Division File
HFD-110/JAdvani/01/27/98
HFD-110/CSO
District
HFD-810/CHoiberg

R/D Init by: JShort/

J.V. Advani, Review Chemist
filename: **N20-895**

1/30/98

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

PHARMACOLOGY REVIEWS

OVERVIEW PGS 1-25

NDA #20-895

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

VIAGRA® Tablets (Sildenafil Citrate)

Pfizer Pharmaceutical Production Corporation
Ringaskiddy
County Cork, Ireland

Reviewers:
Estela A. Barry, M.S.
Albert F. DeFelice, Ph.D.
Thomas Papoian, Ph.D.

Division of Cardio-Renal Drug Products (HFD-110)
Center for Drug Evaluation and Research
Food and Drug Administration

January 23, 1998

Table of Contents

Table of Contents (cont.)

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Estela A. Barry, M.S.
Albert F. DeFelice, Ph.D.
Thomas Papoian, Ph.D.

Introduction

SUBMISSION DATE: 9/29/97
CENTER RECEIPT DATE: 9/30/97
REVIEWERS RECEIPT DATE: 10/2/97

SPONSOR: Pfizer Pharmaceuticals Production Corporation
Ringaskiddy, County Cork, Ireland

DRUG:

Code Name: UK-92,480 (free base); UK-92,480-10 (citrate salt)

Generic Name: Sildenafil citrate

Trade Name: Viagra

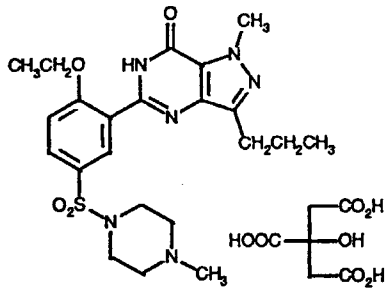
Chemical Name: 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)phenylsulfonyl]-4-methylpiperazine citrate salt

CAS Registry Number: 171,599-83-0

Molecular Formula: $C_{22}H_{30}N_6O_4S \cdot C_6H_8O_7$

Molecular Weight: 666.7 (citrate salt)

Structure:



PHARMACOLOGIC CLASS: Cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) inhibitor.

PROPOSED CLINICAL INDICATION: Treatment of male erectile dysfunction

FORMULATION AND DOSE: 25 mg, 50 mg and 100 mg tablets of sildenafil are adjusted according to potency of sildenafil. The formulation per 25 mg tablet in mg/unit is as follows: 35.12 mg sildenafil citrate (based on a potency of 71.2%); microcrystalline cellulose NF; dibasic calcium phosphate; croscarmellose sodium; magnesium stearate; Blue (contains: , purified water. The proposed dose is 50 mg taken about 60 min before sexual activity. The dose may be increased to 100 mg or decreased to 25 mg. The maximum recommended human dose (MRHD) is 100 mg (= 1.4 mg/kg based on a 70 kg man).

ROUTE OF ADMINISTRATION: Oral

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

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

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

[Note: Because sildenafil possesses antithrombotic, antivasospactic, and coronary vasodilator properties when given intravenously (i.v.), the original indication in humans was for angina pectoris. Therefore, some of the preclinical studies in animals were performed using the i.v. route of administration.]

1. PHARMACOLOGY

1.1 Overview:

Stimulation of penile autonomic innervation releases nitric oxide which activates guanylate cyclase and enhances levels of cyclic guanosine monophosphate (cGMP). The cGMP relaxes cavernosal and vascular smooth muscle and causes tumescence. Preserving cGMP, which is normally hydrolyzed by type 5 phosphodiesterase (PDE5), might be expected to facilitate initiation and/or maintenance of erection. Sponsor has documented sildenafil's potent and selective inhibition of PDE5 - the only cGMP- specific PDE in human corpora cavernosa - and propose the agent for treatment of erectile dysfunction.

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

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

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

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

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

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

1.2. Activities related to mechanism of action.

Sponsor studied effects of sildenafil on corpus cavernosal smooth muscle *in vitro* and *in vivo*, and - given the tissue distribution of PDE isoenzymes - on the cardiovascular and gastro-intestinal systems, on bleeding and clotting times, and also on the retina as part of the pre-clinical evaluation of this agent.

1.2.1 Effect on PDE isoenzymes *in vitro*

Effects of sildenafil on each of the PDE isoenzymes was determined using human corpora cavernosa, cardiac ventricle and skeletal muscle as well as rabbit, dog and human platelets, rat diaphragm and kidney, and human, dog and rat retina . Potency and selectivity are shown in Table 1:

Table 1

The effects of sildenafil on the activities of phosphodiesterase (PDE) isoenzymes isolated from a variety of tissues and species

PDE isoenzyme	Source	n	Geometric mean IC_{50} value with 95% Confidence Interval.
PDE1	Human cardiac ventricle	6	280.0 nM (229 - 337)
	Rat kidney	2	430.0 nM *
	Rat diaphragm	5	218.0 nM (123 - 386)
PDE2	Human *corpora cavernosa*	5	68.0 mcM (31.6 - 146.3)
	Rat kidney	3	>100 mcM
	Rat diaphragm	3	32.8 mcM (18.8 - 57.2)
PDE3	Human *corpora cavernosa*	4	16.2 mcM (9.5 - 27.8)
	Human platelet	3	41.2 mcM (26.1 - 65.0)
	Rabbit platelet	3	48.0 mcM (24.0 - 98.0)
PDE4	Human skeletal muscle	3	7.2 mcM (4.5 - 11.5)
	Rat kidney	1	19.0 mcM *
	Rat diaphragm	3	6.3 mcM (5.3 - 7.5)
PDE5	Human *corpora cavernosa*	15	3.5 nM (2.5 - 4.8)
	Human platelet	3	6.1 nM (3.0 - 12.6)
	Rabbit platelet	4	3.9 nM (3.6 - 4.1)
	Dog platelet	1	4.8 nM *
	Rat diaphragm	5	1.8 nM (0.7 - 4.6)
PDE6	Human retina - cone	6	34.1 nM (24.5 - 47.4)
	Human retina - rod	6	37.5 nM (29.0 - 48.5)
	Dog retina - cone	11	26.9 nM (19.4 - 37.1)
	Dog retina - rod	9	58.2 nM (46.1 - 73.4)
	Rat retina - cone	4	26.9 nM (17.2 - 42.2)
	Rat retina - rod	5	67.4 nM (56.3 - 80.8)

n = Number of samples or experiments
IC_{50} = Concentration required to inhibit enzyme activity by 50%

* Results are geometric means, except for values where n=2 which are arithmetic means

Sildenafil selectively, and potently, inhibited PDE5 in human and other tissue with mean IC50 values ranging from ca. 2 to 6 nM. Retinal PDE6 is also susceptible, being blocked at approx. 10-fold higher concentrations. In contrast , sildenafil exhibited only modest activity against the cGMP-degrading calcium/calmodulin- dependent PDE1 found in human cardiac ventricles and the indicated rat tissues. That is, sildenafil exhibited ca. 80-fold greater potency for the human PDE5 compared with human and rat PDE1 isoenzymes. Consistent with demonstrating preferential activity against cGMP hydrolyzing PDE enzymes, sildenafil weakly inhibited the cGMP-stimulated PDE2 isoenzyme isolated from human corpora cavernosa (IC50 68.0 µM) and rat diaphragm and rat kidney (IC_{50}s 32.8 and >100 µM, respectively). Likewise, sildenafil is a weak inhibitor of cGMP-inhibited PDE3 found in the human corpora cavernosa (IC_{50} 16.2 µM) and human (IC_{50} 41.

μM) and rabbit (48.0 μM) platelets. Sildenafil only weakly inhibited the cAMP specific PDE4 isoenzyme found in human skeletal muscle (IC_{50} 7.2 μM) and rat kidney (IC_{50} 19.0 μM) and rat diaphragm (IC_{50} 6.3 μM). Accordingly, for human enzymes, sildenafil has a >1000 fold selectivity for PDE5 over PDE2, PDE3, PDE4; an 80-fold selectivity over cardiac ventricular PDE1; and the least selectivity (approx. 10-fold) vs. retinal PDE6.

1.2.2 Functional effects in *Corpus Cavernosum*.

Sildenafil dose-relatedly enhanced the relaxant effect of tissue NO released by electrical stimulation, methacholine, and/or sodium nitroprusside in isolated rabbit and human cavernosal strips. It had no direct relaxing activity in either rabbit or human cavernosal smooth muscle. Intravenously, it potently enhanced the tumescent
(i.e., cavernosal pressor) effect of pelvic nerve stimulation or intracavernosal sod. nitroprusside injection at dosages devoid of appreciable cardiovascular effect:

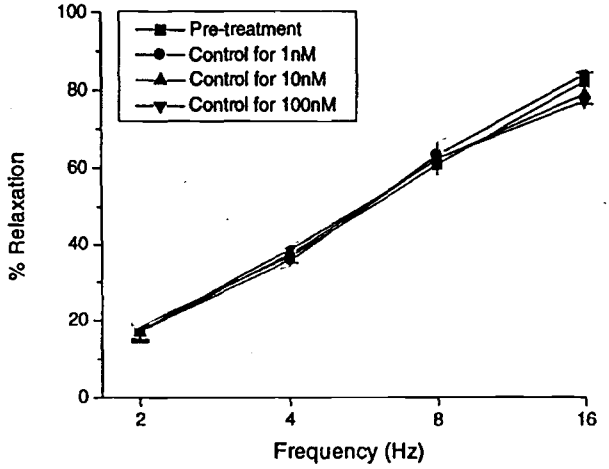
1.2.2.1 *In Vitro assays*

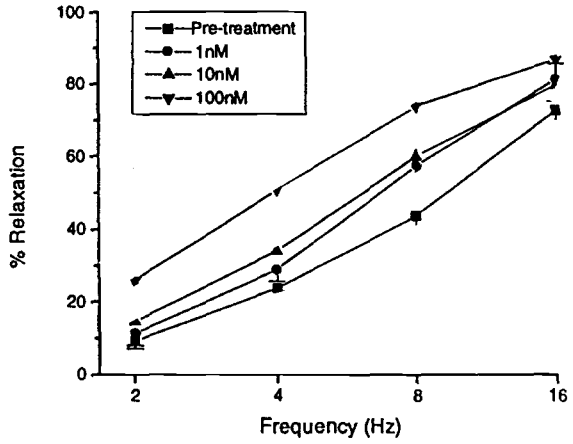
1.2.2.1.1. Rabbit:

In an assay of rabbit cavernosal strips pre-contracted with phenylephrine and made to relax with NO/cGMP-dependent stimuli , i.e. electrical field stimulation (EFS), sod. nitroprusside, or methacholine , sildenafil significantly and dose-relatedly enhanced the relaxant effect of such stimuli: threshold and maximum observed effect occurred at sildenafil levels of <10nM and 100 nM, respectively (Fig.1; Table 2).

Figure 1

The effects of sildenafil (Lower Panel) and vehicle (Upper Panel) on the relaxation of phenylephrine (10 mcM)-contracted rabbit isolated *corpus cavernosal* strips induced by electrical field stimulation (EFS: 2, 4, 8 and 16 Hz, 0.2 msec, 10 V for 10 sec).





Each point represents the mean, ± S.E.M., of the results obtained from 8 to 9 strips.

Parallel straight lines were fitted to percentage relaxation *vs* log frequency plots for each tissue and the significance of the differences in intercepts at 2Hz between pre-treatment and treatment responses were examined using Student's t-test for paired data. For the treated tissues (Lower Panel), the differences between the EFS responses for 1, 10 and 100 nM sildenafil treatments and pre-treatment were significant; $P<0.05$, $P<0.01$, $P<0.001$, respectively. For the vehicle-treated (time-matched control) tissues there were no significant differences between the lines.

Vehicle	=	0.6% v/v lactic acid, 50 mcl
Pre-treatment	=	Responses prior to addition of sildenafil or vehicle
%		Percentage

Table 2

The effects of sildenafil on the relaxation of phenylephrine (10 mcM)-contracted rabbit isolated *corpus cavernosal* strips induced by sodium nitroprusside (SNP).

Treatment	n	Mean, (95% Confidence Interval) IC_{50}s for SNP (mcM)	Treatment	n	Mean, (95% Confidence Interval) IC_{50}s for SNP (mcM)
Pre-treatment	10	6.35 (3.41 - 11.83)	Pre-treatment	10	6.15 (3.04 - 12.46)
Time matched control	8	12.36 (7.46 - 20.47)	Sildenafil 10 nM	10	4.94 (3.24 - 7.53)**
Time matched control	10	10.39 (6.34 - 17.04)	Sildenafil 100 nM	10	1.07 (0.74 - 1.56)***
Time matched control	8	9.77 (6.16 - 15.50)	Sildenafil 1 mcM	7	0.77 (0.44 - 1.36)***

n = Number of tissues
Vehicle = 0.6 % v/v lactic acid, 50 mcl
Pre-treatment = Responses prior to addition of sildenafil or vehicle
IC_{50} = Concentration required to produce a 50% relaxation of the phenylephrine-induced contraction

Significance of difference from time-matched control ** P <0.01, ***P<0.001
(Analysis of Covariance adjusted for pre-treatment values)

The effects of sildenafil on the relaxation of phenylephrine (10 mcM)-contracted rabbit isolated *corpus cavernosal* strips induced by methacholine.

Treatment	n	Mean, (95% Confidence Intervals) IC_{50}s for methacholine (nM)	Treatment	n	Mean, (95% Confidence Intervals) IC_{50}s for methacholine (nM)
Time matched control	6	38.0 (21.4 - 67.6)	Sildenafil 1 nM	6	23.4 (10.5 - 53.7)
Time matched control	7	49.0 (30.2 - 77.6)	Sildenafil 10 nM	7	20.0 (9.3 -41.7)*
Time matched control	7	40.7 (27.5 - 60.3)	Sildenafil 100 nM	7	11.7 (6.3 - 21.9)**
Time matched control	7	43.7 (29.5 - 64.6)	Sildenafil 1 mcM	7	9.8 (5.6 - 16.2)***

n = Number of tissues
Vehicle = 0.6 % v/v lactic acid, 50 mcl
IC_{50} = Concentration required to produce a 50% relaxation of the phenylephrine-induced contraction

Significance of difference from time-matched control *P<0.05, ** P <0.01, ***P<0.001
(Student's Independent t-test)

At higher concentrations (IC50 = ca. 500 nM) sildenafil *per se* also relaxed such tissue - an effect completely prevented by the NO synthase inhibitor L-n- nitroarginine. Again, endogenous NO/cGMP is implicated as a sildenafil mechanism. The non-selective PDE inhibitor papaverine relaxed this tissue even in the presence of L-n- nitroarginine.
Accordingly, sildenafil relaxes corpus cavernosal smooth muscle *in vitro* by enhancing the effect of dilators mobilizing the endogenous NO/cGMP cascade. Sildenafil 100 nM caused a near maximal effect since 1 μM did not cause a marked further potentiation of EFS-, SNP- or methacholine-induced relaxation.

1.2.2.1.2. Human:

Sildenafil also potentiated relaxation of pre-contracted human isolated corpora cavernosa induced by EFS in a dose-related manner and over a similar concentration range active on rabbit isolated corpora cavernosa (ca. 1-100 nM).As in the rabbit, the electrical stimulus relaxes via endogenous NO release. Again, sildenafil had no direct muscle relaxant activity.

1.2.2.2. *In situ assays* : intracavernosal pressure of anesthetized dogs.

1.2.2.2.1. Pelvic nerve stimulation:

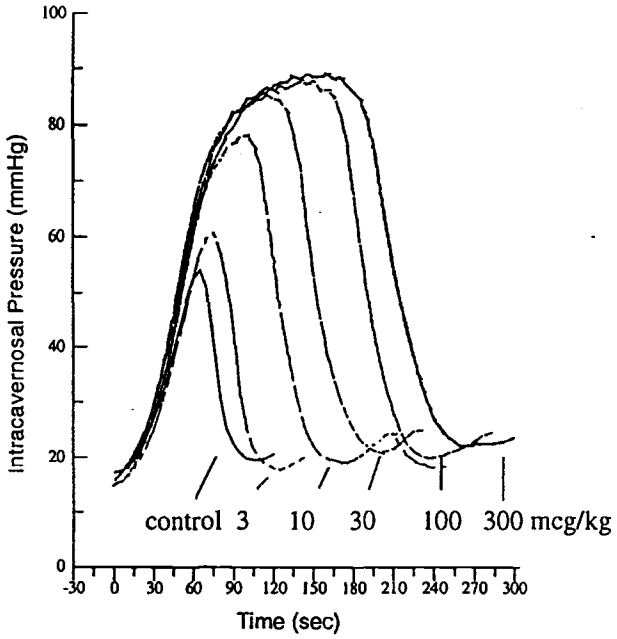
Sildenafil enhanced the increase in intracavernosal pressure elicited by stimulation of the pelvic nerve. A cavernosal pressure of ca. 30% of mean blood pressure achieved by electrical stimulation *per se* (which releases NO) was promoted, dose-relatedly, to up to 70% of systemic blood pressure by pre-treatment with 10 to 100 µg/Kg iv. of sildenafil. Blood sildenafil levels averaged 14 and 49 ng/ml after 30 and 100 µg/Kg dosages, respectively. Over this dose range, mean arterial BP, HR, and LV pressure were not appreciably affected. LV dP/dT index of contractility, elevated approx. 10% at all dosages, was, accordingly, not dose-relatedly or appreciably affected.

1.2.2.2.2. Intracavernosal sod. nitroprusside (SNP):

Injection of SNP (1 to 5 µg) into the corpora cavernosa produced an initial transient fall in pressure (<10 mm Hg) followed by a slowly developing rise which returned to baseline within 5 min. or less. Sildenafil (3 to 300 µg/kg iv.) increased the magnitude and duration of the tumescent response to SNP in a dose-related manner and the mean dose (95% CI) required to produce a response 50% of the maximum was 16.2 (11.1 - 23.6) µg/kg iv.(figure 2.).

Figure 2

A representative figure showing the effects on intracavernosal pressure of sodium nitroprusside (SNP) injected into the *corpus cavernosum* either alone (control) or following intravenous doses of sildenafil in the anaesthetised dog.



Neither blood pressure nor heart rate of these anesthetized dogs was appreciably affected over this dose range. Thus 10-300 μ g/Kg of sildenafil selectively enhances cavernosal sensitivity to purinergic (i.e., cGMp-mediated) stimuli.

In vitro and *in vivo* studies *in toto* indicate that sildenafil enhances NO→cGMP stimulation of the corpus cavernosum rather than acting as a direct smooth muscle relaxant.

1.2.3 Functional effects on other tissues expressing PDE5 enzyme.

In addition to being located in human corpora cavernosa, PDE5 occurs in platelets and muscle (vascular; skeletal; gastrointestinal). Functional consequences of blocking the enzyme in such tissues were investigated *in vitro* and *in vivo*::

1.2.3.1 Platelet aggregation/disaggregation

Sponsor assayed effect of sildenafil alone and combined with the NO donor SNP on platelet disaggregation *in vitro* and on agonist-induced platelet aggregation *in vitro* and *ex vivo* . Platelet rich plasma (PRP) prepared from rabbit and human blood was used. Sildenafil *per se* had no effect on agonist-induced platelet aggregation or on platelet disaggregation, but potentiated the platelet anti-aggregatory and disaggregatory effects of SNP *in vitro* and *ex vivo*. The *ex vivo* effect was maintained on sub-acute (5 days) dosing and there was no evidence of tachyphylaxis or the development of tolerance.

1.2.3.1.1. *In vitro* studies on rabbit and human platelet aggregation :

1.2.3.1.1.1. Rabbit:

Sildenafil alone, at a concentration of 1 μM, had no effect on agonist-induced (e.g. PAF) platelet aggregation but did significantly potentiate the platelet anti-aggregatory activity of 3 μM SNP (a NO donor) against 5 different thrombotic stimuli (Table 3).

Table 3

Effects of sildenafil alone and in combination with sodium nitroprusside (SNP) on platelet aggregation produced by a variety of agonists in rabbit platelet rich plasma (PRP).

Agonist and concentration	Mean, ± S.E.M., percentage platelet aggregation		Mean, ± S.E.M., percentage inhibition of platelet aggregation by 3 mcM SNP	
	Vehicle	Sildenafil 1 mcM	Vehicle	Sildenafil 1 mcM
PAF 10 nM	59.8 ± 4.4 (n=4)	60.8 ± 2.0 (n=3)	47.7 ± 1.6 (n=4)	75.7 ± 1.5** (n=3)
Collagen 6 mcg/ml	75.3 ± 1.8 (n=3)	71.8 ± 2.4 (n=3)	41.8 ± 1.5 (n=3)	98.5 ± 1.5** (n=3)
ADP 3 mcM	47.5 ± 2.5 (n=3)	46.0 ± 2.2 (n=3)	48.0 ± 0.6 (n=3)	89.0 ± 0.6** (n=3)
U46619 3 mcM	70.3 ± 0.5 (n=3)	68.3 ± 2.3 (n=3)	56.5 ± 3.3 (n=3)	94.2 ± 0.4** (n=3)
A23187 3 mcM	68.0 ± 6.6 (n=4)	71.5 ± 3.9 (n=3)	31.7 ± 3.4 (n=4)	55.7 ± 1.9** (n=3)

n = Number of animals
Vehicle = 10 mM HCl for aggregation studies
Vehicle = Distilled water for inhibition of aggregation studies
PAF = Platelet activating factor
ADP = Adenosine diphosphate
U46619 = Thromboxane A_2 receptor agonist
A23187 = Calcium ionophore

Significance of difference from vehicle group ** P<0.01
(Student's independent t-test)

The potentiating activity of sildenafil to enhance both anti-aggregatory and disaggregatory of SNP on ADP- aggregated platelet -rich plasma was concentration- related over the 10 nM to 1μM range. In further studies, 1 μM sildenafil alone had no platelet anti-aggregatory or disaggregatory activity but significantly reduced mean IC50 and EC50 for SNP-induced platelet anti-aggregation and disaggregation, respectively, from 4.61 to 0.59 μM and from 23.23 to 1.59 μM, respectively., i.e., by about an order of magnitude.

1.2.3.1.1.2. Human:

Sponsor studied platelet- rich human blood aggregated with ADP. Over the concentration range 0.3 to 30 μM, ADP induced a concentration-related aggregation of human platelets, the EC50 being 1.6 μM. This action of ADP was not affected by sildenafil 1 μM. However, the

compound significantly reduced the mean IC50 for SNP-induced platelet anti-aggregation from 2.4 to 0.8 μM.
The dose-response for ability of sildenafil to enhance SNP was investigated in human PRP aggregated with 20 μM ADP. In human PRP, 20 and 40 ng/ml sildenafil (equivalent to ca 40 and 80 nM, respectively) potentiated the platelet anti-aggregatory effects of SNP compared with the vehicle (distilled water). The mean (95% CI) shifts in the SNP IC50 were 1.7 (1.4 - 2.1)-fold and 3.0 (2.7 - 3.3)-fold for 20 and 40 ng/ml, respectively. Functional activity in human platelets was comparable with that observed in rabbit platelets. Accordingly, sildenafil *per se* had no effect on aggregation of either human or rabbit platelets provoked by ADP, or provoked by any of 4 other thrombotic stimuli - including PAF or collagen - in rabbits. However, the compound consistently and significantly potentiated the platelet anti-aggregatory effects of the NO donor SNP *in vitro* (rabbit and man) and ex vivo (rabbit: see below).

1.2.3.1.2. *Ex vivo* studies on rabbit platelet aggregation

1.2.3.1.2.1. Acute intravenous administration:

Effects on the platelet anti-aggregatory activity of SNP were studied using platelet rich plasma (PRP) prepared from blood of anesthetized rabbits which had been treated with the compound. In these experiments, PRP was aggregated with 4 μM ADP in the presence and absence of SNP. Sildenafil potentiated the anti-aggregatory effects of SNP measured ex vivo: In PRP obtained from anaesthetized rabbits treated with 0.1 mg/kg sildenafil iv., the mean (± S.E.M) IC_{50} for the anti-aggregatory effects of SNP was significantly reduced from the control value of 9.98 ± 1.49 to 2.80 ±0.39 μM in samples taken 30 min. after sildenafil administration . In samples taken 120 min. after dosing, the IC_{50} (5.64 ± 0.56 μM) was still significantly reduced . These results indicate that a single iv. dose of sildenafil (0.1 mg/Kg) can appreciably potentiate the anti-aggregatory effect of SNP in the rabbit, and with a duration of action consistent with its plasma half-life (approximately 1h) in this species.

1.2.3.1.2.2. Sub-acute(6 days) oral dosing: rabbit

Study protocol was similar to that of the acute iv. study except rabbits were dosed bid for 6 days at 1 mg/Kg , blood was drawn 2 hr. after the first dose on day 1 and 6, and the SNP-potentiating activity of sildenafil compared for any evidence of tolerance . In the day 1 and day 6 vehicle-treated groups, the geometric mean (95% CI) IC_{50}s for the anti-aggregatory effect of SNP were almost the same - 7.2 (4.0 - 13.0) and 7.0 (3.7 - 13.3) μM, respectively. In the sildenafil-treated groups, the IC_{50}s for SNP for days 1 and 6 were 1.7 (0.9 - 3.3) and 1.2 (0.7 - 1.8) μM, respectively. The IC_{50}s in the drug-treated groups were significantly different from their respective vehicle controls.
Thus, the ability of sildenafil to potentiate the platelet anti-aggregatory activity of SNP was maintained following 5 days b.i.d. dosing, with no evidence of tachyphylaxis or tolerance. - at least of a high 1 mg/Kg dose.

1.2.3.2 Antithrombotic activity:

Because sildenafil enhanced platelet anti- aggregatory effect of sod. nitroprusside *in vitro* and *ex vivo* , effects on thrombus formation were assessed in a small *in vivo* study of four anesthetized rabbits with stenosed damaged l. carotid arteries. Blood flow through such arteries is subject to cyclic flow reductions; such blood flow variations are said to reflect platelet thrombus formation. Results: Doses of 0.01 and 0.03 mg/Kg iv., and 1, 3, and 10 mg/Kg i.d. were said to afford, at the higher doses, complete reversal of the cyclical flow variations in 1 to 3 of the four rabbits tested, accompanied, however, by 5 to 20 mm Hg reductions in mean blood pressure. Since this

was a pilot study, full dose response and comprehensive hemodynamic data are lacking and the results are only suggestive.

1.2.3.3. Bleeding and clotting time:

1.2.3.3.1. Rat:

Effect on bleeding time was assessed 15 min. post-dose using the rat tail bleeding method. Clotting times were measured 20 to 25 min. post-dose using blood collected from a cannulated jugular vein. At 0.1 mg/kg iv., sildenafil did not affect either bleeding or clotting time. Increasing the dose to 0.3 mg/kg iv. increased the bleeding time by approximately 60%, although the change was not statistically significant, without appreciably affecting the clotting time. The positive control (aspirin :10 mg/kg iv.), as expected from its known pharmacological properties, markedly prolonged the bleeding time, and produced a modest (approximately 30%) increase in the clotting time. Thus, sildenafil did not affect blood clotting time at 0.1 and 0.3 mg/kg iv. but did increase bleeding time at the higher dose although less than that observed after aspirin.

1.2.3.3.2. Rabbit:

The effects of sildenafil, heparin and aspirin on bleeding time were assessed 20, 40 and 60 min. post-dose using the ear of anesthetized rabbits and a Simplate bleeding time device. Sildenafil (1 mg/kg iv.) and heparin (150 IU/kg iv.), significantly prolonged the bleeding time with mean increases of 51 ± 8 and 102 ± 17 sec, respectively (control ≅ 120 sec.) The combination of sildenafil and heparin had a greater effect than either agent alone but the increase (145 ± 37 sec) was additive rather than synergistic and there was no evidence of an interaction between the two agents. Aspirin alone, at 1 and 10 mg/kg iv. (equivalent to clinically-used low and high dose aspirin, respectively), tended to slightly prolong bleeding time, although the effect was not significant. The combination of sildenafil with each of the 2 doses of aspirin also did not significantly affect bleeding time when compared with sildenafil alone. Consequently, there was no evidence of an interaction between the 2 agents.

Accordingly, a high intravenous dose of sildenafil increased bleeding time in the rabbit ear preparation. This effect was additive with that of heparin, and there was no evidence of an interaction between sildenafil and heparin - or aspirin. The dose which prolonged bleeding time (1 mg/Kg) was 33 to 100 -fold greater than an anti-thrombotic dose (0.01-0.03 mg/Kg iv.) in that species , and 10 to 100 X greater than the doses (10-100 µg/Kg iv.) producing a targeted cavernosal effect in the dog. A similar trend was observed in the rat at a 3-fold lower dose (0.3 mg/Kg), but the compound had no effect on blood clotting times in this species. Effects are consistent with the identified actions of sildenafil on platelets in the absence of an effect on the clotting cascade.

1.2.3.4 Cardiovascular effects:

Effects of sildenafil on vascular smooth muscle, which contains PDE5, and on the intact cardiovascular system were investigated using both *in vitro* and *in vivo* models. These include isolated vascular preparations (e.g. canine coronary artery, rabbit aorta), dog cardiac trabeculae, conscious Okamoto spontaneously hypertensive rats (SHR) and hemodynamic studies in anesthetized and intact conscious dogs.

1.2.3.4.1. Isolated artery (dog; rabbit)

cGMP content: In canine isolated coronary artery sections, incubation for 5 min. with sildenafil (10 and 100 nM) increased the tissue levels of cGMP, but not cAMP, in a concentration-dependent manner up to approx. 4x basal levels.

Contractility of rabbit aorta: Aorta rings were contracted with phenylephrine (PE); EC_{50} value was 0.33 μM (95% CI: 0.20 - 0.52). Sildenafil (1 μM) *per se* had no effect on the PE concentration-contraction curve , but the PE curve was shifted to the right by 1 μM glyceryl trinitrate (GTN) to a PE EC_{50} value of 1.65 (1.05 - 2.59). A combination of GTN (1 μM) and sildenafil (1 μM) markedly increased the EC_{50} for phenylephrine to 6.85 (4.33 - 10.84). Comparison of the ratio of EC_{50} values for vehicle vs. sildenafil (1.36) and vehicle vs. GTN (5.0) alone, and vehicle vs. sildenafil + GTN (20.8) suggests that the actions of sildenafil and GTN are synergistic rather than additive. Accordingly, sildenafil enhances the relaxation of vascular smooth muscle *in vitro* provoked by guanylate cyclase/cGMP (recruited in these studies by GTN), but shows no direct activity.

Contractility of rabbit coronary artery:
Sponsor measured the perfusion pressure in rabbit Langendorff hearts with air embolus-damaged coronary arteries to determine whether sildenafil could antagonize endothelin (ET-1)- induced vasoconstriction. Sildenafil produced significant concentration-related inhibitions of the response to ET-1 ranging from 14 ± 4% at 100 nM to 64 ± 5% at 30 μM. Accordingly, sildenafil does not appreciably block contractions of isolated vascular smooth muscle produced by ET-1, or PE.

1.2.3.4.2. Dog cardiac trabeculae:

Sponsor compared effects of isoprenaline (β -agonist), sildenafil (up to 10 μM), and the selective PDE3 inhibitor milrinone on tension generated by field-stimulated isolated cardiac (presumably l. ventricular) trabeculae carnae. Milrinone (0.1 to 60 μM)increased the developed tension up to a level equivalent to 61% of the isoprenaline maximum response. In contrast, sildenafil had no effect on the contractility of the trabeculae up to a concentration (10 μM) which is 100-300 fold higher than that which inhibits PDE5 in the isolated human and rabbit corpus cavernosum (section 1.1.2.1 above). Absence of positive inotropic activity is consistent with it's weak PDE3- inhibiting activity (i.e., IC50 of ca. 30 μM in human cavernosal and platelet tissue).

1.2.3.4.3. Effect on blood pressure of spontaneously hypertensive (SH) rat:

At doses (0.03 and 0.1 mg/Kg iv.) which are active on the corpus cavernosum in the anaesthetized dog i.e., which enhance the cavernosal pressor response to pelvic nerve stimulation, sildenafil had no effect on blood pressure and heart rate in SHR. However, higher iv. doses (0.3 and 1.0 mg/Kg) as well as a 10 mg / Kg oral dose did decrease blood pressure by approx. 25 mm Hg without any consistent dose-related tachycardia. Antihypertensive effect of the oral dose persisted for at least 3 hours. Such vasodepressor activity is consistent with vasodilatation resulting from a cGMP enhancement in vascular smooth muscle and, therefore, from the known actions of sildenafil.

1.2.3.4.4. Hemodynamic effects in conscious normotensive dogs.

In a study of 5-6 conscious instrumented dogs, a variety of hemodynamic variables were monitored along with plasma drug levels following oral administration. A dose of 0.1 mg/Kg, which afforded a mean sildenafil plasma level of 13.8 ng/ml, did not provoke any remarkable hemodynamic effect in the conscious dog. Higher doses (0.3; 1.0 mg/Kg) achieving plasma levels of 34 to 115 ng/ml produced only modest effects (e.g., slight changes in heart rate, LVEDP, and LV dP/dT) consistent with vasodilation and reductions in cardiac preload and/or afterload resulting from cGMP enhancement in vascular smooth muscle. Another study at up to 3 mg/Kg (plasma drug levels not monitored, but max. of 350 ng/ml expected) revealed very modest changes of up to only 10-20% in a variety of parameters including total systemic vasc. resistance(- 15%), cardiac output (+ 16%), LV dP/dT (+ 5%), and heart rate(+ 25%); QT interval decreased by only 16 msec.

As noted above in dog pelvic nerve stimulation studies, the threshold plasma sildenafil level for concentration-related cavernosal effects was approx. 5-15 ng/ml with near maximal effect at ca. 50 ng/ml. Accordingly, there are no outstanding or unexpected hemodynamic changes in dogs at up to at least 10 X the blood levels achieving desired cavernosal effects in that species.

1.2.3.4.5. Hemodynamic effects in atropinized and ganglion/ adrenoceptor-blocked dogs.

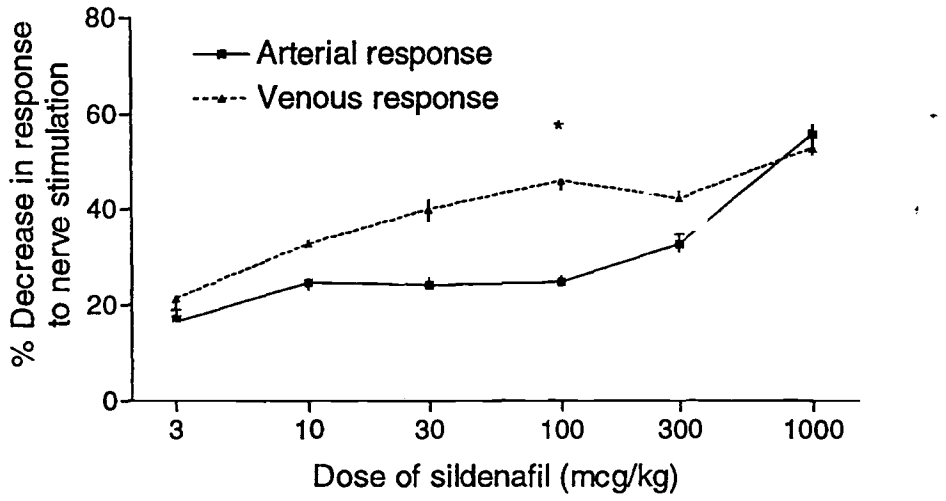
In 4 dogs treated with sildenafil and compared with time-related control animals (n=3), a statistically significant reduction in a standard noradrenaline pressor response (ca. 100 mm Hg) of 19.2 ± 0.5 mm Hg was noted 5 min. after administration of 3 mg/kg sildenafil iv. The pressor response to noradrenaline returned with time but was still significantly reduced up to 2.5 h post-dose (7.5 ± 1.7 mm Hg). This duration of action is consistent with the plasma half-life in the dog of 5.2 h (see Metabolism and Pharmacokinetics Section). In the ganglion- and α/β -adrenoceptor-blocked dog, there was no evidence of a direct effect of sildenafil on heart rate. In the single dog dosed with 10 mg/kg iv., MABP fell acutely by 33 mm Hg, but heart rate increased by only 2 bpm. Likewise, in the 4 dogs given 3 mg/kg, MABP fell by a mean of 36 ± 3.9 mm Hg, while the heart rate increased by only 2 to 3 bpm. Accordingly, high sildenafil doses produced marked falls in blood pressure in pharmacologically sympathectomized animals with no tachycardic effect. The PDE-3 blocker milrinone was not tested in this model for corresponding activities.

1.2.3.4.6. Arterio-venous dilator activity in the anesthetized dog.

Sponsor determined the arterio-venous balance of sildenafil 's vasodilator activity vis a vis the mixed nitrate (isosorbide dinitrate, ISDN) and arterial calcium antagonist (diltiazem) classes of vasodilator. The arterial and venous responses to electrical stimulation of the lumbar sympathetic chains in perfused arterial and venous segments in the hind limbs of the anaesthetized, β-adrenoceptor-blocked, atropinised dog were used in this assessment: Sildenafil displayed dose-related arterio-venous dilator properties (Fig.3) at intravenous doses (3-300 μg/Kg) similar to those active on the corpus cavernosum in the anaesthetized dog (10-100 μg/Kg). Its arterio-venous profile resembled that of ISDN - i.e., approx. equipotent on both arterial and venous sides .

Figure 3

The effects of intravenously-administered sildenafil on the hind limb arterial and venous vasoconstrictor responses to electrical stimulation of the lumbar sympathetic chains of β-adrenoceptor-blocked and atropinised anaesthetised dogs (n=4)



n = Number of animals
% = Percentage

Results are expressed as mean, ± S.E.M., values

Dogs were β-adrenoceptor blocked and atropinised by the intravenous administration of propranolol (2 mg/kg) and atropine (1 mg/kg)

Electrical stimulation parameters for the lumbar sympathetic chains were 3 Hz (left chain), 8 Hz (right chain), 0.5 msec, supramaximal voltage (30 V)

Significance of difference between responses * P <0.05
(Student's independent t-test)

However, over the dose ranges studied, sildenafil inhibited neural vasoconstriction up to approx. 50% Vs ≥ 80% for ISDN. In this regard, both agents differed from that of the arterio-selective diltiazem which selectively blocked neural arterial vasoconstriction a max. of 100%.
g. Hemodynamic effects in anesthetized cats:
At all doses tested (0.3,1, and 3 mg/Kg iv.), sildenafil provoked marked but short-lasting (ca. 5 min.) drops in mean BP (ca. 40 %), LV systolic pressure (ca. 25%), and LV dP/dT (20%) which were stat. signif. but not dose-related, and an 18% increase in heart rate at the highest dose.

1.2.3.5 Gastro-intestinal effects:

1.2.3.5.1. *In vitro:*

Sponsor studied effects of sildenafil on GI tract since PDE5 and PDE1 are expressed in such smooth muscle:

Concentrations of sildenafil which were maximally effective on the corpus cavernosum in vitro (100 nM) had no effect on electrically stimulated rat esophagus, histamine-stimulated guinea

pig ileum, or carbachol-stimulated mouse ileum. However, higher concentrations (1 μM) relaxed pre-contracted mouse and rat ileum with equivalent potency. This concentration had no effect on the dog isolated lower oesophageal sphincter, although at 10 μM, sildenafil caused a significant relaxation of this preparation. The latter effect was blocked by the NO synthase inhibitor, L-nitroarginine, suggesting sildenafil acts to potentiate endogenous NO.

1.2.3.5.2. *In vivo:*

At oral doses up to and including 10 mg/kg, sildenafil did not affect basal gastric acid secretion or gastrointestinal propulsive activity in the rat. Histamine , cimetidine, and morphine behaved as expected of their known pharmacology. Consequently, the compound did not affect gastrointestinal function in controlled studies in this species.

1.2.3.6. Retinal effects:

It is reported that illuminated rhodopsin, the visual pigment of the rod, stimulates retinal PDE6 via the G-protein, transducin. As levels of cGMP decrease, cGMP-gated ion channels close and hyperpolarize photoreceptors. Hyperpolarisation induced by blue light was recorded in vitro in dark-adapted dog retina by measuring the transretinal potential via bilateral silver/silver chloride electrodes. In addition, in situ electrical activity of the dog dark-adapted retina in response to a light stimulus was detected using a corneal electrode, and the resultant electroretinogram analyzed.

Results:

Dose-related actions of sildenafil on phototransduction were detected *in vitro* (fig 4) as well as *in vivo* (fig. 5; Table 4). Such effects were observed *in vitro* at concentrations of 0.3-100 μM (IC50 = 3μM). The slowing of the rate of hyperpolarisation is consistent with the compound opposing the normal physiological response to light. This change , the slowing of the rate of repolarisation, and the change in the duration of response have all been observed with other non-selective PDE inhibitors, and is expected of sildenafil. according to sponsor.

Figure 4

A representative figure to show the effects of sildenafil on the response of the dog dark-adapted isolated retina to a 50 msec blue light challenge. Each curve represents the average response to 7 consecutive light challenges.

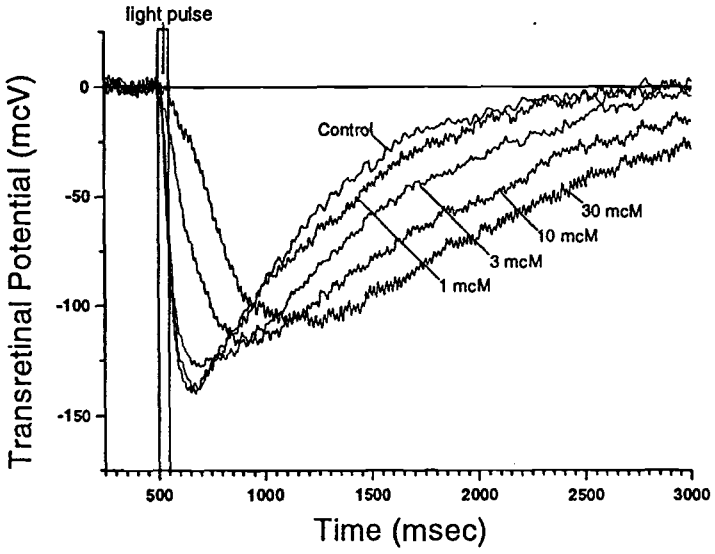
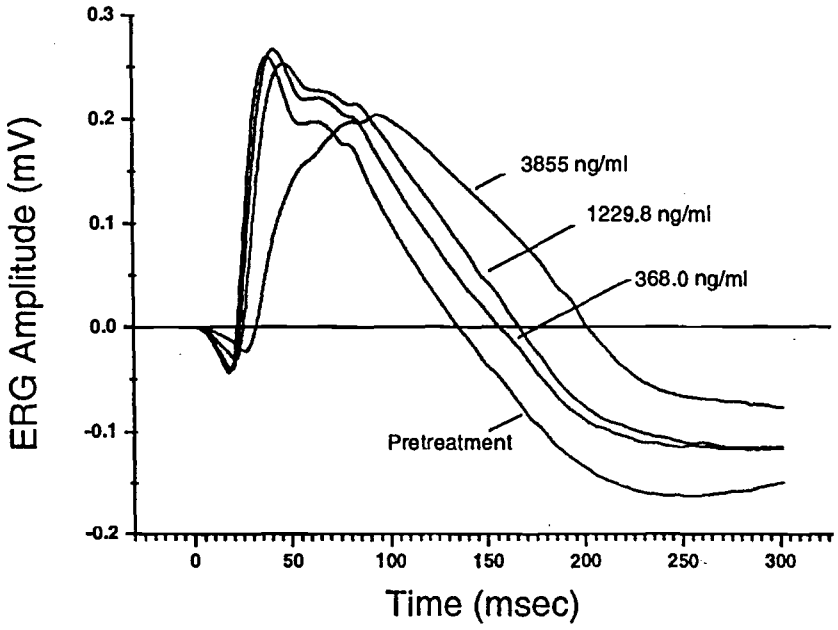


Figure 5

A representative figure to show the effects of intravenously-administered sildenafil on the electroretinogram (ERG) of the dark-adapted anaesthetised dog evoked by a flash (50 mcsec) of blue light. Each curve represents the average response to 5 consecutive light challenges.



In vivo , electroretinogram changes occurred at serum sildenafil concentrations of 368-3855 ng/ml with a suggestion that the dose-response may begin at lower threshold level of 100-200 ng/ml. Implicit times of the a- and b-waves (the most sensitive marker of effects according to sponsor) were increased by approximately 10% at a mean total plasma concentration of 368.0 ± 24.0 ng/ml. As noted above, cavernosally -active plasma levels achieved following bolus doses of 30 µg/kg iv. of sildenafil were 13.7 ± 1.2 ng/ml.
Thus, plasma concentrations which are active *in vivo* on the retina are nearly 27-fold higher than those active on the corpus cavernosum in the anaesthetized dog. However, a more conservative safety ratio (e.g., min. retinally-active conc./ max. cavernosally - active conc.) would be approx. 4 (i.e., 200 ng per ml/50 ng per ml).

Table 4

The effects of intravenously-administered sildenafil and vehicle on the electroretinogram (ERG) of the dark-adapted anaesthetised dog (n=5). The ERG was evoked using a flash (50 mcsec) of blue light.

Mean, ± S.E.M., plasma concentration (ng/ml)	a-wave amplitude (mV)		a-wave implicit time (msec)		b-wave amplitude (mV)		b-wave implicit time (msec)		Slope of the b-wave mcV/msec)	
	Sildenafil	Vehicle	Sildenafil	Vehicle	Sildenafil	Vehicle	Sildenafil	Vehicle	Sildenafil	Vehicle
Pre-dose values	-0.045 ± 0.006	-0.042 ± 0.004	+19.36 ± 0.53	+19.75 ± 0.91	+0.30 ± 0.03	+0.30 ± 0.03	+41.51 ± 1.47	+42.92 ± 1.25	+13.87 ± 1.43	+12.82 ± 1.21
Control	-2.0 ± 2.6	+12.8 ± 5.0	+1.3 ± 0.6	+0.3 ± 1.4	+3.5 ± 2.0	+3.0 ± 3.0	+0.2 ± 1.0	+1.5 ± 0.5	+4.4 ± 2.2	+1.5 ± 0.5
113.2 ± 7.6	-7.1 ± 1.6	-0.2 ± 7.7	+3.5 ± 0.6	+3.3 ± 1.2	+5.5 ± 1.5	+2.1 ± 1.5	+3.2 ± 0.3	+3.0 ± 0.7	+2.5 ± 1.1	+1.5 ± 2.3
368.0 ± 24.0	-16.2 ± 4.1	-0.7 ± 6.2	+8.9 ± 1.2	+4.7 ± 1.7	+6.8 ± 2.1	+1.5 ± 1.9	+9.5 ± 1.1	+4.8 ± 1.1	-3.0 ± 0.9	-1.0 ± 2.6
1229.8 ± 62.7	-50.5 ± 2.4	-3.9 ± 7.9	+14.1 ± 3.3	+5.4 ± 1.7	+1.2 ± 4.6	+0.9 ± 2.6	+23.4 ± 2.9	+5.8 ± 1.1	-21.9 ± 3.2	-3.4 ± 3.0
3855.0 ± 7.0	-53.3 ± 2.7	-9.2 ± 11.0	+45.3 ± 4.6	+6.7 ± 2.3	-21.0 ± 4.3	+0.1 ± 2.9	+125.6 ± 10.6	+7.5 ± 1.3	-72.9 ± 8.7	-8.2 ± 3.3

n = Number of animals
Vehicle = Acidified saline
Control = No treatment

. = Decrease
+ = Increase
Pre-dose values are the absolute values for each parameter expressed in the units indicated
Sildenafil was infused intravenously to achieve steady state plasma concentrations. The compound was infused at increasing concentrations (3.3 mcg/kg/min for 30 min plus 50 mcg/kg/min for the first 2 min) from 30 to 60, (10 mcg/kg/min for 30 min plus 100 mcg/kg/min for the first 2 min) from 60 to 90, (33 mcg/kg/min for 30 min plus 350 mcg/kg/min for the first 2 min) from 90 to 120 and (100 mcg/kg/min for 30 min plus 800 mcg/kg/min for the first 2 min) from 120 to 150 min.
Results are mean, ± S.E.M., percentage changes from pre-dose values based on the average of 5 consecutive ERG readings taken pre-dose and at steady state plasma levels

1.3 General / Safety Pharmacology

1.3.1 *In vitro* receptor binding profile:

Displacement of the binding of appropriate radioligands to brain tissue membranes in vitro was used as an index of affinity for adrenoceptors, adenosine (A_1 and A_2), dopamine (D_1 and D_2), histamine (H_1),5-HT_1, 5-HT_2, muscarinic and opioid receptors, dihydropyridine, verapamil and diltiazem calcium channel binding sites and benzodiazepine binding sites: At concentrations up to 10 µM, sildenafil displayed little affinity for α and β adrenoceptors, dopamine (D_1 and D_2), histamine (H_1), 5-HT_1, 5-HT_2, muscarinic and opioid receptors and verapamil and benzodiazepine binding sites (Table 5).

Table 5

Summary of radioligand binding affinity of sildenafil for receptors and binding sites *in vitro.*

Receptor type/ binding site	Standard agent	IC_{50} (nM) of:	
		Standard	Sildenafil
α_1-adrenoceptor	Prazosin	0.51 ± 0.04	>10,000
α_2-adrenoceptor	UK-14,304	1.68 ± 0.10	>10,000
β-adrenoceptor	Propranolol	4.6*	>10,000
Porcine Adenosine A_1	Cyclohexyladenosine	4.0 ± 0.3	5300 ± 1400
Rat Adenosine A_1	Cyclohexyladenosine	0.88	51% inhibition at 100 nM, 100% at 10000 nM
Porcine Adenosine A_2	Cyclopentyladenosine	1082 ± 56	300 ± 71
Human Adenosine A_{2a}	NECA	24.5	48% inhibition at 100 nM, 87% at 10000 nM
Human Adenosine A_{2b}	NECA	36.1	86% inhibition at 10000 nM
Human Adenosine A_3	NECA	16.3	32% inhibition at 10000 nM
Dopamine D_1	SCH-23,390	1.5 ± 0.2	>10,000
Dopamine D_2	Butaclamol	14.8 ± 3.0	>10,000
Histamine H_1	Pyrilamine	3.7*	>10,000
5-HT_1	5-HT	1.71 ± 0.40	>10,000
5-HT_2	Ketanserin	4.6*	>10,000
Muscarinic	Atropine	3.7*	>10,000
Opioid	Naloxone	2.7 ± 0.3	>10,000
Dihydropyridine	Nitrendipine	0.44 ± 0.11	51% inhibition at 10,000nM
Verapamil	Verapamil	405 ± 140	>10,000
Diltiazem	Diltiazem	84 ± 18	3400 ± 340
Benzodiazepine	Flunitrazepam	4.26*	>10,000

All values represent the mean, ± S.E.M., of at least 3 experiments or the average* of 2 experiments

IC_{50} = Concentration required to reduce the degree of specific binding by 50%

Weak affinity was seen at porcine adenosine A_1 receptors (IC_{50} 5.3 μM), human adenosine A_{2b} (86% inhibition at 10 μM) and A_3 (32% inhibition at 10 μM) receptors and dihydropyridine (51% inhibition at 10 μM) and diltiazem (IC_{50} 3.4 μM) binding sites. In comparison with the mean IC_{50} of 3.5 nM for inhibition of PDE5 in human corpus cavernosum, it is unlikely that these weak binding affinities have biological relevance at efficacious doses. However, sildenafil had moderate affinity for the rat adenosine A_1 (IC_{50} approximately 100 nM), porcine adenosine A_2 (IC_{50} 300 nM) and human adenosine A_{2a} (48% inhibition at 100 nM and 87% inhibition at 10 μM) receptors, although the concentrations are still 28 to 85 times higher than that required to inhibit the human PDE5 enzyme found in the corpus cavernosum .

Receptor affinity profile and selectivity is consistent with a threshold for noteworthy cardiovascular , gastrointestinal, or central effects occurring at least an order of magnitude higher dosages and/or blood levels than those achieving targeted cavernosal effects in the dog - the species in which the broadest spectrum of bioassays were performed.

1.3.2 Effects on Electroconversion in the Anaesthetized Dog.

This was one of the more important safety assays performed by the sponsor. Since enzyme and receptor selectivity is never absolute, sildenafil can block PDE3 as well as the targeted PDE5, and the PDE3 inhibitor milrinone is pro-arrhythmogenic in humans and dog models:
Effect of sildenafil on the electroconversion of ventricular fibrillation to sinus rhythm was investigated in pentobarbitone-anaesthetised dogs. Ventricular fibrillation was induced by high frequency electrical stimulation(PES) of an electrode positioned in the right ventricle, and the fibrillating hearts were cardioverted using DC shock applied through direct contact electrodes positioned on either side of the chest wall. A large bolus intravenous injection of 3 mg/kg sildenafil (N= 4 dogs) - vs. an equivalent volume of vehicle (acidified saline, pH 2.66; N= 4 dogs) - neither reduced the duration (train) of high frequency electrical stimulation required to induce ventricular fibrillation (Table 6), nor augment the energy required to defibrillate the hearts (Table 7) during the 3 hour experimental protocol.

Table 6

The effects of intravenously-administered vehicle (Panel A) and sildenafil (Panel B) on the duration (train) of high frequency electrical stimulation (40 Hz, 1 msec, 4 V) required to induce ventricular fibrillation in the hearts of pentobarbitone-anaesthetised dogs (n=4).

Panel A Vehicle (Acidified saline (mean, ± S.E.M., pH 2.66 ± 0.15), 10ml plus 2ml wash-in i.v.)

Dog number	Meaned duration (sec) control 0 to 60 min	Meaned duration (sec) 60 to 120 min	Percentage change from control	Meaned duration (sec) 120 to 180 min	Percentage change from control	Meaned duration (sec) 60 to 180 min	Percentage change from control
OFE2	0.525	0.550	+4.8	0.600	+14.3	0.575	+9.5
OFF2	0.600	0.575	-4.2	0.600	0.0	0.588	-2.0
OGC3	0.525	0.500	-4.8	0.450	-14.3	0.475	-9.5
OFC2	0.700	0.500	-28.6	0.675	-3.6	0.588	-16.0
Mean percentage change ± S.E.M.			-8.2 ± 7.1		-0.9 ± 5.9		-4.5 ± 5.5

Panel B Sildenafil (3 mg/kg i.v.)

Dog number	Meaned duration (sec) control 0 to 60 min	Meaned duration (sec) 60 to 120 min	Percentage change from control	Meaned duration (sec) 120 to 180 min	Percentage change from control	Meaned duration (sec) 60 to 180 min	Percentage change from control
OEP3	0.575	0.575	0.0	0.575	0.0	0.575	0.0
OFC4	0.600	0.475	-20.8	0.525	-12.5	0.500	-1.7
OFE1	0.575	0.525	-8.7	0.575	0.0	0.550	-4.4
OGD1	0.550	0.500	-9.1	0.425	-22.7	0.462	-16.0
Mean percentage change ± S.E.M.			-9.7 ± 4.3		-8.8 ± 5.5		-5.5 ± 3.6

\- = Decrease
\+ = Increase

Table 7

The effects of intravenously-administered vehicle (Panel A) and sildenafil (Panel B) on the energy (Joules) required to defibrillate the hearts of pentobarbitone-anaesthetised dogs (n=4).

Panel A Vehicle (Acidified saline (mean, ± S.E.M., pH 2.66 ± 0.15), 10 ml plus 2 ml wash-in i.v.)

Dog number	Meaned energy (Joules) control 0 to 60 min	Meaned energy (Joules) 60 to 120 min	Percentage change from control	Meaned energy (Joules) 120 to 180 min	Percentage change from control	Meaned energy (Joules) 60 to 180 min	Percentage change from control
OFE2	25.0	25.0	0.0	22.5	-10.0	23.8	-4.8
OFF2	25.0	22.5	-10.0	25.0	0.0	23.8	-4.8
OGC3	20.0	22.5	+12.5	22.5	+12.5	22.5	+12.5
OFC2	22.5	17.5	-22.2	22.5	0.0	20.0	-11.1
Mean percentage change ± S.E.M.			-4.9 ± 7.4		+0.6 ± 4.6		-2.0 ± 5.1

Panel B Sildenafil (3 mg/kg i.v.)

Dog number	Meaned energy (Joules) control 0 to 60 min	Meaned energy (Joules) 60 to 120 min	Percentage change from control	Meaned energy (Joules) 120 to 180 min	Percentage change from control	Meaned energy (Joules) 60 to 180 min	Percentage change from control
OEP3	20.0	25.0	+25.0	20.0	0.0	22.5	+12.5
OFC4	22.5	20.0	-11.1	22.5	0.0	21.2	-5.6
OFE1	27.5	22.5	-18.2	28.8	+4.5	25.6	-6.9
OGD1	17.5	20.0	+14.3	17.5	0.0	18.8	+7.1
Mean percentage change ± S.E.M.			+2.5 ± 10.2		+1.1 ± 1.1		+1.8 ± 4.8

\- = Decrease
\+ = Increase

Accordingly, sildenafil did not facilitate induction of fibrillation or impede cardioversion at a dosage (3 mg/Kg iv.,) approx. 30 to 100 times cavernosally active dosages (30-100 µg/Kg iv. in the same species. Unfortunately , a positive control such as the PDE3 blocker milrinone was not included in this bioassay [Milrinone was proarrhythmogenic in a dog model of PES-provoked V fib; such data has been published by it's author, Dr. Lucchesi].

1.3.3 Effects on nervous system

1.3.3.1 Autonomic nervous system.

In the standard anesthetized cat nictitating membrane preparation (electrical stimulation of the pre-ganglionic superior cervical sympathetic nerve), sildenafil (0.3, 1 and 3 mg/kg iv.) did not reveal any sympathomimetic or ganglion stimulating or blocking activity as evidenced by no change in muscle tension (basal , or neuronally stimulated).

1.3.3.2 Central nervous system

The effects of sildenafil on the central and peripheral nervous systems were assessed from acute studies of effects on the appearance and behaviour of rats and mice, the performance of mice on a rotating rod, alcohol- and pentobarbitone-induced sleeping times in mice, and analgesia assays (tail flick and acetic acid-induced abdominal constriction in mice).

Results: Behaviorally, sildenafil was tolerated by rats (up to 300 mg/Kg) and mice (up to 10 mg/Kg) following single oral and intravenous administration, respectively, and following multiple dose oral administration (30 mg/kg t.i.d./5 days) - despite peripheral erythema indicative of vasodilation. In chlorpromazine-controlled mouse studies, the compound did not a) display sedative activity; b). interact meaningfully with alcohol or pentobarbitone; or c) impair motor co-ordination (rotarod test) in the mouse at 10 mg/Kg p.o. Somatic function in the cat. Sildenafil was not analgesic in mice following multiple dosing over 5 days at 30 mg/ Kg t.i.d.

1.3.4. Hemodynamic effects:

1.3.4.1 Rats and dogs

These are described above under: Effects related to inhibition of PDE (1.2.3.4 : Cardiovascular effects)

1.3.4.2 Effects on Histaminergic, Cholinergic, and Adrenergic agonists in cat.

At up to 3 mg/Kg iv., sildenafil revealed no evidence of any consistent compound-related antagonistic effects on the cardiovascular responses to histamine, acetylcholine, phenylephrine or isoprenaline in the anesthetized cat. This is consistent with absence of any affinity of this compound - at 10 μM - for these receptor types in *in vitro* radioligand binding studies (see above 1.3.1), and with the minimal systemic hemodynamic effects at cavernosa - specific blood sildenafil levels.

1.3.5 Effects on the Gastrointestinal tract *in vivo.*

As indicated above (Effects related to inhibition of PDE : section **1.2.3.5**) sponsor reported no remarkable functional changes either *in vitro* or *in vivo* in bioassays involving rat, mouse, guinea pig, or dog .

1.3.6 Respiratory system

Activity of sildenafil, vehicle (acidified saline) and morphine on blood gas tensions and blood pH were assessed in rats implanted with arterial and venous cannulae. As expected of it's central depressant activity, morphine (4 mg/kg iv.) decreased arterial blood pH and pO2 and increased pCO2. In contrast, sildenafil (3 mg/kg iv.) did not significantly affect any marker of respiratory function in the conscious rat.

1.3.7 Renal function

The effects of sildenafil on renal function were investigated. Sildenafil, by its cGMP PDE inhibitory mechanism, increases tissue cGMP levels, and consequently may enhance effects of atrial natriuretic factor. Effects on urinary pH, excretion of fluid and electrolytes, and concentrations of electrolytes, over a 5 h period after dosing, were monitored in conscious, normotensive female rats given an oral saline load. Furosemide significantly increased the mean total urinary excretion of fluid, Na+,K+,Cl-, and significantly reduced urinary pH and concentrations of electrolytes. In contrast, sildenafil (1, 3 and 10 mg/kg p.o.) reduced urinary pH and the mean total excretions of fluid, Na+, K+ and Cl- to essentially the same extent at all dose levels, although the changes did not always reach statistical significance. Accordingly, at doses of 1 mg/kg p.o. and above, sildenafil has antidiuretic activity in the rat. In my experience, this would be expected in view of the compound's vaso-depressor activity at the dosages tested (at 10 mg/Kg,blood pressure of the SH rat was reduced by 25 mmHg for at least 3 hours: **1.2.3.4** c., above).

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

PHARMACOLOGY REVIEWS

TOXICOLOGY PAGES 26-48

2. TOXICOLOGY

2.1. Acute Toxicology

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

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

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

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

Results:

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

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

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

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

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

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

In these studies, data reported show that the minimal lethal dose (MLDL) level is between 500-1000 mg/kg in mice and between 300-500 mg/kg in rats. In rats, the severity of clinical signs in females and the mortality which occurred in females only, suggest a sex-linked difference in the sensitivity to acute effects of UK-92,480.

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

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

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

It was concluded that i.v. administration of UK-92,480-10 to mice at 20 mg/kg and to rats at 10 mg/kg produced no evidence of acute toxicity.

2.2. Subchronic/Chronic Toxicology

2.2.1. Rats

2.2.1.1. Oral

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

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

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

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

Palpebral (eyelid) closure and chromodacryorrhea (bloody tears) were observed in the 150 and 500 mg/kg groups. Dyspnea and salivation occurred in the 500 mg/kg groups.

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

There were significant increases in absolute and relative liver weights in the high dose (500 mg/kg) males and in the mid (150 mg/kg) and high (500 mg/kg) dose females (Figure 6). Microscopically, this correlated with an increased incidence of hepatic centrilobular hypertrophy (Table 8). This change was considered to be an adaptive process since it has been found in other cases of liver enzyme induction.

Figure 6

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

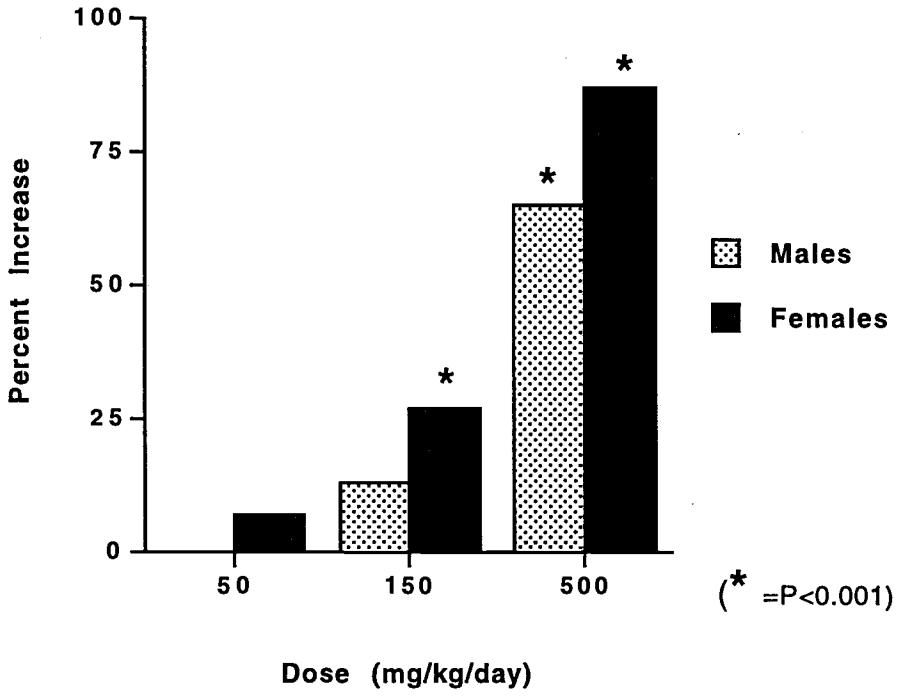


Table 8

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

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

Plasma drug concentration ratios of UK-92,480 and the major pharmacologically active metabolite, UK-103,320, showed that males were exposed mostly to the metabolite (Figure 7A), while females were exposed mostly to the unchanged drug (Figure 7B). In males, ratios of metabolite to drug, particularly on Day 9, were lower with increasing dose, indicating that metabolism was saturable.

Figure 7A

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

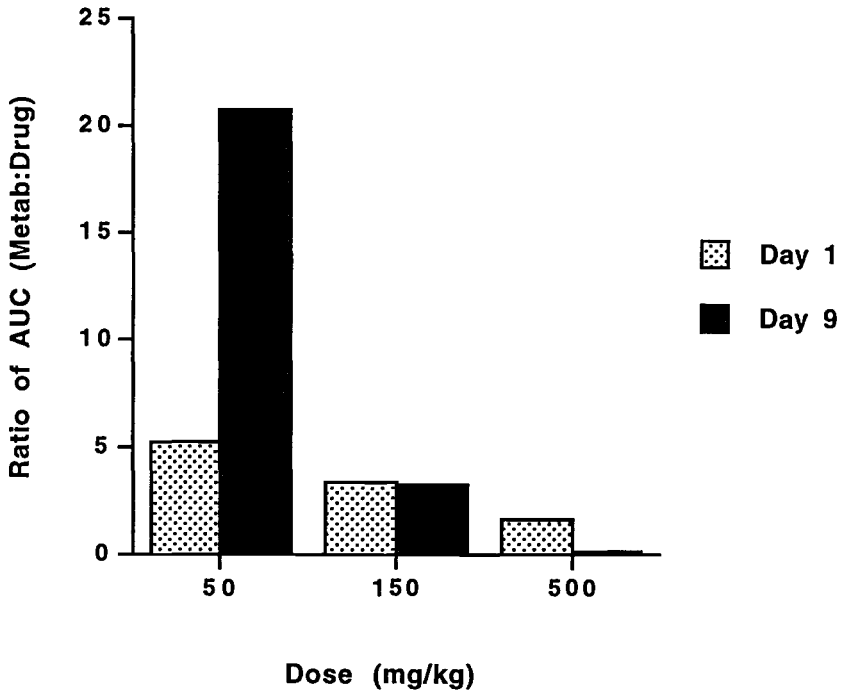
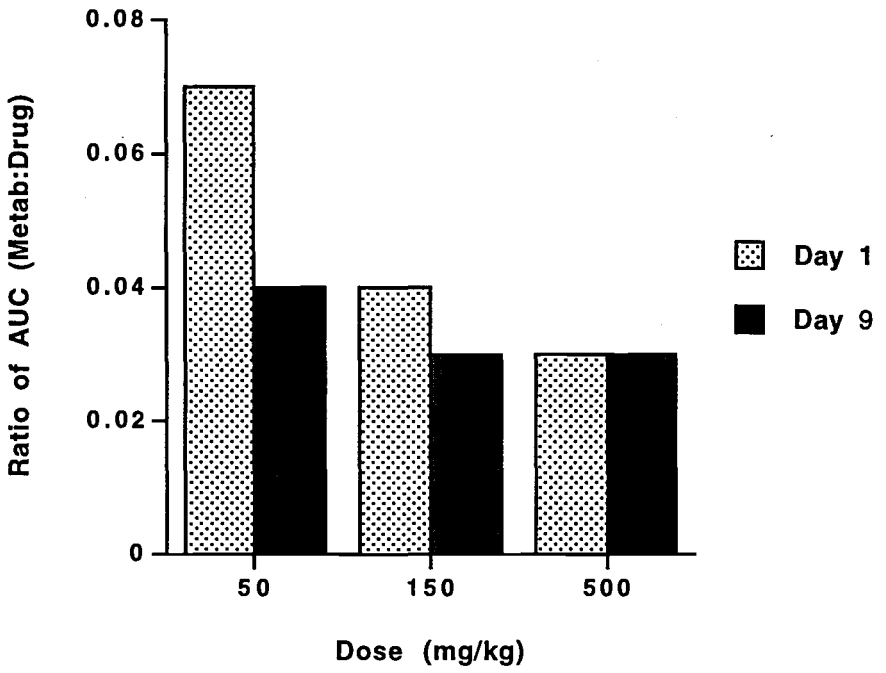


Figure 7B

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



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

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

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

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

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

Results:

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

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

A number of clinical signs and histopathological changes as well as variations in food and water consumption, biochemical parameters, platelets and heart and kidney weights, were seen in treated animals, in most instances at MD and HD doses. Although these observations were not considered to be of toxicological importance by sponsor, a remarkable finding reported consisted of testicular atrophy in some male rats in all groups. The lesion involved less than 10% of the seminiferous tubules but in 1 HD rat where most of the tubules were affected. No remarkable changes in mean absolute testicular was reported. The dose of 10 mg/kg appeared to be the no-adverse effect level (NOAEL).

N-demethylation of UK-92,480 to UK-103,320 was found to be an important route of UK-92,480 biotransformation in male rats. The transformation rate is sex-dependent, females being exposed predominantly to the unchanged drug and males to an almost equal balance of drug and metabolite.

PLASMA DRUG CONCENTRATION:

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

UK-92,480:

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

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

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

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

UK-92,340:

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

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

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

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

UK-103,320:

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

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

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

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

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

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

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

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

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

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

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

Results:

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

A trend towards a reduced body weight gain was seen at the HD. In the MD and HD groups, there were statistically significant mild to moderate, dose-related increases in absolute and/or relative liver weight, the increases being more prominent in the females.

Decreases of plasma bilirubin and triglycerides, and increases in plasma urea, total proteins and cholesterol were seen at HD (See under Plasma Chemistry below, table prepared by sponsor on page 38.) These changes were suggested to sponsor as drug-induced metabolic changes in the liver.

Increased liver weight was associated with mild centrilobular hypertrophy as seen in the 1-mo study. Thyroid hypertrophy occurred at HD in both sexes and at a lower incidence in MD males. This change was considered by sponsor to be a secondary phenomenon related to increased hepatic clearance of thyroid hormone. Hypertrophy and increase in weight of the zona glomerulosa of the adrenal gland was seen at a dose-related incidence at MD and HD.

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

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

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

Table 1 - Plasma Chemistry

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

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

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

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

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

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

PLASMA DRUG CONCENTRATION:

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

UK-92,480:

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

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

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

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

UK-103,320:

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

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

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

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

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

Ratio of mean AUC's (metabolite:drug)

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

-: Not calculated

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

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

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

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

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

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

2.2.1.2. Intravenous

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

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

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

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

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

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

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

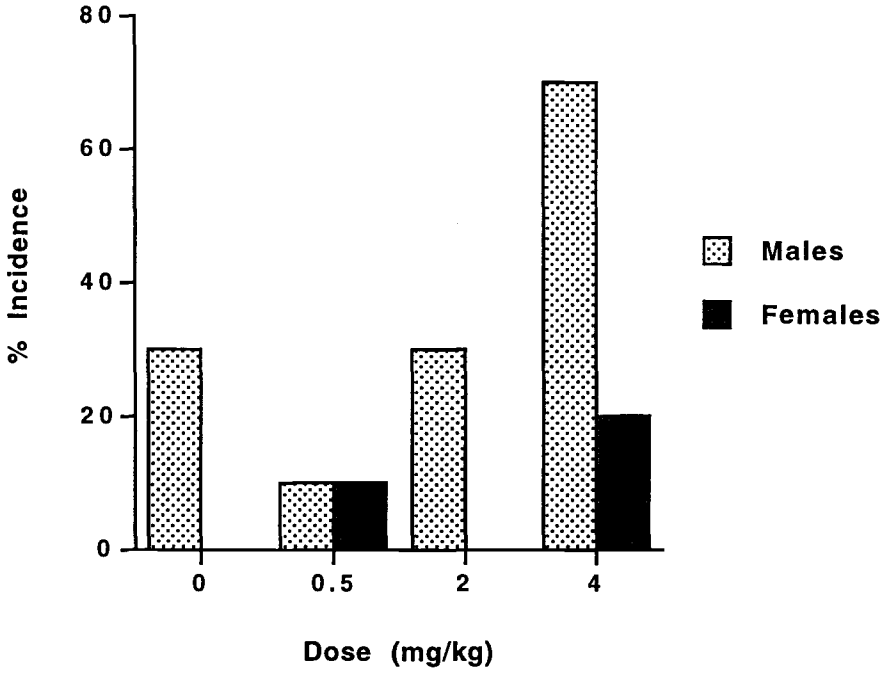
Male and female Sprague-Dawley rats (10/sex/group; 267 gms for males and 187 gms for females) were injected with UK-92,480 (lot no. 953-27) i.v. at 0.5, 2, or 4 mg/kg/day for 28 days. Controls received vehicle (5% mannitol solution). Rats were observed for clinical signs and weighed once per week. An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

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

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

Figure 8

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



2.2.2. Dogs

2.2.2.1. Oral

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

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

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

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

No deaths were reported. Altered clinical signs were observed and consisted of emesis and salivation in the high dose (100 mg/kg) group, conjuctival redness in the mid (30 mg/kg) and high (100 mg/kg) dose groups, and lacrimation in all dose groups.

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

Figure 9

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

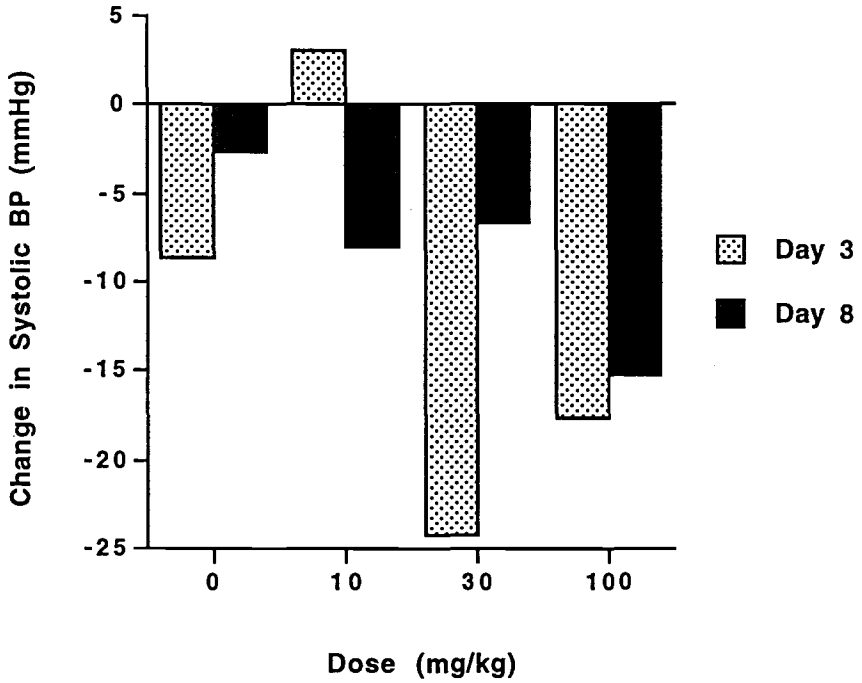
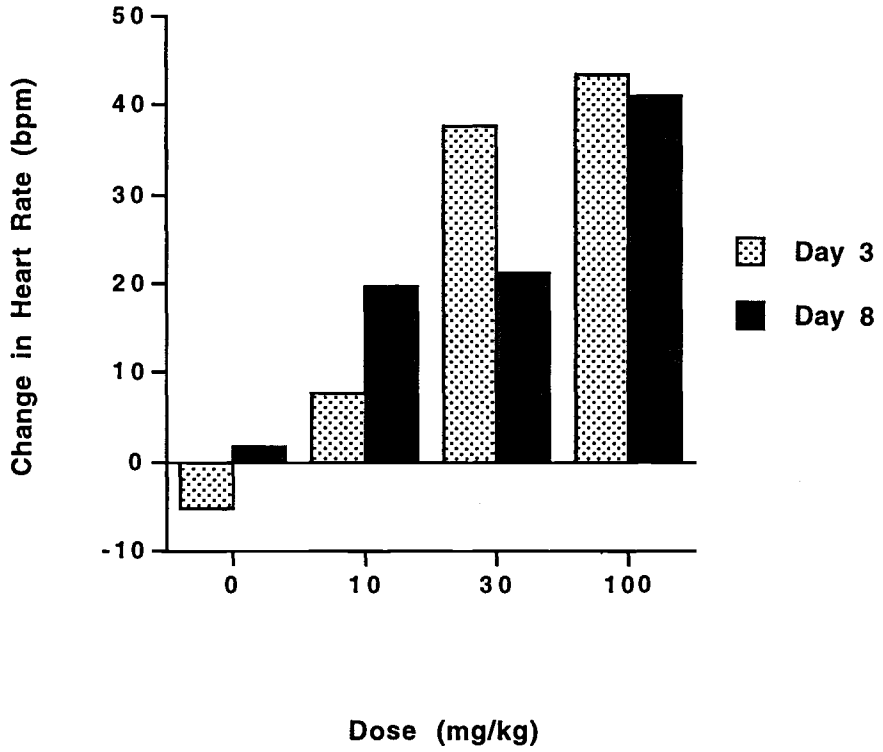


Figure 10

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



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

Table 8

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

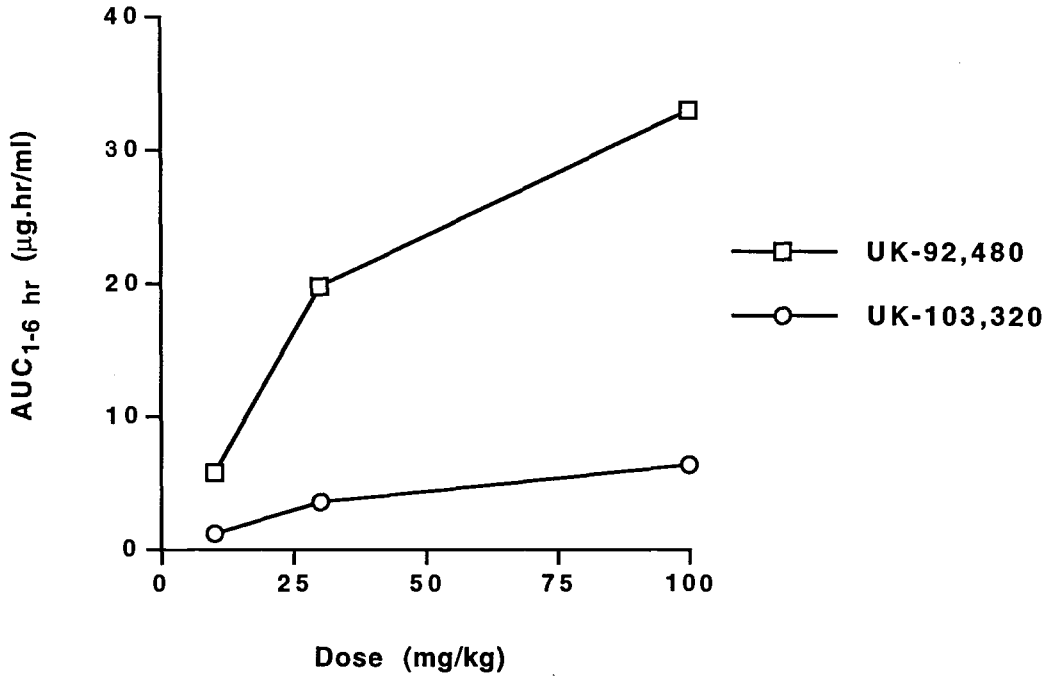
	PQ Interval (msec)			QT Interval (msec)		
Dose (mg/kg)	**Day -1**	**Day 3**	**Day 8**	**Day -1**	**Day 3**	**Day 8**
0	93	93	89	186	205	190
10	104	108	105	202	193	197
30	102	107	103	195	190	195
100	110	**96**	**87**	197	**177**	**179**

Two dogs in the high dose group showed a 45% and 65% increase in plasma cholesterol when compared to pre-dose values. Relative liver weights were slightly increased 30, 28, and 27% in the 10, 30, and 100 mg/kg groups, respectively when compared to the single control dog, and may reflect individual variation. Microscopic analysis found a focal arteritis in the right coronary artery of one high dose female. Although such lesions may occur spontaneously in Beagle dogs, it has been associated in dogs with PDE3 inhibitors.

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

Figure 11

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



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

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

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

Results:

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

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

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

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

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

Results:

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

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

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

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

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

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

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

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

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

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

PLASMA DRUG CONCENTRATION:

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

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

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

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

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

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

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

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

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

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

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

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

No deaths were reported. Clinical signs in 2/4 high-dose males were noted. These included: pain, arched back, hyperthermia, increased salivation, and tremor. Also, redness of the conjunctiva in these high-dose males was thought to be due to the vasodilatory properties of the drug. There were no treatment-related effects of body weight or blood pressure. There were no noteworthy drug-related changes in hematology, clinical chemistry, or urinalysis.

Heart rates were increased 2 hours after treatment as measured on several days (Table 9). The increased heart rates may have been a compensatory response to the vasodilatory effects of the drug.

Table 9

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

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

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

ECG results showed that there were increases in P amplitude (atrial contraction) and decreases in PQ (time between atrial and ventricular contraction) and QT (time between the beginning of ventricular contraction and repolarization) intervals in the high-dose dogs (Table 10). These changes correlated with the increases in heart rates observed. The changes were within the sponsor's historical range for dogs, and were not considered toxicologically significant.

Table 10

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

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

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

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

On microscopic examination, a periarteritis was observed in 3/4 high-dose males, 1/4 high-dose females. and 1/4 low-dose females. It was characterized by a mononuclear infiltrate in the adventitia and media accompanied by intimal proliferation and fragmentation of the internal elastic lamina. In females, the periarteritis was focal and restricted to a coronary vessel, while in affected males it involved the heart and other organs. Other microscopic findings included accumulation of hemosiderin in the liver of one high-dose male.

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

Figure 12

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

The major toxicological finding of this study was the occurrence of a periarteritis in 3/4 high-dose (50 mg/kg) males. Periarteritis was also found in a previous 6-month toxicity study in 2/4 male dogs treated with 50 mg/kg (Study No. 91099). This condition, also known as idiopathic febrile necrotizing arteritis, occurs spontaneously on a rare occasion in Beagle dogs. Clinical pathology changes in this syndrome include neutrophilia, high fibrinogen levels, anemia, increased alkaline phosphatase, and decreased sodium and chloride. These changes were found in the high-dose male dogs, but in none of the controls indicating that these effects were drug-related. It was concluded that the NOAEL for this study in dogs was 10 mg/kg/day.

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

Table 11

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

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

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

Table 12

Human and Dog Plasma Protein Binding

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

Comparison of the dog AUCs for total drug exposure (sum of unbound UK-92,480 and UK-103,320 AUCs) as a multiple of the maximum recommended human dose (MRHD) of 100 mg is shown in Table . The unbound AUCs were calculated by multiplying the total bound and unbound AUC (Table 11) by the fraction unbound (Table 12). As shown in Table 13, the total of unbound AUCs in dogs given 50 mg/kg/day was 48.9X the AUC of men given a single dose of 100 mg. This value represents a relatively large safety margin with respect to the possible development of drug-induced arteritis in man. Systemic exposure (sum of unbound AUCs) in dogs to 10 mg/kg (NOAEL) was 7.8X the human exposure at 100 mg (1.4 mg/kg).

Table 13

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

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

2.2.2.2. Intravenous

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

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

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

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

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

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

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

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

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

No deaths were reported. Clinical signs consisted of emesis and salivation were unrelated to treatment. No effects were reported on pupil size or pupillary reflex to light. There were no drug-related effects on cardiovascular parameters (ECG, BP, HR). No other drug-related effects were noted. It was concluded that UK-92,480 given to dogs i.v. at up to 4 mg/kg/day for 28 days produced no evidence of toxicity.

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

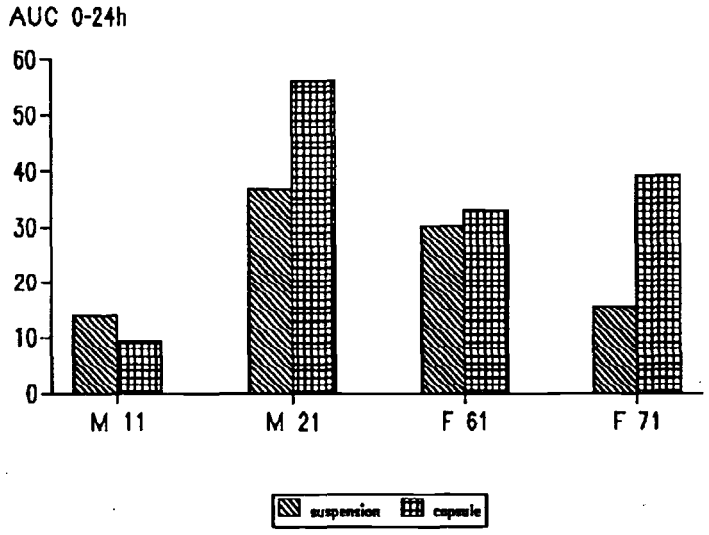
Early toxicity studies were conducted with the free base (UK-92,480) while further toxicity studies and clinical trials were conducted with the citrate salt (UK-92,480-10). This study assessed in dogs the oral bioequivalence of a suspension of the base and of capsules of the citrate salt.

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

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

Figure 13

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



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

2.2.3. Mice

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

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

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

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

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

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

RESULTS

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

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

PHARMACOLOGY REVIEWS

TOXICOLOGY PAGES 49-65

Incidence of mortality
(number of animals found dead or sacrificed as moribund)

	Cause of death	Dose (mg/kg)									
		Control		10		50		100		200	
		M	F	M	F	M	F	M	F	M	F
Main groups (n=10)	gavage accident	0	1	0	1	0	0	1	0	1	0
	gastro-intestinal dilation	0	0	0	0	3	0	2	1	2	1
	unknown	0	0	0	0	0	0	0	0	0	1
Pharmacokinetic groups (n=12)	not determined*	-	-	0	1	2	1	0	1	1	2

n : number of animals for each dose level and each sex.
* : the cause of the death in the pharmacokinetic groups is not routinely determined.

Noteworthy signs of toxicity at 50 mg/kg and above included prostration/decreased activity, dyspnea/noisy respiration and swollen abdomen; these signs were also noted in animals prior to death.

Bodyweight/bodyweight gain of drug treated mice were reduced compared controls.

Mean body weight and body weight gain in male mice: differences (%) in treated animals relative to controls at the end of the study (day 91)

Dose (mg/kg)	body weight gain	body weight
10	-10	-2
50	-40*	-9
100	-17	-5
200	-24	-5

* = statistically significant at p=0.05

The most remarkable sign of toxicity reported in the animals that died was g.i. dilatation at 50 mg/kg and above. Mortality was the consequence of marked g.i. dilatation. A few mice showed colonies of Gram-positive cocci within the lumen of dilated g.i. segments. Other remarkable signs included mild to marked hemoconcentration in moribund mice, fatty change in liver, and atrophy of adipose tissues, decrease in body weight gain (M at the 3 highest doses; not dose-related), dose-related decrease in plasma triglycerides, and increases in plasma cholesterol.

On day 63 of study, the range of maximal UK-92,480/UK 103,320 and $AUC_{1\text{-}5hr}$ were dose-related.

Changes in plasma lipids (% from controls)

Dose (mg/kg)	Triglycerides (males)	Cholesterol (females)
10	+4	+11
50	-9	+46 **
100	-48 **	+37 *
200	-33 **	+32

*, ** = statistically significant at p=0.05 and 0.01, respectively.
Figures in bold denote mean values outside the laboratory range for untreated animals.

Range of maximal UK-103,320 concentrations and mean $AUC_{(1-5h)}$

Dose (UK-92,480-10) (mg/kg)	Concentrations (ug/ml)	Mean AUC (µg.h/ml)
10		not calculated
50		1.07
100		3.20
200		4.68

Range of maximal UK-92,480 concentrations and mean $AUC_{(1-5h)}$

Dose (UK-92,480-10) (mg/kg)	Concentrations (µg/ml)	Mean AUC (µg.h/ml)
10		not calculated
50		3.02
100		9.43
200		13.82

In the drug sponsor conclusions, there were no statements regarding what was considered the a MTD of UK-92,480-10 or doses that might have been selected for the toxicity/carcinogenicity study in mouse. The NOAEL appears to < the LD-10 mg/kg.

Drug sponsor conducted a 2nd study 3-mo mouse repeat dose study which is summarized below (Study No. 94101).

2.3.3.2. 3-month oral (gavage) exploratory toxicity study in CD1 mice (study No. 94101; Vol. 1.30 pp. 7704-7929):

(Sprague-Dawley albino mice, CD-1 [Crl:COBS-VAF-CD1(ICR)BR) (GLP Study No. 94101 conducted in Pfizer Centre de Recherche, Amboise, France, Study starting date: 05-10-94 and ended Feb./Mar. 1995)

The aim of this 2nd study was to obtain further information to aid in the estimate the maximum non lethal dose of UK-92,480-10 so to select doses for the 2-year toxicity/carcinogenicity study in mice. Further, drug sponsor stated that the HD (100 mg/kg/day) was used to confirm the findings of the previous mouse study.

Material and methods were described in the study report. Briefly, the main study consisted of 4 treatment groups of 10 mice/sex/group given 0, 20, 40, and 100 UK-92,480-10 mg/kg/day by gavage for 90 or 91 days. As in the previous 3-mo study, the drug was suspended in a 0.5% sol. of methylcellulose containing 0.1% Tween 80 and controls received the vehicle.

For plasma drug or metabolite levels determination, a supplemental group of 3 mice/sex/dose were treated concurrently with similar doses of UK-92,480 used in the main study. The mice were 6 weeks old and weighed ~25 to 30 g.

During the study, mice were observed daily for signs of toxicity and clinical signs, body weight/food consumption, and mortality. In the main study, hematology, and clinical chemistry were performed at the end of the study. Mice were then sacrificed, necropsied and a number of organs weighed. Plasma drug/metabolite plasma concentrations were determined on day 63 of study at 1, 3, 5 and 8 hrs postdosing. Necropsy was performed on all drug treated mice in the main study. A number of organs were weighed and histopathological examinations were carried out on a range of tissues from mice found dead, sacrificed moribund or at scheduled sacrifice.

RESULTS

Drug related mortality occurred in 1 MD F (sacrificed moribund on day 48 of study); remarkable signs of toxicity before sacrifice included prostration, swollen abdomen, dyspnea. One HD F was found dead on day 89; post mortem signs of toxicity were g.i. dilation. Two other deaths recorded were no considered drug related (Control 1 F sacrificed moribund on day 57 of study with leukemia, 1 LD-M sacrificed moribund on day 17 showed hepatic abscess), and 1 HD M from the supplemental group showed g.i. dilatation, but was not considered drug related by drug sponsor.

There were no remarkable changes reported for hematology, body weight, and food consumption.

Plasma drug/demethylated metabolite determinations showed that the Cmax occurred 1 hr after dosing (determined on day 63); the concentrations of these compounds decreased thereafter to values below the limit of the assay (0.04µg/ml) after 3 hrs for the LD, and 5 hrs for the MD and HD groups.

The $AUC_{0\text{-}8hr}$ values of both UK-92,480 and the demethylated metabolite of the parent drug- UK-103,320 increased superproportionally with doses of the parent drug.

Since UK-103,320, is pharmacologically equipotent to the parent compound, the systemic exposure to these two compounds after oral administration of UK-92,480 is of importance.

Mean plasma concentration of the metabolite were highest a 1 hr after dosing and decreased rapidly. No sex difference was noted in plasma levels of the metabolite, thus data were of both sexes were combined for analysis.

Range of highest UK-92,480 concentrations and mean $AUC_{(0\text{-}8h)}$ values on day 63

Dose (UK-92,480-10) (mg/kg)	Concentrations (µg/ml)	Mean $AUC_{(0\text{-}8h)}$ (µg.h/ml)
20		0.46
40		1.97
100		7.26

Range of highest UK-103,320 concentrations and mean $AUC_{(0\text{-}8h)}$ values on day 63

Dose (UK-92,480-10) (mg/kg)	Concentrations (µg/ml)	Mean $AUC_{(0\text{-}8h)}$ (µg.h/ml)
20		NC
40		0.74
100		2.60

NC : not calculated

Postmortem Observations

The only remarkable effect noted was an increase in both absolute/relative liver weights when compared to control. However, drug sponsor asserted that these increase in values were within the level of their historical control values.

In the mice unscheduled deaths, the most remarkable findings reported were g.i. dilation in some MD and HD F. This findings was also reported in the previous 3-mo oral toxicity study in mice. Two other mice dying of causes unrelated to treatment showed macroscopic/microscopic abnormalities (1 LD M with nodules in liver, spleen, thymus and kidney sacrificed moribund; histologic correlates of macroscopic findings were described. One control F showed enlarged spleen consistent with lymphoblastic leukemia diagnosed cytologically; this pathologic condition was reported by drug sponsor to occur occasionally in young mice.)

Terminal sacrifice animals showed no treatment related findings.

Drug sponsor concluded that the findings in this study confirmed those of the previous mouse study in that the only treatment related effects were mortality and g.i. dilation at doses of 40 mg/kg/day and above.

2.3. Carcinogenicity

2.3.1. Rats

2.3.1.1. Twenty-four month oral toxicity and carcinogenicity study in Sprague Dawley rats (Study No. 94092; Vol. 1.19-1.25 pp. 2620-5147):

Testing Facility: Pfizer, Centre de Recherche, Amboise Cedex, France
Study Number: 94092
Study Date(s): 10/11/94 to 10/10/96
GLP Compliance: Yes

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

NUMBER OF RATS:
- Main study:
  - Control 1 (C1): 60 males and 60 females
  - Control 2 (C2): 60 males and 60 females
  - Low Dose (LD): 60 males and 60 females
  - Middle Dose (MD): 60 males and 60 females
  - High Dose (HD): 60 males and 60 females

- Groups for plasma drug level determinations:
  - Low Dose (LD): 7 males and 7 females
  - Middle Dose (MD): 7 males and 7 females
  - High Dose (HD): 7 males and 7 females

RAT DOSE LEVELS* (mg/kg/day):
Rat Low Dose: 1.5
Rat Middle Dose: 5.0
Rat High Dose: 60.0
- *Dose adjusted during study

BASIS FOR DOSES SELECTED:
- MTD: The dose of 60 mg/kg/day chosen for the two year rat study was based on data from several toxicity and pharmacokinetic studies: (1) doses above 60 mg/kg resulted in mortality and hypertrophy of several organs, (2) a dose of 60 mg/kg for 6 months resulted in similar

adaptive responses and a moderate decrease in body weight gain (-9% in males and -7% in females), and (3) the sums of the AUC levels for free parent and metabolite in rats given 60 mg/kg for 14 days were 27X and 40X for male and female rats, respectively, the human exposure at the maximum recommended dose of 100 mg/day. It should be noted that after two years of treatment in the rat, the multiple of the human exposure was reduced to 18X and 21X for males and females, respectively. This may been due to liver enzyme induction and hypertrophy resulting in increased metabolism of the drug.

PRIOR FDA DOSE CONCURRENCE: No

RAT CARCINOGENICITY: Negative (males and females)

RAT TUMOR FINDINGS:

Tumors were analyzed using the Peto's death rate method for fatal tumors and prevalence analysis for incidental tumors (Peto *et al.*, 1980). According to the sponsor, the only statistically significant finding was an increased proliferation in thyroid follicular cells in male rats treated at the high dose of 60 mg/kg/day (combined incidence of hyperplasia, adenoma, and carcinoma; P = 0.0056 for positive trend using the Peto analysis; Table 14). A combined statistical analysis was performed as recommended for a multistage model of carcinogenesis in which thyroid follicular hyperplasia, adenoma, and carcinoma represent a morphological progression from hyperplasia to neoplasia (McConnell *et al.*, 1986). Other proliferative and neoplastic changes in males and females were observed with similar frequencies in the treated and untreated groups.

Table 14

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

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

In a separate study to assess the relationship between liver enzyme induction and thyroxin clearance, female rats were given either vehicle or UK-92,480 orally at 200 mg/kg for 29 days. Results showed that treatment produced an increase in liver and thyroid weights, thyroid follicular cell hypertrophy, increased hepatic UDP-glucuronyl transferase (UDPGT) activity, increased TSH, decreased T3 and T4 hormones, and an increased clearance of exogenous thyroxin. These results were thought to be consistent with the view that the thyroid hypertrophy found in treated rats was due to induction of hepatic UDPGT which increased the clearance of thyroid hormone and caused a compensatory increase in plasma TSH which, in turn, stimulated the thyroid gland.

Evidence for such a mechanism at the 60 mg/kg dose was not presented, however. Additional experiments assessing induction of genes coding for specific hepatic enzymes, such as UDPGT-specific mRNA levels, would have been able to detect gene induction at the 60 mg/kg dose if such a mechanism were responsible for the thyroid hypertrophy observed in treated rats.

RAT STUDY COMMENTS:

Mortality: No drug-related increase in mortality was found (Table 15). Survival in the treated male groups appeared to be higher when compared to the untreated male controls and to all female groups.

Table 15

Percent Mortality and Percent Survival

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

Body Weights: Mean body weights are shown in Figure 14A (males) and Figure 14B (females). Percent changes in mean body weight gains in male and female rats are shown in Table 16 (Day 1 and Day 723). Results showed that high dose males (60 mg/kg/day) gained 11.0% less weight than controls, while mid- and high dose females gained 17.0% and 15.7% less weight, respectively than controls.

Figure 14A (Sponsor's Figure 8)

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

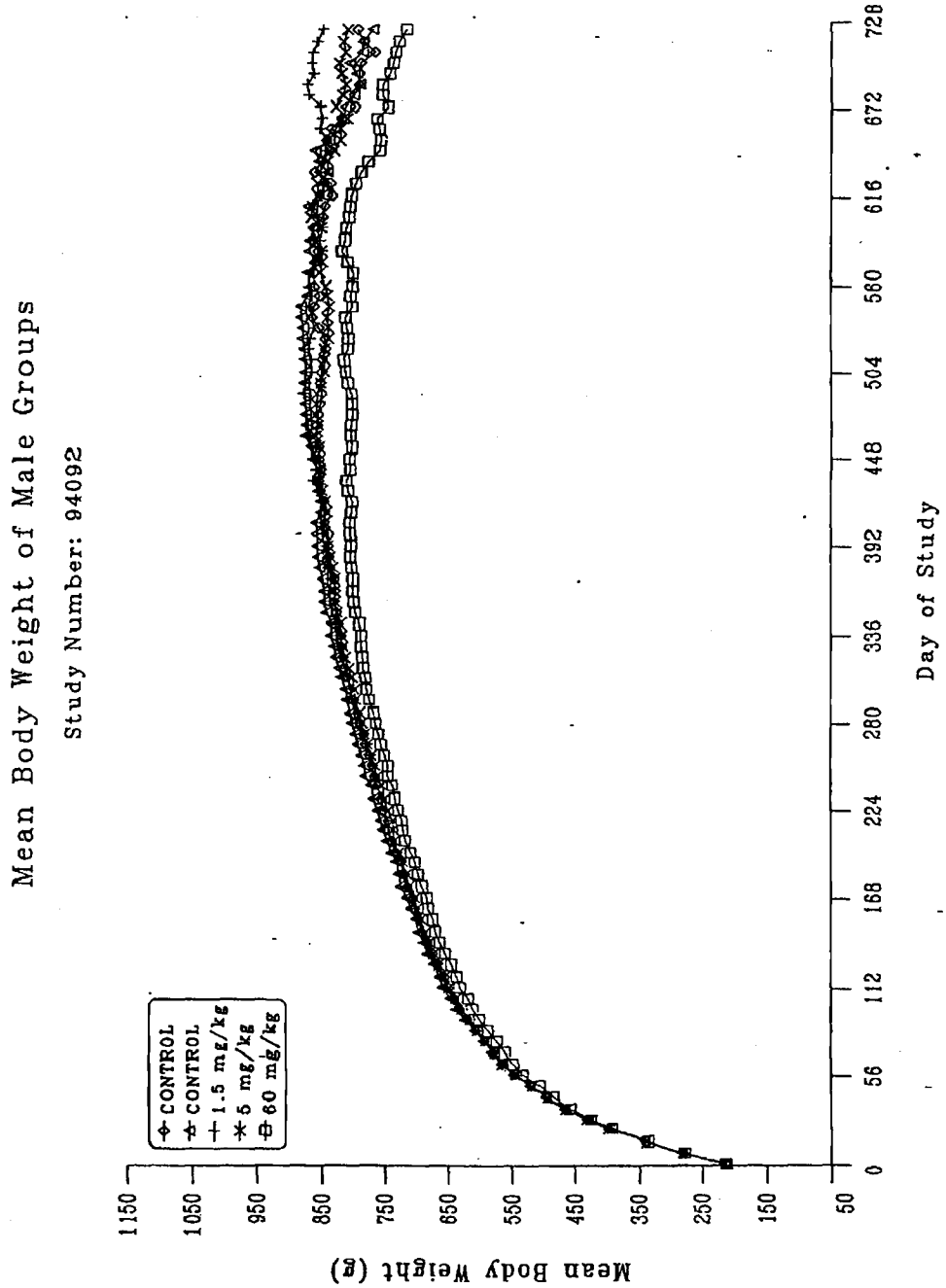


Figure 14B (Sponsor's Figure 9)

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

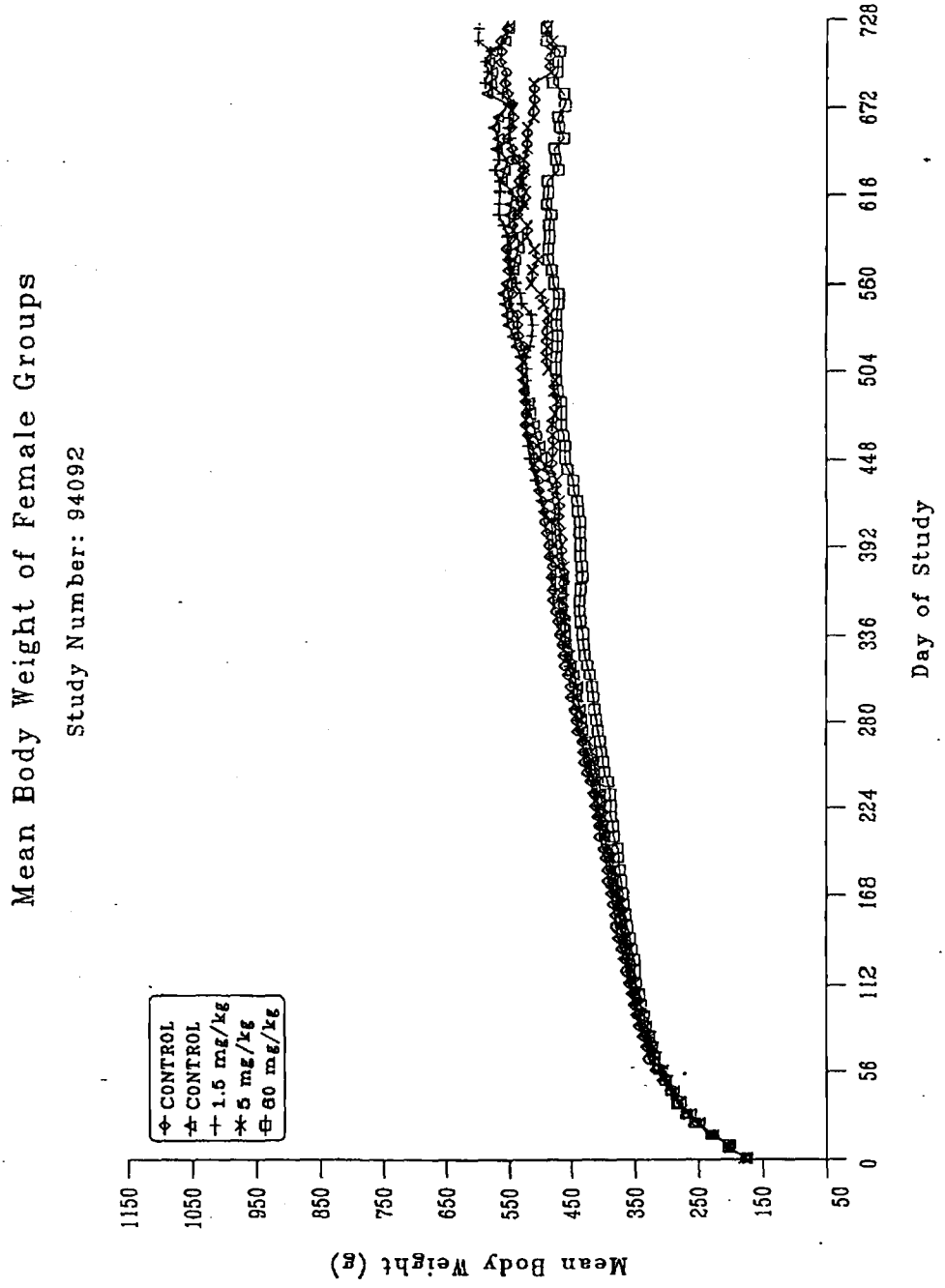


Table 16

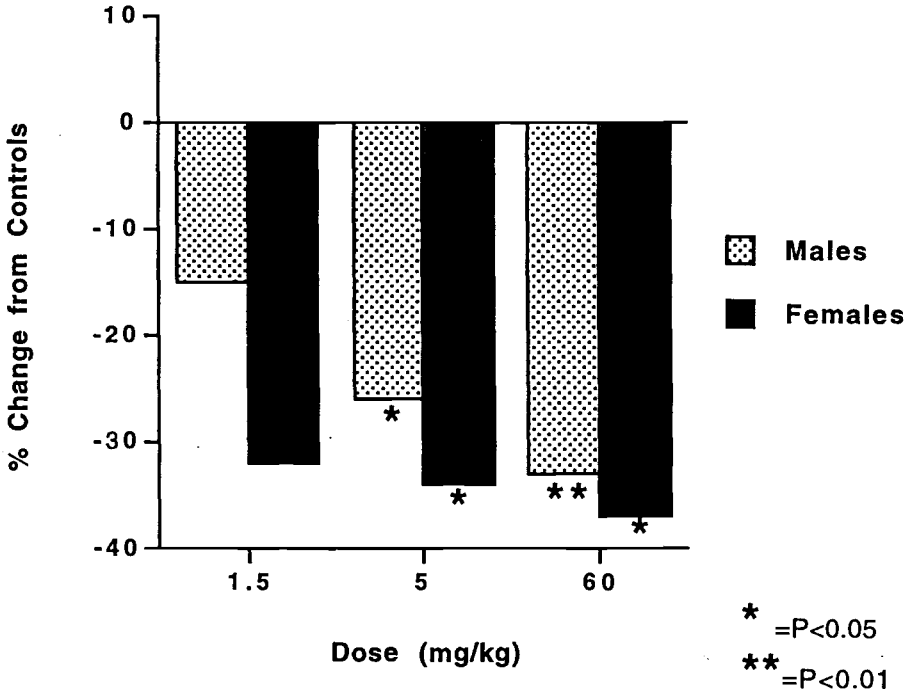
Effect of UK-92,480 on Mean Body Weight Gain in Rats

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

Non-Neoplastic Pathology: The only consistent change that was reported was a dose-related decrease in plasma bilirubin in both sexes which was statistically significant ($P < 0.01$ and 0.05) at the mid and high doses (Figure 15).

Figure 15

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



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

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

Plasma drug levels (AUCs) for UK-92,480 (parent drug) and UK-103,320 (major metabolite) were determined from supplementary rats on Day 366. Mean systemic exposures ($AUC_{1\text{-}8\ hr}$) to UK-92,480 and UK-103,320 are shown in Figure 16A (males) and Figure 16B (females). As can be seen, exposure to UK-92,480 and UK-103,320 was dose-proportional in both sexes. However, males were exposed mostly to the metabolite UK-103,320, whereas females were exposed mostly to the parent drug UK-92,480.

Figure 16A

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

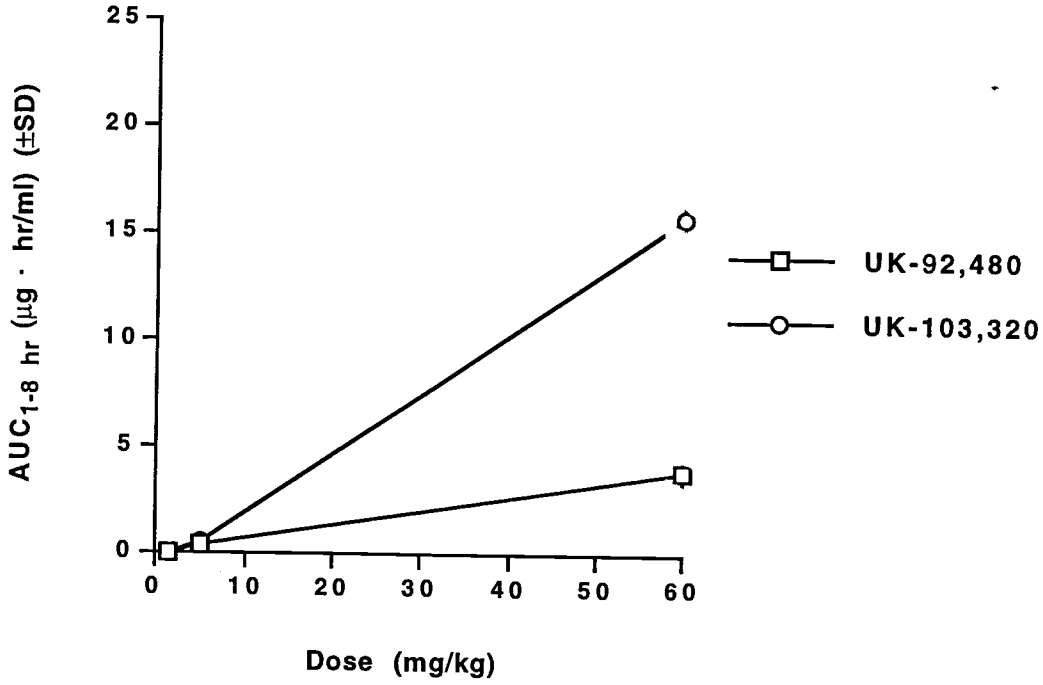
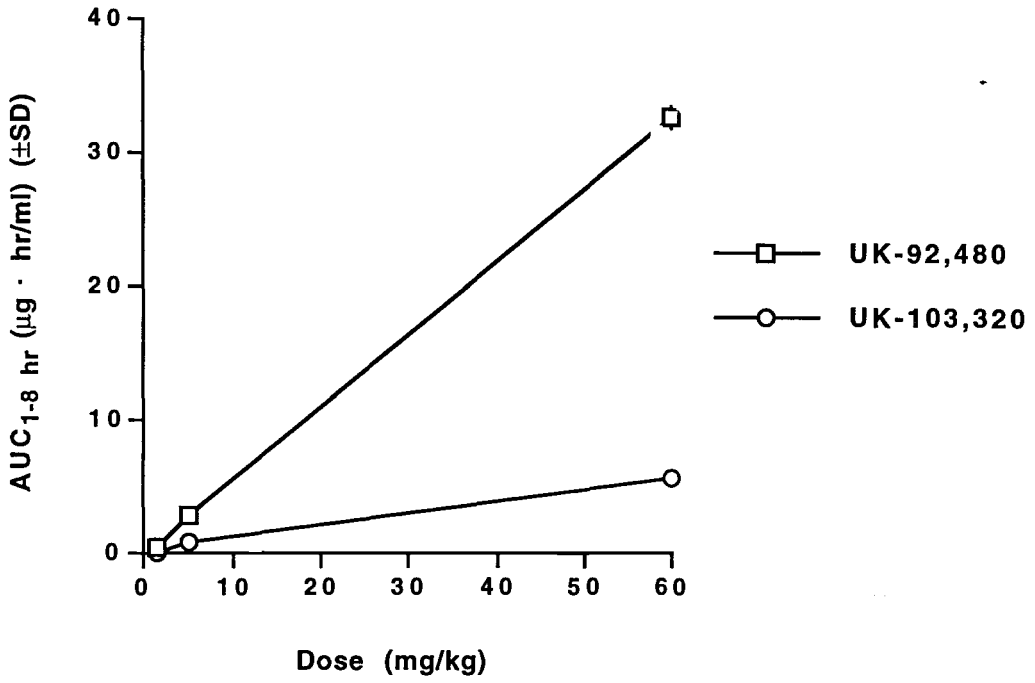


Figure 16B

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



Comparative $AUCs_{(1-24\ hr)}$ for UK-92,480 and UK-103,320 between normal male human volunteers given the maximum recommended human dose (MRHD) of 100 mg (=1.43 mg/kg based on a 70 kg man) and male and female rats given 60 mg/kg/day are shown in Table 17 (values represent total drug, bound and unbound). [Note: Since the human study (#148-228) used pharmacokinetic sampling times up to 24 hours post-dose, the rat $AUCs_{(1-8\ hr)}$ were recalculated by this reviewer (T. Papoian) using the linear trapezoidal rule also to 24 hours post-dose which was past the last quantifiable plasma drug concentration.]

Table 17

Comparative Total $AUCs_{(1-24\ hr)}$ (Total Bound and Unbound) for UK-92,480 and UK-103,320 Between Male Humans and Male and Female Rats

Species	Dose	UK-92,480 $AUC_{(1-24hr)}$ (µg·hr/ml)	UK-103,320 $AUC_{(1-24hr)}$ (µg·hr/ml)
Man	100 mg/70 kg	1.667	0.756
Rat (male)	60 mg/kg/day	6.621	29.385
Rat (female)	60 mg/kg/day	54.25	11.379

Since pharmacologic activity for sildenafil (UK-92,480) and its active metabolite (UK-103,320) is represented by the unbound fraction, the percentage of plasma protein binding for both human and rat is shown in Table 18.

Table 18

Human and Rat Plasma Protein Binding

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

Comparison of the male and female rat $AUCs_{(1\text{-}24\ hr)}$ for total drug exposure (sum of unbound UK-92,480 and UK-103,320 AUCs) as a multiple of the maximum recommended human dose (MRHD) of 100 mg is shown in Table 19. The unbound AUCs were calculated by multiplying the total bound and unbound AUC (Table 17) by the fraction unbound (Table 18). As shown, the total of unbound $AUCs_{(1\text{-}24\ hr)}$ in male and female rats given 60 mg/kg/day was ~34X and ~38X, respectively the $AUC_{(1\text{-}24\ hr)}$ of men given a single dose of 100 mg.

Table 19

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

Species	**Unbound UK-92,480 AUC (μg·hr/ml)**	**Unbound UK-103,320 AUC (μg·hr/ml)**	**Total of Unbound AUCs (μg·hr/ml)**	**Multiple of MRHD**
Man	0.067	0.038	0.105	--
Rat (male)	0.331	3.232	3.563	**33.9X**
Rat (female)	2.713	1.252	3.965	**37.8X**

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

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

Systemic exposure to total unbound drug (sum of the parent drug UK-92,480 and the principle pharmacologically active metabolite UK-103,320) was calculated to be 34X and 38X the maximum recommended human dose of 100 mg in male and female rats, respectively. These results suggest that the lack of a carcinogenic effect in rats was not due to inadequate systemic exposure to sildenafil. A statistical review of tumor incidence in the rat study by the Division of Biometrics is pending.

2.3.1.2. 104-week oral (gavage) carcinogenicity study in the rat (aborted) (Study No. 911/002; Vol. 1.25 pp. 5148-5235):

Testing Facility: Pfizer, Centre de Recherche, Amboise Cedex, France
Study Number: 911/002
Study Date(s): 7/12/94 to 9/5/94
GLP Compliance: Yes (?)

Between days 18 and 25 after initiation of treatment, 46/61 of the high dose males died. Autopsy revealed necrotizing and inflammatory changes with hemorrhage in the GI tract suggesting a necrotizing event about two days before death. The drug formulations were analyzed and pharmacokinetics determined in the surviving animals. Although low levels of UK-92,480 were found, high levels of another substance with a MW of 270 containing fluorine but no nitrogen was detected (MW of UK-92,480 free base is 475 and contains nitrogen but no fluorine).

Discussions with the technician revealed that a highly cytotoxic compound from another client was mistakenly issued by the pharmacy for the week 3 preparation. It is not clear why only the high dose males died and not the high dose females, although an explanation was offered: "The cytotoxic compound did not suspend well in the vehicle used in the present study and the heterogenicity in the different dosing portions explain why only males of the high dose were affected." The study was invalidated and terminated at week 8 on 8/31/94.

2.3.1.3. One-month exploratory study in rats (Study No. 94085; Vol. 1.30 pp. 7658-7703):

Testing Facility: Pfizer, Centre de Recherche, Amboise Cedex, France
Study Number: 94085
Study Date(s): 8/9/94 to 9/5/94
GLP Compliance: Not mentioned

This study was initiated to determine the cause of death seen in male rats in an ongoing carcinogenicity study (Study No. 911/002). It was subsequently determined that the cause of death in the previous study was due to dosing of the animals with a cytotoxic compound from another company, and not with UK-92,480.

Male Sprague-Dawley rats (10/group; 209 gms) were given UK-92,480 (batch no. R108) orally by gavage at 60 or 120 mg/kg/day for 28 days. Controls received vehicle (0.5% methylcellulose with 0.1% Tween 80). Rats were observed for mortality and clinical signs. Body weights and food consumption were recorded. Blood was taken on Day 9 for plasma drug concentrations. After the last dose, a necropsy was performed which consisted of a gross exam, and weights of several organs.

No deaths were reported. Significant organ weight changes were seen in the livers and kidneys only at the high dose of 120 mg/kg/day (+16% and +10%, respectively). No other effects were noted.

Mean plasma concentrations (µg/ml) for the parent UK-92,480 and the active metabolite UK-103,320 one hour after dosing are shown in Table 20.

Table 20

Mean Plasma Concentrations (μg/ml) of UK-92,480 and UK-103,320 in Male Rats

Dose (mg/kg/day)	UK-92,480	UK-103,320
60	0.21	2.01
120	1.05	3.29

It was concluded that UK-92,480 when given orally to rats at doses up to 120 mg/kg/day for 28 days produced minimal adverse effects in male rats. These results were consistent with other toxicity studies in male rats.

2.3.1.4. Pharmacokinetic study in rats (Study No. 94067; Vol. 1.31 pp. 7930-7938):

(GLP Study No. 94067 conducted in Pfizer Centre de Recherche, Amboise, France. Study dates: 6/9/94 to 6/23/94.)

5 rats (8 wks old, 208-419 g)/sex/group were given repeated single dose of 60 mg/kg/day of UK-92,480-10 (dissolved in vehicle- 0.5% aq. sol. of methylcellulose/0.1% Tween 80), p.o. (by gavage) for 14 days. Drug sol. was given in a volume of 10 mg/kg.

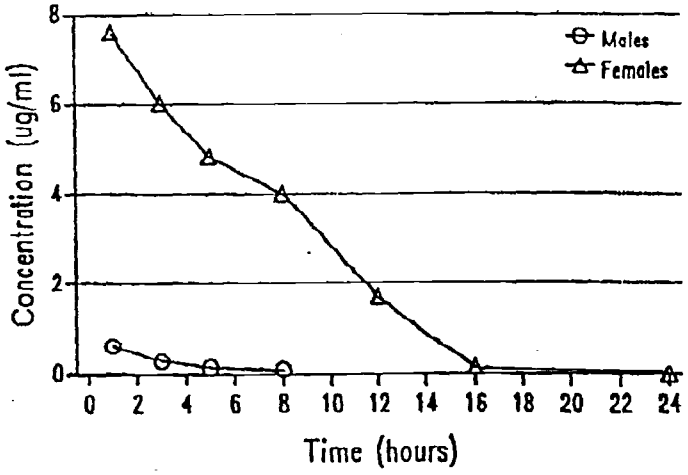
Observations and Measurements: Drug plasma levels of the drug/demethyl metabolite were determined on day 14 of study on all rats at 1, 3, 5, 8, 12, 16 and 24 hours post-dosing. Different volumes of blood were obtained from anesthetized rats from orbital sinus or aorta for clinical chemistry determination; samples were kept on ice until centrifuged to obtain plasma. Plasma analysis for UK-92,480 and metabolite UK-103,320 were done by an HPLC assay.

RESULTS

Clinical observations: No rats died, and no adverse clinical signs were reported.

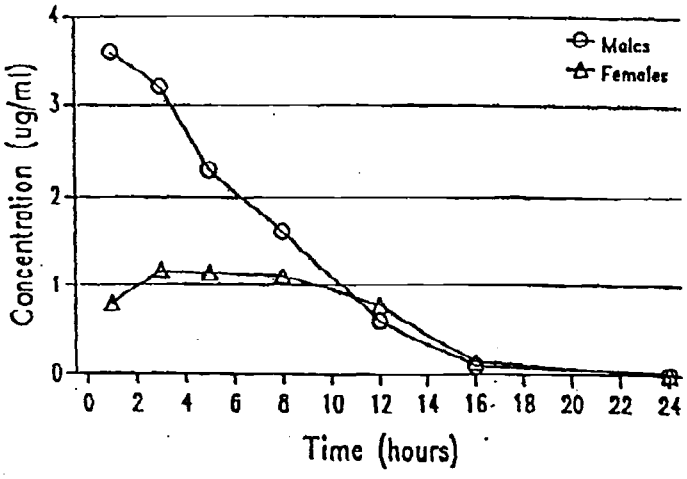
Plasma concentrations of UK-90,480: In F, plasma UK-92,480 levels were much higher in M; the highest individual concentrations were reported at 1-3 hours post-dosing. Levels of the drug in plasma ranged from 0.36 to 1.18 μg/ml in M, and from 5.81 to 9.98 μg/ml in F.

Variations of mean plasma UK-92,480 concentrations with time on day 14



UK-103,320 (Demethylated Metabolite of UK-92,480): Maximal plasma UK-103,320 levels were noted at 1-3 hours in M and 3-5 hours in F; the values reported ranged from 3.25-3.89 µg/ml in M, and 1.04-1.57 µg/ml for F. Mean conc. of the metabolite declined rapidly thereafter in M, and remained sustained during the first 8 hrs after treatment in F.

Variations of mean plasma UK-103,320 concentrations with time on day 14



In the drug sponsor conclusions, there were no statements regarding what was considered the a MTD of UK-92,480-10 or doses that might have been selected for the toxicity/carcinogenicity study in mouse. The NOAEL appears to be the LD-10 mg/kg.

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

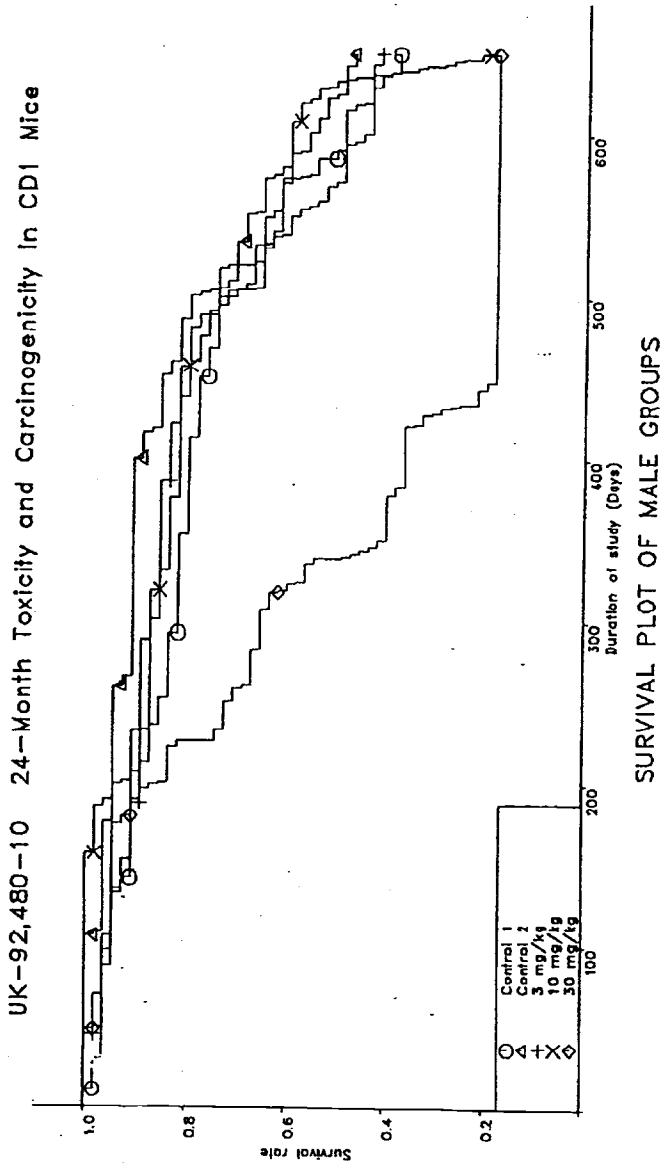
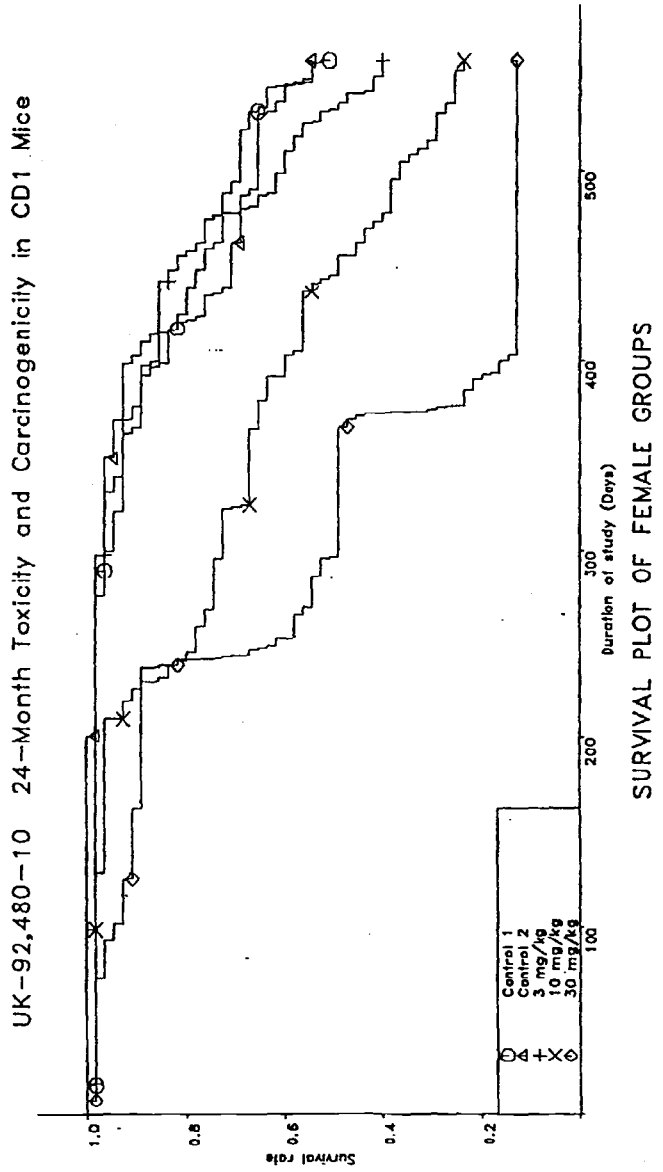


Figure 17B (Sponsor's Figure 2)

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

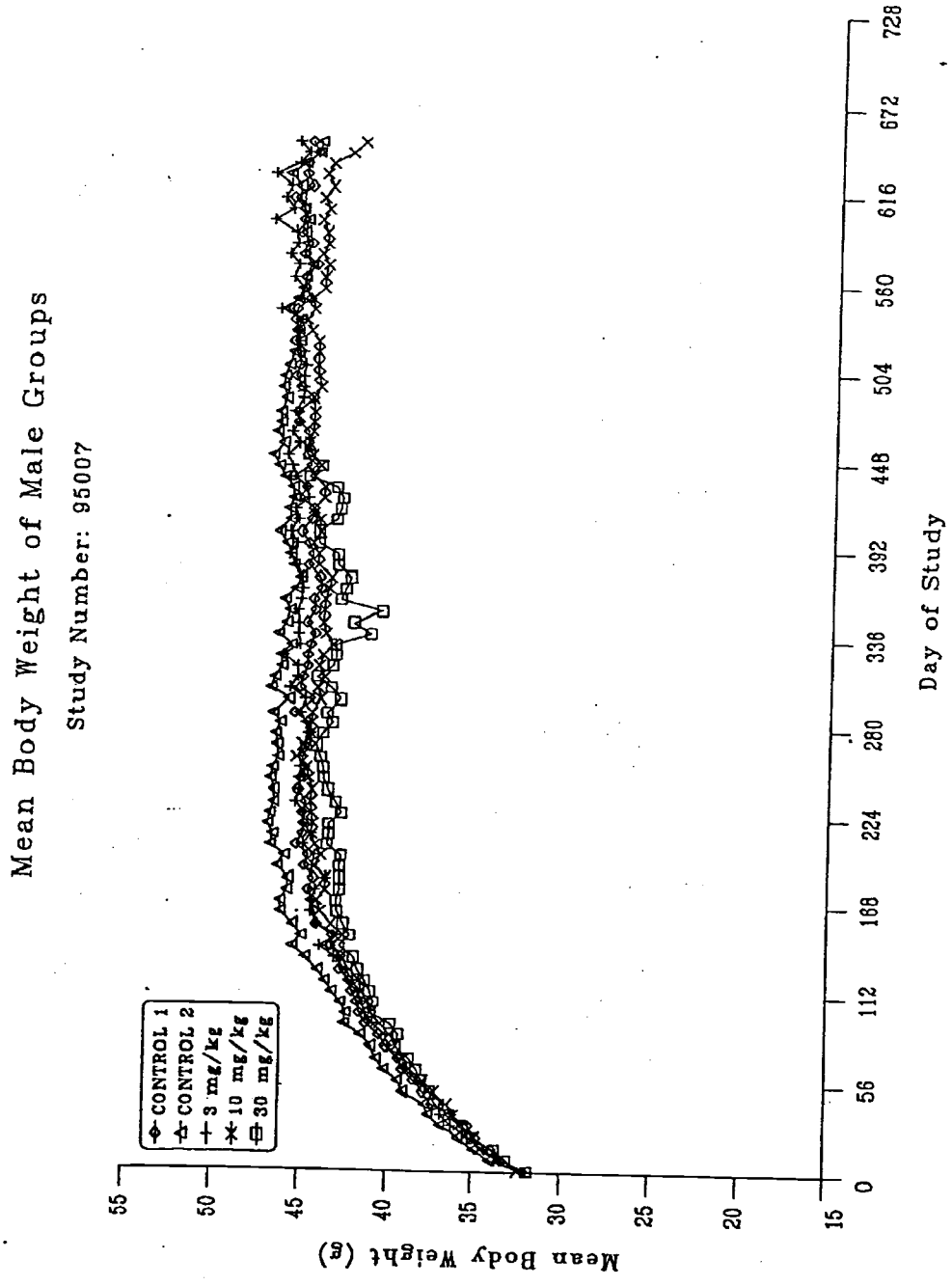
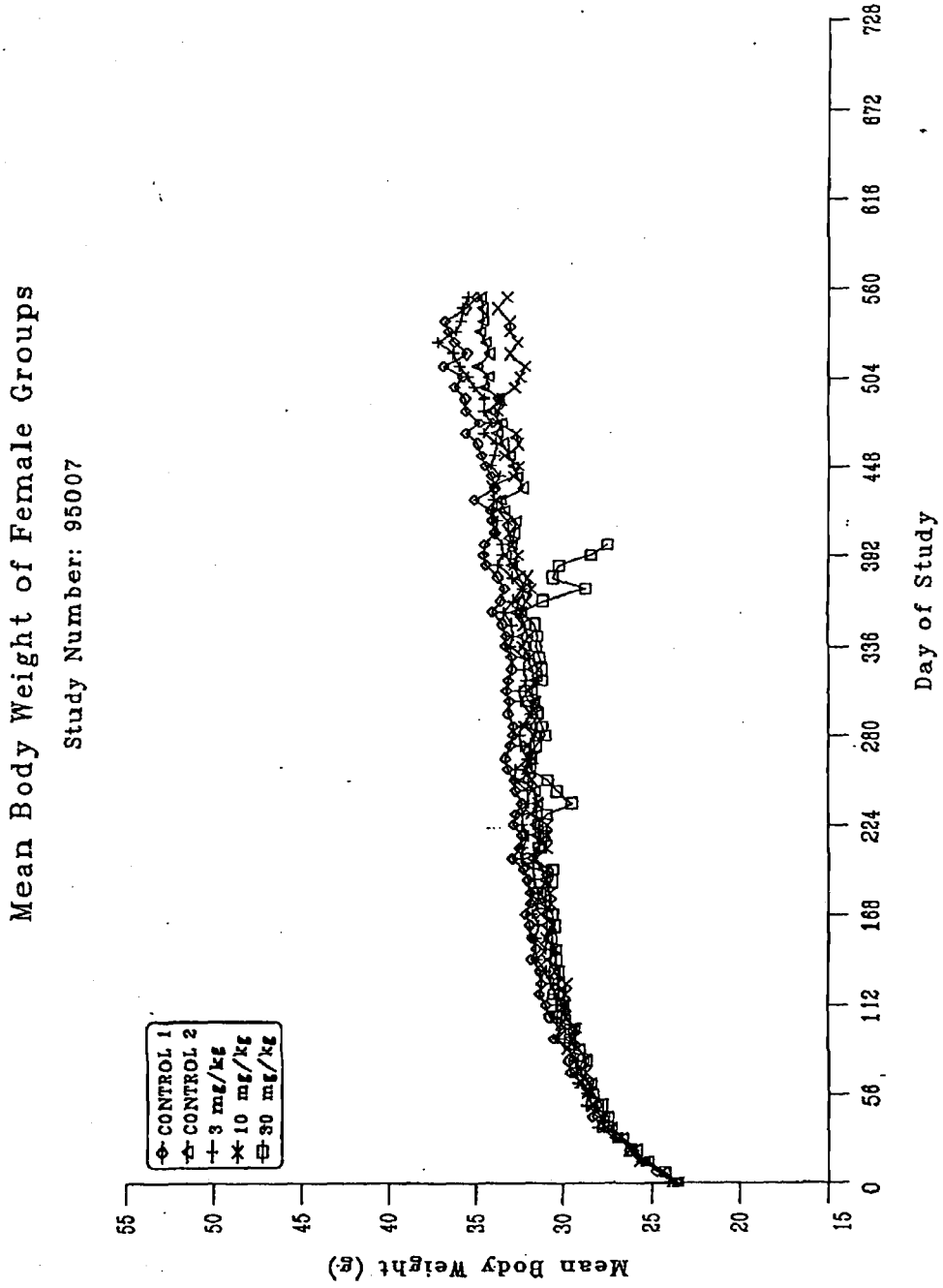


Figure 18B (Sponsor's Figure 4)

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)

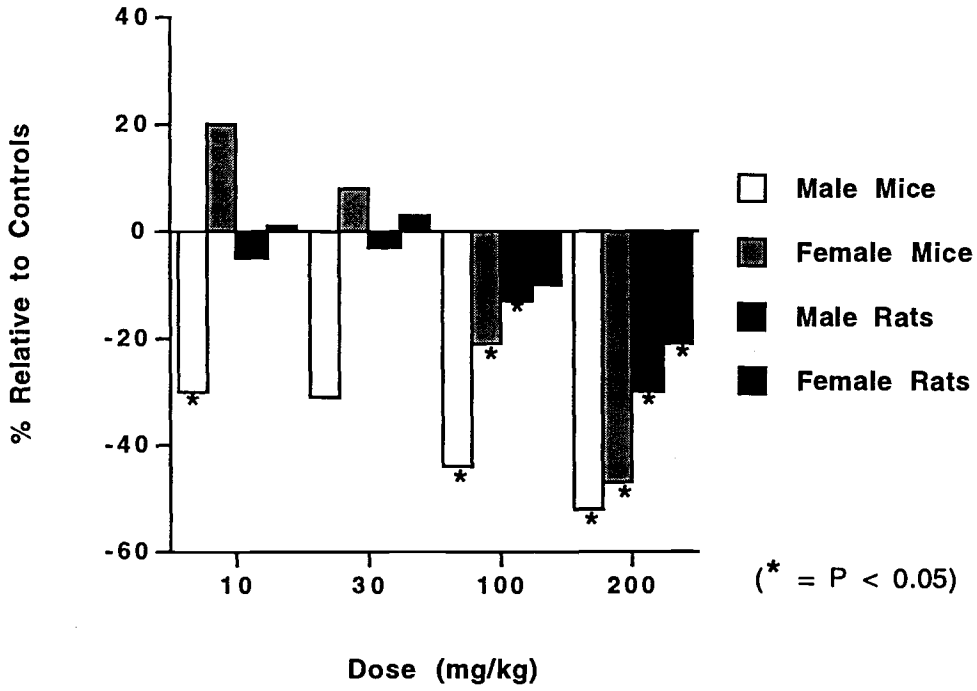


Figure 20A

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

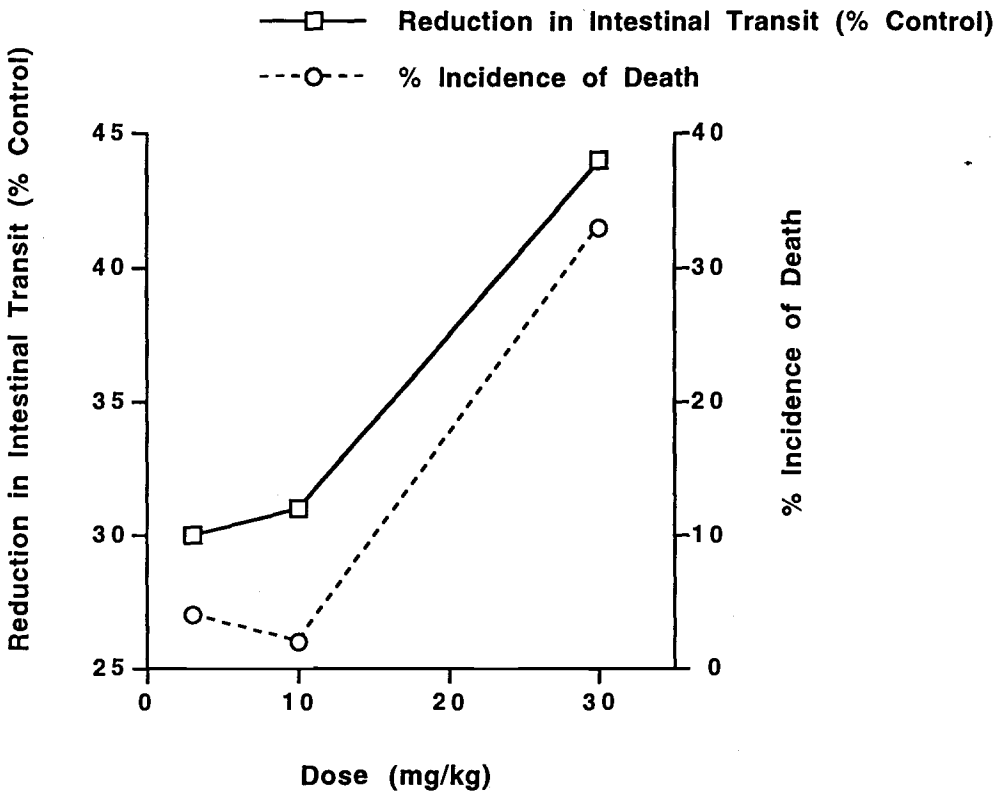
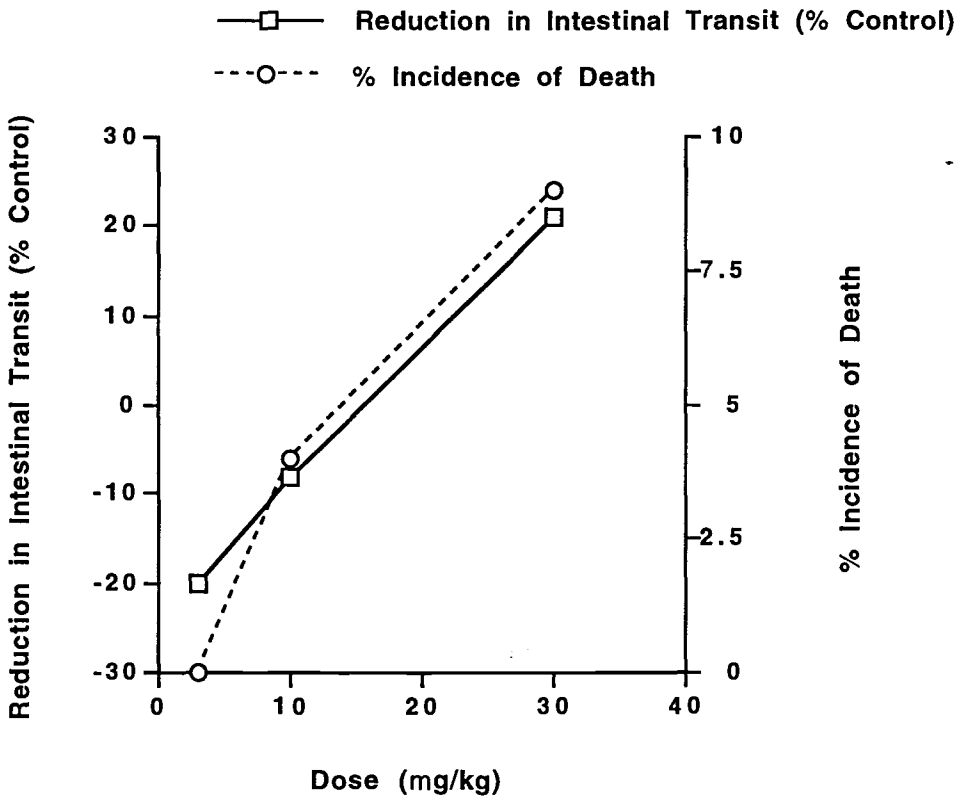


Figure 20B

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)

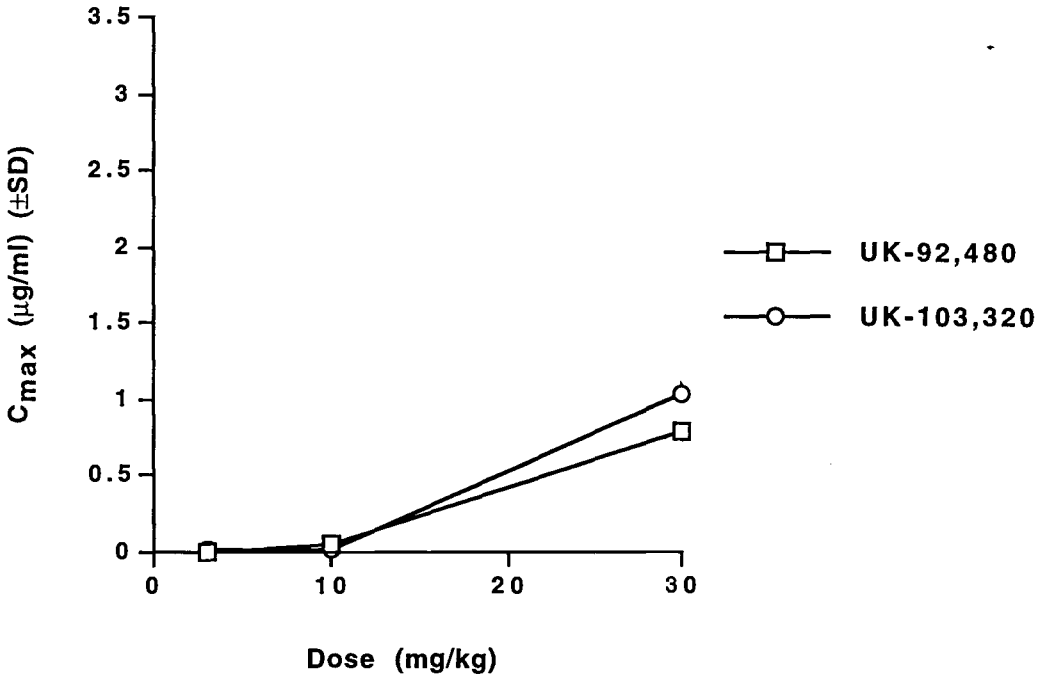


Figure 21B

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.

---

* 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 maternal toxicity 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 by oral 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.

* 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.

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

PHARMACOLOGY REVIEWS

TOXICOLOGY
PHARMACOKINETICS
PAGES 91-116

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(3.7)	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

* 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 Concentrations in maternal plasma, amniotic fluid and fetuses
Drug Safety Evaluation — Study number: 95044
Centre de Recherche - Amboise - France — Data for scheduled date : 06-Jun-95
Rabbit/New Zealand White — Study start date: 16-May-95

Dose	Animal Number	Maternal plasma Pre-dose	1H	3H	5H	AUC 1-5h	Amniotic fluid	Fetuses 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.)

* 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/Concentration 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 X 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	113	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	96	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 — Cmpd. Lot Number: R-1
Legal Book Number: 20757-25 — Test Date: 1/10/91

* 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

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	98	97	8	
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 — Test Date: 1/8/91
Legal Book Number: 20757-12 — S9 Source: R901016 Aroclor-Induced
Cmpd. Lot Number: R-1

ppt: precipitation occured 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	2.8	26.0
	4	3.0	24.0
	5	Dec	
Mean (± SD)		2.5 ± 0.53	26.8 ± 2.5
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
Mean (± SD)		0.14 ± 0.09	33.0 ± 1.2
High Dose 2000 mg/kg/d	1	0.1	33.0
	2	0.2	30.0
	3	0.0	29.0
	4	0.3	28.5
	5	0.1	30.0
Mean (± SD)		0.14 ± 0.11	30.1 ± 1.7

Data Location:18813-77-79
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	34.0
	2	0.1	46.0
	3	0.0	44.0
	4	0.2	47.0
	5	0.1	40.0
Mean (± SD)		0.12 ± 0.08	42.2 ± 5.3
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	1.6	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	38.0
Mean (± SD)		0.16 ± 0.09	33.0 ± 3.7
Mid Dose 1000 mg/kg/d	1	0.2	27.5
	2	0.1	33.0
	3	0.0	42.5
	4	0.1	32.0
	5	0.3	29.0
Mean (± SD)		0.14 ± 0.11	32.2 ± 6.0
High Dose 2000 mg/kg/d	1	0.1	29.0
	2	0.1	28.0
	3	0.2	31.0
	4	0.1	28.0
	5	0.1	34.0
Mean (± SD)		0.14 ± 0.05	28.6 ± 3.0

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.

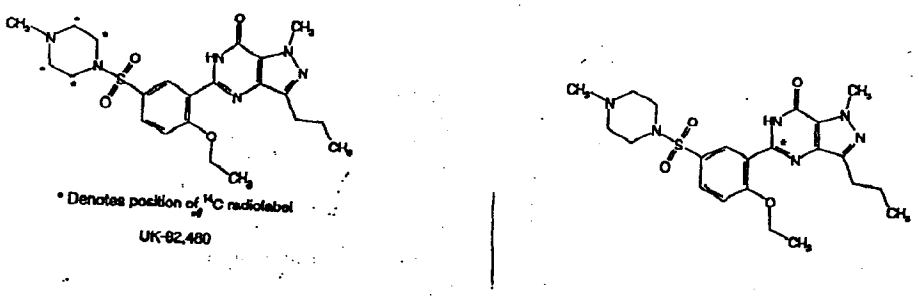
3. PHARMACOKINETICS (ADME)

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.

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

PHARMACOLOGY REVIEWS

PHARMACOKINETICS SUMMARY/EVALUATION PAGES 117- 142

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

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

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

3.3. Repeat Dose Pharmacokinetics

3.3.1. Mouse (CD-1)

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

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

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

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

3.3.2. Rat (Sprague-Dawley)

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

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

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

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

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

3.3.3. Beagles

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

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

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

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

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

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

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

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

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

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

NC indicates parameter not calculable

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

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

3.4. Absorption

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

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

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

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

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

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

3.5. Distribution

3.5.1. Tissues

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

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

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

TISSUE	TIME (h)			
	0.1 male µg eq/g	1 male µg eq/g	6h male µg eq/g	24 male µg eq/g
Adipose tissue				
Brown fat	4.12	0.62	0.23	0.22
White fat	blq	0.37	0.18	blq
Adrenal gland	9.9	1.45	0.30	0.22
Blood	1.33	0.46	0.20	0.12
Bone marrow	3.83	0.97	0.32	0.25
Brain	0.67	blq	0.18	blq
Cardiac muscle	3.39	0.54	0.21	0.08
Eye (retina)	5.82	2.26	1.45	1.45
Harderian gland	4.85	3.2	0.73	0.33
Liver	8.64	3.88	1.31	0.62
Lung	7.23	1.18	0.45	0.12
Nasal/optic sinus	1.68	0.36	0.23	0.25
Pancreas	4.24	1.02	0.55	0.15
Preputial gland	4.05	1.45	0.50	0.27
Pineal gland	3.88	0.73	0.23	0.18
Pituitary gland	5.21	1.89	0.23	0.18
Prostate gland	2.18	0.38	0.23	0.22
Salivary gland	4.36	0.82	0.36	0.18
Seminal vesicle	blq	0.36	0.18	0.21
Skeletal muscle	2.1	0.38	0.18	0.08
Skin (epidermis)	1.73	0.35	0.18	0.08
Sebaceous gland	1.45	0.33	0.20	0.23
Spleen	2.91	0.67	0.50	0.18
Substantia nigra	nm	1.43	0.32	nm
Testis	0.64	0.32	0.18	0.08
Thymus gland	2.45	0.55	0.20	0.22
Thyroid gland	3.6	0.87	0.36	0.33
Upper limit of quantification	23.3	11.6	5.82	2.9
Lower limit of quantification	0.36	0.18	0.091	0.045

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

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

TISSUE	TIME (h)			
	0.1 female µg eq/g	1 female µg eq/g	6h female µg eq/g	24 female µg eq/g
Adipose tissue				
Brown fat	2.15	2.42	1.38	0.44
White fat	blq	1.09	0.36	0.08
Adrenal gland	3.44	2.11	0.87	0.26
Blood	0.32	0.36	0.36	0.22
Bone marrow	0.94	1.35	0.64	0.36
Brain	blq	blq	blq	blq
Cardiac muscle	1.49	1.41	0.45	0.22
Eye (retina)	2.08	2.45	2.01	2.01
Harderian gland	0.99	5.43	1.45	0.85
Liver	2.48	4.03	2.06	0.70
Lung	2.69	3.37	1.64	0.20
Nasal/optic sinus	0.18	0.34	0.55	0.18
Pancreas	1.57	2.12	1.21	0.32
Pineal gland	2.20	2.38	1.31	0.34
Pituitary gland	2.66	3.11	1.09	0.34
Salivary gland	0.89	1.98	1.11	0.29
Skeletal muscle	0.53	0.63	0.36	0.13
Skin (epidermis)	0.36	0.65	blq	blq
Sebaceous gland	0.19	0.44	blq	blq
Spleen	0.93	2.31	0.73	0.35
Substantia nigra	0.32	alq	0.45	0.53
Thymus gland	0.69	1.13	0.73	0.40
Thyroid gland	2.30	1.59	1.38	0.16
Upper limit of quantification	11.63	11.6	11.6	5.8
Lower limit of quantification	0.091	0.18	0.18	0.045

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

3.5.2. Plasma Protein Binding

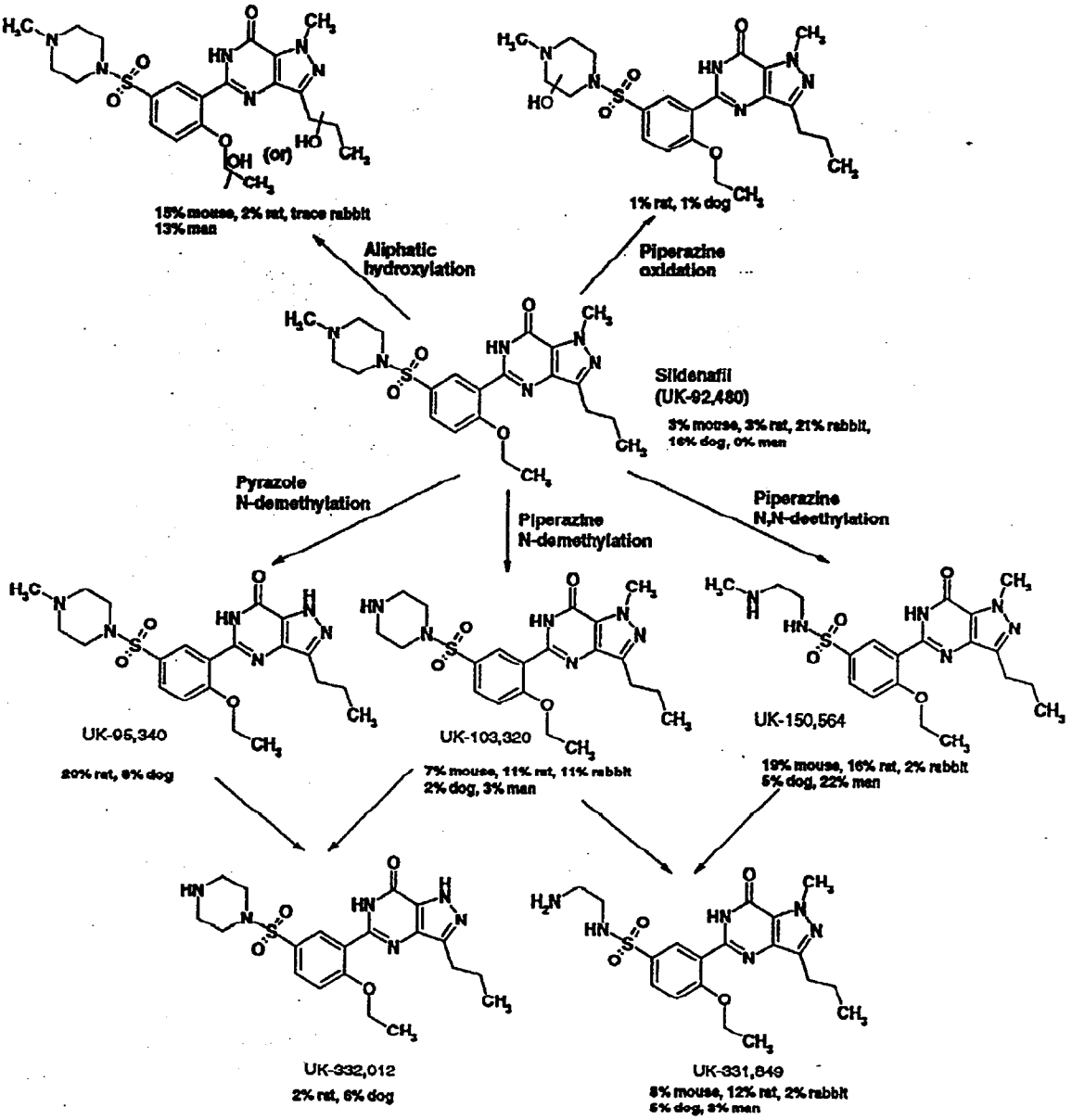
The binding of 14C-UK-92,480 in plasma of mouse, rat, rabbit, dog and man has been measured in vitro using equilibrium dialysis. Plasma protein binding of UK-92,480 was independent of total drug concentration over the range 0.01-10 µg/ml. The extent of binding to plasma proteins of mouse ~ 94%, ~ rat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

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

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

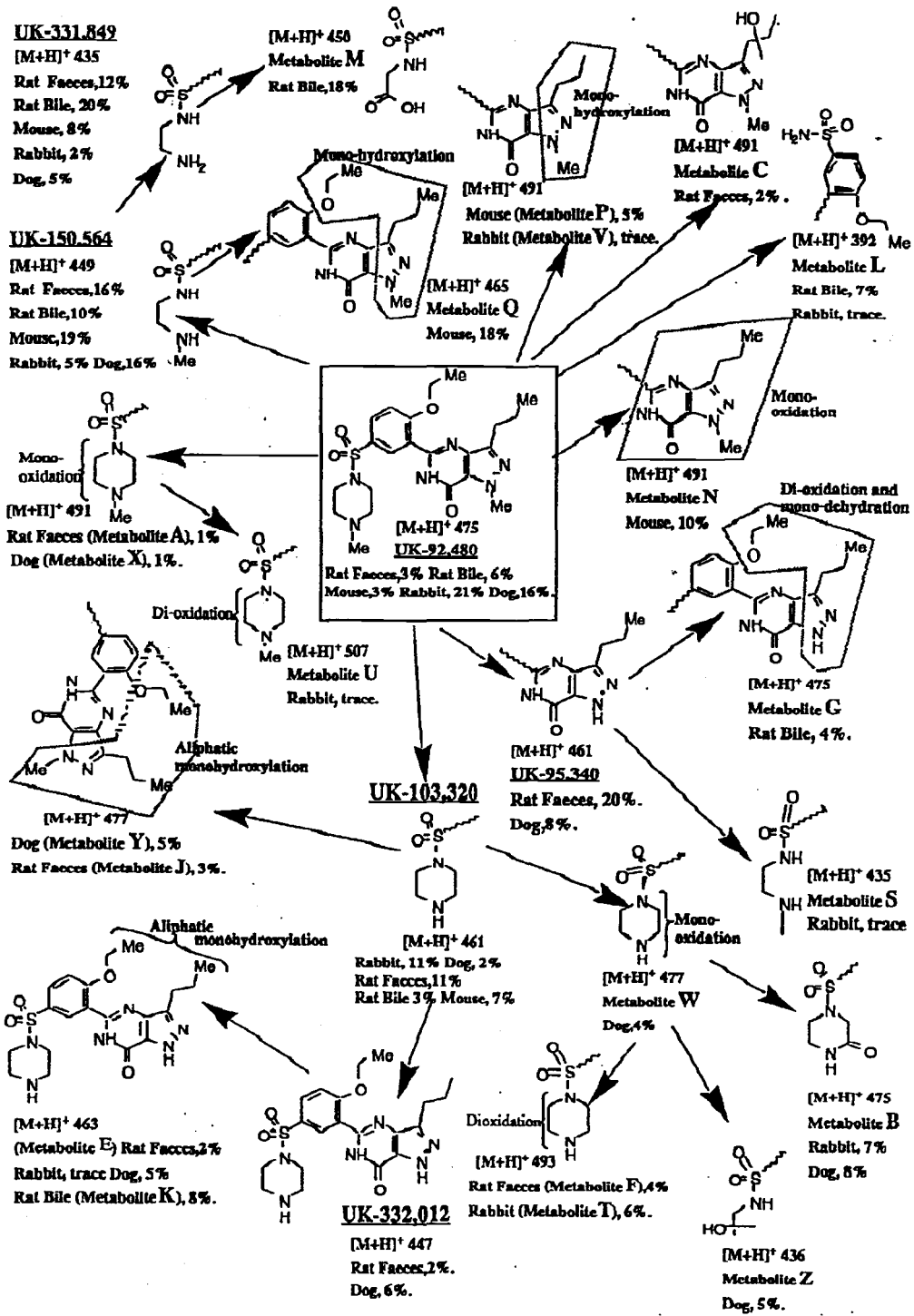
3.6. Metabolism

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



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

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



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

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

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

3.6.1 Circulating Metabolites

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

3.6.2. Biologic activity of UK-103,320

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

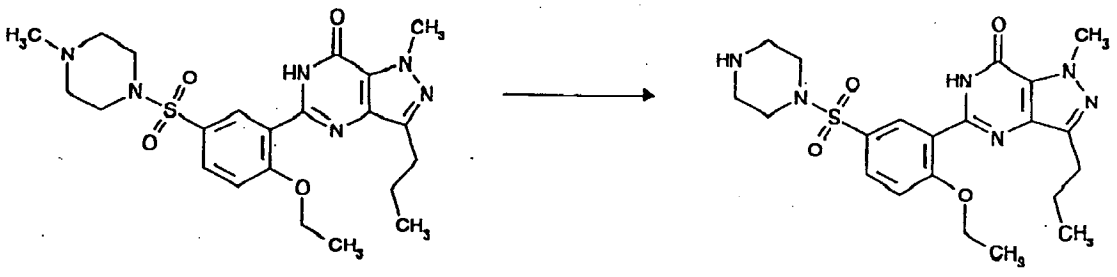
In pharmacology studies using anesthetized rats and dogs, UK-103.320 (0.03 to 3 mg/kg **iv**) was reported as producing dose-related transient falls in MABP; the hypotensive responses were accompanied by tachycardia. (The duration of these effects were reported ~5 min and effects were seen only in some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

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

3.6.3. In vitro Metabolism:

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

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



UK-92,480 UK-103,320

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

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

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

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

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

3.7. Excretion

3.7.1. General

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

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

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

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

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

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

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

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

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

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

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

4. LABELING (Package Insert)

The following changes to the package insert are recommended:

Carcinogenesis, Mutagenesis, Impairment of Fertility

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

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

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

Pregnancy, Nursing Mothers and Pediatric Use

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

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

5. OVERALL SUMMARY AND EVALUATION

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

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

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

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

5.1. Pharmacology

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

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

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

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

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

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

5.2. Toxicology

5.2.1. Acute Toxicology

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

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

5.2.2. Subchronic/Chronic Toxicology

5.2.2.1. Rats

5.2.2.1.1. Oral

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

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

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

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

5.2.2.1.2. Intravenous

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

5.2.2.2. Dogs

5.2.2.2.1. Oral

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

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

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

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

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

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

5.2.2.2.2. Intravenous

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

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

5.2.3. Carcinogenicity

5.2.3.1. Rat

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

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

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

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

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

5.2.3.2. Mouse

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

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

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

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

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

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

5.2.4. Reproduction Toxicity

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

5.2.4.1. Rat

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

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

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

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

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

5.2.4.2. Rabbit

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

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

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

5.2.5. Genetic Toxicity

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

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

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

5.3. Pharmacokinetics (ADME)

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

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

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

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

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

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

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

5.4. Conclusions

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

6. RECOMMENDATIONS

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

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

1-23-98

Estela A. Barry, M.S.
Pharmacologist

1/23/98

Thomas Papoian, Ph.D.
Pharmacologist

Albert F. DeFelice, Ph.D.
Pharmacology Team Leader 1/23/98

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

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

STATISTICAL REVIEW AND EVALUATION

FEB 4 1998

Statistical Review and Evaluation

Carcinogenicity Review of Sildenafil in Rats and Mice

DATE:

NDA #: 20-895, Animal Carcinogenicity Studies.

DATE BY CDER: October 14, 1997.

DRUG NAME: Sildenafil (Viagra).

SPONSOR: Pfizer Inc.

INDICATION FOR THE HUMAN USE: Male Erectile Dysfunction.

DOSES USED: For the male and female rats; two controls and sildenafil at1.5, 5 & 60 mg/kg/day. For the male and female mice; two controls and sildenafil at 3, 10 & 30 mg/kg/day.

DOCUMENTS REVIEWED: Carcinogenicity Volume with Document Identification **BS**, and the Review of Dr. Thomas Papoian (dated 1/29/98), the pharmacology reviewer in HFD-110.

This review has been discussed with Dr. Thomas Papoian, the pharmacology reviewer and with Dr. Albert Defelice, the pharmacology Team Leader from the Division of Cardio-Renal Drug Products (**HFD-110**) of the FDA.

Organization

The review's organization consists of **four** sections and eachc with subsections as follows:

Section 1: Introduction

Section 2: Rat Study

2.1. Study Design

2.2. Sponsor's Analysis and Conclusion for Males and Females.

2.2.1. Mortality/Survival Analysis

2.2.2. Tumor Trend Analysis

2.3. Reviewer's analysis and Conclusion

2.3.1. Male Rats

2.3.1.1. Mortality/Survival Analysis

2.3.1.2. Tumor Trend Analysis

2.3.2. Female Rats

2.3.2.1. Mortality/Survival Analysis

2.3.2.2. Tumor Trend Analysis

Section 3: Mouse Study

3.1. Study Design

3.2. Sponsor's Analysis and Conclusion for Males and Females.

3.2.1. Mortality/Survival Analysis

3.2.2. Tumor Trend Analysis

3.3. Reviewer's analysis and Conclusion

3.3.1. Male Mice

3.3.1.1. Mortality/Survival Analysis

3.3.1.2. Tumor Trend Analysis

2.3.2. Female Mice

3.3.2.1. Mortality/Survival Analysis

3.3.2.2. Tumor Trend Analysis

Section 4: Reviewer's Conclusion

1. INTRODUCTION

Sildenafil (UK-92, 480) with the trade name of **Viagra** is a competitive and selective inhibitor of cyclic GMP specific phosphodiesterase type-5. For the human use, this drug is being developed for the treatment of male erectile dysfunction.

The Toxicity and Carcinogenicity of sildenafil submission contains: study on male and female Sprague-Dawley rats and study in male and female CD1 Mice.

The aims of the animal studies were to assess the chronic toxicity and potential carcinogenicity of sildenafil when administered orally to the rats and mice for two years.

2. RAT STUDY

2.1. Study Design

The following describes the design and the conduct of the study for both male and female rats.

The study title is "24-Month Oral Toxicity and Carcinogenicity Study in Sprague-Dawley Rats."

The study duration was 24 months and consisted of five treatment groups with 60 animals in each treatment group.

During the study, the rats were observed daily for mortality and clinical signs. Rates were weighed and examined for the presence of palpable masses, once a week. The food consumption was measured weekly for the first six months and monthly there after. Water consumption was measured every two months. Histopathological examinations were performed on numerous tissues recovered from dead animals during the study and those that were sacrificed or killed as moribund.

The terminal sacrifice for the male and female rates started at week 104 and went through week 105.

The study doses were two controls and sildenafil at **1.5, 5.0,** and **60.0 mg/kg/day** during the **24** months. According to Dr. Papoian's calculations of the AUC (review was completed on 01/29/98), the dose of 60.0 mg/kg/day is about 36 fold, for the male rats and about 40 fold , for the female rats higher than the human dose. So, the dose level of 60.0 mg/kg/day is high enough to meets the standard for a phrnacokinetics endpoint (25-fold ratio of rodent to human plasma AUC).

2.2. Sponsor's Analysis and Conclusion

The sponsor's analyses include: mortality analysis, tumor analysis, body weight analysis, clinical signs analysis, analysis on clinical laboratory measures, and plasma concentration analysis. Those of concern are discussed below.

2.2.1. Mortality/Survival Analysis

The following table presents the sponsor's summary of the percent mortality during the study.

Table I_2.2.1: Percent (%) of Mortality/Survival During the Study and Median Survival Time.

		Males				Females			
		Cont. 1+2	1.5 mg	5 mg	60 mg	Cont. 1+2	1.5 mg	5 mg	60 mg
Median Survival Time (**Day**)		629	652.5¤	669.0¤	630.0	604.0	583.5	556.5	615.5
Mortality/ Survival (%)	Found Dead	56.7	43.3	30.0	48.3	34.2	33.3	30.0	55.0
	Sacrificed as Moribund	25.0	15.0	35.0	21.7	45.0	41.7	48.3	30.0
	Survived at the End	18.3	41.7	35.0	30.0	20.8	25.0	21.7	15.0
	Total	100	100.0	100.0	100.0	100.0	100.0	100.0	100.0

¤: Statistically Significant at α=0.05.

For the male rats the survival time for the low and medium doses were statistically significantly higher than that in the controls. However, the sponsor concluded that "in the absence of an effect at the high dose, we consider that overall there was no effect of the treatment on the survival times."

Comment: As will be seen later, the sponsor's conclusion is verified from this reviewer's results.

2.2.2. Tumor Trend Analysis

There was a slight apparent increase in proliferative changes in the thyroid of the males in the sildenafil treated groups, affecting C-cells and follicular cells. This was not seen in treated females. For the male rates the results are summarized in **Table II_2.2.2**.

The sponsor concluded that "The increase in C-cell proliferative changes in treated males was not dose-related and not statistically significant." The sponsor also stated that the incidence observed is with the range of in-house historical data.

Comment: From the reviewer's tumor trend (as a function of the dose) analysis, this kind of tumor is categorized as a "common tumor," with the associated P-values of P = 0.0266 for the exact test and P = 0.0134 for the asymptotic test. Thus, by the FDA's rule the trend is not statistically

significant[1].

Table II_2.2.2: Incidences of Thyroid Proliferative Changes in Male Rats

		Cont. 1	Cont. 2	1.5 mg	5 mg	60 mg
C-cell	Hyperplasia	1	5	6	7	3
	Adenoma	2	4	7	9	7
	Carcinoma	0	0	1	1	1
	Combined ⊡	3	8	14	15	10
Follicular Cells	Hyperplasia	0	1	3	1	5
	Adenoma	4	0	0	2	5
	Carcinoma	1	1	0	2	0
	Combined ⊡	5	2	3	5	10

⊡: Rats bearing more than one of the above are counted only once.

Overall, the sponsor's analyses demonstrated that:

- The mortality/survival analysis has demonstrated that there is no dose-response relationship in survival.
- The tumor trend analysis has demonstrated that there is no dose-response relationship in survival.

2.3. Reviewer's Analysis and Conclusion

The discussion will be presented for male and female rats separately. We also will confine ourselves to the discussion of the results from the mortality/survival and the tumor trend analysis.

---

[1]- By the FDA's Guidance for Industry on the Statistical Aspects of Design, Analysis, and Interpretation of Animal Carcinogenicity Studies:

i. Tumors with the spontaneous incidence rate of > 1% (based on historical observations or based on observations in the control groups) are considered as **common**. Then, the choice of significance level will be $\alpha' = 0.01$ for the pairwise comparisons and $\alpha' = 0.005$.

ii. Tumors with the spontaneous incidence rate of ≤ 1% are considered as **rare**. Then, the level of significance should be set at $\alpha' = 0.05$ for the pairwise comparisons and at $\alpha' = 0.025$ for the trend analysis.

2.3.1. Male Rats

2.3.1.1. Mortality/Survival Analysis

Table III_2.3.1.1. displays the distribution of the number and the percent of the male rats which either died or were terminally sacrificed after the week 103, the end of study (during week 104-105).

TABLE III_2.3.1.1. Distribution of Number of Male Rats Died or Terminally Sacrificed

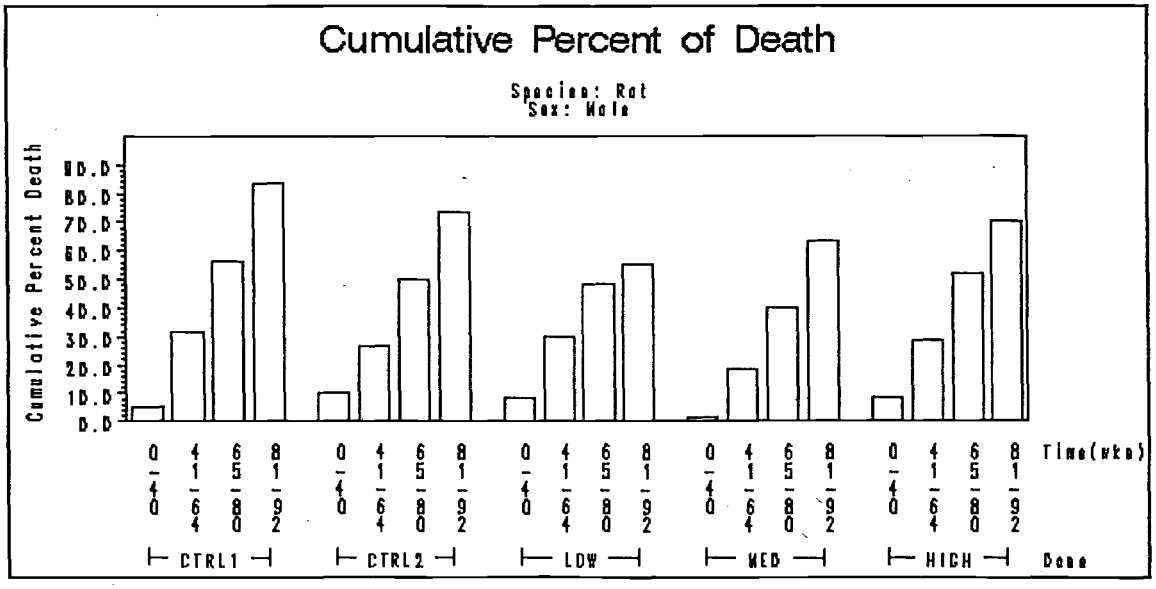
Week	Control 1			Control 2			1.5 mg/kg/day			5 mg/kg/day			60 mg/kg/day			Total No. Died
	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	
0-52	60	3	5.0	60	6	10.0	60	5	8.3	60	1	1.7	60	5	8.3	20
53-78	57	16	26.7	54	10	16.7	55	13	21.7	59	10	16.6	55	12	20.0	61
79-91	41	15	25.7	44	14	23.3	42	11	18.3	49	13	21.7	43	14	23.4	67
92-103	26	16	26.2	14	14	23.3	31	4	6.7	36	14	23.3	29	11	18.3	59
Terminal■ Sacrifice	10	10	16.7	16	16	26.7	27	27	45.0	22	22	36.7	18	18	30.0	93

■: The number of animals terminally sacrificed is the same as the number of animals survived after week 103.

From the last column of the Table III_2.3.1.1., it appears that there is no dose related trend with respect to the animal survival at the end of the study.

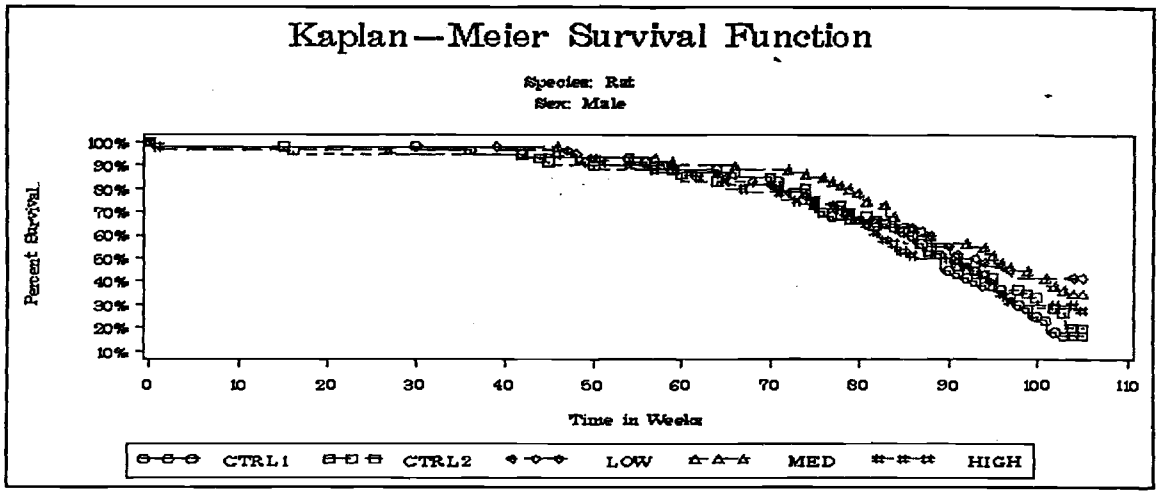
Figure I_2.3.1.1. presents the cumulative distribution of the percent of rat death, up to week 103.

FIGURE I_2.3.1.1. Cumulative Distribution of Percent of Male Rats Died During the Study



Kaplan-Meirer estimates of the survival function is presented in **Figure II_2.3.1.1.**. The graph shows the homogeneity of the survival distributions in the 5 treatment groups.

FIGURE II_2.3.1.1. Kaplan-Meirer Estimate of the Survival Function for the Male Rats



A Dose-Mortality Trend test was also performed, using both Cox Regression and Non-Parameteric Kruskal-Wallis methods. The results are summarized in **Table IV_2.3.1.1.** and the resulted P-values indicating of no evidence of statistically significant dose related mortality trend.

TABLE IV_2.3.1.1. Dose Related Mortality Trend Test for Male Rats

Method	Time Adjusted Trend-Test	Statistics	P-Value
Cox	Dose-Mortality Trend	0.08	0.7837
	Departure From Trend	7.47	0.0583
	Homogeneity	7.55	0.1097
Kruskal-Wallis	Dose-Mortality Trend	0.39	0.5332
	Departure From Trend	4.57	0.2061
	Homogeneity	4.96	0.2915

2.3.1.2. Tumor Trend Analysis

The results for the tumor trend analysis are summarized in Tables **I_ A-Male-Rats** and **II_A-Male-Rats** of the **Appendix A-Male-Rats.** From the review of the results, the pharmacology reviewer might be interested in the following cases, summarized in **Table V_2.3.1.2.**.

TABLE V_2.3.1.2. Noticeable Tumor Trends in Male

ORGAN TYPE	TUMOR TYPE	Number of Incidences Per Treatment					Incidental or Fatal	Rare or Common ¤	Trend Analysis P-Values	
		Cot 1	Cot 2	1.5 mg	5 mg	60 mg			Exact	Asymp-totic
Kidney	B-Renal Tubule Adenoma	0	0	0	0	2	IN	R	0.0390	0.0023
Skin and Adnexa	B-Lipoma	0	0	0	0	1	IN	R	0.1935	0.0210
Skin and Adnexa	B-Lymphangioma	0	0	0	0	1	IN	R	0.1935	0.0210
Thyroid	B-Follicular Cell Adenoma	4	0	0	0	0	IN	C	0.0265	0.0132

¤ : Considered as a common tumor if the incidental rate is > 1% historically or in Control Group.

Now, by the FDA's rule (see Footnote 1 on Page 5), one may draw the following conclusions.

- The tumor observed in the thyroid is of a **common type,** with the P=0.0266 for the exact and P=0.0134 for the asymptotic test. Thus, as compared to $\alpha = 0.005$, both exact and asymptotic tests suggest that there is no statistically significant dose related trend.

- The tumor observed in the kidney is of **rare type,** with the P=0.0390 for the exact and P=0.0023 for the asymptotic test. Now, as compared to α=0.025 the exact test suggests no statistically significant dose related trend; whereas, the asymptotic test suggests, a statistically significant dose related trend.

- The tumors observed in the skin, for both B-Lipoma and B-Lymphangioma, are of **rare type,** with the P=0.0193 for the exact and P=0.0210 for the asymptotic test. Now, as compared to α=0.025 the exact test suggests no statistically significant dose related trend; whereas, the asymptotic test suggests, a statistically significant dose related trend.

To resolve the contradictory conclusions, for the case of kidney and skin tumors, this reviewer conducted the Fisher Exact Test, comparing the high dose (for which the tumors were observed) with the two controls combined. The resulting P-values are P=0.110 for the kidney and P=0.333 for the skin tumors. Therefore, one may conclude that: **no statistically significant dose related trend is suggested.**

2.3.2. Female Rat

2.3.2.1. Mortality/Survival Analysis

Table VI_2.3.2.1. displays the distribution of the number and the percent of the female rats that they either died or were terminally sacrificed after the week 103 (during week 104-105, the end of the study).

TABLE VI_2.3.2.1. Distribution of Number of female Rats Died or Terminally Sacrificed

Week	Control 1			Control 2			1.5 mg/kg/day			5 mg/kg/day			60 mg/kg/day			Total No. Died
	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	
0-52	60	1	1.7	60	2	13.3	60	4	6.7	60	5	8.3	60	5	8.3	17
53-78	59	17	28.3	58	15	25.0	56	19	31.6	55	21	35.0	55	16	26.7	88
79-91	42	17	28.3	43	19	31.7	37	13	21.7	34	16	26.7	39	16	26.7	81
92-103	25	11	16.4	24	11	18.3	24	8	13.3	18	5	8.3	23	13	21.6	48
Terminal■ Sacrifice	14	14	23.3	13	13	21.7	16	16	26.7	13	13	21.7	10	10	16.7	66

■: The number of animals terminally sacrificed is the same as the number of animals survived after week 103.

Here as well, the last column of the Table VI_2.3.2.1. shows that there is no dose related trend with respect to the animal survival at the end of the study.

Figure III_2.3.2.1. presents the cumulative distribution of the percent death during the study.

FIGURE III_2.3.2.1. Cumulative Distribution of Percent of Female Rats Died During the Study

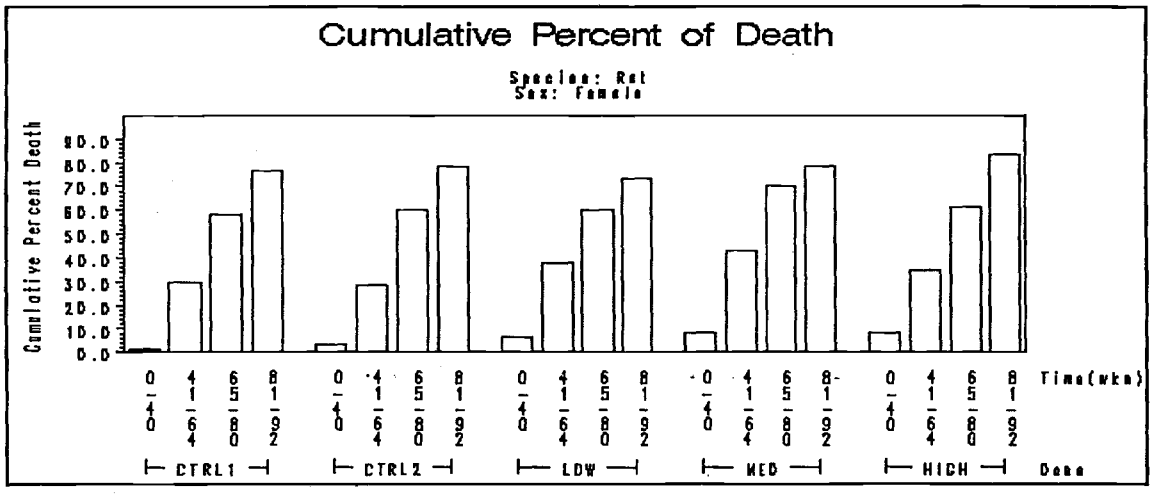
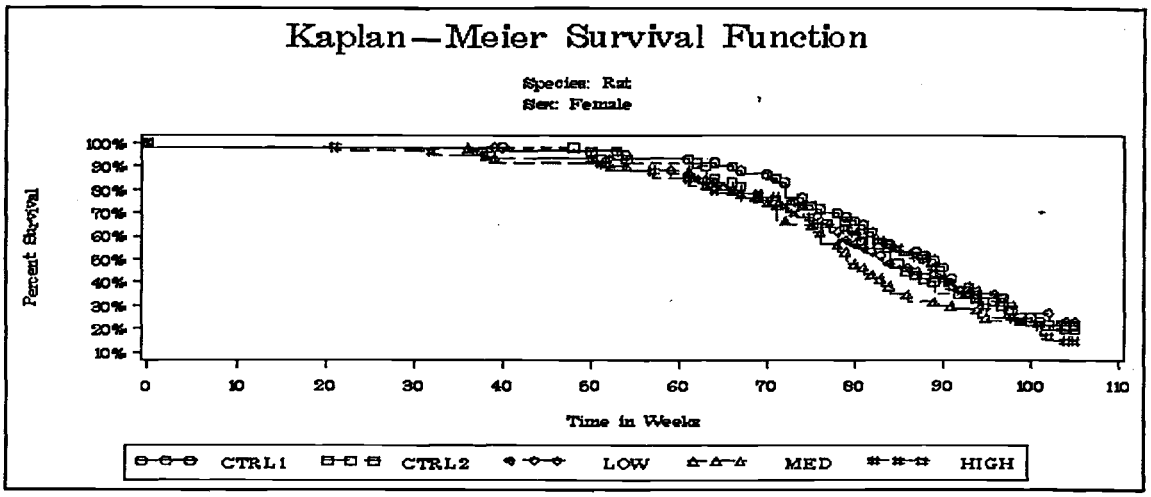


Figure IV_2.3.2.1. shows the Kaplan-Meirer estimates of the survival function for the five treatment groups. The graph demonstrates the homogeneity of the five treatment groups with respect to the survival distribution.

FIGURE IV_2.3.2.1. Kaplan-Meirer Estimate of the Survival Function for the Female Rats



The results for the Cox Regression and the Non-Parameteric Kruskal-Wallis methods on the dose-mortality trend are summarized in **Table VII_2.3.2.1.** For both methods the resulting high p-values suggest that there is a lack of statistically significant dose related trend.

TABLE VII_2.3.2.1. Dose Related Mortality Trend Test for the Female Rats

Method	Time Adjusted Trend-Test	Statistics	P-Value
Cox	Dose-Mortality Trend	0.35	0.5560
	Departure From Trend	1.39	0.7083
	Homogeneity	1.73	0.7844
Kruskal-Wallis	Dose-Mortality Trend	0.13	0.7234
	Departure From Trend	3.00	0.3921
	Homogeneity	3.12	0.5376

2.3.2.2. Tumor Trend Analysis

The results for the tumor trend analysis are summarized in Tables **I_ B-Female-Rats** and **II_B-Female-Rats** of the **Appendix B-Female-Rats.** From the results it appears that no statistically significant dose related trend is suggested.

In conclusion, this reviewer's results verified the sponsor's findings for which:

- There is no statistically significant evidence of a dose related mortality trend.

- There is no statistically significant evidence of a dose related tumor trend.

3. MOUSE STUDY

3.1. Study Design

Following is the description of the study design and the conduct of the study for both male and female mice.

The study is titled "24-Month Oral Toxicity and Carcinogenicity Study in CD1 Mice."

The study duration was 24 months and consisted of 5 treatment groups with 55 mice in each treatment group.

During the study, mice were observed daily for mortality and clinical signs. Animals were weighed and examined for the presence of palpable masses once a week. The food consumption was measured weekly for the first six month and monthly then after. Water consumption was measured every two months for about one year. Histopathological examinations were performed on a wide range of tissues recovered from the dead animals during the study and those that were sacrificed or killed as moribund.

The treatment groups consist of two controls and sildenafil at **3, 10.0,** and **30.0 mg/kg/day** during the study.

Due to high mortality the following procedure was adopted: The mice in the control groups and those in the low and medium dose groups were treated up to 649 to 650 days for the males and up to 558 to 562 for the females. The high dose mice were treated up to day 453 for males and up to day 404 for females.

3.2. Sponsor's Analysis and Conclusion

The sponsor's analyses include: mortality analysis, tumor analysis, body weight analysis, clinical signs analysis, analysis on clinical laboratory measures, and plasma concentration analysis. Those of concern are discussed below.

3.2.1. Mortality/Survival Analysis

The following table presents the sponsor's summary of the percent mortality during the study. From the table it appears that at the high dose (30 mg/kg/day), a compound-related increase in mortality was observed after seven months in males and after four months in female groups. In order to maximize the duration of treatment and ensure a sufficient number of animals for subsequent analyses, when the survival at the high dose fell below 20%, the mice were sacrificed.

For the 10 mg dose groups, a dose related increases in mortality was observed after seven months in female mice, but for the male mice the mortality was the same as those in controls and in the low dose groups.

Table VIII_3.2.1: Percent (%) of Mortality/Survival During the Study and Day of Terminal Sacrifice.

		Males				Females			
		Cont. 1+2	3 mg	10 mg	30 mg	Cont. 1+2	3 mg	10 mg	30 mg
Day of Terminal Sacrifice		650	650	650	454	559	559	559	405
Mortality/ Survival (%)	Found Dead	42.7	45.4	47.3	41.8	30.0	41.8	58.2	50.9
	Sacrificed as Moribund	14.5	12.7	30.9	40.0	15.5	18.2	18.2	36.4
	Survived at the End	42.8	41.9	18.8	9.2	54.5	40.0	23.6	12.7
	Total	100	100.0	100.0	100.0	100.0	100.0	100.0	100.0

□: Statistically Significant at α=0.05.

Comment: As will be seen later, these findings are verified from the reviewer's analyses.

3.2.2. Tumor Trend Analysis

There was no statistically significantly dose related tumor trends, for both male and female mice.

Comment: This assertion is granted from the results of this reviewer's analysis.

Overall, the sponsor's analyses demonstrated that:

- The mortality/survival analysis has demonstrated a dose related increases in mortality for the high dose, for both male and female mice and a dose related increases in mortality for the medium dose for the female mice.

- There is no treatment related tumor trend.

3.3. Reviewer's Analysis and Conclusion

The discussion will be presented for male and female mice separately. For the tumor trend evaluation two sets of analyses will be performed. For the first set, the analysis will include all five dose groups. The second set excludes the high dose (30 mg/kg/day). The rational for this is that, because of high mortality and, hence, early termination in the high dose group, the potential of observing dose related tumors in the high dose group is eliminated. Thus, the second set of analysis is to determine if there is a dose related trend in tumor growth in the absence of the high dose.

3.3.1. Male Mice

3.3.1.1. Mortality/Survival Analysis

Table IX_3.3.1.1. displays the distribution of the number and the percent of the mice which either died or were terminally sacrificed. The week intervals are chosen because the mice were begun to be terminally sacrificed at week 93 for the animals in the two controls, the low, and the medium dose groups and at the beginning of week 65 for the animals in the high dose group. From the results, it appears that there was a higher mortality in the high dose as compared to the other doses.

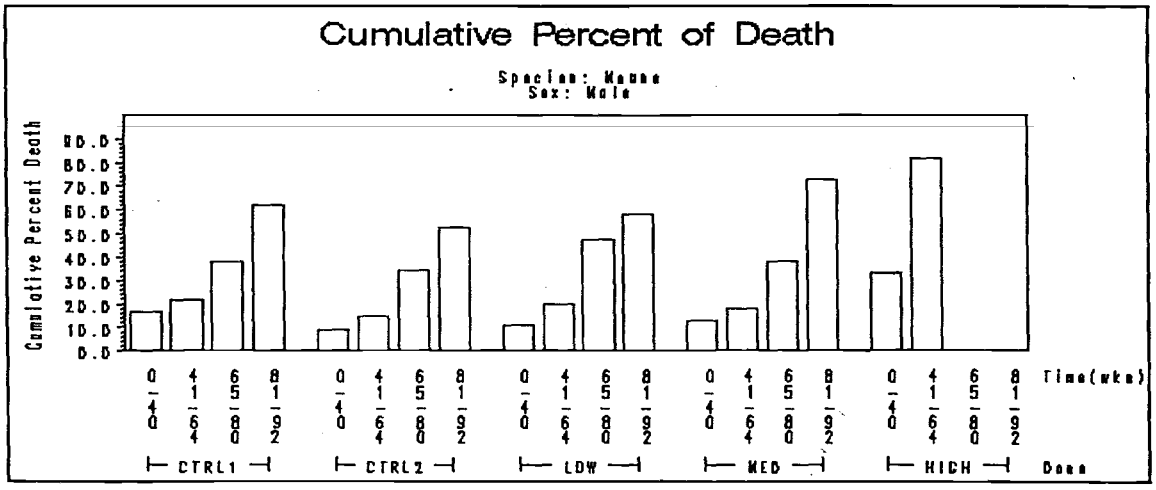
TABLE IX_3.3.1.1. Distribution of Number of Male Mice Died or Terminally Sacrificed

Week	Control 1			Control 2			3 mg/kg/day			10 mg/kg/day			30 mg/kg/day			Total No. Died
	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	
0-40	55	9	16.4	55	5	9.1	55	6	10.9	55	7	12.7	55	18	32.7	45
41-64	46	3	5.4	50	3	5.4	49	5	9.1	48	3	5.5	27	27	49.1	41
65-80	43	9	16.4	47	11	20.0	44	15	27.3	45	11	20.0	10♂	10♂	18.2	56
81-92	34	13	23.6	36	10	18.2	29	6	10.9	34	19	34.5	0	0	0	48
Terminal■ Sacrifice	21	21	38.2	26	26	47.3	23	23	41.8	15	15	27.3	0	0	0	85

■: The number of animals terminally sacrificed is the same as the number of animals survived after week 92.
♂: The mice in high dose group were terminally sacrificed at the beginning of week 65.

Figure V_3.3.1.1. presents the cumulative distribution of percent death during the study.

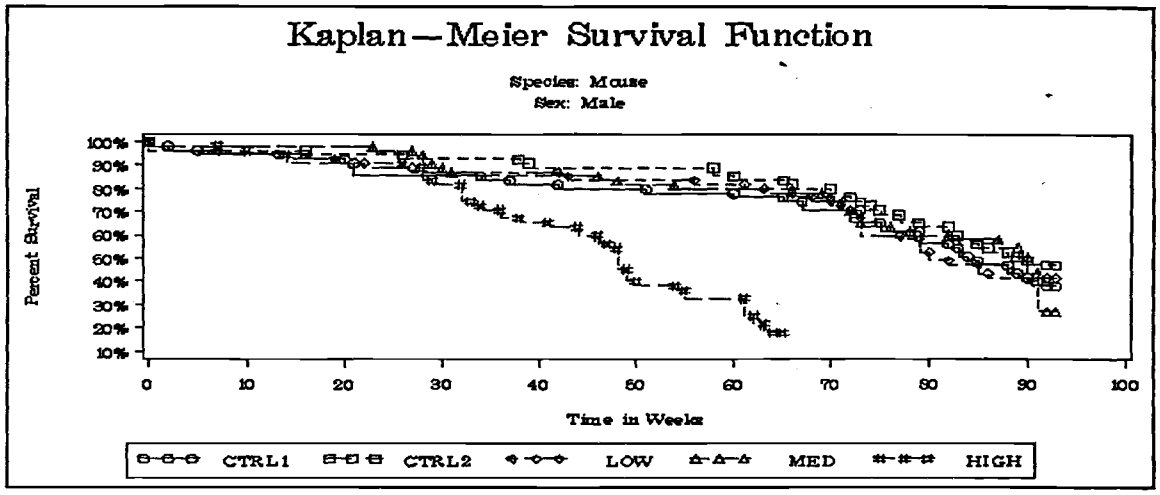
FIGURE V_3.3.1.1. Cumulative Distribution of Percent of Male Mice Died During the Study



Statistical Carcinogenicity Reviewer: Kooros Mahjoob

Further analysis consists of the Kaplan-Meirer estimates of the survival function, presented in **Figure VI_3.3.1.1.**.

FIGURE VI_3.3.1.1. Kaplan-Meirer Estimate of the Survival Function for the Male Mice



The graph shows that the mortality rate in the high dose is substantially higher than those in the other dose groups.

Dose-Mortality Trend Test was also performed, using both Cox Regression and Non-Parameteric Kruskal-Wallis methods. The results are summarized in **Table X_3.3.1.1.**

TABLE X_3.3.1.1. Dose Related Mortality Trend Test for Male Mice

Method	Time Adjusted Trend-Test		Statistics	P-Value
Cox	Dose-Mortality Trend:	All Doses Included	75.90	0.0000
		High Dose Excluded	1.37	0.2422
	Departure From Trend:	All Doses Included	15.86	0.0012
		High Dose Excluded	0.93	0.6293
	Homogeneity:	All Doses Included	91.76	0.0000
		High Dose Excluded	2.29	0.5136
Kruskal-Wallis	Dose-Mortality Trend:	All Doses Included	66.85	0.0000
		High Dose Excluded	0.48	0.4880
	Departure From Trend:	All Doses Included	10.56	0.0144
		High Dose Excluded	1.06	0.5893
	Homogeneity	All Doses Included	77.41	0.0000
		High Dose Excluded	1.54	0.6734

The analysis was performed for the case where all doses were included as well as for the case where the high dose was exclude. The results show that the mortality rate in the high dose is statistically significantly higher than the mortality rate in the other doses. After exclusion of the high dose, there is no statistically significant difference among doses with respect to the mortality rate.

The conclusion is that due to the high mortality for the male mice in the 30 mg/kg/day dose group, one may conclude that the dose level of 30.0 mg/kg/day is high enough to meets the standard for a "Maximum Tolerated Dose (**MTD**)."

3.3.1.2. Tumor Trend Analysis

The results for the tumor trend analysis for the two sets of analyses (with and without exclusion of the high dose from the analysis) are summarized in **Tables I_C-Male-Mice, II_C-Male-Mice, III_C-Male-Mice** and **IV_C-Male-Mice** of **Appendix C-Male-Mice**. From the results presented in those table one may conclude that:

- When all doses are included, Table I_C-Male-Mice indicates that no statistically significant treatment dependent tumor trend is suggested.
- When the high dose is excluded from the analysis, Table III_C-Male-Mice indicates that, here as well, no statistically significant treatment dependent tumor trend is suggested.

3.3.2. Female Mice

3.3.2.1. Mortality/Survival Analysis

Table XI_3.3.2.1. displays the distribution of the number and the percent of the female mice which either died or were terminally sacrificed.

TABLE XI_3.3.2.1. Distribution of Number of Female Mice Died or Terminally Sacrificed

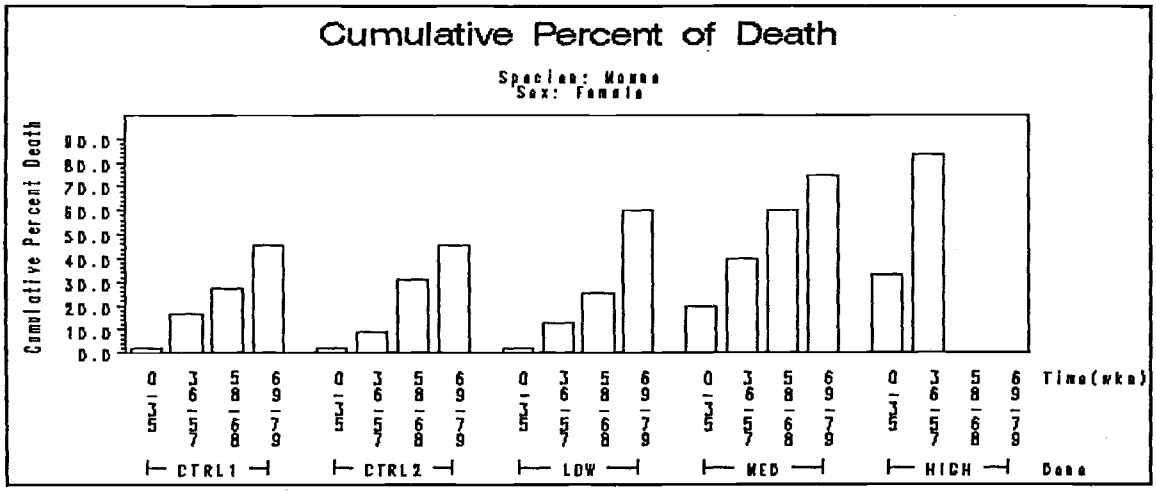
Week	Control 1			Control 2			3 mg/kg/day			10 mg/kg/day			30 mg/kg/day			Total No. Died
	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	
0-35	55	1	1.8	55	1	1.8	55	1	1.8	55	11	20.0	55	18	32.7	32
36-57	54	8	14.5	54	4	7.3	54	6	10.9	44	11	20.0	37	28	50.9	57
58-68	46	6	10.9	50	12	21.8	48	7	12.7	33	11	20.0	9♀	9♀	16.4	45
69-79	40	10	18.2	38	8	14.5	41	19	34.5	22	8	14.5				45
Terminal■ Sacrifice	30	30	54.5	30	30	54.5	22	22	40.0	14	14	25.5				96

■: The number of animals terminally sacrificed is the same as the number of animals survived after week 79.
♀: The mice in high dose group were terminally sacrificed at the beginning of week 58.

The week intervals in Table XI_3.3.2.1. are chosen because the mice were begun to be terminally sacrificed at week 80 for the animals in the two controls, the low, and the medium dose groups and at beginning of week 58 for the animals in the high dose group. From the table, it appears that there was a higher mortality in the medium and in the high dose groups as compared to the other doses.

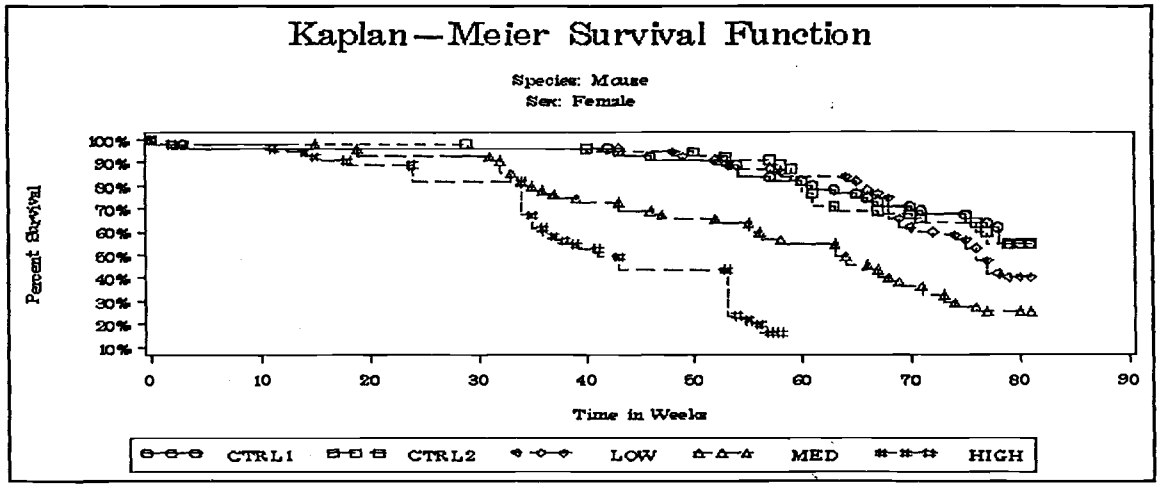
Figure VII_3.3.2.1. presents the cumulative distribution of animals' death during the study with exclusion of the terminal sacrifices.

FIGURE VII_3.3.2.1. Cumulative Distribution of Number of Female Mice Died During the Study



Further analysis consists of Kaplan-Meirer estimates of the survival function which is presented in **Figure VIII_3.3.2.1.**.

FIGURE VIII_3.3.2.1. Kaplan-Meirer Estimate of the Survival Function for the Female Mice



Statistical Carcinogenicity Reviewer: Kooros Mahjoob

The graph shows that the mortality rate in the medium and high dose groups was substantially higher than those in the other dose groups.

Dose-Mortality Trend Test was performed, using the Cox Regression and the Non-Parameteric Kruskal-Wallis methods. The analysis was carried for the case where all doses were included as well as for the case where the high dose was exclude. The results are summarized in **Table XII_3.3.2.1.**

TABLE XII_3.3.1.1. Dose Related Mortality Trend Test for Female Mice

Method	Time Adjusted Trend-Test		Statistics	P-Value
Cox	Dose-Mortality Trend:	All Doses Included	123.21	0.0000
		High Dose Excluded	22.53	0.0000
	Departure From Trend:	All Doses Included	1.19	0.7559
		High Dose Excluded	0.12	0.9418
	Homogeneity:	All Doses Included	124.4	0.0000
		High Dose Excluded	22.65	0.0000
Kruskal-Wallis	Dose-Mortality Trend	All Doses Included	119.25	0.0000
		High Dose Excluded	26.15	0.0000
	Departure From Trend	All Doses Included	0.596	0.8979
		High Dose Excluded	0.72	0.6960
	Homogeneity	All Doses Included	119.84	0.0000
		High Dose Excluded	26.87	0.0000

The results show that the mortality rates in the high dose, and possibly in the medium dose, are statistically significantly higher than the mortality rate in the other doses. After exclusion of the high dose, the results show that the mortality rate in the medium dose is also statistically significantly higher than the other doses.

One also concludes that due to the high mortality for the female mice in the dose groups of 10 mg/kg/day and 30 mg/kg/day, one may conclude that the dose level of 10 mg and 30 mg are high enough to meet the standard for a "Maximum Tolerated Dose (**MTD**)."

3.3.2.2. Tumor Trend Analysis

The results for the tumor trend analysis for the two sets of analyses (with and without exclusion of the high dose from the analysis) are summarized in Tables **I_D-Female-Mice, II_D-Female-Mice, III_D-Female-Mice** and **IV_C-Female-Mice** of **Appendix D-Female-Mice**. From the results, one may conclude that:

- When all doses are included, Table I_D-Female-Mice indicates that no statistically significant treatment dependent tumor trend is suggested.

- When the high dose is excluded from the analysis, Table III_D-Female-Mice indicates that, here as well, no statistically significant treatment dependent tumor trend is suggested.

4. REVIEWER'S CONCLUSION

Overall,

- For both the male and female rats, there was no evidence of a statistically significant treatment related mortality trend.
- For both the male and female rats, there was no evidence of a statistically significant treatment related tumor trend.
- For the male mice, the sildenafil dose of 30 mg/kg/day was toxic and demonstrated a statistically significantly higher mortality rate, as compared to the other doses.
- For the female mice, the sildenafil doses of 10 mg/kg/day and 30 mg/kg/day were toxic and demonstrated a statistically significantly higher mortality rate, as compared to the other doses.
- For both the male and female mice, there was no evidence of a statistically significant treatment related tumor trend. This finding was the same for the analyses with inclusion and without inclusion of the dose 30 mg/kg/day.

Kooros Mahjoob, Ph.D.
Mathematical Statistician

Concur: Dr. Chi 2/4/98

This review consists of 18 pages which include text, 12 tables and 6 Figures. There are 4 appendices attached in the back (Appendix A-Male Rats, 9 pgaes; Appendix B-Female Rats, 11 pgaes; Appendix C-Male Mice, 10 pgaes; and Appendix D-Female Mice, 8 pgaes).

CC:

Arch. NDA 20-895/Sildenafil.

HFD-110
HFD-110/Dr. Defelicy
HFD-110/Dr. Papoian
HFD-110/Mr. Buehler
HFD-344/Dr. Barton
HFD-710/Dr. Chi
HFD-710/Dr. Mahjoob
HFD-710/Chron.

K. Mahjoob: 4-5301:Biometrics 1/Team 1:km.

Statistical Carcinogenicity Reviewer: Kooros Mahjoob

NDA 20-895

Sildenafil

Appendix A-Male-Rats

Male Rats

NDA 20-895, Sildenafil
Appendix A-Male-Rats

Table I_A-Male-Rats

Test for Positive Dose-Response (Tumor) Linear Trend
Species: Rat 18:08 Friday, January 23, 1998
Sex: Male
Sorted by: Organ Name

Organ Code	Organ Name	Tumor Code	Tumor Name	Exact-P	Asymp-P	AsyCor-P
45	Abdomen	327	B-LIPOMA	0.5672	0.6964	0.7036
6	Adrenal	122	B-CORTICAL ADENOMA	0.6817	0.6554	0.6584
6	Adrenal	134	B-PHEOCHROMOCYTOMA	0.8353	0.8275	0.8283
6	Adrenal	353	M-CORTICAL CARCINOMA	1.0000	0.7076	0.7149
6	Adrenal	228	M-PHEOCHROMOCYTOMA, MALIG	0.8524	0.7819	0.7840
59	Bone, unsp.	411	B-OSTEOMA	0.7204	0.6951	0.7027
27	Brain	196	B-GLIOMA	1.0000	0.7076	0.7149
27	Brain	177	M-ASTROCYTOMA	0.6199	0.5723	0.5758
27	Brain	406	M-MALIGNANT RETICULOSIS	0.7204	0.6951	0.7027
43	Jejunum	202	M-ADENOCARCINOMA	1.0000	0.7180	0.7250
1	Kidney	373	B-LIPOMA	0.7204	0.6951	0.7027
1	Kidney	187	B-RENAL TUBULE ADENOMA	0.0390	0.0023	0.0024 ✓
1	Kidney	382	M-LIPOSARCOMA	1.0000	0.7103	0.7178
3	Liver	218	B-HEPATOCELLULAR ADENOMA	0.9029	0.8751	0.8764
3	Liver	330	M-CHOLANGIOCARCINOMA	1.0000	0.7076	0.7149
3	Liver	337	M-HEPATOCELLULAR CARCINOM	0.5486	0.6213	0.6245
32	Lungs	241	B-BRONCHIOLAR-ALVEOLAR AD	0.5745	0.7640	0.7678
32	Lungs	164	M-BRONCHIOLAR-ALVEOLAR CA	0.2487	0.3475	0.3514
32	Lungs	333	X-OSTEOSARCOMA	0.6552	0.7309	0.7361
38	Lymphoreticular	53	M-GRANULOCYTIC LEUKAEMIA	0.7258	0.6988	0.7018
38	Lymphoreticular	315	M-HISTIOCYTIC SARCOMA	1.0000	0.8260	0.8294
38	Lymphoreticular	11	M-LARGE GRANULAR CELL LYM	0.7993	0.7290	0.7313
38	Lymphoreticular	252	M-LYMPHOBLASTIC LYMPHOMA	0.8683	0.8112	0.8146
38	Lymphoreticular	212	M-LYMPHOMA, NOS	0.3431	0.1441	0.1476
20	Mesenteric node	345	B-HAEMANGIOMA	0.6571	0.7265	0.7316
19	Pancreas	257	B-ACINAR ADENOMA	0.3719	0.3229	0.3254
19	Pancreas	144	B-ISLET CELL ADENOMA	0.8003	0.8217	0.8228
9	Parathyroid	369	B-ADENOMA	0.5070	0.3323	0.3367
7	Pituitary	6	B-ADENOMA, PARS DISTALIS	0.3515	0.3556	0.3550
7	Pituitary	127	M-CARCINOMA, PARS DISTALI	1.0000	0.7871	0.7915
50	Seminal vesicle	231	M-SQUAMOUS CELL CARCINOMA	1.0000	0.7172	0.7243
30	Skin and adnexa	328	B-ADENOMA, MAMMARY GLAND	1.0000	0.7176	0.7249
30	Skin and adnexa	343	B-BASAL CELL TUMOUR	0.4754	0.4136	0.4178
30	Skin and adnexa	146	B-FIBROADENOMA, MAMMARY G	0.3501	0.3761	0.3801
30	Skin and adnexa	78	B-FIBROMA	0.8100	0.8405	0.8430
30	Skin and adnexa	243	B-FIBROUS HISTIOCYTOMA	0.9022	0.8460	0.8474
30	Skin and adnexa	181	B-HAIR FOLLICLE TUMOUR	0.4622	0.4464	0.4490
30	Skin and adnexa	427	B-LIPOMA	0.1935	0.0210	0.0220 ✓
30	Skin and adnexa	425	B-LYMPHANGIOMA	0.1935	0.0210	0.0220 ✓
30	Skin and adnexa	260	B-SEBACEOUS GLAND ADENOMA	0.5758	0.6985	0.7057
30	Skin and adnexa	403	B-SQUAMOUS CELL PAPILLOMA	0.6384	0.5251	0.5290
30	Skin and adnexa	290	M-ADENOCARCINOMA, MAMMARY	0.2803	0.2481	0.2522
30	Skin and adnexa	238	M-FIBROSARCOMA	0.8220	0.8348	0.8375
30	Skin and adnexa	267	M-FIBROUS HISTIOCYTOMA, M	0.4740	0.7200	0.7253

Source: E:\RAT\m_rat.fil

NDA 20-895, Sildenafil
Appendix A-Male-Rats

Table I_A-Male-Rats
(Continued)

Test for Positive Dose-Response (Tumor) Linear Trend
Species: Rat 18:08 Friday, January 23, 1998
Sex: Male
Sorted by: Organ Name

Organ Code	Organ Name	Tumor Code	Tumor Name	Exact-P	Asymp-P	AsyCor-P
30	Skin and adnexa	234	M-HAEMANGIOPERICYTOMA	0.4070	0.6352	0.6433
30	Skin and adnexa	259	M-SEBACEOUS/SQUAMOUS CELL	0.5967	0.6857	0.6934
30	Skin and adnexa	223	M-SQUAMOUS CELL CARCINOMA	0.8168	0.8313	0.8340
18	Spleen	347	B-HAEMANGIOMA	1.0000	0.7176	0.7249
18	Spleen	379	M-HAEMANGIOSARCOMA	0.5263	0.7231	0.7283
13	Testes	180	B-INTERSTITIAL CELL ADENO	0.3979	0.3549	0.3578
13	Testes	349	M-MESOTHELIOMA, MALIGNANT	1.0000	0.6980	0.7061
47	Thorax	485	M-FIBROUS HISTIOCYTOMA, M	0.6092	0.6822	0.6900
47	Thorax	214	M-HAEMANGIOSARCOMA	1.0000	0.7150	0.7223
31	Thymus	426	M-THYMOMA, EPITHELIAL PRE	0.1935	0.0210	0.0220
31	Thymus	209	M-THYMOMA, LYMPHOCYTIC PR	0.7799	0.7731	0.7777
8	Thyroid	141	B-C-CELL ADENOMA	0.1887	0.2159	0.2172
8	Thyroid	170	B-FOLLICULAR CELL ADENOMA	0.0266	0.0134	0.0136 ✓
8	Thyroid	254	M-C-CELL CARCINOMA	0.1893	0.2485	0.2524
8	Thyroid	375	M-FOLLICULAR CELL CARCINO	0.7232	0.8288	0.8316

Source: E:\RAT\m_rat.fil

Appendix A-Male Rats

Table II_A_Male_Rats

Analysis of Carcinogenic Potential in Male Rat
Test of Dose-Response (Tumor) Positive Linear Trend
Ted Guo, PH.D, CDER/FDA
Run Date & Time: January 29, 1998 (17:30)
Source: E:\RAT\m_rat.fil

Note: Dose Levels Included: CTRL1 CTRL2 LOW MED HIGH (0 0 1.5 5 60)
Missing value in Tumor-Caused Death is treated as tumor not causing death
Tumor Type: IN: Incidental (nonfatal) tumor, FA: Fatal tumor.

ORGAN/TISSUE NAME AND TUMOR NAME	(ORG#) (TMR#)	TUMOR TYPES	TIME STRATA	ROW NO.	2xC_CONTINGENCY -----TABLE-----	EXACT PROB =PR(STATISTIC.GE.OBSERVED)	ASYMP TOTIC	ASYMP(CONTI NUITY CORR)
Kidney	(1)	IN	65-80	1	0 0 0 0 1	0.0390	0.0023	0.0024
B-RENAL TUBULE ADENOMA	(187)	IN	65-80	2	15 14 11 13 13			
		IN	81-92	1	0 0 0 0 1			
		IN	81-92	2	16 14 4 14 10			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 0 0 0 2			
Kidney	(1)	IN	93-93	1	0 0 1 0 0	0.7204	0.6951	0.7027
B-LIPOMA	(373)	IN	93-93	2	10 16 26 22 18			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 0 1 0 0			
Kidney	(1)	FA	102	1	0 1 0 0 0	1.0000	0.7103	0.7178
M-LIPOSARCOMA	(382)	FA	102	2	14 19 27 25 19			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 1 0 0 0			
Testes	(13)	IN	65-80	1	0 0 0 0 1	0.3979	0.3549	0.3578
B-INTERSTITIAL CELL ADENO	(180)	IN	65-80	2	15 14 11 13 13			
		IN	81-92	1	0 2 0 0 1			
		IN	81-92	2	16 12 4 14 10			
		IN	93-93	1	1 0 2 1 0			
		IN	93-93	2	9 16 25 21 18			
Spontaneous tumor rate 3% in ctrl. - Total				-	1 2 2 1 2			
Testes	(13)	FA	98	1	0 1 0 0 0	1.0000	0.6980	0.7061
M-MESOTHELIOMA, MALIGNANT	(349)	FA	98	2	20 24 27 28 19			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 1 0 0 0			
Spleen	(18)	IN	93-93	1	1 0 0 0 0	1.0000	0.7176	0.7249
B-HAEMANGIOMA	(347)	IN	93-93	2	9 16 27 22 18			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1 0 0 0 0			
Spleen	(18)	IN	93-93	1	0 0 1 0 0	0.5263	0.7231	0.7283
M-HAEMANGIOSARCOMA	(379)	IN	93-93	2	10 16 26 22 18			
		FA	101	1	0 0 0 1 0			
		FA	101	2	15 20 27 26 19			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 0 1 1 0			
Pancreas	(19)	IN	41-64	1	1 0 2 0 1	0.8003	0.8217	0.8228
B-ISLET CELL ADENOMA	(144)	IN	41-64	2	15 10 11 10 11			
		IN	65-80	1	1 0 0 3 1			
		IN	65-80	2	14 14 11 10 13			
		IN	81-92	1	4 2 0 1 1			
		IN	81-92	2	12 12 4 13 10			
		IN	93-93	1	2 4 5 7 2			
		IN	93-93	2	8 12 22 15 16			
Spontaneous tumor rate 12% in ctrl. - Total				-	8 6 7 11 5			

Pancreas	(19)	IN	41-64	1	0	0	1	0	0	0.3719	0.3229	0.3254
B-ACINAR ADENOMA	(257)	IN	41-64	2	16	10	12	10	12			
		IN	81-92	1	2	0	0	0	2			
		IN	81-92	2	14	14	4	14	9			
		IN	93-93	1	2	0	3	1	1			
		IN	93-93	2	8	16	24	21	17			
Spontaneous tumor rate 3%	in ctrl. - Total			-	4	0	4	1	3			
Mesenteric node	(20)	IN	81-92	1	0	1	0	1	0	0.6571	0.7265	0.7316
B-HAEMANGIOMA	(345)	IN	81-92	2	15	13	4	12	10			
Spontaneous tumor rate LE 1%	in ctrl. - Total			-	0	1	0	1	0			
Brain	(27)	IN	93-93	1	0	1	0	1	0	0.6199	0.5723	0.5758
M-ASTROCYTOMA	(177)	IN	93-93	2	10	15	27	21	18			
		FA	75	1	0	0	1	0	0			
		FA	75	2	47	48	44	52	45			
		FA	86	1	0	0	0	0	1			
		FA	86	2	37	38	39	41	31			
		FA	91	1	0	1	0	0	0			
		FA	91	2	27	31	33	36	30			
		FA	93	1	0	1	0	0	0			
		FA	93	2	25	28	31	34	28			
Spontaneous tumor rate 3%	in ctrl. - Total			-	0	3	1	1	1			
Brain	(27)	IN	81-92	1	1	0	0	0	0	1.0000	0.7076	0.7149
B-GLIOMA	(196)	IN	81-92	2	15	14	4	14	11			
Spontaneous tumor rate LE 1%	in ctrl. - Total			-	1	0	0	0	0			
Brain	(27)	IN	93-93	1	0	0	1	0	0	0.7204	0.6951	0.7027
M-MALIGNANT RETICULOSIS	(406)	IN	93-93	2	10	16	26	22	18			
Spontaneous tumor rate LE 1%	in ctrl. - Total			-	0	0	1	0	0			
Liver	(3)	IN	41-64	1	0	1	1	0	0	0.9029	0.8751	0.8764
B-HEPATOCELLULAR ADENOMA	(218)	IN	41-64	2	16	9	12	10	12			
		IN	65-80	1	0	0	1	1	0			
		IN	65-80	2	15	14	10	12	14			
		IN	81-92	1	3	0	0	0	0			
		IN	81-92	2	13	14	4	14	11			
		IN	93-93	1	0	2	2	1	1			
		IN	93-93	2	10	14	25	21	17			
Spontaneous tumor rate 5%	in ctrl. - Total			-	3	3	4	2	1			
Liver	(3)	IN	81-92	1	1	0	0	0	0	1.0000	0.7076	0.7149
M-CHOLANGIOCARCINOMA	(330)	IN	81-92	2	15	14	4	14	11			
Spontaneous tumor rate LE 1%	in ctrl. - Total			-	1	0	0	0	0			
Liver	(3)	IN	65-80	1	0	0	1	0	0	0.5486	0.6213	0.6245
M-HEPATOCELLULAR CARCINOM	(337)	IN	65-80	2	15	14	10	13	14			
		IN	81-92	1	0	0	0	2	0			
		IN	81-92	2	16	14	4	12	11			
		IN	93-93	1	1	1	1	0	1			
		IN	93-93	2	9	15	26	22	17			
Spontaneous tumor rate 2%	in ctrl. - Total			-	1	1	2	2	1			
Skin and adnexa	(30)	IN	65-80	1	0	0	1	0	0	0.3501	0.3761	0.3801
B-FIBROADENOMA, MAMMARY G	(146)	IN	65-80	2	15	13	10	13	14			
		IN	93-93	1	0	1	0	0	0			
		IN	93-93	2	10	15	27	22	18			
		FA	54	1	0	0	0	0	1			
		FA	54	2	57	53	55	59	54			
		FA	97	1	0	0	0	1	0			
		FA	97	2	22	25	28	28	21			
Spontaneous tumor rate LE 1%	in ctrl. - Total			-	0	1	1	1	1			
Skin and adnexa	(30)	IN	65-80	1	0	1	1	0	1	0.4622	0.4464	0.4490
B-HAIR FOLLICLE TUMOUR	(181)	IN	65-80	2	15	12	10	13	13			
		IN	81-92	1	2	1	0	0	0			
		IN	81-92	2	14	13	4	14	11			
		IN	93-93	1	1	2	0	3	2			
		IN	93-93	2	9	14	27	19	16			
Spontaneous tumor rate 6%	in ctrl. - Total			-	3	4	1	3	3			

Appendix A-Male Rats: Table II_Male Rats (Continued)

Skin and adnexa	(30)	IN 81-92	1	0	1	1	0	0	0.8168	0.8313	0.8340
M-SQUAMOUS CELL CARCINOMA	(223)	IN 81-92	2	16	13	3	14	11			
		IN 93-93	1	0	0	0	1	0			
		IN 93-93	2	10	16	27	21	18			
		FA 87	1	0	1	0	0	0			
		FA 87	2	36	37	38	38	31			
Spontaneous tumor rate 2%	in ctrl. -	Total	-	0	2	1	1	0			
Skin and adnexa	(30)	FA 66	1	0	0	0	1	0	0.4070	0.6352	0.6433
M-HAEMANGIOPERICYTOMA	(234)	FA 66	2	53	49	51	54	50			
Spontaneous tumor rate LE 1%	in ctrl. -	Total	-	0	0	0	1	0			
Skin and adnexa	(30)	IN 81-92	1	1	0	0	0	0	0.8220	0.8348	0.8375
M-FIBROSARCOMA	(238)	IN 81-92	2	15	13	4	14	11			
		FA 57	1	0	0	0	1	0			
		FA 57	2	55	53	55	58	54			
		FA 83	1	0	0	1	0	0			
		FA 83	2	39	40	39	45	37			
		FA 93	1	0	1	0	0	0			
		FA 93	2	25	28	31	34	28			
Spontaneous tumor rate 2%	in ctrl. -	Total	-	1	1	1	1	0			
Skin and adnexa	(30)	IN 81-92	1	2	0	0	0	1	0.9022	0.8460	0.8474
B-FIBROUS HISTIOCYTOMA	(243)	IN 81-92	2	14	14	4	14	10			
		IN 93-93	1	3	2	1	0	0			
		IN 93-93	2	7	14	26	22	18			
		FA 57	1	0	0	0	1	0			
		FA 57	2	55	53	55	58	54			
		FA 90	1	1	0	0	0	0			
		FA 90	2	30	32	36	36	31			
Spontaneous tumor rate 7%	in ctrl. -	Total	-	6	2	1	1	1			
Skin and adnexa	(30)	FA 74	1	0	0	1	0	0	0.5967	0.6857	0.6934
M-SEBACEOUS/SQUAMOUS CELL	(259)	FA 74	2	50	48	46	53	45			
Spontaneous tumor rate LE 1%	in ctrl. -	Total	-	0	0	1	0	0			
Skin and adnexa	(30)	IN 65-80	1	0	0	1	0	0	0.5758	0.6985	0.7057
B-SEBACEOUS GLAND ADENOMA	(260)	IN 65-80	2	15	13	10	13	14			
Spontaneous tumor rate LE 1%	in ctrl. -	Total	-	0	0	1	0	0			
Skin and adnexa	(30)	IN 41-64	1	0	0	1	0	0	0.4740	0.7200	0.7253
M-FIBROUS HISTIOCYTOMA, M	(267)	IN 41-64	2	16	10	12	10	12			
		FA 102	1	0	0	0	1	0			
		FA 102	2	14	20	27	24	19			
Spontaneous tumor rate LE 1%	in ctrl. -	Total	-	0	0	1	1	0			
Skin and adnexa	(30)	IN 81-92	1	0	0	0	1	1	0.2803	0.2481	0.2522
M-ADENOCARCINOMA, MAMMARY	(290)	IN 81-92	2	16	14	4	13	10			
		IN 93-93	1	1	0	0	0	0			
		IN 93-93	2	9	16	27	22	18			
Spontaneous tumor rate LE 1%	in ctrl. -	Total	-	1	0	0	1	1			
Skin and adnexa	(30)	IN 93-93	1	0	1	0	0	0	1.0000	0.7176	0.7249
B-ADENOMA, MAMMARY GLAND	(328)	IN 93-93	2	10	15	27	22	18			
Spontaneous tumor rate LE 1%	in ctrl. -	Total	-	0	1	0	0	0			
Skin and adnexa	(30)	IN 65-80	1	0	0	1	0	0	0.4754	0.4136	0.4178
B-BASAL CELL TUMOUR	(343)	IN 65-80	2	15	13	10	13	14			
		IN 81-92	1	0	0	0	0	1			
		IN 81-92	2	16	14	4	14	10			
		IN 93-93	1	1	0	1	0	0			
		IN 93-93	2	9	16	26	22	18			
Spontaneous tumor rate LE 1%	in ctrl. -	Total	-	1	0	2	0	1			
Skin and adnexa	(30)	IN 93-93	1	2	0	2	0	1	0.6384	0.5251	0.5290
B-SQUAMOUS CELL PAPILLOMA	(403)	IN 93-93	2	8	16	25	22	17			
Spontaneous tumor rate 2%	in ctrl. -	Total	-	2	0	2	0	1			
Skin and adnexa	(30)	IN 93-93	1	0	0	0	0	1	0.1935	0.0210	0.0220
B-LYMPHANGIOMA	(425)	IN 93-93	2	10	16	27	22	17			
Spontaneous tumor rate LE 1%	in ctrl. -	Total	-	0	0	0	0	1			

Appendix A-Male Rats: Table II_Male Rats (Continued)

Skin and adnexa	(30)	IN 93-93	1	0	0	0	0	1	0.1935	0.0210	0.0220
B-LIPOMA	(427)	IN 93-93	2	10	16	27	22	17			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	0	0	0	1			
Skin and adnexa	(30)	IN 65-80	1	1	0	1	0	0	0.8100	0.8405	0.8430
B-FIBROMA	(78)	IN 65-80	2	14	13	10	13	14			
		IN 81-92	1	0	0	0	1	0			
		IN 81-92	2	16	14	4	13	11			
		FA 74	1	1	0	0	0	0			
		FA 74	2	49	48	47	53	45			
Spontaneous tumor rate 2% in ctrl. - Total			-	2	0	1	1	0			
Thymus	(31)	IN 65-80	1	0	1	0	0	0	0.7799	0.7731	0.7777
M-THYMOMA, LYMPHOCYTIC PR	(209)	IN 65-80	2	15	13	11	13	14			
		IN 81-92	1	0	0	1	0	0			
		IN 81-92	2	16	14	3	14	11			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	1	1	0	0			
Thymus	(31)	IN 93-93	1	0	0	0	0	1	0.1935	0.0210	0.0220
M-THYMOMA, EPITHELIAL PRE	(426)	IN 93-93	2	10	16	27	22	17			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	0	0	0	1			
Lungs	(32)	IN 65-80	1	0	0	0	0	1	0.2487	0.3475	0.3514
M-BRONCHIOLAR-ALVEOLAR CA	(164)	IN 65-80	2	15	14	11	13	13			
		IN 81-92	1	0	1	0	1	0			
		IN 81-92	2	16	13	4	13	11			
		IN 93-93	1	0	0	0	1	0			
		IN 93-93	2	10	16	27	21	18			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	1	0	2	1			
Lungs	(32)	IN 65-80	1	0	0	0	1	0	0.5745	0.7640	0.7678
B-BRONCHIOLAR-ALVEOLAR AD	(241)	IN 65-80	2	15	14	11	12	14			
		IN 81-92	1	0	1	0	1	0			
		IN 81-92	2	16	13	4	13	11			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	1	0	2	0			
Lungs	(32)	IN 93-93	1	0	0	0	1	0	0.6552	0.7309	0.7361
X-OSTEOSARCOMA	(333)	IN 93-93	2	10	16	27	21	18			
		FA 98	1	1	0	0	0	0			
		FA 98	2	19	25	27	28	19			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1	0	0	1	0			
Lymphoreticular	(38)	IN 81-92	1	1	0	0	0	0	0.7993	0.7290	0.7313
M-LARGE GRANULAR CELL LYM	(11)	IN 81-92	2	15	13	4	14	11			
		IN 93-93	1	1	1	0	0	1			
		IN 93-93	2	9	14	27	21	17			
		FA 66	1	1	0	0	0	0			
		FA 66	2	52	50	51	55	50			
		FA 100	1	0	1	0	0	0			
		FA 100	2	17	20	27	27	19			
		FA 104	1	0	1	0	1	0			
		FA 104	2	10	15	27	21	18			
Spontaneous tumor rate 5% in ctrl. - Total			-	3	3	0	1	1			
Lymphoreticular	(38)	FA 16	1	0	1	0	0	0	0.3431	0.1441	0.1476
M-LYMPHOMA, NOS	(212)	FA 16	2	60	58	60	60	59			
		FA 102	1	0	0	0	0	1			
		FA 102	2	14	20	27	25	18			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	1	0	0	1			
Lymphoreticular	(38)	IN 93-93	1	0	0	1	0	0	0.8683	0.8112	0.8146
M-LYMPHOBLASTIC LYMPHOMA	(252)	IN 93-93	2	10	16	26	22	18			
		FA 39	1	0	0	1	0	0			
		FA 39	2	58	58	59	60	58			
		FA 98	1	0	1	0	0	0			
		FA 98	2	20	24	27	28	19			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	1	2	0	0			

Appendix A-Male Rats: Table II_Male Rats (Continued)

Lymphoreticular	(38)	IN	93-93	1	0	1	0	0	0	1.0000	0.8260	0.8294
M-HISTIOCYTIC SARCOMA	(315)	IN	93-93	2	10	15	27	22	18			
		FA	97	1	1	0	0	0	0			
		FA	97	2	21	25	28	29	21			
		FA	98	1	1	0	0	0	0			
		FA	98	2	19	25	27	28	19			
Spontaneous tumor rate 3% in ctrl. - Total				-	2	1	0	0	0			
Lymphoreticular	(38)	IN	93-93	1	0	0	2	0	0	0.7258	0.6988	0.7018
M-GRANULOCYTIC LEUKAEMIA	(53)	IN	93-93	2	10	16	25	22	18			
		FA	75	1	1	0	0	0	0			
		FA	75	2	46	48	46	52	45			
		FA	76	1	1	0	0	0	0			
		FA	76	2	43	45	45	52	44			
		FA	77	1	1	0	0	0	0			
		FA	77	2	41	45	45	51	44			
		FA	79	1	0	0	1	0	0			
		FA	79	2	41	44	41	49	43			
		FA	80	1	0	0	0	0	1			
		FA	80	2	40	42	41	48	41			
		FA	86	1	0	0	0	1	0			
		FA	86	2	37	38	39	40	32			
Spontaneous tumor rate 3% in ctrl. - Total				-	3	0	3	1	1			
Jejunum	(43)	IN	65-80	1	0	1	0	0	0	1.0000	0.7180	0.7250
M-ADENOCARCINOMA	(202)	IN	65-80	2	15	13	11	13	14			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	1	0	0	0			
Abdomen	(45)	IN	65-80	1	0	0	1	0	0	0.5672	0.6964	0.7036
B-LIPOMA	(327)	IN	65-80	2	15	14	10	13	14			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	1	0	0			
Thorax	(47)	FA	79	1	0	1	0	0	0	1.0000	0.7150	0.7223
M-HAEMANGIOSARCOMA	(214)	FA	79	2	41	43	42	49	43			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	1	0	0	0			
Thorax	(47)	FA	88	1	0	0	1	0	0	0.6092	0.6822	0.6900
M-FIBROUS HISTIOCYTOMA, M	(485)	FA	88	2	34	34	36	38	31			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	1	0	0			
Seminal vesicle	(50)	FA	50	1	0	1	0	0	0	1.0000	0.7172	0.7243
M-SQUAMOUS CELL CARCINOMA	(231)	FA	50	2	55	54	55	59	57			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	1	0	0	0			
Bone, unsp.	(59)	IN	93-93	1	0	0	1	0	0	0.7204	0.6951	0.7027
B-OSTEOMA	(411)	IN	93-93	2	10	16	26	22	18			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	1	0	0			
Adrenal	(6)	IN	41-64	1	1	0	1	0	0	0.6817	0.6554	0.6584
B-CORTICAL ADENOMA	(122)	IN	41-64	2	15	10	12	10	12			
		IN	65-80	1	1	0	1	0	0			
		IN	65-80	2	14	14	10	13	14			
		IN	93-93	1	0	1	0	1	1			
		IN	93-93	2	10	14	26	21	16			
Spontaneous tumor rate 3% in ctrl. - Total				-	2	1	2	1	1			
Adrenal	(6)	IN	41-64	1	0	1	3	1	0	0.8353	0.8275	0.8283
B-PHEOCHROMOCYTOMA	(134)	IN	41-64	2	16	9	10	9	12			
		IN	65-80	1	2	2	0	1	1			
		IN	65-80	2	13	12	11	12	13			
		IN	81-92	1	3	2	2	3	4			
		IN	81-92	2	13	12	2	11	7			
		IN	93-93	1	4	4	9	4	2			
		IN	93-93	2	6	11	17	18	15			
Spontaneous tumor rate 15% in ctrl. - Total				-	9	9	14	9	7			
Adrenal	(6)	IN	65-80	1	0	2	0	0	0	0.8524	0.7819	0.7840
M-PHEOCHROMOCYTOMA, MALIG	(228)	IN	65-80	2	15	12	11	13	14			
		IN	81-92	1	1	1	0	0	0			
		IN	81-92	2	15	13	4	14	11			
		IN	93-93	1	1	0	3	0	1			

Appendix A-Male Rats: Table II_Male Rats (Continued)

		IN 93-93	2	9	15	23	22	16		
Spontaneous tumor rate 4% in ctrl. - Total			-	2	3	3	0	1		
Adrenal	(6)	IN 81-92	1	0	1	0	0	0	1.0000	0.7076 0.7149
M-CORTICAL CARCINOMA	(353)	IN 81-92	2	16	13	4	14	11		
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	1	0	0	0		
Pituitary	(7)	FA 81	1	1	0	0	0	0	1.0000	0.7871 0.7915
M-CARCINOMA, PARS DISTALI	(127)	FA 81	2	38	41	40	46	39		
		FA 92	1	0	1	0	0	0		
		FA 92	2	26	29	30	35	29		
Spontaneous tumor rate 2% in ctrl. - Total			-	1	1	0	0	0		
Pituitary	(7)	IN 0-40	1	0	1	0	0	1	0.3515	0.3556 0.3550
B-ADENOMA, PARS DISTALIS	(6)	IN 0-40	2	3	5	5	1	4		
		IN 41-64	1	2	4	4	0	2		
		IN 41-64	2	8	3	6	5	7		
		IN 65-80	1	3	6	8	2	6		
		IN 65-80	2	8	3	1	1	5		
		IN 81-92	1	4	10	0	4	5		
		IN 81-92	2	3	2	1	1	0		
		IN 93-93	1	9	10	17	18	15		
		IN 93-93	2	1	5	8	3	2		
		FA 54	1	1	0	0	0	0		
		FA 54	2	55	53	53	57	54		
		FA 60	1	0	1	0	0	0		
		FA 60	2	53	51	52	54	52		
		FA 61	1	0	0	0	0	1		
		FA 61	2	53	51	52	54	51		
		FA 64	1	0	1	0	0	0		
		FA 64	2	53	50	52	54	50		
		FA 65	1	0	0	0	0	1		
		FA 65	2	52	49	50	54	49		
		FA 68	1	0	0	1	0	0		
		FA 68	2	51	49	48	53	47		
		FA 71	1	1	1	0	0	1		
		FA 71	2	49	48	47	53	46		
		FA 72	1	0	0	0	1	0		
		FA 72	2	49	48	46	52	46		
		FA 74	1	2	0	0	1	0		
		FA 74	2	47	48	45	51	44		
		FA 75	1	1	0	0	0	0		
		FA 75	2	45	47	44	51	44		
		FA 76	1	1	0	0	0	0		
		FA 76	2	42	44	43	51	43		
		FA 77	1	0	0	0	1	0		
		FA 77	2	41	44	43	49	43		
		FA 78	1	0	0	1	1	0		
		FA 78	2	40	44	42	48	43		
		FA 79	1	0	0	0	1	0		
		FA 79	2	40	43	41	47	42		
		FA 80	1	0	0	0	1	0		
		FA 80	2	39	41	40	46	41		
		FA 81	1	0	0	0	1	0		
		FA 81	2	39	41	40	45	39		
		FA 82	1	0	0	1	0	1		
		FA 82	2	38	40	39	44	37		
		FA 83	1	0	1	0	1	0		
		FA 83	2	38	38	39	43	36		
		FA 84	1	0	0	0	3	0		
		FA 84	2	38	38	38	40	34		
		FA 85	1	0	1	0	0	1		
		FA 85	2	37	37	38	40	32		
		FA 86	1	1	0	0	2	0		
		FA 86	2	35	37	38	38	31		
		FA 87	1	0	1	0	0	0		
		FA 87	2	35	36	37	37	30		
		FA 88	1	0	0	0	1	0		
		FA 88	2	33	33	36	36	30		
		FA 89	1	1	0	0	0	0		
		FA 89	2	31	32	35	35	30		

Appendix A-Male Rats: Table II_Male Rats (Continued)

		FA 90	1	1	0	0	0	0		
		FA 90	2	30	32	35	35	30		
		FA 91	1	0	1	1	0	0		
		FA 91	2	27	31	31	35	30		
		FA 92	1	1	0	0	2	1		
		FA 92	2	25	30	30	33	28		
		FA 93	1	1	0	1	0	1		
		FA 93	2	24	29	29	33	27		
		FA 94	1	0	0	1	1	0		
		FA 94	2	24	27	28	32	27		
		FA 95	1	0	0	0	1	0		
		FA 95	2	23	26	28	31	26		
		FA 96	1	0	0	1	2	2		
		FA 96	2	23	25	27	28	22		
		FA 97	1	1	0	0	0	2		
		FA 97	2	21	25	27	28	19		
		FA 99	1	1	1	0	1	0		
		FA 99	2	17	21	26	26	19		
		FA 100	1	2	0	0	0	0		
		FA 100	2	15	21	26	26	19		
		FA 101	1	1	0	0	0	0		
		FA 101	2	14	20	26	26	19		
		FA 102	1	1	1	0	1	0		
		FA 102	2	13	19	26	23	19		
		FA 103	1	1	0	0	1	0		
		FA 103	2	10	17	26	21	18		
		FA 104	1	0	1	1	0	0		
		FA 104	2	10	15	25	21	18		
		FA 105	1	0	0	0	0	1		
		FA 105	2	8	10	21	16	13		
Spontaneous tumor rate 64%	in ctrl.	- Total	-	36	41	37	47	41		
Thyroid	(8)	IN 41-64	1	1	0	3	1	3	0.1887	0.2159 0.2172
B-C-CELL ADENOMA	(141)	IN 41-64	2	15	10	10	9	9		
		IN 65-80	1	0	1	0	2	2		
		IN 65-80	2	15	13	11	11	12		
		IN 81-92	1	0	0	1	0	0		
		IN 81-92	2	16	14	3	14	11		
		IN 93-93	1	1	3	3	6	2		
		IN 93-93	2	9	13	24	16	16		
Spontaneous tumor rate 5%	in ctrl.	- Total	-	2	4	7	9	7		
Thyroid	(8)	IN 41-64	1	0	0	0	0	1	0.0266	0.0134 0.0136
B-FOLLICULAR CELL ADENOMA	(170)	IN 41-64	2	16	10	13	10	11		
		IN 65-80	1	0	0	0	0	1		
		IN 65-80	2	15	14	11	13	13		
		IN 81-92	1	1	0	0	0	1		
		IN 81-92	2	15	14	4	14	10		
		IN 93-93	1	3	0	0	2	2		
		IN 93-93	2	7	16	27	20	16		
Spontaneous tumor rate 3%	in ctrl.	- Total	-	4	0	0	2	5		
Thyroid	(8)	IN 41-64	1	0	0	1	0	0	0.1893	0.2485 0.2524
M-C-CELL CARCINOMA	(254)	IN 41-64	2	16	10	12	10	12		
		IN 93-93	1	0	0	0	1	1		
		IN 93-93	2	10	16	27	21	17		
Spontaneous tumor rate LE 1%	in ctrl.	- Total	-	0	0	1	1	1		
Thyroid	(8)	IN 93-93	1	1	1	0	2	0	0.7232	0.8288 0.8316
M-FOLLICULAR CELL CARCINO	(375)	IN 93-93	2	9	15	27	20	18		
Spontaneous tumor rate 2%	in ctrl.	- Total	-	1	1	0	2	0		
Parathyroid	(9)	IN 81-92	1	1	0	0	0	0	0.5070	0.3323 0.3367
B-ADENOMA	(369)	IN 81-92	2	9	9	3	12	10		
		IN 93-93	1	0	1	0	0	1		
		IN 93-93	2	9	13	22	19	15		
Spontaneous tumor rate 2%	in ctrl.	- Total	-	1	1	0	0	1		

Appendix A-Male Rats: Table II_Male Rats (Continued)

NDA 20-895

Sildenafil

Appendix B-Female-Rats

Female Rats

NDA 20-895, Sildenafil
Appendix B-Female-Rats

Table I_B-Female-Rats

Test for Positive Dose-Response (Tumor) Linear Trend
Species: Rat 18:08 Friday, January 23, 1998
Sex: Female
Sorted by: Organ Name

Organ Code	Organ Name	Tumor Code	Tumor Name	Exact-P	Asymp-P	AsyCor-P
45	Abdomen	327	B-LIPOMA	0.4649	0.3375	0.3417
45	Abdomen	474	B-PARAGANGLIOMA	0.3485	0.6021	0.6113
6	Adrenal	122	B-CORTICAL ADENOMA	0.8376	0.8545	0.8555
6	Adrenal	134	B-PHEOCHROMOCYTOMA	0.6008	0.6710	0.6730
6	Adrenal	353	M-CORTICAL CARCINOMA	0.3228	0.4029	0.4070
27	Brain	476	B-GRANULAR CELL TUMOUR	0.1515	0.0092	0.0098
27	Brain	177	M-ASTROCYTOMA	0.9255	0.8288	0.8318
24	Colon	362	M-ADENOCARCINOMA	0.1919	0.0204	0.0215
22	Duodenum	463	B-LEIOMYOMA	1.0000	0.6903	0.6986
43	Jejunum	202	M-ADENOCARCINOMA	1.0000	0.6903	0.6986
1	Kidney	373	B-LIPOMA	1.0000	0.7123	0.7196
1	Kidney	187	B-RENAL TUBULE ADENOMA	0.3485	0.6021	0.6113
1	Kidney	447	M-NEPHROBLASTOMA	0.3880	0.6362	0.6442
3	Liver	218	B-HEPATOCELLULAR ADENOMA	0.8281	0.7748	0.7769
3	Liver	337	M-HEPATOCELLULAR CARCINOM	1.0000	0.7418	0.7479
38	Lymphoreticular	315	M-HISTIOCYTIC SARCOMA	0.3728	0.2818	0.2861
38	Lymphoreticular	11	M-LARGE GRANULAR CELL LYM	0.2609	0.2771	0.2811
38	Lymphoreticular	252	M-LYMPHOBLASTIC LYMPHOMA	0.3918	0.6353	0.6433
38	Lymphoreticular	397	M-SMALL LYMPHOCYTIC LYMPH	1.0000	0.7730.	0.7777
15	Ovaries	459	B-HAEMANGIOMA	0.2708	0.0507	0.0527
15	Ovaries	433	B-SEX CORD/STROMAL TUMOUR	0.6838	0.8253	0.8283
15	Ovaries	372	M-SCHWANNOMA, MALIGNANT	0.3412	0.1422	0.1458
15	Ovaries	355	M-SEX CORD/STROMAL TUMOUR	1.0000	0.7745	0.7792
19	Pancreas	257	B-ACINAR ADENOMA	0.6177	0.4520	0.4562
19	Pancreas	144	B-ISLET CELL ADENOMA	0.3496	0.4509	0.4533
19	Pancreas	431	B-MIXED ACINAR-ISLET CELL	0.5938	0.6586	0.6676
9	Parathyroid	369	B-ADENOMA	0.4342	0.2113	0.2154
7	Pituitary	6	B-ADENOMA, PARS DISTALIS	0.4403	0.4473	0.4479
7	Pituitary	127	M-CARCINOMA, PARS DISTALI	0.5347	0.6505	0.6532
29	Skeletal muscle	388	M-CHONDROSARCOMA	1.0000	0.7432	0.7493
30	Skin and adnexa	328	B-ADENOMA, MAMMARY GLAND	0.6281	0.6508	0.6529
30	Skin and adnexa	146	B-FIBROADENOMA, MAMMARY G	0.8369	0.8374	0.8369
30	Skin and adnexa	243	B-FIBROUS HISTIOCYTOMA	0.1276	0.0611	0.0624
30	Skin and adnexa	181	B-HAIR FOLLICLE TUMOUR	0.3875	0.6363	0.6442
30	Skin and adnexa	427	B-LIPOMA	0.7835	0.8332	0.8358
30	Skin and adnexa	290	M-ADENOCARCINOMA, MAMMARY	0.8307	0.8408	0.8416
30	Skin and adnexa	291	M-CARCINOMA ARISING IN FI	0.9627	0.9616	0.9620
30	Skin and adnexa	296	M-CARCINOSARCOMA, MAMMARY	1.0000	0.7125	0.7197
30	Skin and adnexa	238	M-FIBROSARCOMA	0.3810	0.6306	0.6388
30	Skin and adnexa	267	M-FIBROUS HISTIOCYTOMA, M	0.8301	0.7436	0.7490
30	Skin and adnexa	470	M-LIPOSARCOMA	0.5909	0.6647	0.6733
30	Skin and adnexa	481	M-MYCOSIS FUNGOIDES	0.3962	0.3476	0.3503
30	Skin and adnexa	259	M-SEBACEOUS/SQUAMOUS CELL	0.3620	0.1540	0.1576
30	Skin and adnexa	223	M-SQUAMOUS CELL CARCINOMA	0.5784	0.7126	0.7181

Source: E:\RAT\f_rat.fil

NDA 20-895, Sildenafil
Appendix B-Female-Rats

Table I_A-Male-Rats
(Continued)

Test for Positive Dose-Response (Tumor) Linear Trend
Species: Mouse 15:05 Monday, February 2, 1998
Sex: Female
Sorted by: Organ Name

Organ Code	Organ Name	Tumor Code	Tumor Name	Exact-P	Asymp-P	AsyCor-P
56	Abdomen	348	B-HAEMANGIOMA	0.1458	0.0118	0.0170
7	Adrenal	285	B-SUBCAPSULAR CELL ADENOM	1.0000	0.7319	0.7771
37	Harderian gland	258	B-ADENOMA	0.9535	0.9104	0.9156
1	Kidney	266	B-RENAL TUBULE ADENOMA	1.0000	0.7319	0.7771
3	Liver	332	B-HAEMANGIOMA	0.4710	0.4597	0.5145
33	Lungs	154	B-BRONCHIOLAR-ALVEOLAR AD	0.3570	0.3467	0.3542
33	Lungs	149	M-BRONCHIOLAR-ALVEOLAR CA	0.3670	0.3767	0.3872
58	Lymphoreticular	244	M-HISTIOCYTIC SARCOMA	0.3001	0.3299	0.3445
58	Lymphoreticular	234	M-IMMUNOBLASTIC LYMPHOMA	0.5625	0.2620	0.2759
58	Lymphoreticular	183	M-LYMPHOBLASTIC LYMPHOMA	0.2908	0.2812	0.2875
16	Ovaries	209	B-CYSTADENOMA	0.4794	0.4707	0.4818
16	Ovaries	246	B-SEX CORD/STROMAL TUMOUR	0.7605	0.7184	0.7461
16	Ovaries	407	B-TERATOMA	1.0000	0.8073	0.8438
31	Skin and adnexa	259	M-ADENOCARCINOMA, MAMMARY	1.0000	0.7310	0.7783
31	Skin and adnexa	204	M-FIBROUS HISTIOCYTOMA, M	1.0000	0.8073	0.8438
19	Spleen	330	B-HAEMANGIOMA	0.3750	0.4028	0.4594
22	Stomach	272	B-SQUAMOUS CELL PAPILLOMA	1.0000	0.7319	0.7771
22	Stomach	270	M-SQUAMOUS CELL CARCINOMA	1.0000	0.8114	0.8381
32	Thymus	359	M-THYMOMA, EPITHELIAL PRE	1.0000	0.8517	0.8710
9	Thyroid	331	M-FOLLICULAR CELL CARCINO	0.6047	0.7032	0.7181
17	Uterus	264	B-HAEMANGIOMA	1.0000	0.7310	0.7783
17	Uterus	287	B-LEIOMYOMA	0.2585	0.1731	0.1951
17	Uterus	284	M-STROMAL CELL SARCOMA	0.8448	0.7974	0.8086
18	Vagina	282	B-LEIOMYOMA	0.8400	0.7765	0.7890

Appendix B-Female Rats

Table II_B_Female_Rats

Analysis of Carcinogenic Potential in Female Rats
Test of Dose-Response (Tumor) Positive Linear Trend
Ted Guo, PH.D, CDER/FDA
Run Date & Time: January 29, 1998 (17:55)
Source: E:\RAT\f_rat.fil

Note: Dose Levels Included: CTRL1 CTRL2 LOW MED HIGH (0 0 1.5 5 60)
Missing value in Tumor-Caused Death is treated as tumor not causing death
Tumor Type: IN: Incidental (nonfatal) tumor, FA: Fatal tumor.

ORGAN/TISSUE NAME AND TUMOR NAME	(ORG#) (TMR#)	TUMOR TYPES	TIME STRATA	ROW NO.	2xC_CONTINGENCY -----TABLE-----	EXACT PROB =PR(STATISTIC.GE.OBSERVED)	ASYMP TOTIC	ASYMP(CONTI NUITY CORR)
Kidney	(1)	IN	93-93	1	0 0 0 1 0	0.3485	0.6021	0.6113
B-RENAL TUBULE ADENOMA	(187)	IN	93-93	2	14 13 16 12 10			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 0 0 1 0			
Kidney	(1)	IN	65-80	1	0 1 0 0 0	1.0000	0.7123	0.7196
B-LIPOMA	(373)	IN	65-80	2	17 18 13 16 16			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 1 0 0 0			
Kidney	(1)	FA	72	1	0 0 0 1 0	0.3880	0.6362	0.6442
M-NEPHROBLASTOMA	(447)	FA	72	2	51 45 46 43 46			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 0 0 1 0			
Ovaries	(15)	IN	93-93	1	1 0 0 0 0	1.0000	0.7745	0.7792
M-SEX CORD/STROMAL TUMOUR	(355)	IN	93-93	2	13 13 16 13 10			
		FA	91	1	1 0 0 0 0			
		FA	91	2	27 24 25 19 25			
Spontaneous tumor rate 2% in ctrl. - Total				-	2 0 0 0 0			
Ovaries	(15)	IN	41-64	1	0 1 0 0 0	0.3412	0.1422	0.1458
M-SCHWANNOMA, MALIGNANT	(372)	IN	41-64	2	17 13 18 21 15			
		FA	57	1	0 0 0 0 1			
		FA	57	2	57 55 54 54 54			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 1 0 0 1			
Ovaries	(15)	IN	65-80	1	0 0 0 1 0	0.6838	0.8253	0.8283
B-SEX CORD/STROMAL TUMOUR	(433)	IN	65-80	2	17 19 12 15 16			
		IN	81-92	1	0 1 1 0 0			
		IN	81-92	2	11 10 7 5 13			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 1 1 1 0			
Ovaries	(15)	IN	81-92	1	0 0 0 0 1	0.2708	0.0507	0.0527
B-HAEMANGIOMA	(459)	IN	81-92	2	11 11 8 5 12			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 0 0 0 1			
Uterus	(16)	IN	41-64	1	1 0 0 0 1	0.3357	0.1361	0.1395
M-STROMAL CELL SARCOMA	(303)	IN	41-64	2	16 15 18 21 15			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1 0 0 0 1			
Uterus	(16)	IN	41-64	1	0 0 0 0 1	0.1084	0.0438	0.0448
B-GRANULAR CELL TUMOUR	(321)	IN	41-64	2	17 15 18 21 15			
		IN	65-80	1	0 0 0 0 1			
		IN	65-80	2	17 19 13 16 15			
		IN	93-93	1	1 0 1 0 0			
		IN	93-93	2	13 13 15 13 10			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1 0 1 0 2			

Uterus	(16)	IN 81-92	1	0	0	0	0	1	0.2708	0.0507	0.0527
B-HAEMANGIOMA	(389)	IN 81-92	2	11	11	8	5	12			
Spontaneous tumor rate LE 1% in ctrl. - Total		-		0	0	0	0	1			
Uterus	(16)	IN 41-64	1	1	0	0	0	0	0.7537	0.7110	0.7136
B-ENDOMETRIAL STROMAL POL	(393)	IN 41-64	2	16	15	18	21	16			
		IN 65-80	1	0	1	0	1	0			
		IN 65-80	2	17	18	13	15	16			
		IN 81-92	1	3	0	0	0	0			
		IN 81-92	2	8	11	8	5	13			
		IN 93-93	1	0	0	0	0	1			
		IN 93-93	2	14	13	16	13	9			
Spontaneous tumor rate 4% in ctrl. - Total		-		4	1	0	1	1			
Uterus	(16)	IN 81-92	1	0	0	0	1	0	0.3750	0.6766	0.6834
M-SARCOMA, N.O.S.	(443)	IN 81-92	2	11	11	8	4	13			
Spontaneous tumor rate LE 1% in ctrl. - Total		-		0	0	0	1	0			
Uterus	(16)	IN 93-93	1	0	0	1	0	0	0.5909	0.6647	0.6733
M-CARCINOMA	(468)	IN 93-93	2	14	13	15	13	10			
Spontaneous tumor rate LE 1% in ctrl. - Total		-		0	0	1	0	0			
Uterus	(16)	IN 93-93	1	0	1	0	0	0	1.0000	0.6903	0.6986
B-ADENOMA	(479)	IN 93-93	2	14	12	16	13	10			
Spontaneous tumor rate LE 1% in ctrl. - Total		-		0	1	0	0	0			
Uterus	(16)	IN 93-93	1	1	0	0	0	0	1.0000	0.6903	0.6986
B-LEIOMYOMA	(486)	IN 93-93	2	13	13	16	13	10			
Spontaneous tumor rate LE 1% in ctrl. - Total		-		1	0	0	0	0			
Vagina	(17)	FA 61	1	0	0	1	0	0	0.5837	0.6887	0.6963
M-LEIOMYOSARCOMA	(437)	FA 61	2	56	56	50	54	52			
Spontaneous tumor rate LE 1% in ctrl. - Total		-		0	0	1	0	0			
Vagina	(17)	FA 76	1	0	0	0	1	0	0.4563	0.7216	0.7267
M-SCHWANNOMA, MALIGNANT	(440)	FA 76	2	43	44	42	38	40			
		FA 79	1	0	0	1	0	0			
		FA 79	2	41	43	35	34	38			
Spontaneous tumor rate LE 1% in ctrl. - Total		-		0	0	1	1	0			
Vagina	(17)	IN 93-93	1	0	0	0	0	1	0.1515	0.0092	0.0098
B-SQUAMOUS CELL PAPILLOMA	(475)	IN 93-93	2	14	13	16	13	9			
Spontaneous tumor rate LE 1% in ctrl. - Total		-		0	0	0	0	1			
Pancreas	(19)	IN 41-64	1	0	0	0	1	0	0.3496	0.4509	0.4533
B-ISLET CELL ADENOMA	(144)	IN 41-64	2	17	15	19	20	16			
		IN 65-80	1	0	1	1	0	0			
		IN 65-80	2	17	18	11	16	16			
		IN 81-92	1	0	0	0	1	1			
		IN 81-92	2	11	11	7	4	12			
		IN 93-93	1	0	3	0	2	1			
		IN 93-93	2	13	10	16	11	8			
Spontaneous tumor rate 3% in ctrl. - Total		-		0	4	1	4	2			
Pancreas	(19)	IN 81-92	1	0	1	0	0	1	0.6177	0.4520	0.4562
B-ACINAR ADENOMA	(257)	IN 81-92	2	11	10	7	5	12			
		IN 93-93	1	1	1	0	0	0			
		IN 93-93	2	12	12	16	13	9			
Spontaneous tumor rate 3% in ctrl. - Total		-		1	2	0	0	1			
Pancreas	(19)	IN 93-93	1	0	0	1	0	0	0.5938	0.6586	0.6676
B-MIXED ACINAR-ISLET CELL	(431)	IN 93-93	2	13	13	15	13	9			
Spontaneous tumor rate LE 1% in ctrl. - Total		-		0	0	1	0	0			
Duodenum	(22)	IN 93-93	1	1	0	0	0	0	1.0000	0.6903	0.6986
B-LEIOMYOMA	(463)	IN 93-93	2	13	13	16	13	10			
Spontaneous tumor rate LE 1% in ctrl. - Total		-		1	0	0	0	0			

Appendix B-Female Rats: Table II_Female Rats (Continued)

Colon	(24)	FA	95	1	0	0	0	0	1	0.1919	0.0204	0.0215
M-ADENOCARCINOMA	(362)	FA	95	2	21	20	22	17	18			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	0	0	1			
Brain	(27)	FA	66	1	0	1	0	0	0	0.9255	0.8288	0.8318
M-ASTROCYTOMA	(177)	FA	66	2	55	50	50	49	48			
		FA	75	1	1	0	0	0	0			
		FA	75	2	45	44	44	40	42			
		FA	87	1	0	0	1	0	0			
Spontaneous tumor rate 2% in ctrl. - Total				-	1	1	1	0	0			
Brain	(27)	IN	93-93	1	0	0	0	0	1	0.1515	0.0092	0.0098
B-GRANULAR CELL TUMOUR	(476)	IN	93-93	2	14	13	16	13	9			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	0	0	1			
Skeletal muscle	(29)	IN	81-92	1	1	0	0	0	0	1.0000	0.7432	0.7493
M-CHONDROSARCOMA	(388)	IN	81-92	2	10	11	8	4	13			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1	0	0	0	0			
Liver	(3)	IN	41-64	1	0	0	0	1	0	0.8281	0.7748	0.7769
B-HEPATOCELLULAR ADENOMA	(218)	IN	41-64	2	17	15	19	20	16			
		IN	65-80	1	0	1	0	0	0			
		IN	65-80	2	17	18	13	16	16			
		IN	81-92	1	2	1	0	0	0			
		IN	81-92	2	9	10	8	5	13			
		IN	93-93	1	0	2	1	0	1			
		IN	93-93	2	14	11	15	13	9			
Spontaneous tumor rate 5% in ctrl. - Total				-	2	4	1	1	1			
Liver	(3)	IN	81-92	1	0	1	0	0	0	1.0000	0.7418	0.7479
M-HEPATOCELLULAR CARCINOM	(337)	IN	81-92	2	11	10	8	5	13			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	1	0	0	0			
Skin and adnexa	(30)	IN	0-40	1	0	1	0	0	0	0.8369	0.8374	0.8369
B-FIBROADENOMA, MAMMARY G	(146)	IN	0-40	2	1	1	4	5	5			
		IN	41-64	1	2	1	7	5	3			
		IN	41-64	2	14	13	11	16	13			
		IN	65-80	1	4	6	2	3	5			
		IN	65-80	2	10	6	9	11	10			
		IN	81-92	1	4	2	3	2	4			
		IN	81-92	2	5	8	5	2	7			
		IN	93-93	1	7	8	11	11	5			
		IN	93-93	2	7	5	4	2	5			
		FA	67	1	0	0	1	0	0			
		FA	67	2	54	50	49	47	48			
		FA	69	1	0	1	0	0	0			
		FA	69	2	53	48	47	46	48			
		FA	74	1	1	0	0	0	0			
		FA	74	2	49	45	45	39	42			
		FA	79	1	0	1	0	0	0			
		FA	79	2	42	42	37	33	39			
		FA	80	1	1	2	0	0	0			
		FA	80	2	40	38	35	31	38			
		FA	81	1	0	2	0	0	0			
		FA	81	2	40	36	34	28	37			
		FA	83	1	0	0	1	0	0			
		FA	83	2	37	35	31	25	36			
		FA	84	1	1	1	1	0	0			
		FA	84	2	36	33	30	24	35			
		FA	86	1	0	1	0	0	0			
		FA	86	2	34	28	29	23	33			
		FA	87	1	1	0	0	0	0			
		FA	87	2	33	27	28	21	33			
		FA	89	1	0	0	0	1	0			
		FA	89	2	31	25	27	20	30			
		FA	90	1	0	0	0	0	1			
		FA	90	2	30	24	26	19	27			
		FA	93	1	0	0	0	0	1			

Appendix B-Female Rats: Table II_Female Rats (Continued)

			FA 93	2	25	21	24	18	21		
			FA 94	1	1	0	0	0	0		
			FA 95	1	0	0	0	1	0		
			FA 95	2	21	20	22	16	20		
			FA 96	1	0	1	0	0	0		
			FA 96	2	21	19	22	15	18		
			FA 100	1	1	0	0	0	0		
			FA 100	2	19	15	18	14	14		
			FA 102	1	0	0	0	0	1		
			FA 102	2	14	15	18	14	12		
			FA 104	1	0	0	1	0	0		
			FA 104	2	14	13	15	13	10		
Spontaneous tumor rate 42%		in ctrl. -	Total	-	23	27	27	23	20		
Skin and adnexa	(30	)	IN 65-80	1	0	0	0	1	0	0.3875	0.6363 0.6442
B-HAIR FOLLICLE TUMOUR	(181	)	IN 65-80	2	17	19	13	14	16		
Spontaneous tumor rate LE 1%		in ctrl. -	Total	-	0	0	0	1	0		
Skin and adnexa	(30	)	IN 93-93	1	0	0	0	1	0	0.5784	0.7126 0.7181
M-SQUAMOUS CELL CARCINOMA	(223	)	IN 93-93	2	14	13	16	12	10		
			FA 99	1	0	1	0	0	0		
			FA 99	2	20	16	18	15	15		
Spontaneous tumor rate LE 1%		in ctrl. -	Total	-	0	1	0	1	0		
Skin and adnexa	(30	)	FA 66	1	0	0	0	1	0	0.3810	0.6306 0.6388
M-FIBROSARCOMA	(238	)	FA 66	2	55	51	50	47	48		
Spontaneous tumor rate LE 1%		in ctrl. -	Total	-	0	0	0	1	0		
Skin and adnexa	(30	)	IN 93-93	1	0	0	0	0	1	0.1276	0.0611 0.0624
B-FIBROUS HISTIOCYTOMA	(243	)	IN 93-93	2	14	13	16	13	9		
			FA 64	1	1	0	0	0	0		
			FA 64	2	55	54	51	50	49		
			FA 90	1	0	0	0	0	1		
			FA 90	2	30	24	26	19	27		
			FA 93	1	0	0	1	0	0		
			FA 93	2	25	21	23	18	22		
Spontaneous tumor rate LE 1%		in ctrl. -	Total	-	1	0	1	0	2		
Skin and adnexa	(30	)	IN 65-80	1	0	1	0	0	1	0.3620	0.1540 0.1576
M-SEBACEOUS/SQUAMOUS CELL	(259	)	IN 65-80	2	17	18	13	15	15		
Spontaneous tumor rate LE 1%		in ctrl. -	Total	-	0	1	0	0	1		
Skin and adnexa	(30	)	IN 93-93	1	1	0	0	0	0	0.8301	0.7436 0.7490
M-FIBROUS HISTIOCYTOMA, M	(267	)	IN 93-93	2	13	13	15	13	10		
			FA 104	1	0	0	1	0	0		
			FA 104	2	14	13	15	13	10		
Spontaneous tumor rate LE 1%		in ctrl. -	Total	-	1	0	1	0	0		
Skin and adnexa	(30	)	IN 0-40	1	0	0	0	0	1	0.8307	0.8408 0.8416
M-ADENOCARCINOMA, MAMMARY	(290	)	IN 0-40	2	1	2	4	4	3		
			IN 41-64	1	5	0	1	1	1		
			IN 41-64	2	11	14	17	18	14		
			IN 65-80	1	5	1	0	5	1		
			IN 65-80	2	11	16	13	10	15		
			IN 81-92	1	1	1	2	0	3		
			IN 81-92	2	9	7	6	5	10		
			IN 93-93	1	2	2	5	8	1		
			IN 93-93	2	12	10	11	5	9		
			FA 37	1	0	0	0	1	0		
			FA 37	2	60	60	60	57	58		
			FA 51	1	0	0	0	0	1		
			FA 51	2	59	58	59	55	55		
			FA 61	1	0	0	0	0	1		
			FA 64	1	0	0	1	0	0		
			FA 64	2	56	54	50	50	49		
			FA 67	1	1	1	0	0	0		
			FA 67	2	53	49	50	47	48		
			FA 70	1	0	0	0	1	0		

Appendix B-Female Rats: Table II_Female Rats (Continued)

		FA 70	2	53	46	46	45	47		
		FA 72	1	0	0	0	1	0		
		FA 72	2	51	45	46	42	46		
		FA 79	1	0	1	0	0	0		
		FA 79	2	42	42	37	33	39		
		FA 87	1	0	1	0	0	0		
		FA 87	2	34	26	28	21	33		
		FA 90	1	1	0	0	0	0		
		FA 90	2	29	24	26	19	28		
		FA 92	1	0	1	0	0	0		
		FA 92	2	25	23	24	18	23		
		FA 96	1	0	1	0	0	0		
		FA 96	2	21	19	22	15	18		
		FA 101	1	1	0	0	0	0		
		FA 101	2	14	15	18	14	14		
		FA 102	1	0	1	0	0	0		
		FA 102	2	14	14	18	14	13		
		FA 104	1	0	1	0	0	0		
		FA 104	2	14	12	16	13	10		
Spontaneous tumor rate 23%	in ctrl.	- Total	-	16	11	9	17	9		
Skin and adnexa	(30)	IN 41-64	1	0	0	1	0	0	0.9627	0.9616 0.9620
M-CARCINOMA ARISING IN FI	(291)	IN 41-64	2	17	15	18	21	16		
		IN 65-80	1	3	1	2	1	0		
		IN 65-80	2	13	17	10	13	16		
		IN 81-92	1	0	1	1	0	1		
		IN 81-92	2	11	10	7	5	12		
		IN 93-93	1	2	0	1	3	0		
		IN 93-93	2	12	13	15	10	10		
		FA 80	1	0	1	0	0	0		
		FA 80	2	41	39	35	31	38		
		FA 82	1	0	0	0	1	0		
		FA 82	2	39	35	33	26	36		
		FA 87	1	1	0	0	0	0		
		FA 87	2	33	27	28	21	33		
		FA 89	1	0	0	1	0	0		
		FA 89	2	31	25	26	21	30		
Spontaneous tumor rate 8%	in ctrl.	- Total	-	6	3	6	5	1		
Skin and adnexa	(30)	FA 54	1	1	0	0	0	0	1.0000	0.7125 0.7197
M-CARCINOSARCOMA, MAMMARY	(296)	FA 54	2	57	57	56	54	55		
Spontaneous tumor rate LE 1%	in ctrl.	- Total	-	1	0	0	0	0		
Skin and adnexa	(30)	IN 65-80	1	1	0	0	0	0	1.0000	0.7128 0.7199
M-SQUAMOUS CELL CARCINOMA	(319)	IN 65-80	2	16	19	13	15	16		
Spontaneous tumor rate LE 1%	in ctrl.	- Total	-	1	0	0	0	0		
Skin and adnexa	(30)	IN 41-64	1	0	1	0	0	0	0.6281	0.6508 0.6529
B-ADENOMA, MAMMARY GLAND	(328)	IN 41-64	2	17	14	19	21	16		
		IN 65-80	1	0	0	1	1	1		
		IN 65-80	2	17	19	12	14	15		
		IN 81-92	1	0	1	0	0	1		
		IN 81-92	2	11	10	8	5	12		
		IN 93-93	1	2	3	1	2	0		
		IN 93-93	2	12	10	15	11	10		
Spontaneous tumor rate 6%	in ctrl.	- Total	-	2	5	2	3	2		
Skin and adnexa	(30)	IN 81-92	1	0	0	0	1	0	0.7835	0.8332 0.8358
B-LIPOMA	(427)	IN 81-92	2	11	11	8	4	13		
		IN 93-93	1	2	0	0	0	0		
		IN 93-93	2	12	13	16	13	10		
		FA 76	1	0	0	1	0	0		
		FA 76	2	44	44	43	38	41		
Spontaneous tumor rate 2%	in ctrl.	- Total	-	2	0	1	1	0		

Appendix B-Female Rats: Table II_Female Rats (Continued)

Skin and adnexa	(30)	IN 93-93	1	0	0	1	0	0	0.5909	0.6647	0.6733
M-LIPOSARCOMA	(470)	IN 93-93	2	14	13	15	13	10			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	0	1	0	0			
Skin and adnexa	(30)	IN 41-64	1	1	0	0	0	0	0.3962	0.3476	0.3503
M-MYCOSIS FUNGOIDES	(481)	IN 41-64	2	16	15	19	21	16			
		IN 81-92	1	0	0	0	0	2			
		IN 81-92	2	11	11	8	5	11			
		IN 93-93	1	2	1	1	0	0			
		IN 93-93	2	12	12	15	13	10			
		FA 79	1	0	0	0	1	0			
		FA 79	2	42	43	37	32	39			
Spontaneous tumor rate 3% in ctrl. - Total			-	3	1	1	1	2			
Thymus	(31)	IN 41-64	1	1	0	0	1	1	0.7410	0.6854	0.6883
M-THYMOMA, LYMPHOCYTIC PR	(209)	IN 41-64	2	16	15	19	20	15			
		IN 65-80	1	1	0	0	0	0			
		IN 65-80	2	16	19	13	16	16			
		IN 81-92	1	1	1	0	0	0			
		IN 81-92	2	10	10	8	5	13			
		IN 93-93	1	1	0	0	0	0			
		IN 93-93	2	13	13	16	13	10			
Spontaneous tumor rate 4% in ctrl. - Total			-	4	1	0	1	1			
Thymus	(31)	FA 81	1	0	1	0	0	0	1.0000	0.7154	0.7224
M-SCHWANNOMA, MALIGNANT	(374)	FA 81	2	40	37	34	29	37			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	1	0	0	0			
Thymus	(31)	IN 81-92	1	0	0	0	0	1	0.2708	0.0507	0.0527
M-CARCINOMA, NOS	(391)	IN 81-92	2	11	11	8	5	12			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	0	0	0	1			
Thymus	(31)	IN 41-64	1	0	0	1	0	0	0.8513	0.7561	0.7612
M-THYMOMA, EPITHELIAL PRE	(426)	IN 41-64	2	17	15	18	21	16			
		IN 93-93	1	1	0	0	0	0			
		IN 93-93	2	13	13	16	13	10			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1	0	1	0	0			
Lymphoreticular	(38)	IN 81-92	1	0	0	0	1	0	0.2609	0.2771	0.2811
M-LARGE GRANULAR CELL LYM	(11)	IN 81-92	2	11	11	8	4	13			
		IN 93-93	1	0	1	0	0	0			
		IN 93-93	2	14	12	16	13	10			
		FA 21	1	0	0	0	0	1			
		FA 21	2	60	60	60	60	59			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	1	0	1	1			
Lymphoreticular	(38)	FA 38	1	0	0	0	1	0	0.3918	0.6353	0.6433
M-LYMPHOBLASTIC LYMPHOMA	(252)	FA 38	2	60	60	60	57	58			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	0	0	1	0			
Lymphoreticular	(38)	FA 61	1	1	0	0	0	0	0.3728	0.2818	0.2861
M-HISTIOCYTIC SARCOMA	(315)	FA 61	2	56	56	53	54	53			
		FA 96	1	0	0	1	0	0			
		FA 96	2	21	20	21	15	18			
		FA 98	1	0	0	0	0	1			
		FA 98	2	20	18	20	15	17			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1	0	1	0	1			
Lymphoreticular	(38)	IN 65-80	1	0	1	0	0	0	1.0000	0.7730	0.7777
M-SMALL LYMPHOCYTIC LYMPH	(397)	IN 65-80	2	17	18	13	16	16			
		IN 93-93	1	0	1	0	0	0			
		IN 93-93	2	14	12	16	13	10			
Spontaneous tumor rate 2% in ctrl. - Total			-	0	2	0	0	0			
Jejunum	(43)	IN 93-93	1	0	1	0	0	0	1.0000	0.6903	0.6986
M-ADENOCARCINOMA	(202)	IN 93-93	2	14	12	16	13	10			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	1	0	0	0			

Appendix B-Female Rats: Table II_Female Rats (Continued)

Abdomen	(45)	IN	41-64	1	0	0	0	0	1	0.4649	0.3375	0.3417
B-LIPOMA	(327)	IN	41-64	2	17	15	19	21	15			
		IN	93-93	1	0	2	1	0	0			
		IN	93-93	2	14	11	15	13	10			
Spontaneous tumor rate 2%	in ctrl. -	Total		-	0	2	1	0	1			
Abdomen	(45)	IN	93-93	1	0	0	0	1	0	0.3485	0.6021	0.6113
B-PARAGANGLIOMA	(474)	IN	93-93	2	14	13	16	12	10			
Spontaneous tumor rate LE 1%	in ctrl. -	Total		-	0	0	0	1	0			
Adrenal	(6)	IN	0-40	1	0	0	1	0	0	0.8376	0.8545	0.8555
B-CORTICAL ADENOMA	(122)	IN	0-40	2	1	2	3	5	5			
		IN	41-64	1	1	0	2	0	0			
		IN	41-64	2	16	15	17	21	16			
		IN	65-80	1	1	0	2	0	2			
		IN	65-80	2	16	19	11	16	14			
		IN	81-92	1	2	3	2	1	1			
		IN	81-92	2	9	8	6	4	12			
		IN	93-93	1	0	1	1	3	0			
		IN	93-93	2	14	12	15	10	10			
Spontaneous tumor rate 7%	in ctrl. -	Total		-	4	4	8	4	3			
Adrenal	(6)	IN	41-64	1	0	0	1	0	1	0.6008	0.6710	0.6730
B-PHEOCHROMOCYTOMA	(134)	IN	41-64	2	17	15	18	21	15			
		IN	65-80	1	0	2	1	1	0			
		IN	65-80	2	17	17	12	15	16			
		IN	81-92	1	1	1	0	0	1			
		IN	81-92	2	10	10	8	5	12			
		IN	93-93	1	0	2	0	3	0			
		IN	93-93	2	14	11	16	10	10			
Spontaneous tumor rate 5%	in ctrl. -	Total		-	1	5	2	4	2			
Adrenal	(6)	IN	81-92	1	0	1	1	0	0	0.3228	0.4029	0.4070
M-CORTICAL CARCINOMA	(353)	IN	81-92	2	11	10	7	5	13			
		IN	93-93	1	0	0	0	1	1			
		IN	93-93	2	14	13	16	12	9			
Spontaneous tumor rate LE 1%	in ctrl. -	Total		-	0	1	1	1	1			
Pituitary	(7)	IN	65-80	1	0	1	0	0	0	0.5347	0.6505	0.6532
M-CARCINOMA, PARS DISTALI	(127)	IN	65-80	2	17	17	13	15	15			
		IN	93-93	1	0	0	0	1	0			
		IN	93-93	2	13	12	16	12	10			
		FA	63	1	0	0	0	0	1			
		FA	63	2	56	54	51	53	49			
		FA	78	1	0	0	0	1	0			
		FA	78	2	43	42	39	36	38			
		FA	80	1	0	0	0	1	0			
		FA	80	2	41	39	35	31	37			
		FA	85	1	0	1	0	0	0			
		FA	85	2	34	31	29	23	33			
		FA	94	1	0	1	0	0	0			
		FA	94	2	23	19	23	18	21			
		FA	104	1	1	0	0	0	0			
		FA	104	2	13	12	16	13	10			
Spontaneous tumor rate 3%	in ctrl. -	Total		-	1	3	0	3	1			
Pituitary	(7)	IN	0-40	1	0	1	0	0	0	0.4403	0.4473	0.4479
B-ADENOMA, PARS DISTALIS	(6)	IN	0-40	2	1	0	0	3	5			
		IN	41-64	1	6	1	3	1	2			
		IN	41-64	2	2	3	2	6	2			
		IN	65-80	1	7	7	4	5	1			
		IN	65-80	2	0	5	1	2	3			
		IN	81-92	1	4	5	2	1	5			
		IN	81-92	2	0	1	0	0	1			
		IN	93-93	1	11	11	16	12	8			
		IN	93-93	2	3	1	0	1	1			
		FA	39	1	0	0	1	1	0			
		FA	39	2	60	59	59	56	56			
		FA	50	1	0	1	0	0	0			

Appendix B-Female Rats: Table II_Female Rats (Continued)

FA	50	2	59	57	59	56	56
FA	52	1	0	0	3	1	0
FA	52	2	59	57	56	55	54
FA	53	1	0	1	0	0	0
FA	53	2	59	56	56	55	54
FA	54	1	0	1	0	1	0
FA	54	2	58	55	56	54	54
FA	57	1	0	0	0	0	1
FA	57	2	57	55	56	54	53
FA	59	1	0	0	2	0	0
FA	59	2	57	55	54	54	52
FA	61	1	0	0	0	1	2
FA	61	2	57	55	53	53	50
FA	62	1	0	1	1	0	0
FA	62	2	56	54	51	53	50
FA	63	1	0	1	0	2	1
FA	63	2	56	53	51	51	49
FA	64	1	0	3	0	1	0
FA	64	2	56	50	51	50	48
FA	67	1	0	0	2	1	0
FA	67	2	54	49	48	47	47
FA	69	1	0	2	1	0	1
FA	69	2	53	46	46	47	46
FA	70	1	1	0	0	1	0
FA	70	2	52	45	46	46	46
FA	71	1	1	1	0	1	1
FA	71	2	51	44	46	44	45
FA	72	1	1	0	0	2	1
FA	72	2	50	44	46	42	44
FA	73	1	0	0	1	0	2
FA	73	2	50	44	45	40	41
FA	74	1	3	1	1	0	0
FA	74	2	47	43	44	40	41
FA	75	1	1	0	0	1	1
FA	75	2	45	43	44	39	40
FA	76	1	1	0	3	1	2
FA	76	2	43	43	41	38	38
FA	77	1	0	0	1	0	0
FA	77	2	43	42	39	37	38
FA	78	1	1	0	2	2	0
FA	78	2	42	42	37	35	38
FA	79	1	1	1	1	1	1
FA	79	2	41	41	36	33	37
FA	80	1	0	0	1	2	1
FA	80	2	41	39	34	30	36
FA	81	1	1	0	1	0	1
FA	81	2	39	37	33	29	35
FA	82	1	1	0	1	0	0
FA	82	2	38	34	32	28	35
FA	83	1	0	1	0	1	1
FA	83	2	37	33	32	25	34
FA	84	1	2	0	1	2	1
FA	84	2	35	33	30	23	33
FA	85	1	0	2	0	0	0
FA	85	2	34	30	29	23	33
FA	86	1	0	1	1	1	0
FA	86	2	34	27	28	22	32
FA	87	1	1	0	0	0	2
FA	87	2	33	26	28	21	30
FA	88	1	1	1	0	0	1
FA	88	2	31	24	27	21	29
FA	89	1	1	1	0	1	1
FA	89	2	30	23	27	20	28
FA	90	1	0	0	1	0	0
FA	90	2	30	23	25	19	27
FA	91	1	2	0	1	1	2
FA	91	2	26	23	24	18	23
FA	92	1	0	2	0	0	1
FA	92	2	25	21	24	18	22

Appendix B-Female Rats: Table II_Female Rats (Continued)

		FA 93	1	2	0	0	0	0		
		FA 93	2	23	20	24	18	22		
		FA 94	1	1	0	1	1	0		
		FA 94	2	22	20	22	17	21		
		FA 95	1	0	0	0	1	1		
		FA 95	2	21	19	22	16	19		
		FA 97	1	1	1	1	0	0		
		FA 97	2	20	17	20	15	18		
		FA 98	1	0	1	2	0	2		
		FA 98	2	20	16	18	15	16		
		FA 99	1	0	1	0	1	1		
		FA 99	2	20	15	18	14	14		
		FA 100	1	3	0	0	0	0		
		FA 100	2	17	14	18	14	14		
		FA 101	1	0	0	0	0	1		
		FA 101	2	15	14	18	14	13		
		FA 102	1	0	0	2	0	1		
		FA 102	2	14	14	16	14	12		
		FA 103	1	0	0	0	1	0		
		FA 103	2	14	12	16	13	10		
		FA 104	1	0	0	0	0	1		
		FA 104	2	14	12	16	13	9		
Spontaneous tumor rate 86%	in ctrl.	- Total	-	54	49	57	48	47		
Thyroid	(8)	IN 41-64	1	1	1	1	1	0	0.7008	0.6837	0.6855
B-C-CELL ADENOMA	(141)	IN 41-64	2	16	14	18	20	16		
		IN 65-80	1	0	1	1	1	0		
		IN 65-80	2	17	18	12	15	16		
		IN 81-92	1	1	1	0	0	0		
		IN 81-92	2	10	10	8	5	13		
		IN 93-93	1	1	3	4	1	3		
		IN 93-93	2	12	10	12	12	7		
Spontaneous tumor rate 8%	in ctrl.	- Total	-	3	6	6	3	3		
Thyroid	(8)	IN 41-64	1	0	0	0	1	1	0.2694	0.2457	0.2497
B-FOLLICULAR CELL ADENOMA	(170)	IN 41-64	2	17	15	19	20	15		
		IN 65-80	1	0	1	0	0	0		
		IN 65-80	2	17	18	13	16	16		
Spontaneous tumor rate LE 1%	in ctrl.	- Total	-	0	1	0	1	1		
Thyroid	(8)	IN 93-93	1	0	0	1	0	0	0.6000	0.6666	0.6751
M-C-CELL CARCINOMA	(254)	IN 93-93	2	13	13	15	13	10		
Spontaneous tumor rate LE 1%	in ctrl.	- Total	-	0	0	1	0	0		
Thyroid	(8)	IN 81-92	1	0	1	0	0	0	1.0000	0.7418	0.7479
M-FOLLICULAR CELL CARCINO	(375)	IN 81-92	2	11	10	8	5	13		
Spontaneous tumor rate LE 1%	in ctrl.	- Total	-	0	1	0	0	0		
Parathyroid	(9)	IN 65-80	1	0	1	0	0	1	0.4342	0.2113	0.2154
B-ADENOMA	(369)	IN 65-80	2	12	11	10	9	13		
Spontaneous tumor rate LE 1%	in ctrl.	- Total	-	0	1	0	0	1		

Appendix B-Female Rats: Table II_Female Rats (Continued)

NDA 20-895

Sildenafil

Appendix C-Male-Mice

Male Mice

NDA 20-895, Sildenafil
Appendix C-Male-Mice

Table I_C-Male-Mice
Case Where All Doses Are Included

Test for Positive Dose-Response (Tumor) Linear Trend
Species: Mouse 15:05 Monday, February 2, 1998
Sex: Male
Sorted by: Organ Name

Organ Code	Organ Name	Tumor Code	Tumor Name	Exact-P	Asymp-P	AsyCor-P
56	Abdomen	348	B-HAEMANGIOMA	0.3958	0.1132	0.1349
56	Abdomen	381	M-HAEMANGIOSARCOMA	0.5043	0.5457	0.5919
7	Adrenal	321	B-CORTICAL ADENOMA	0.8742	0.8472	0.8589
7	Adrenal	285	B-SUBCAPSULAR CELL ADENOM	0.1765	0.0215	0.0295
54	Bone, uns.	98	M-OSTEOSARCOMA	0.1667	0.0181	0.0202
28	Brain	306	M-ASTROCYTOMA	1.0000	0.7810	0.8156
23	Duodenum	389	M-ADENOCARCINOMA	1.0000	0.7555	0.7969
37	Harderian gland	258	B-ADENOMA	0.4884	0.4732	0.4872
1	Kidney	266	B-RENAL TUBULE ADENOMA	0.3768	0.3070	0.3326
3	Liver	332	B-HAEMANGIOMA	0.1385	0.0838	0.0939
3	Liver	356	B-HEPATOCELLULAR ADENOMA	0.8330	0.8230	0.8290
3	Liver	309	M-HAEMANGIOSARCOMA	0.7168	0.6997	0.7301
3	Liver	318	M-HEPATOCELLULAR CARCINOM	0.8953	0.8627	0.8695
33	Lungs	154	B-BRONCHIOLAR-ALVEOLAR AD	0.9704	0.9627	0.9639
33	Lungs	149	M-BRONCHIOLAR-ALVEOLAR CA	0.4082	0.4033	0.4096
58	Lymphoreticular	244	M-HISTIOCYTIC SARCOMA	0.2572	0.2230	0.2326
58	Lymphoreticular	234	M-IMMUNOBLASTIC LYMPHOMA	1.0000	0.8496	0.8581
58	Lymphoreticular	183	M-LYMPHOBLASTIC LYMPHOMA	0.3860	0.3328	0.3430
12	Oesophagus	398	B-SQUAMOUS CELL PAPILLOMA	0.4043	0.1173	0.1396
8	Pituitary	354	B-ADENOMA, PARS DISTALIS	1.0000	0.8211	0.8472
27	Prostate	403	M-ADENOCARCINOMA	0.4524	0.4575	0.5116
31	Skin and adnexa	375	B-FIBROUS HISTIOCYTOMA	0.3750	0.4311	0.4494
31	Skin and adnexa	204	M-FIBROUS HISTIOCYTOMA, M	0.5441	0.6216	0.6432
19	Spleen	330	B-HAEMANGIOMA	1.0000	0.8259	0.8369
14	Testes	391	B-HAEMANGIOMA	0.7351	0.7446	0.7708
14	Testes	371	B-INTERSTITIAL CELL ADENO	0.9493	0.8899	0.8954
9	Thyroid	402	B-FOLLICULAR CELL ADENOMA	0.5434	0.6271	0.6667
26	Urinary bladder	370	B-TRANSITIONAL CELL PAPIL	1.0000	0.7589	0.7992

Appendix C-Male Mice

Table II_C_Male_Mice
Case Where All Doses Are Included

Analysis of Carcinogenic Potential in Male Mouse
Test of Dose-Response (Tumor) Positive Linear Trend
Ted Guo, PH.D, CDER/FDA
Run Date & Time: January 29, 1998 (15:46)
Source: E:\MOUSE\m_mouse3.fil

Note: Dose Levels Included: CTRL1 CTRL2 LOW MED HIGH (0 0 3 10 30)
Missing value in Tumor-Caused Death is treated as tumor not causing death
Tumor Type: IN: Incidental (nonfatal) tumor, FA: Fatal tumor.

ORGAN/TISSUE NAME AND TUMOR NAME	(ORG#) (TMR#)	TUMOR TIME TYPES STRATA	ROW NO.	2xC_CONTINGENCY -----TABLE-----	EXACT PROB =PR(STATISTIC.GE.OBSERVED)	ASYMP TOTIC	ASYMP(CONTI NUITY CORR)
Kidney	(1	) IN 81-92	1	0 0 0 1 0	0.3768	0.3070	0.3326
B-RENAL TUBULE ADENOMA	(266	) IN 81-92	2	13 10 6 18 0			
		IN 93-93	1	0 1 1 0 0			
		IN 93-93	2	21 25 22 15 0			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0 1 1 1 0			
Oesophagus	(12	) IN 81-92	1	0 0 0 1 0	0.4043	0.1173	0.1396
B-SQUAMOUS CELL PAPILLOMA	(398	) IN 81-92	2	12 10 6 18 0			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0 0 0 1 0			
Testes	(14	) IN 65-80	1	0 0 2 0 0	0.9493	0.8899	0.8954
B-INTERSTITIAL CELL ADENO	(371	) IN 65-80	2	9 11 13 11 10			
		IN 93-93	1	3 1 0 0 0			
		IN 93-93	2	18 25 23 15 0			
Spontaneous tumor rate 4% in ctrl. - Total			-	3 1 2 0 0			
Testes	(14	) IN 81-92	1	1 0 0 0 0	0.7351	0.7446	0.7708
B-HAEMANGIOMA	(391	) IN 81-92	2	12 10 6 19 0			
		IN 93-93	1	0 0 1 0 0			
		IN 93-93	2	21 26 22 15 0			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1 0 1 0 0			
Spleen	(19	) IN 65-80	1	0 1 0 0 0	1.0000	0.8259	0.8369
B-HAEMANGIOMA	(330	) IN 65-80	2	9 10 15 11 10			
		IN 93-93	1	0 1 0 0 0			
		IN 93-93	2	21 25 23 15 0			
Spontaneous tumor rate 2% in ctrl. - Total			-	0 2 0 0 0			
Duodenum	(23	) IN 93-93	1	1 0 0 0 0	1.0000	0.7555	0.7969
M-ADENOCARCINOMA	(389	) IN 93-93	2	20 26 23 14 0			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1 0 0 0 0			
Urinary bladder	(26	) IN 93-93	1	1 0 0 0 0	1.0000	0.7589	0.7992
B-TRANSITIONAL CELL PAPIL	(370	) IN 93-93	2	20 26 23 15 0			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1 0 0 0 0			

Prostate	(27)	IN	93-93	1	0	0	1	0	0	0.4524	0.4575	0.5116
M-ADENOCARCINOMA	(403)	IN	93-93	2	20	26	22	15	0			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	1	0	0			
Brain	(28)	FA	73	1	1	0	0	0	0	1.0000	0.7810	0.8156
M-ASTROCYTOMA	(306)	FA	73	2	40	42	39	39	0			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1	0	0	0	0			
Liver	(3)	IN	93-93	1	0	0	1	0	0	0.7168	0.6997	0.7301
M-HAEMANGIOSARCOMA	(309)	IN	93-93	2	21	26	22	15	0			
		FA	84	1	1	0	0	0	0			
		FA	84	2	29	33	27	33	0			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1	0	1	0	0			
Liver	(3)	IN	65-80	1	0	1	0	0	0	0.8953	0.8627	0.8695
M-HEPATOCELLULAR CARCINOM	(318)	IN	65-80	2	9	9	14	11	10			
		IN	81-92	1	0	1	0	0	0			
		IN	81-92	2	12	9	6	18	0			
		IN	93-93	1	2	0	1	0	0			
		IN	93-93	2	19	26	22	15	0			
		FA	79	1	0	1	0	0	0			
		FA	79	2	36	37	33	34	0			
		FA	80	1	0	0	1	0	0			
		FA	80	2	34	36	31	34	0			
		FA	83	1	1	0	0	0	0			
		FA	83	2	30	35	27	33	0			
		FA	92	1	0	0	0	1	0			
		FA	92	2	22	27	24	23	0			
Spontaneous tumor rate 5% in ctrl. - Total				-	3	3	2	1	0			
Liver	(3)	IN	93-93	1	0	0	1	1	0	0.1385	0.0838	0.0939
B-HAEMANGIOMA	(332)	IN	93-93	2	21	26	22	14	0			
		FA	90	1	1	0	0	0	0			
		FA	90	2	23	28	24	30	0			
		FA	91	1	0	0	0	1	0			
		FA	91	2	23	27	24	27	0			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1	0	1	2	0			
Liver	(3)	IN	65-80	1	0	1	1	0	0	0.8330	0.8230	0.8290
B-HEPATOCELLULAR ADENOMA	(356)	IN	65-80	2	9	9	14	11	10			
		IN	81-92	1	0	0	1	1	0			
		IN	81-92	2	13	10	5	17	0			
		IN	93-93	1	4	5	3	1	0			
		IN	93-93	2	17	21	20	14	0			
		FA	79	1	0	1	0	0	0			
		FA	79	2	36	37	33	34	0			
		FA	92	1	0	0	0	1	0			
		FA	92	2	22	27	24	23	0			
Spontaneous tumor rate 10% in ctrl. - Total				-	4	7	5	3	0			
Skin and adnexa	(31)	FA	56	1	0	0	1	0	0	0.5441	0.6216	0.6432
M-FIBROUS HISTIOCYTOMA, M	(204)	FA	56	2	43	50	46	45	19			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	1	0	0			
Skin and adnexa	(31)	IN	65-80	1	0	0	0	1	0	0.3750	0.4311	0.4494
B-FIBROUS HISTIOCYTOMA	(375)	IN	65-80	2	9	11	15	10	10			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	0	1	0			
Lungs	(33)	IN	41-64	1	0	0	0	0	1	0.4082	0.4033	0.4096
M-BRONCHIOLAR-ALVEOLAR CA	(149)	IN	41-64	2	3	3	5	3	26			
		IN	65-80	1	0	0	1	2	0			
		IN	65-80	2	9	10	12	9	10			
		IN	93-93	1	1	5	3	2	0			
		IN	93-93	2	20	21	20	13	0			
		FA	73	1	0	0	1	0	0			

Appendix C-Female Mice: Table II_Male Mice (Continued)

			FA 73	2	41	42	38	39	0		
			FA 77	1	0	0	1	0	0		
			FA 77	2	36	39	36	35	0		
			FA 79	1	0	1	0	0	0		
			FA 79	2	36	37	33	34	0		
			FA 82	1	1	0	0	0	0		
			FA 82	2	33	36	29	34	0		
			FA 85	1	0	1	0	0	0		
			FA 85	2	28	32	27	33	0		
			FA 86	1	0	1	0	0	0		
			FA 86	2	27	30	26	33	0		
			FA 89	1	0	0	0	1	0		
			FA 89	2	26	29	24	31	0		
			FA 90	1	0	0	0	1	0		
			FA 90	2	24	28	24	29	0		
Spontaneous tumor rate 9%	in ctrl.	-	Total	-	2	8	6	6	1		
Lungs	(33	)	IN 41-64	1	0	0	1	0	0	0.9704	0.9627 0.9639
B-BRONCHIOLAR-ALVEOLAR AD	(154	)	IN 41-64	2	3	3	4	3	27		
			IN 65-80	1	1	1	1	2	0		
			IN 65-80	2	8	10	14	9	10		
			IN 81-92	1	4	0	1	4	0		
			IN 81-92	2	9	10	5	15	0		
			IN 93-93	1	4	7	5	0	0		
			IN 93-93	2	17	19	18	15	0		
Spontaneous tumor rate 15%	in ctrl.	-	Total	-	9	8	8	6	0		
Harderian gland	(37	)	IN 81-92	1	1	1	2	2	0	0.4884	0.4732 0.4872
B-ADENOMA	(258	)	IN 81-92	2	12	9	4	17	0		
			IN 93-93	1	2	1	3	1	0		
			IN 93-93	2	19	25	20	14	0		
Spontaneous tumor rate 5%	in ctrl.	-	Total	-	3	2	5	3	0		
Bone, uns.	(54	)	FA 38	1	0	0	0	0	1	0.1667	0.0181 0.0202
M-OSTEOSARCOMA	(98	)	FA 38	2	46	52	49	48	38		
Spontaneous tumor rate LE 1%	in ctrl.	-	Total	-	0	0	0	0	1		
Abdomen	(56	)	IN 81-92	1	0	0	0	1	0	0.3958	0.1132 0.1349
B-HAEMANGIOMA	(348	)	IN 81-92	2	13	10	6	18	0		
Spontaneous tumor rate LE 1%	in ctrl.	-	Total	-	0	0	0	1	0		
Abdomen	(56	)	FA 86	1	0	0	1	0	0	0.5043	0.5457 0.5919
M-HAEMANGIOSARCOMA	(381	)	FA 86	2	27	31	25	33	0		
Spontaneous tumor rate LE 1%	in ctrl.	-	Total	-	0	0	1	0	0		
Lymphoreticular	(58	)	IN 41-64	1	0	0	0	0	1	0.3860	0.3328 0.3430
M-LYMPHOBLASTIC LYMPHOMA	(183	)	IN 41-64	2	3	3	5	3	26		
			IN 81-92	1	0	0	1	0	0		
			IN 81-92	2	13	10	4	19	0		
			IN 93-93	1	1	2	0	2	0		
			IN 93-93	2	20	24	23	13	0		
			FA 66	1	0	1	0	0	0		
			FA 66	2	42	45	44	45	0		
			FA 67	1	1	0	0	0	0		
			FA 67	2	41	45	43	44	0		
			FA 79	1	0	0	1	0	0		
			FA 79	2	36	38	32	34	0		
			FA 86	1	0	0	1	0	0		
			FA 86	2	27	31	25	33	0		
Spontaneous tumor rate 5%	in ctrl.	-	Total	-	2	3	3	2	1		
Lymphoreticular	(58	)	IN 0-40	1	1	0	0	0	0	1.0000	0.8496 0.8581
M-IMMUNOBLASTIC LYMPHOMA	(234	)	IN 0-40	2	8	5	6	7	18		
Spontaneous tumor rate LE 1%	in ctrl.	-	Total	-	1	0	0	0	0		

Appendix C-Female Mice: Table II_Male Mice (Continued)

Lymphoreticular	(58	)	IN 65-80	1	0	0	1	0	0	0.2572	0.2230	0.2326
M-HISTIOCYTIC SARCOMA	(244	)	IN 65-80	2	9	10	14	11	10			
			FA 64	1	0	0	0	0	1			
			FA 64	2	43	47	44	45	11			
			FA 65	1	0	1	0	0	0			
			FA 65	2	43	46	44	45	10			
			FA 82	1	1	0	0	0	0			
			FA 82	2	33	36	29	34	0			
Spontaneous tumor rate 2%	in ctrl. - Total			-	1	1	1	0	1			
Adrenal	(7	)	IN 93-93	1	0	0	0	1	0	-0.1765	0.0215	0.0295
B-SUBCAPSULAR CELL ADENOM	(285	)	IN 93-93	2	21	26	23	14	0			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	0	1	0			
Adrenal	(7	)	IN 81-92	1	1	1	0	0	0	0.8742	0.8472	0.8589
B-CORTICAL ADENOMA	(321	)	IN 81-92	2	12	9	6	19	0			
			IN 93-93	1	0	1	3	0	0			
			IN 93-93	2	21	25	20	15	0			
Spontaneous tumor rate 3%	in ctrl. - Total			-	1	2	3	0	0			
Pituitary	(8	)	IN 81-92	1	0	1	0	0	0	1.0000	0.8211	0.8472
B-ADENOMA, PARS DISTALIS	(354	)	IN 81-92	2	13	9	4	19	0			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	1	0	0	0			
Thyroid	(9	)	IN 81-92	1	0	0	1	0	0	0.5434	0.6271	0.6667
B-FOLLICULAR CELL ADENOMA	(402	)	IN 81-92	2	12	9	5	19	0			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	1	0	0			

Appendix C-Female Mice: Table II_Male Mice (Continued)

NDA 20-895, Sildenafil
Appendix C-Male-Mice

Table III_C-Male-Mice

Case Where High Dose Is Excluded

Test for Positive Dose-Response (Tumor) Linear Trend
Species: Mouse 15:05 Monday, February 2, 1998
Sex: Male
Sorted by: Organ Name

Organ Code	Organ Name	Tumor Code	Tumor Name	Exact-P	Asymp-P	AsyCor-P
56	Abdomen	348	B-HAEMANGIOMA	0.3958	0.1132	0.1349
56	Abdomen	381	M-HAEMANGIOSARCOMA	0.5043	0.5457	0.5919
7	Adrenal	321	B-CORTICAL ADENOMA	0.8742	0.8472	0.8589
7	Adrenal	285	B-SUBCAPSULAR CELL ADENOM	0.1765	0.0215	0.0295
28	Brain	306	M-ASTROCYTOMA	1.0000	0.7810	0.8156
23	Duodenum	389	M-ADENOCARCINOMA	1.0000	0.7555	0.7969
37	Harderian gland	258	B-ADENOMA	0.4884	0.4732	0.4872
1	Kidney	266	B-RENAL TUBULE ADENOMA	0.3768	0.3070	0.3326
3	Liver	332	B-HAEMANGIOMA	0.1385	0.0838	0.0939
3	Liver	356	B-HEPATOCELLULAR ADENOMA	0.7510	0.7369	0.7470
3	Liver	309	M-HAEMANGIOSARCOMA	0.7168	0.6997	0.7301
3	Liver	318	M-HEPATOCELLULAR CARCINOM	0.8714	0.8466	0.8563
33	Lungs	154	B-BRONCHIOLAR-ALVEOLAR AD	0.7960	0.7852	0.7924
33	Lungs	149	M-BRONCHIOLAR-ALVEOLAR CA	0.2512	0.2368	0.2455
58	Lymphoreticular	244	M-HISTIOCYTIC SARCOMA	0.8882	0.8367	0.8536
58	Lymphoreticular	234	M-IMMUNOBLASTIC LYMPHOMA	1.0000	0.7834	0.8169
58	Lymphoreticular	183	M-LYMPHOBLASTIC LYMPHOMA	0.5659	0.5449	0.5607
12	Oesophagus	398	B-SQUAMOUS CELL PAPILLOMA	0.4043	0.1173	0.1396
8	Pituitary	354	B-ADENOMA, PARS DISTALIS	1.0000	0.8211	0.8472
27	Prostate	403	M-ADENOCARCINOMA	0.4524	0.4575	0.5116
31	Skin and adnexa	375	B-FIBROUS HISTIOCYTOMA	0.2391	0.0461	0.0597
31	Skin and adnexa	204	M-FIBROUS HISTIOCYTOMA, M	0.4973	0.5192	0.5681
19	Spleen	330	B-HAEMANGIOMA	1.0000	0.8655	0.8846
14	Testes	391	B-HAEMANGIOMA	0.7351	0.7446	0.7708
14	Testes	371	B-INTERSTITIAL CELL ADENO	0.9245	0.8885	0.8987
9	Thyroid	402	B-FOLLICULAR CELL ADENOMA	0.5434	0.6271	0.6667
26	Urinary bladder	370	B-TRANSITIONAL CELL PAPIL	1.0000	0.7589	0.7992

Appendix C-Male Mice

Table IV_C_Male_Mice
Case Where the High Dose is Excluded

Analysis of Carcinogenic Potential in Male Mouse
Test of Dose-Response (Tumor) Positive Linear Trend
Ted Guo, PH.D, CDER/FDA
Run Date & Time: January 29, 1998 (16:26)
Source: E:\MOUSE\m_mouse3.fil

Note: Dose Levels Included: CTRL1 CTRL2 LOW MED (0 0 3 10)
Missing value in Tumor-Caused Death is treated as tumor not causing death
Tumor Type: IN: Incidental (nonfatal) tumor, FA: Fatal tumor.

ORGAN/TISSUE NAME AND TUMOR NAME	(ORG#) (TMR#)	TUMOR TIME TYPES STRATA	ROW NO.	2xC_CONTINGENCY -----TABLE-----	EXACT PROB =PR(STATISTIC.GE.OBSERVED)	ASYMP TOTIC	ASYMP(CONTI NUITY CORR)
Kidney	(1)	IN 81-92	1	0 0 0 1	0.3768	0.3070	0.3326
B-RENAL TUBULE ADENOMA	(266)	IN 81-92	2	13 10 6 18			
		IN 93-93	1	0 1 1 0			
		IN 93-93	2	21 25 22 15			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0 1 1 1			
Oesophagus	(12)	IN 81-92	1	0 0 0 1	0.4043	0.1173	0.1396
B-SQUAMOUS CELL PAPILLOMA	(398)	IN 81-92	2	12 10 6 18			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0 0 0 1			
Testes	(14)	IN 65-80	1	0 0 2 0	0.9245	0.8885	0.8987
B-INTERSTITIAL CELL ADENO	(371)	IN 65-80	2	9 11 13 11			
		IN 93-93	1	3 1 0 0			
		IN 93-93	2	18 25 23 15			
Spontaneous tumor rate 4% in ctrl. - Total			-	3 1 2 0			
Testes	(14)	IN 81-92	1	1 0 0 0	0.7351	0.7446	0.7708
B-HAEMANGIOMA	(391)	IN 81-92	2	12 10 6 19			
		IN 93-93	1	0 0 1 0			
		IN 93-93	2	21 26 22 15			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1 0 1 0			
Spleen	(19)	IN 65-80	1	0 1 0 0	1.0000	0.8655	0.8846
B-HAEMANGIOMA	(330)	IN 65-80	2	9 10 15 11			
		IN 93-93	1	0 1 0 0			
		IN 93-93	2	21 25 23 15			
Spontaneous tumor rate 2% in ctrl. - Total			-	0 2 0 0			
Duodenum	(23)	IN 93-93	1	1 0 0 0	1.0000	0.7555	0.7969
M-ADENOCARCINOMA	(389)	IN 93-93	2	20 26 23 14			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1 0 0 0			
Urinary bladder	(26)	IN 93-93	1	1 0 0 0	1.0000	0.7589	0.7992
B-TRANSITIONAL CELL PAPIL	(370)	IN 93-93	2	20 26 23 15			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1 0 0 0			
Prostate	(27)	IN 93-93	1	0 0 1 0	0.4524	0.4575	0.5116
M-ADENOCARCINOMA	(403)	IN 93-93	2	20 26 22 15			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0 0 1 0			
Brain	(28)	FA 73	1	1 0 0 0	1.0000	0.7810	0.8156

M-ASTROCYTOMA	(306)	FA 73	2	40	42	39	39		
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1	0	0	0		
Liver	(3)	IN 93-93	1	0	0	1	0	0.7168	0.6997 0.7301
M-HAEMANGIOSARCOMA	(309)	IN 93-93	2	21	26	22	15		
		FA 84	1	1	0	0	0		
		FA 84	2	29	33	27	33		
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1	0	1	0		
Liver	(3)	IN 65-80	1	0	1	0	0	0.8714	0.8466 0.8563
M-HEPATOCELLULAR CARCINOM	(318)	IN 65-80	2	9	9	14	11		
		IN 81-92	1	0	1	0	0		
		IN 81-92	2	12	9	6	18		
		IN 93-93	1	2	0	1	0		
		IN 93-93	2	19	26	22	15		
		FA 79	1	0	1	0	0		
		FA 79	2	36	37	33	34		
		FA 80	1	0	0	1	0		
		FA 80	2	34	36	31	34		
		FA 83	1	1	0	0	0		
		FA 83	2	30	35	27	33		
		FA 92	1	0	0	0	1		
		FA 92	2	22	27	24	23		
Spontaneous tumor rate 5% in ctrl. - Total			-	3	3	2	1		
Liver	(3)	IN 93-93	1	0	0	1	1	0.1385	0.0838 0.0939
B-HAEMANGIOMA	(332)	IN 93-93	2	21	26	22	14		
		FA 90	1	1	0	0	0		
		FA 90	2	23	28	24	30		
		FA 91	1	0	0	0	1		
		FA 91	2	23	27	24	27		
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1	0	1	2		
Liver	(3)	IN 65-80	1	0	1	1	0	0.7510	0.7369 0.7470
B-HEPATOCELLULAR ADENOMA	(356)	IN 65-80	2	9	9	14	11		
		IN 81-92	1	0	0	1	1		
		IN 81-92	2	13	10	5	17		
		IN 93-93	1	4	5	3	1		
		IN 93-93	2	17	21	20	14		
		FA 79	1	0	1	0	0		
		FA 79	2	36	37	33	34		
		FA 92	1	0	0	0	1		
		FA 92	2	22	27	24	23		
Spontaneous tumor rate 10% in ctrl. - Total			-	4	7	5	3		
Skin and adnexa	(31)	FA 56	1	0	0	1	0	0.4973	0.5192 0.5681
M-FIBROUS HISTIOCYTOMA, M	(204)	FA 56	2	43	50	46	45		
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	0	1	0		
Skin and adnexa	(31)	IN 65-80	1	0	0	0	1	0.2391	0.0461 0.0597
B-FIBROUS HISTIOCYTOMA	(375)	IN 65-80	2	9	11	15	10		
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	0	0	1		
Lungs	(33)	IN 65-80	1	0	0	1	2	0.2512	0.2368 0.2455
M-BRONCHIOLAR-ALVEOLAR CA	(149)	IN 65-80	2	9	10	12	9		
		IN 93-93	1	1	5	3	2		
		IN 93-93	2	20	21	20	13		
		FA 73	1	0	0	1	0		
		FA 73	2	41	42	38	39		
		FA 77	1	0	0	1	0		
		FA 77	2	36	39	36	35		
		FA 79	1	0	1	0	0		
		FA 79	2	36	37	33	34		
		FA 82	1	1	0	0	0		
		FA 82	2	33	36	29	34		
		FA 85	1	0	1	0	0		
		FA 85	2	28	32	27	33		

Appendix C-Male Mice: Table IV_Male Mice (Continued)

		FA	86	1	0	1	0	0	
		FA	86	2	27	30	26	33	
		FA	89	1	0	0	0	1	
		FA	89	2	26	29	24	31	
		FA	90	1	0	0	0	1	
		FA	90	2	24	28	24	29	
Spontaneous tumor rate 9% in ctrl. - Total				-	2	8	6	6	
Lungs	(33)	IN	41-64	1	0	0	1	0	0.7960 0.7852 0.7924
B-BRONCHIOLAR-ALVEOLAR AD	(154)	IN	41-64	2	3	3	4	3	
		IN	65-80	1	1	1	1	2	
		IN	65-80	2	8	10	14	9	
		IN	81-92	1	4	0	1	4	
		IN	81-92	2	9	10	5	15	
		IN	93-93	1	4	7	5	0	
		IN	93-93	2	17	19	18	15	
Spontaneous tumor rate 15% in ctrl. - Total				-	9	8	8	6	
Harderian gland	(37)	IN	81-92	1	1	1	2	2	0.4884 0.4732 0.4872
B-ADENOMA	(258)	IN	81-92	2	12	9	4	17	
		IN	93-93	1	2	1	3	1	
		IN	93-93	2	19	25	20	14	
Spontaneous tumor rate 5% in ctrl. - Total				-	3	2	5	3	
Abdomen	(56)	IN	81-92	1	0	0	0	1	0.3958 0.1132 0.1349
B-HAEMANGIOMA	(348)	IN	81-92	2	13	10	6	18	
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	0	1	
Abdomen	(56)	FA	86	1	0	0	1	0	0.5043 0.5457 0.5919
M-HAEMANGIOSARCOMA	(381)	FA	86	2	27	31	25	33	
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	1	0	
Lymphoreticular	(58)	IN	81-92	1	0	0	1	0	0.5659 0.5449 0.5607
M-LYMPHOBLASTIC LYMPHOMA	(183)	IN	81-92	2	13	10	4	19	
		IN	93-93	1	1	2	0	2	
		IN	93-93	2	20	24	23	13	
		FA	66	1	0	1	0	0	
		FA	66	2	42	45	44	45	
		FA	67	1	1	0	0	0	
		FA	67	2	41	45	43	44	
		FA	79	1	0	0	1	0	
		FA	79	2	36	38	32	34	
		FA	86	1	0	0	1	0	
		FA	86	2	27	31	25	33	
Spontaneous tumor rate 5% in ctrl. - Total				-	2	3	3	2	
Lymphoreticular	(58)	IN	0-40	1	1	0	0	0	1.0000 0.7834 0.8169
M-IMMUNOBLASTIC LYMPHOMA	(234)	IN	0-40	2	8	5	6	7	
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1	0	0	0	
Lymphoreticular	(58)	IN	65-80	1	0	0	1	0	0.8882 0.8367 0.8536
M-HISTIOCYTIC SARCOMA	(244)	IN	65-80	2	9	10	14	11	
		FA	65	1	0	1	0	0	
		FA	65	2	43	46	44	45	
		FA	82	1	1	0	0	0	
		FA	82	2	33	36	29	34	
Spontaneous tumor rate 2% in ctrl. - Total				-	1	1	1	0	
Adrenal	(7)	IN	93-93	1	0	0	0	1	0.1765 0.0215 0.0295
B-SUBCAPSULAR CELL ADENOM	(285)	IN	93-93	2	21	26	23	14	
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	0	1	
Adrenal	(7)	IN	81-92	1	1	1	0	0	0.8742 0.8472 0.8589
B-CORTICAL ADENOMA	(321)	IN	81-92	2	12	9	6	19	
		IN	93-93	1	0	1	3	0	
		IN	93-93	2	21	25	20	15	
Spontaneous tumor rate 3% in ctrl. - Total				-	1	2	3	0	

Appendix C-Male Mice: Table IV_Male Mice (Continued)

Pituitary	(8)	IN 81-92	1	0	1	0	0	1.0000 0.8211 0.8472
B-ADENOMA, PARS DISTALIS	(354)	IN 81-92	2	13	9	4	19	
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	1	0	0	
Thyroid	(9)	IN 81-92	1	0	0	1	0	0.5434 0.6271 0.6667
B-FOLLICULAR CELL ADENOMA	(402)	IN 81-92	2	12	9	5	19	
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	0	1	0	

Appendix C-Male Mice: Table IV_Male Mice (Continued)

NDA 20-895

Sildenafil

Appendix D-Female-Mice

Female Mice

NDA 20-895, Sildenafil
Appendix D-Female-Mice

Table I_D-Female-Mice

Case Where All Doses Are Included

Test for Positive Dose-Response (Tumor) Linear Trend
Species: Mouse 15:05 Monday, February 2, 1998
Sex: Female
Sorted by: Organ Name

Organ Code	Organ Name	Tumor Code	Tumor Name	Exact-P	Asymp-P	AsyCor-P
56	Abdomen	348	B-HAEMANGIOMA	0.1458	0.0118	0.0170
7	Adrenal	285	B-SUBCAPSULAR CELL ADENOM	1.0000	0.7319	0.7771
37	Harderian gland	258	B-ADENOMA	0.9535	0.9104	0.9156
1	Kidney	266	B-RENAL TUBULE ADENOMA	1.0000	0.7319	0.7771
3	Liver	332	B-HAEMANGIOMA	0.4710	0.4597	0.5145
33	Lungs	154	B-BRONCHIOLAR-ALVEOLAR AD	0.3570	0.3467	0.3542
33	Lungs	149	M-BRONCHIOLAR-ALVEOLAR CA	0.3670	0.3767	0.3872
58	Lymphoreticular	244	M-HISTIOCYTIC SARCOMA	0.3001	0.3299	0.3445
58	Lymphoreticular	234	M-IMMUNOBLASTIC LYMPHOMA	0.5625	0.2620	0.2759
58	Lymphoreticular	183	M-LYMPHOBLASTIC LYMPHOMA	0.2908	0.2812	0.2875
16	Ovaries	209	B-CYSTADENOMA	0.4794	0.4707	0.4818
16	Ovaries	246	B-SEX CORD/STROMAL TUMOUR	0.7605	0.7184	0.7461
16	Ovaries	407	B-TERATOMA	1.0000	0.8073	0.8438
31	Skin and adnexa	259	M-ADENOCARCINOMA, MAMMARY	1.0000	0.7310	0.7783
31	Skin and adnexa	204	M-FIBROUS HISTIOCYTOMA, M	1.0000	0.8073	0.8438
19	Spleen	330	B-HAEMANGIOMA	0.3750	0.4028	0.4594
22	Stomach	272	B-SQUAMOUS CELL PAPILLOMA	1.0000	0.7319	0.7771
22	Stomach	270	M-SQUAMOUS CELL CARCINOMA	1.0000	0.8114	0.8381
32	Thymus	359	M-THYMOMA, EPITHELIAL PRE	1.0000	0.8517	0.8710
9	Thyroid	331	M-FOLLICULAR CELL CARCINO	0.6047	0.7032	0.7181
17	Uterus	264	B-HAEMANGIOMA	1.0000	0.7310	0.7783
17	Uterus	287	B-LEIOMYOMA	0.2585	0.1731	0.1951
17	Uterus	284	M-STROMAL CELL SARCOMA	0.8448	0.7974	0.8086
18	Vagina	282	B-LEIOMYOMA	0.8400	0.7765	0.7890

Appendix D-Female Mice

Table II_D_Female_Mice
Case Where All Doses Are Included

Analysis of Carcinogenic Potential in Female Mouse
Test of Dose-Response (Tumor) Positive Linear Trend
Ted Guo, PH.D, CDER/FDA
Run Date & Time: January 29, 1998 (16:50)
Source: E:\MOUSE\f_mouse3.fil
Note: Dose Levels Included: CTRL1 CTRL2 LOW MED HIGH (0 0 3 10 30)
Missing value in Tumor-Caused Death is treated as tumor not causing death
Tumor Type: IN: Incidental (nonfatal) tumor, FA: Fatal tumor.

ORGAN/TISSUE NAME AND TUMOR NAME	(ORG#) (TMR#)	TUMOR TYPES	TIME STRATA	ROW NO.	2xC_CONTINGENCY -----TABLE-----	EXACT PROB =PR(STATISTIC.GE.OBSERVED)	ASYMP TOTIC	ASYMP(CONTI NUITY CORR)
Kidney	(1)	IN	93-93	1	1 0 0 0 0	1.0000	0.7319	0.7771
B-RENAL TUBULE ADENOMA	(266)	IN	93-93	2	29 30 22 14 0			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1 0 0 0 0			
Ovaries	(16)	IN	0-40	1	0 0 0 1 0	0.4794	0.4707	0.4818
B-CYSTADENOMA	(209)	IN	0-40	2	1 1 1 10 18			
		IN	41-64	1	0 0 0 0 1			
		IN	41-64	2	8 4 6 11 27			
		IN	93-93	1	1 0 1 0 0			
		IN	93-93	2	29 30 21 14 0			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1 0 1 1 1			
Ovaries	(16)	IN	93-93	1	2 0 1 0 0	0.7605	0.7184	0.7461
B-SEX CORD/STROMAL TUMOUR	(246)	IN	93-93	2	28 30 21 14 0			
Spontaneous tumor rate 2% in ctrl. - Total				-	2 0 1 0 0			
Ovaries	(16)	IN	81-92	1	1 0 0 0 0	1.0000	0.8073	0.8438
B-TERATOMA	(407)	IN	81-92	2	9 8 19 8 0			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1 0 0 0 0			
Uterus	(17)	FA	77	1	1 0 0 0 0	1.0000	0.7310	0.7783
B-HAEMANGIOMA	(264)	FA	77	2	36 35 29 15 0			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1 0 0 0 0			
Uterus	(17)	IN	65-80	1	1 0 0 0 0	0.8448	0.7974	0.8086
M-STROMAL CELL SARCOMA	(284)	IN	65-80	2	5 12 7 11 9			
		IN	93-93	1	1 0 1 0 0			
		IN	93-93	2	29 30 21 14 0			
Spontaneous tumor rate 2% in ctrl. - Total				-	2 0 1 0 0			
Uterus	(17)	IN	81-92	1	0 0 1 0 0	0.2585	0.1731	0.1951
B-LEIOMYOMA	(287)	IN	81-92	2	10 8 18 8 0			
		IN	93-93	1	1 0 0 1 0			
		IN	93-93	2	29 30 22 13 0			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1 0 1 1 0			
Vagina	(18)	IN	65-80	1	0 1 0 0 0	0.8400	0.7765	0.7890
B-LEIOMYOMA	(282)	IN	65-80	2	6 11 7 11 9			
		IN	81-92	1	0 0 1 0 0			
		IN	81-92	2	10 8 18 8 0			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 1 1 0 0			

Appendix D-Female Mice: Table II_Female Mice (Continued)

Spleen	(19)	IN 93-93	1	0	0	1	0	0	0.3750	0.4028	0.4594
B-HAEMANGIOMA	(330)	IN 93-93	2	30	30	21	14	0			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	0	1	0	0			
Stomach	(22)	IN 93-93	1	1	0	0	0	0	1.0000	0.8114	0.8381
M-SQUAMOUS CELL CARCINOMA	(270)	IN 93-93	2	29	30	22	14	0			
		FA 75	1	1	0	0	0	0			
		FA 75	2	37	36	32	16	0			
Spontaneous tumor rate 2% in ctrl. - Total			-	2	0	0	0	0			
Stomach	(22)	IN 93-93	1	1	0	0	0	0	1.0000	0.7319	0.7771
B-SQUAMOUS CELL PAPILLOMA	(272)	IN 93-93	2	29	30	22	14	0			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1	0	0	0	0			
Liver	(3)	FA 65	1	0	0	1	0	0	0.4710	0.4597	0.5145
B-HAEMANGIOMA	(332)	FA 65	2	43	39	45	27	0			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	0	1	0	0			
Skin and adnexa	(31)	IN 81-92	1	0	1	0	0	0	1.0000	0.8073	0.8438
M-FIBROUS HISTIOCYTOMA, M	(204)	IN 81-92	2	10	7	19	8	0			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	1	0	0	0			
Skin and adnexa	(31)	FA 77	1	1	0	0	0	0	1.0000	0.7310	0.7783
M-ADENOCARCINOMA, MAMMARY	(259)	FA 77	2	36	35	29	15	0			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	1	0	0	0	0			
Thymus	(32)	IN 93-93	1	0	3	0	0	0	1.0000	0.8517	0.8710
M-THYMOMA, EPITHELIAL PRE	(359)	IN 93-93	2	29	27	21	12	0			
Spontaneous tumor rate 3% in ctrl. - Total			-	0	3	0	0	0			
Lungs	(33)	IN 65-80	1	0	0	1	0	0	0.3670	0.3767	0.3872
M-BRONCHIOLAR-ALVEOLAR CA	(149)	IN 65-80	2	6	12	6	11	9			
		IN 93-93	1	1	4	1	3	0			
		IN 93-93	2	29	26	21	11	0			
		FA 48	1	0	0	1	0	0			
		FA 48	2	51	53	52	37	27			
		FA 78	1	0	0	1	0	0			
		FA 78	2	35	33	25	14	0			
Spontaneous tumor rate 5% in ctrl. - Total			-	1	4	4	3	0			
Lungs	(33)	IN 41-64	1	0	0	1	1	0	0.3570	0.3467	0.3542
B-BRONCHIOLAR-ALVEOLAR AD	(154)	IN 41-64	2	8	4	5	10	28			
		IN 65-80	1	0	0	0	0	1			
		IN 65-80	2	6	12	7	11	8			
		IN 81-92	1	0	0	2	1	0			
		IN 81-92	2	10	8	17	7	0			
		IN 93-93	1	2	2	4	1	0			
		IN 93-93	2	28	28	18	13	0			
Spontaneous tumor rate 4% in ctrl. - Total			-	2	2	7	3	1			
Harderian gland	(37)	IN 65-80	1	0	0	1	0	0	0.9535	0.9104	0.9156
B-ADENOMA	(258)	IN 65-80	2	6	11	6	11	9			
		IN 81-92	1	0	0	1	0	0			
		IN 81-92	2	10	8	18	8	0			
		IN 93-93	1	3	4	1	0	0			
		IN 93-93	2	27	26	21	14	0			
Spontaneous tumor rate 6% in ctrl. - Total			-	3	4	3	0	0			
Abdomen	(56)	IN 93-93	1	0	0	0	1	0	0.1458	0.0118	0.0170
B-HAEMANGIOMA	(348)	IN 93-93	2	30	30	22	13	0			
Spontaneous tumor rate LE 1% in ctrl. - Total			-	0	0	0	1	0			
Lymphoreticular	(58)	IN 41-64	1	0	0	0	0	1	0.2908	0.2812	0.2875
M-LYMPHOBLASTIC LYMPHOMA	(183)	IN 41-64	2	8	4	6	10	27			
		IN 65-80	1	0	0	1	0	0			
		IN 65-80	2	6	12	6	11	9			
		IN 93-93	1	1	3	1	0	0			

Appendix D-Female Mice: Table II_Female Mice (Continued)

		IN 93-93	2	29	27	21	14	0		
		FA 24	1	0	0	0	0	1		
		FA 24	2	54	55	54	53	49		
		FA 29	1	0	1	0	0	0		
		FA 29	2	54	54	54	53	49		
		FA 43	1	0	0	0	1	0		
		FA 43	2	53	53	54	40	29		
		FA 79	1	1	0	1	0	0		
		FA 79	2	33	33	22	14	0		
Spontaneous tumor rate 5% in ctrl.		- Total	-	2	4	3	1	2		
Lymphoreticular	(58)	IN 0-40	1	0	0	0	0	1	0.5625	0.2620 0.2759
M-IMMUNOBLASTIC LYMPHOMA	(234)	IN 0-40	2	1	1	1	11	17		
		IN 93-93	1	1	0	0	0	0		
		IN 93-93	2	29	30	22	14	0		
Spontaneous tumor rate LE 1% in ctrl.		- Total	-	1	0	0	0	1		
Lymphoreticular	(58)	IN 93-93	1	1	0	0	1	0	0.3001	0.3299 0.3445
M-HISTIOCYTIC SARCOMA	(244)	IN 93-93	2	29	30	22	13	0		
		FA 40	1	0	1	0	0	0		
		FA 40	2	54	53	54	41	30		
		FA 64	1	0	0	0	1	0		
		FA 64	2	43	39	47	29	0		
		FA 76	1	0	0	1	0	0		
		FA 76	2	37	36	30	16	0		
		FA 78	1	1	0	0	0	0		
		FA 78	2	34	33	26	14	0		
Spontaneous tumor rate 3% in ctrl.		- Total	-	2	1	1	2	0		
Adrenal	(7)	IN 93-93	1	1	0	0	0	0	1.0000	0.7319 0.7771
B-SUBCAPSULAR CELL ADENOM	(285)	IN 93-93	2	29	30	22	14	0		
Spontaneous tumor rate LE 1% in ctrl.		- Total	-	1	0	0	0	0		
Thyroid	(9)	IN 65-80	1	0	0	1	0	0	0.6047	0.7032 0.7181
M-FOLLICULAR CELL CARCINO	(331)	IN 65-80	2	6	11	6	10	9		
Spontaneous tumor rate LE 1% in ctrl.		- Total	-	0	0	1	0	0		

Appendix D-Female Mice: Table II_Female Mice (Continued)

NDA 20-895, Sildenafil
Appendix D-Female-Mice

Table III_D-Female-Mice
Case Where High Dose Is Excluded

Test for Positive Dose-Response (Tumor) Linear Trend
Species: Mouse 15:05 Monday, February 2, 1998
Sex: Female
Sorted by: Organ Name

Organ Code	Organ Name	Tumor Code	Tumor Name	Exact-P	Asymp-P	AsyCor-P
56	Abdomen	348	B-HAEMANGIOMA	0.1458	0.0118	0.0170
7	Adrenal	285	B-SUBCAPSULAR CELL ADENOM	1.0000	0.7319	0.7771
37	Harderian gland	258	B-ADENOMA	0.9415	0.9132	0.9201
1	Kidney	266	B-RENAL TUBULE ADENOMA	1.0000	0.7319	0.7771
3	Liver	332	B-HAEMANGIOMA	0.4710	0.4597	0.5145
33	Lungs	154	B-BRONCHIOLAR-ALVEOLAR AD	0.1611	0.1488	0.1577
33	Lungs	149	M-BRONCHIOLAR-ALVEOLAR CA	0.1414	0.1162	0.1247
58	Lymphoreticular	244	M-HISTIOCYTIC SARCOMA	0.1967	0.1490	0.1628
58	Lymphoreticular	234	M-IMMUNOBLASTIC LYMPHOMA	1.0000	0.7319	0.7771
58	Lymphoreticular	183	M-LYMPHOBLASTIC LYMPHOMA	0.7008	0.6829	0.6984
16	Ovaries	209	B-CYSTADENOMA	0.5126	0.4588	0.4909
16	Ovaries	246	B-SEX CORD/STROMAL TUMOUR	0.7605	0.7184	0.7461
16	Ovaries	407	B-TERATOMA	1.0000	0.8073	0.8438
31	Skin and adnexa	259	M-ADENOCARCINOMA, MAMMARY	1.0000	0.7310	0.7783
31	Skin and adnexa	204	M-FIBROUS HISTIOCYTOMA, M	1.0000	0.8073	0.8438
19	Spleen	330	B-HAEMANGIOMA	0.3750	0.4028	0.4594
22	Stomach	272	B-SQUAMOUS CELL PAPILLOMA	1.0000	0.7319	0.7771
22	Stomach	270	M-SQUAMOUS CELL CARCINOMA	1.0000	0.8114	0.8381
32	Thymus	359	M-THYMOMA, EPITHELIAL PRE	1.0000	0.8517	0.8710
9	Thyroid	331	M-FOLLICULAR CELL CARCINO	0.5000	0.5516	0.5970
17	Uterus	264	B-HAEMANGIOMA	1.0000	0.7310	0.7783
17	Uterus	287	B-LEIOMYOMA	0.2585	0.1731	0.1951
17	Uterus	284	M-STROMAL CELL SARCOMA	0.8059	0.7743	0.7965
18	Vagina	282	B-LEIOMYOMA	0.8000	0.7446	0.7724

Appendix D-Female Mice

Table IV_D_Female_Mice
Case Where the High Dose is Excluded

Analysis of Carcinogenic Potential in Female Mouse
Test of Dose-Response (Tumor) Positive Linear Trend
Ted Guo, PH.D, CDER/FDA
Run Date & Time: January 29, 1998 (17:14)
Source: E:\MOUSE\f_mouse3.fil

Note: Dose Levels Included: CTRL1 CTRL2 LOW MED (0 0 3 10)
Missing value in Tumor-Caused Death is treated as tumor not causing death
Tumor Type: IN: Incidental (nonfatal) tumor, FA: Fatal tumor.

ORGAN/TISSUE NAME AND TUMOR NAME	(ORG#) (TMR#)	TUMOR TYPES	TIME STRATA	ROW NO.	2xC_CONTINGENCY -----TABLE-----	EXACT PROB =PR(STATISTIC.GE.OBSERVED)	ASYMP TOTIC	ASYMP(CONTI NUITY CORR)
Kidney	(1)	IN	93-93	1	1 0 0 0	1.0000	0.7319	0.7771
B-RENAL TUBULE ADENOMA	(266)	IN	93-93	2	29 30 22 14			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1 0 0 0			
Ovaries	(16)	IN	0-40	1	0 0 0 1	0.5126	0.4588	0.4909
B-CYSTADENOMA	(209)	IN	0-40	2	1 1 1 10			
		IN	93-93	1	1 0 1 0			
		IN	93-93	2	29 30 21 14			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1 0 1 1			
Ovaries	(16)	IN	93-93	1	2 0 1 0	0.7605	0.7184	0.7461
B-SEX CORD/STROMAL TUMOUR	(246)	IN	93-93	2	28 30 21 14			
Spontaneous tumor rate 2% in ctrl. - Total				-	2 0 1 0			
Ovaries	(16)	IN	81-92	1	1 0 0 0	1.0000	0.8073	0.8438
B-TERATOMA	(407)	IN	81-92	2	9 8 19 8			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1 0 0 0			
Uterus	(17)	FA	77	1	1 0 0 0	1.0000	0.7310	0.7783
B-HAEMANGIOMA	(264)	FA	77	2	36 35 29 15			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1 0 0 0			
Uterus	(17)	IN	65-80	1	1 0 0 0	0.8059	0.7743	0.7965
M-STROMAL CELL SARCOMA	(284)	IN	65-80	2	5 12 7 11			
		IN	93-93	1	1 0 1 0			
		IN	93-93	2	29 30 21 14			
Spontaneous tumor rate 2% in ctrl. - Total				-	2 0 1 0			
Uterus	(17)	IN	81-92	1	0 0 1 0	0.2585	0.1731	0.1951
B-LEIOMYOMA	(287)	IN	81-92	2	10 8 18 8			
		IN	93-93	1	1 0 0 1			
		IN	93-93	2	29 30 22 13			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1 0 1 1			
Vagina	(18)	IN	65-80	1	0 1 0 0	0.8000	0.7446	0.7724
B-LEIOMYOMA	(282)	IN	65-80	2	6 11 7 11			
		IN	81-92	1	0 0 1 0			
		IN	81-92	2	10 8 18 8			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 1 1 0			
Spleen	(19)	IN	93-93	1	0 0 1 0	0.3750	0.4028	0.4594
B-HAEMANGIOMA	(330)	IN	93-93	2	30 30 21 14			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0 0 1 0			

Stomach	(22)	IN	93-93	1	1	0	0	0	1.0000	0.8114	0.8381
M-SQUAMOUS CELL CARCINOMA	(270)	IN	93-93	2	29	30	22	14			
		FA	75	1	1	0	0	0			
		FA	75	2	37	36	32	16			
Spontaneous tumor rate 2% in ctrl. - Total				-	2	0	0	0			
Stomach	(22)	IN	93-93	1	1	0	0	0	1.0000	0.7319	0.7771
B-SQUAMOUS CELL PAPILLOMA	(272)	IN	93-93	2	29	30	22	14			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1	0	0	0			
Liver	(3)	FA	65	1	0	0	1	0	0.4710	0.4597	0.5145
B-HAEMANGIOMA	(332)	FA	65	2	43	39	45	27			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	1	0			
Skin and adnexa	(31)	IN	81-92	1	0	1	0	0	1.0000	0.8073	0.8438
M-FIBROUS HISTIOCYTOMA, M	(204)	IN	81-92	2	10	7	19	8			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	1	0	0			
Skin and adnexa	(31)	FA	77	1	1	0	0	0	1.0000	0.7310	0.7783
M-ADENOCARCINOMA, MAMMARY	(259)	FA	77	2	36	35	29	15			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1	0	0	0			
Thymus	(32)	IN	93-93	1	0	3	0	0	1.0000	0.8517	0.8710
M-THYMOMA, EPITHELIAL PRE	(359)	IN	93-93	2	29	27	21	12			
Spontaneous tumor rate 3% in ctrl. - Total				-	0	3	0	0			
Lungs	(33)	IN	65-80	1	0	0	1	0	0.1414	0.1162	0.1247
M-BRONCHIOLAR-ALVEOLAR CA	(149)	IN	65-80	2	6	12	6	11			
		IN	93-93	1	1	4	1	3			
		IN	93-93	2	29	26	21	11			
		FA	48	1	0	0	1	0			
		FA	48	2	51	53	52	37			
		FA	78	1	0	0	1	0			
		FA	78	2	35	33	25	14			
Spontaneous tumor rate 5% in ctrl. - Total				-	1	4	4	3			
Lungs	(33)	IN	41-64	1	0	0	1	1	0.1611	0.1488	0.1577
B-BRONCHIOLAR-ALVEOLAR AD	(154)	IN	41-64	2	8	4	5	10			
		IN	81-92	1	0	0	2	1			
		IN	81-92	2	10	8	17	7			
		IN	93-93	1	2	2	4	1			
		IN	93-93	2	28	28	18	13			
Spontaneous tumor rate 4% in ctrl. - Total				-	2	2	7	3			
Harderian gland	(37)	IN	65-80	1	0	0	1	0	0.9415	0.9132	0.9201
B-ADENOMA	(258)	IN	65-80	2	6	11	6	11			
		IN	81-92	1	0	0	1	0			
		IN	81-92	2	10	8	18	8			
		IN	93-93	1	3	4	1	0			
		IN	93-93	2	27	26	21	14			
Spontaneous tumor rate 6% in ctrl. - Total				-	3	4	3	0			
Abdomen	(56)	IN	93-93	1	0	0	0	1	0.1458	0.0118	0.0170
B-HAEMANGIOMA	(348)	IN	93-93	2	30	30	22	13			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	0	1			
Lymphoreticular	(58)	IN	65-80	1	0	0	1	0	0.7008	0.6829	0.6984
M-LYMPHOBLASTIC LYMPHOMA	(183)	IN	65-80	2	6	12	6	11			
		IN	93-93	1	1	3	1	0			
		IN	93-93	2	29	27	21	14			
		FA	29	1	0	1	0	0			
		FA	29	2	54	54	54	53			
		FA	43	1	0	0	0	1			
		FA	43	2	53	53	54	40			
		FA	79	1	1	0	1	0			
		FA	79	2	33	33	22	14			
Spontaneous tumor rate 5% in ctrl. - Total				-	2	4	3	1			
Lymphoreticular	(58)	IN	93-93	1	1	0	0	0	1.0000	0.7319	0.7771
M-IMMUNOBLASTIC LYMPHOMA	(234)	IN	93-93	2	29	30	22	14			
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1	0	0	0			

Appendix D-Female Mice: Table IV_Female Mice (Continued)

Lymphoreticular	(58)	IN	93-93	1	1	0	0	1	0.1967 0.1490 0.1628
M-HISTIOCYTIC SARCOMA	(244)	IN	93-93	2	29	30	22	13	
		FA	40	1	0	1	0	0	
		FA	40	2	54	53	54	41	
		FA	64	1	0	0	0	1	
		FA	64	2	43	39	47	29	
		FA	76	1	0	0	1	0	
		FA	76	2	37	36	30	16	
		FA	78	1	1	0	0	0	
		FA	78	2	34	33	26	14	
Spontaneous tumor rate 3% in ctrl. - Total				-	2	1	1	2	
Adrenal	(7)	IN	93-93	1	1	0	0	0	1.0000 0.7319 0.7771
B-SUBCAPSULAR CELL ADENOM	(285)	IN	93-93	2	29	30	22	14	
Spontaneous tumor rate LE 1% in ctrl. - Total				-	1	0	0	0	
Thyroid	(9)	IN	65-80	1	0	0	1	0	0.5000 0.5516 0.5970
M-FOLLICULAR CELL CARCINO	(331)	IN	65-80	2	6	11	6	10	
Spontaneous tumor rate LE 1% in ctrl. - Total				-	0	0	1	0	

Appendix D-Female Mice: Table IV_Female Mice (Continued)

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

CARCINOGENICITY ASSESSMENT COMMITTEE REPORT

AND

FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET

NDA #20-895

NOV 12 1997

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

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

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

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

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

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

RAT CARCINOGENICITY STUDY: Single study (#94092)

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

NUMBER OF RATS:
- Main study:
  - Control 1 (C1): 60 males and 60 females
  - Control 2 (C2): 60 males and 60 females
  - Low Dose (LD): 60 males and 60 females
  - Middle Dose (MD): 60 males and 60 females
  - High Dose (HD): 60 males and 60 females

- Groups for plasma drug level determinations:
  - Low Dose (LD): 7 males and 7 females
  - Middle Dose (MD): 7 males and 7 females
  - High Dose (HD): 7 males and 7 females

RAT DOSE LEVELS* (mg/kg/day):
Rat Low Dose: 1.5
Rat Middle Dose: 5.0
Rat High Dose: 60.0
- *Dose adjusted during study

BASIS FOR DOSES SELECTED:

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

PRIOR FDA DOSE CONCURRENCE: No

RAT CARCINOGENICITY: Negative (males and females)

RAT TUMOR FINDINGS:

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

Table 1

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

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

In a separate study to assess the relationship between liver enzyme induction and thyroxin clearance, female rats were given either vehicle or UK-92,480 orally at 200 mg/kg for 29 days. Results showed that treatment produced an increase in liver and thyroid weights, thyroid follicular cell hypertrophy, increased hepatic UDP-glucuronyl transferase (UDPGT) activity, increased TSH, decreased T3 and T4 hormones, and an increased clearance of exogenous thyroxin. These results were thought to be consistent with the view that the thyroid hypertrophy found in treated rats was due to induction of hepatic UDPGT which increased the clearance of thyroid hormone and caused a compensatory increase in plasma TSH which, in turn, stimulated the thyroid gland.

Evidence for such a mechanism at the 60 mg/kg dose was not presented, however. Additional experiments assessing induction of genes coding for specific hepatic enzymes, such as UDPGT-specific mRNA levels, would have been able to detect gene induction at the 60 mg/kg dose if such a mechanism were responsible for the thyroid hypertrophy observed in treated rats.

RAT STUDY COMMENTS:

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

Table 2

Percent Mortality and Percent Survival

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

Body Weights: Mean body weights are shown in Figure 1A (males) and Figure 1B (females). Percent changes in mean body weight gains in male and female rats are shown in Table 3 (Day 1 and Day 723). Results showed that high dose males (60 mg/kg/day) gained 11.0% less weight than controls, while mid- and high dose females gained 17.0% and 15.7% less weight, respectively than controls.

Figure 1A (Sponsor's Figure 8)

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

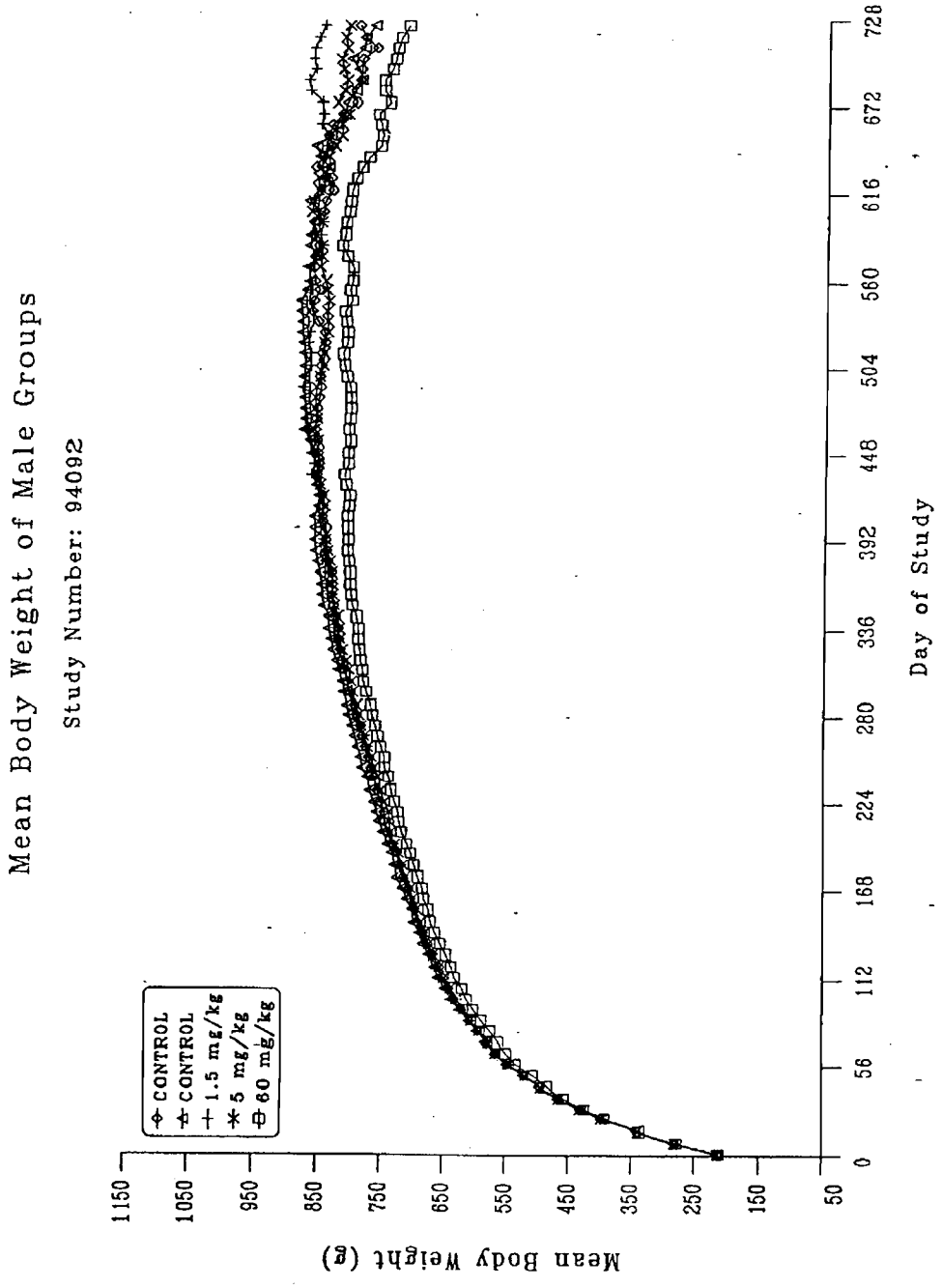


Figure 1B (Sponsor's Figure 9)

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

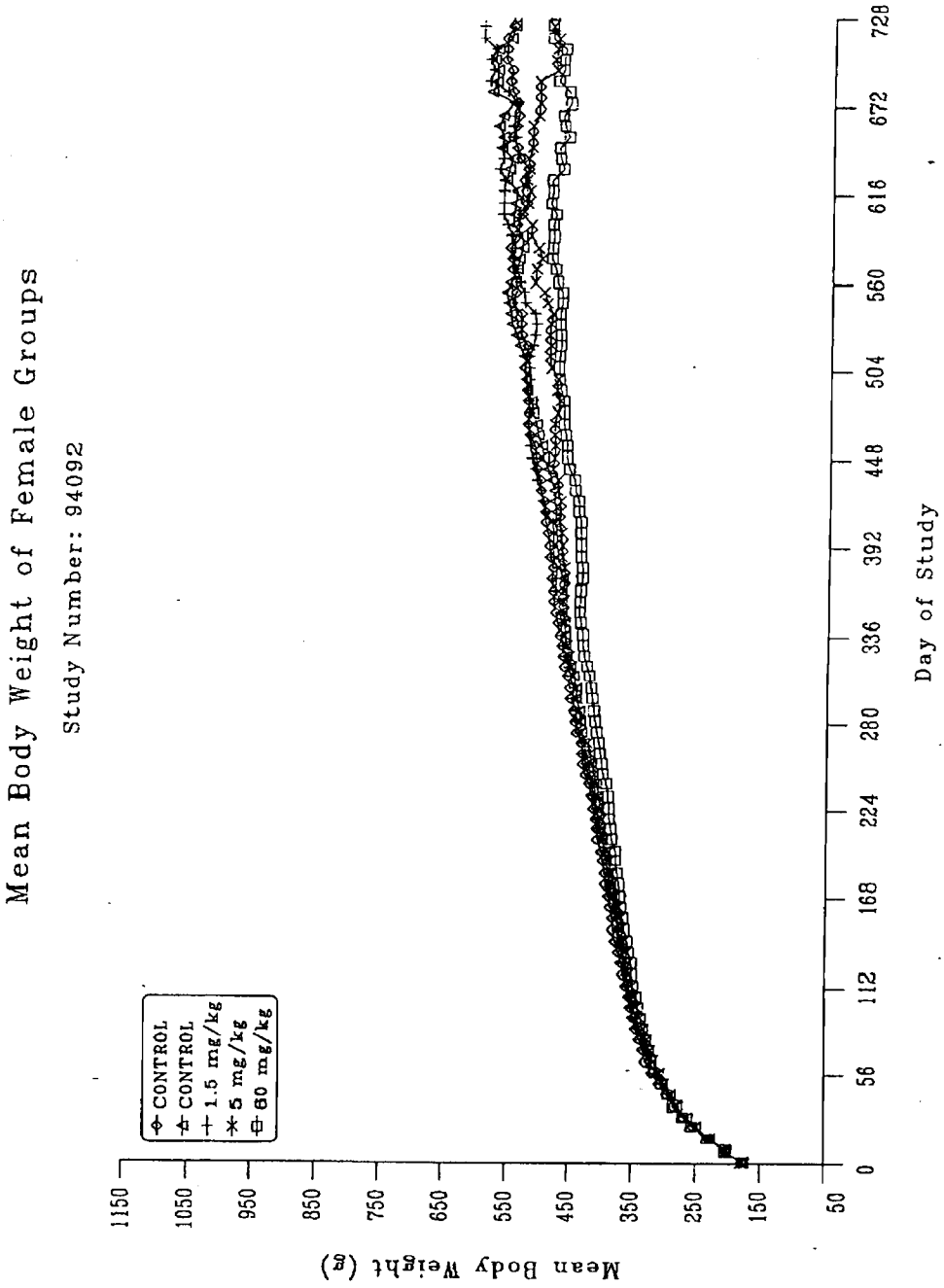


Table 3

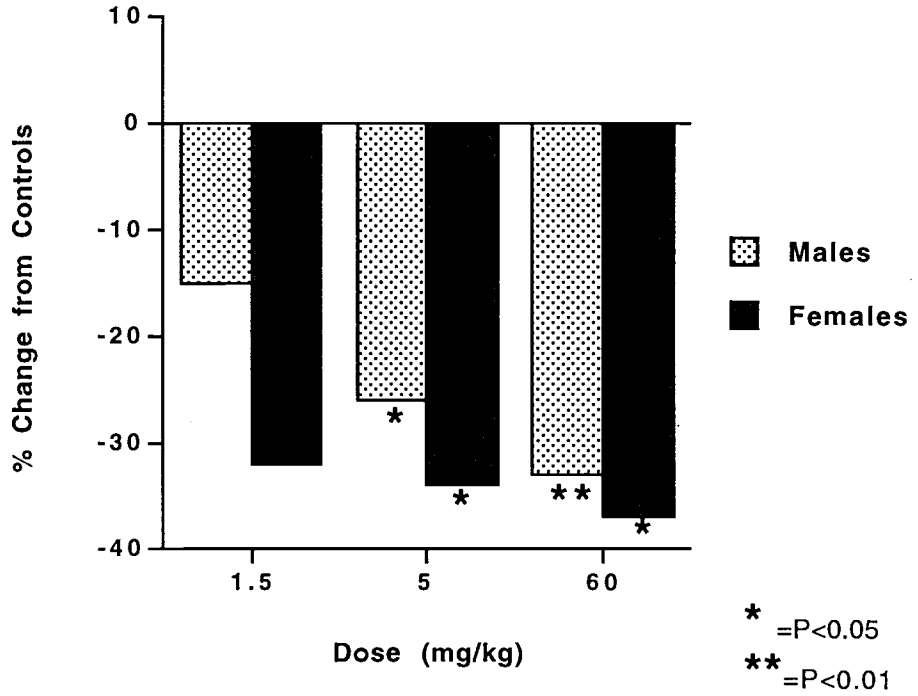
Effect of UK-92,480 on Mean Body Weight Gain in Rats

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

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

Figure 2

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



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

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

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

Figure 3A

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

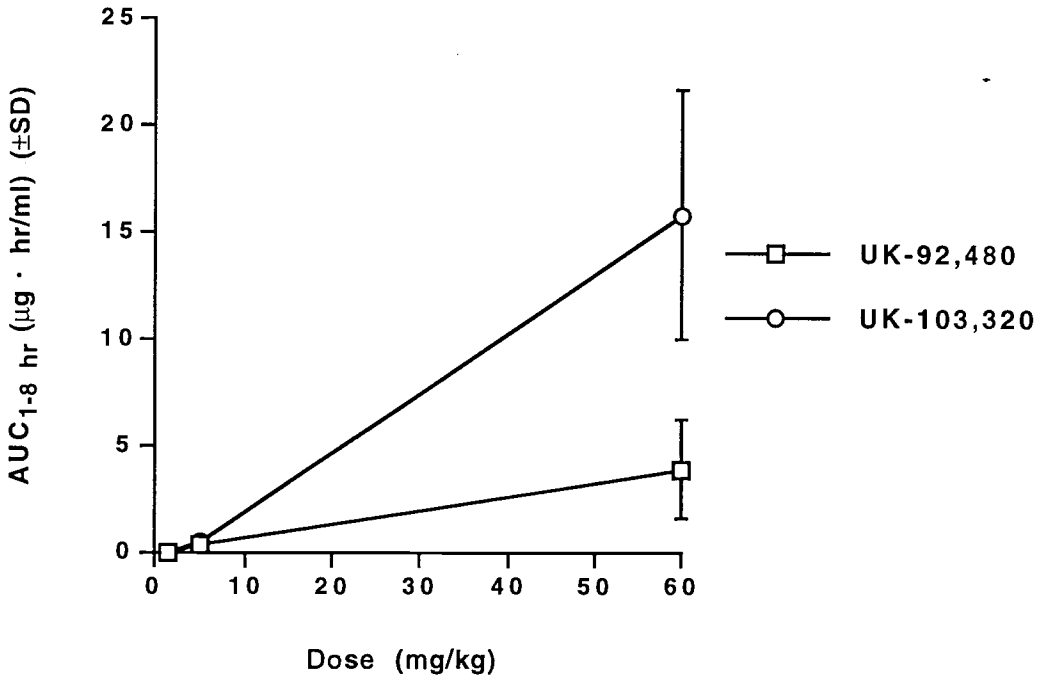
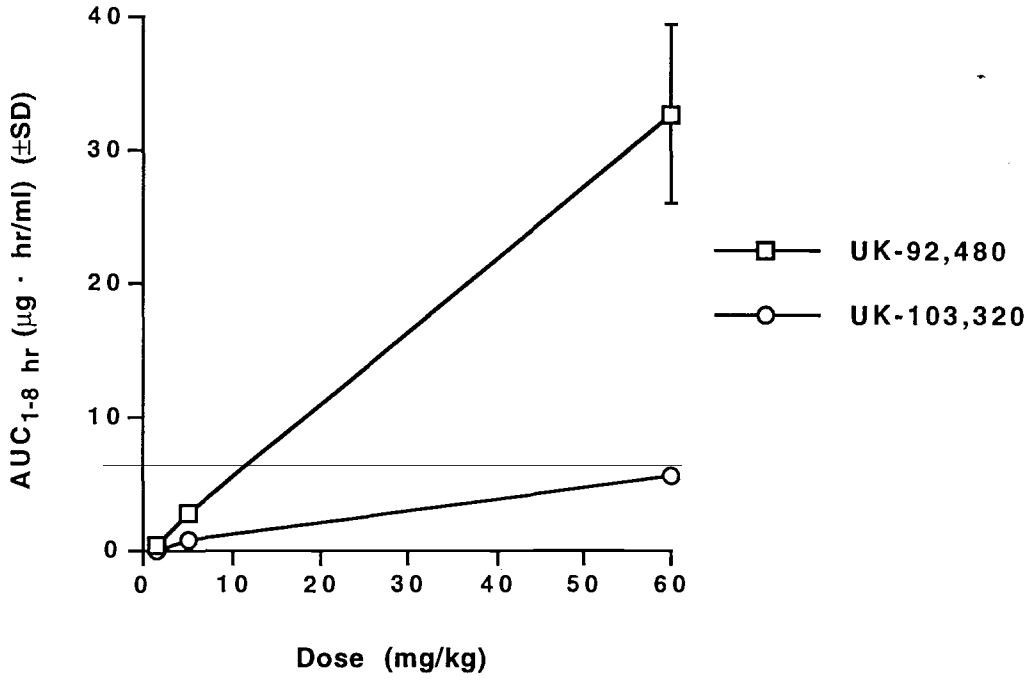


Figure 3B

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



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

Table 4

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

Species	Dose	UK-92,480 AUC (µg·hr/ml)	UK-103,320 AUC (µg·hr/ml)
Man	100 mg/70 kg	1.686	0.801
Rat (male)	60 mg/kg/day	3.902	15.767
Rat (female)	60 mg/kg/day	32.689	5.554

Since pharmacologic activity for sildenafil (UK-92,480) and its active metabolite (UK-103,320) is represented by the unbound fraction, the percentage of plasma protein binding for both human and rat is shown in Table 5.

Table 5

Human and Rat Plasma Protein Binding

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

Comparison of the male and female rat AUCs for total drug exposure (sum of unbound UK-92,480 and UK-103,320 AUCs) as a multiple of the maximum recommended human dose (MRHD) of 100 mg is shown in Table 6. The unbound AUCs were calculated by multiplying the total bound and unbound AUC (Table 4) by the fraction unbound (Table 5). As shown, the total of unbound AUCs in male and female rats given 100 mg/kg/day was 18.0X and 21.0X, respectively the AUC of men given a single dose of 100 mg.

Table 6

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

Species	Unbound UK-92,480 AUC (μg·hr/ml)	Unbound UK-103,320 AUC (μg·hr/ml)	Total of Unbound AUCs (μg·hr/ml)	Multiple of MRHD
Man	0.067	0.040	0.107	--
Rat (male)	0.195	1.734	1.929	**18.0X**
Rat (female)	1.634	0.611	2.245	**21.0X**

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

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

Systemic exposure to total unbound drug (sum of the parent drug UK-92,480 and the principle pharmacologically active metabolite UK-103,320) was calculated to be 18X and 21X the maximum recommended human dose of 100 mg in male and female rats, respectively. Although these values are less than the 25-fold ratio of rodent to human AUC required to qualify as an appropriate endpoint for high dose selection, they do suggest that the lack of a carcinogenic effect in rats was not due to inadequate systemic exposure to sildenafil. A statistical review of tumor incidence in the rat study by the Division of Biometrics is pending.

MOUSE CARCINOGENICITY STUDY: Single study (#95007)

MOUSE STUDY DURATION: 104 weeks
STUDY STARTING DATE: 1/18/95
STUDY ENDING DATE: 10/28/96
MOUSE STRAIN: 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 4A) and in the mid and high dose females (Figure 4B).

Figure 4A (Sponsor's Figure 1)

Survival Plot in Male Mice

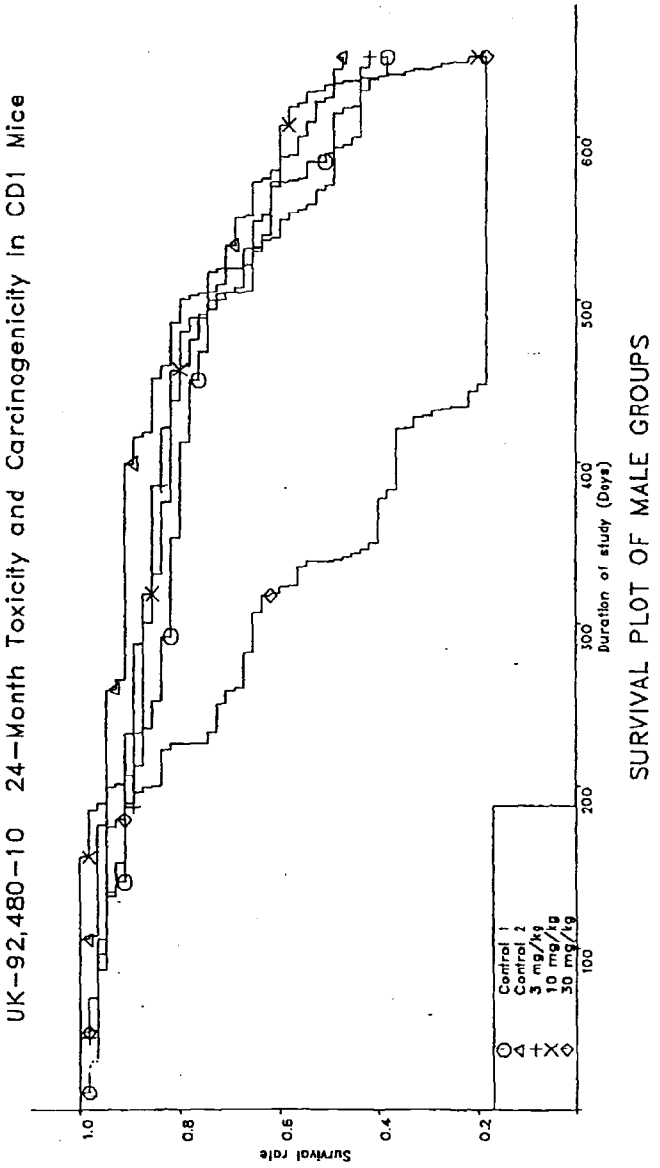
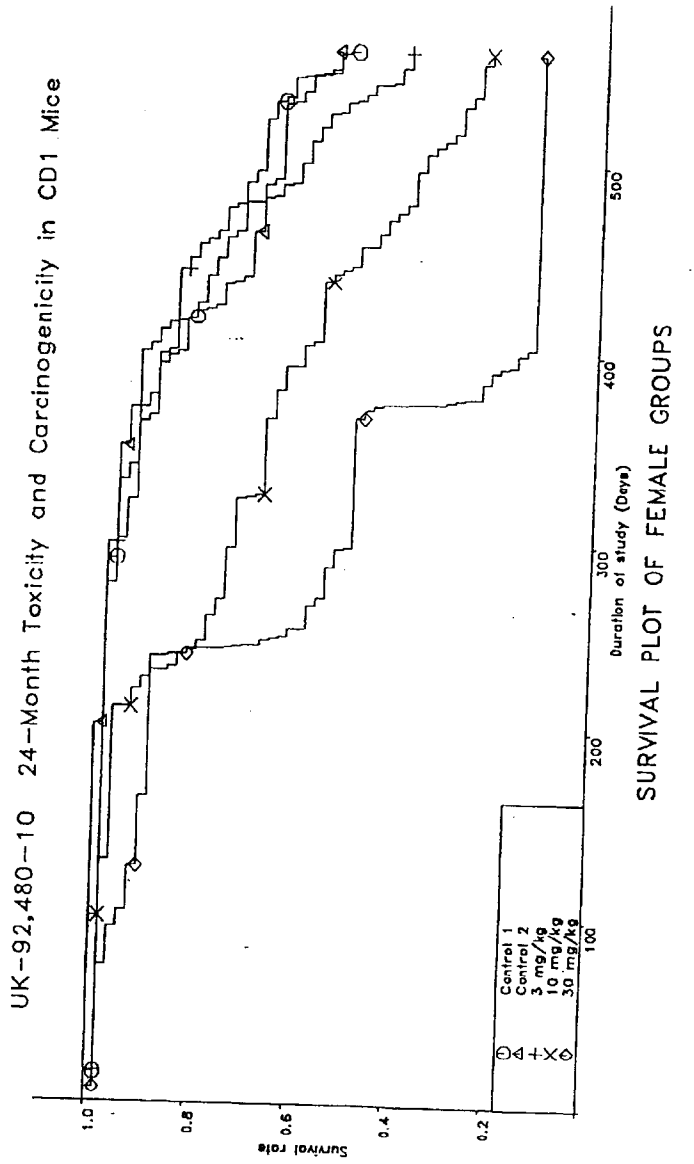


Figure 4B (Sponsor's Figure 2)

Survival Plot in Female Mice



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

Table 7

Times of Sacrifice and Percent Survival at Sacrifice in Mice

		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 5A (males) and Figure 5B (females).

Figure 5A (Sponsor's Figure 3)

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

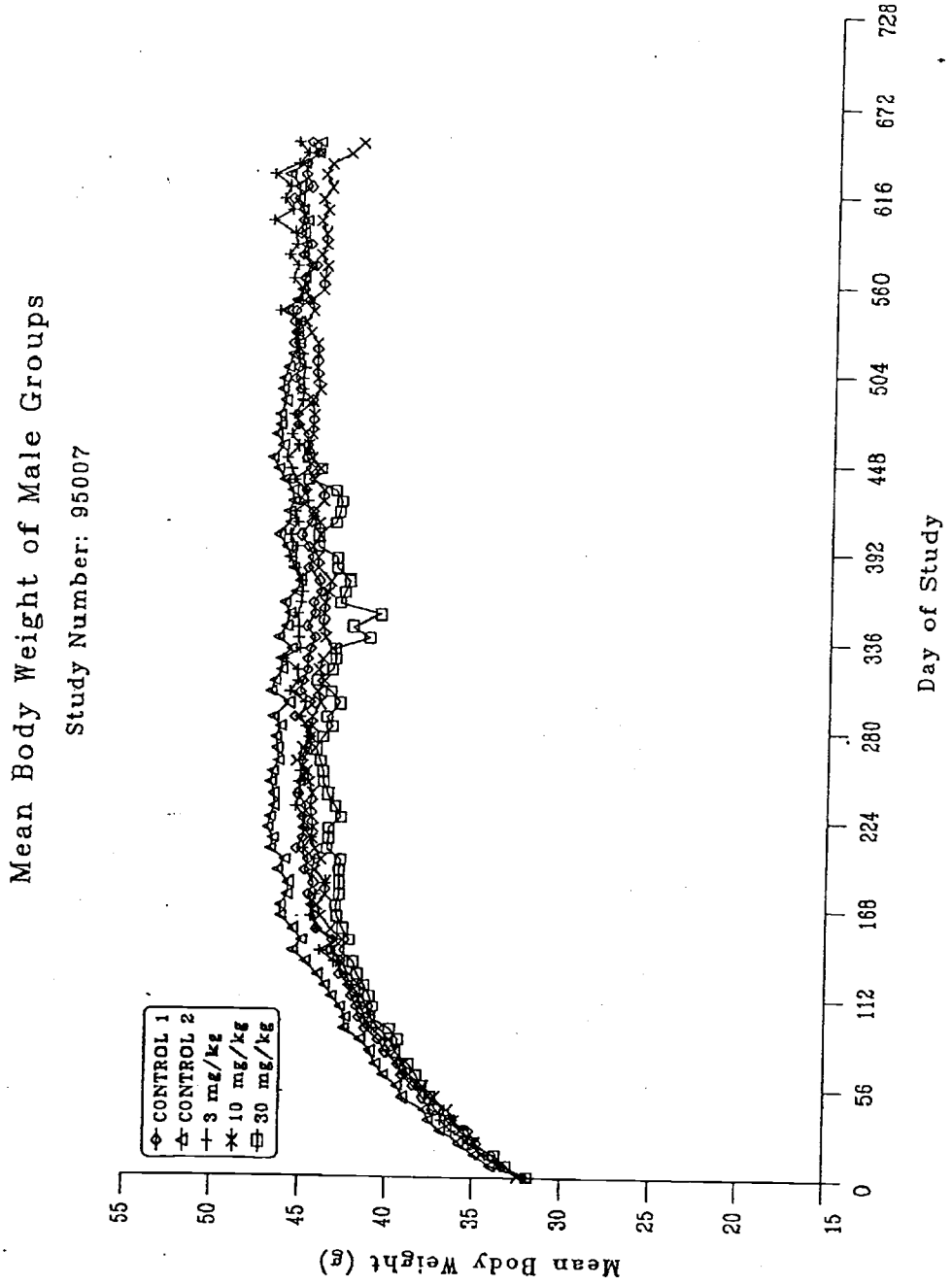
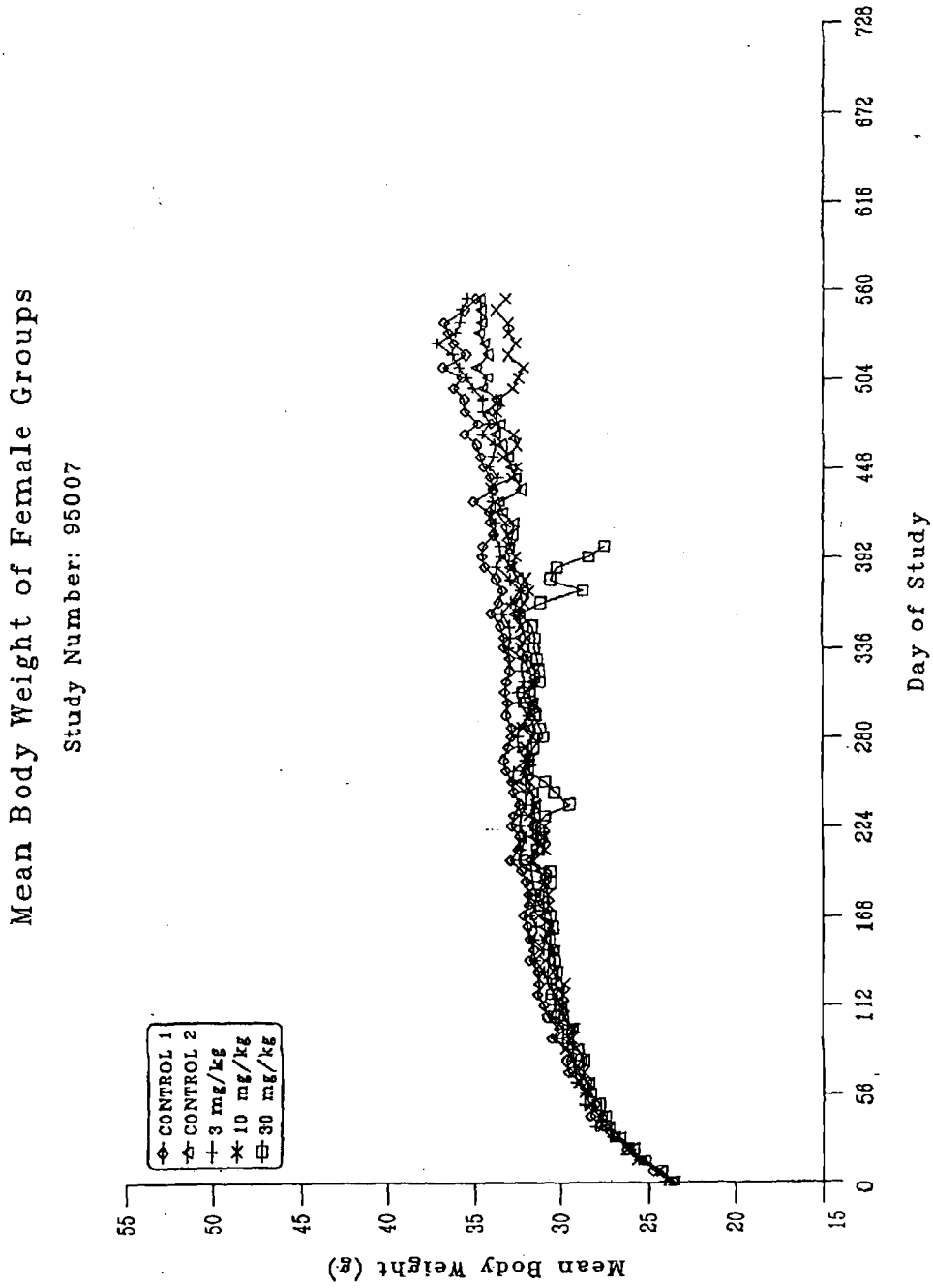


Figure 5B (Sponsor's Figure 4)

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



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

Table 8A

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

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

(* = last weight taken)

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

Table 8B

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

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

(* = last weight taken)

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

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

Table 9

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

Sex	Dose (mg/kg)	Incidence (%)
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 6), 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 7A and 7B).

Figure 6

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

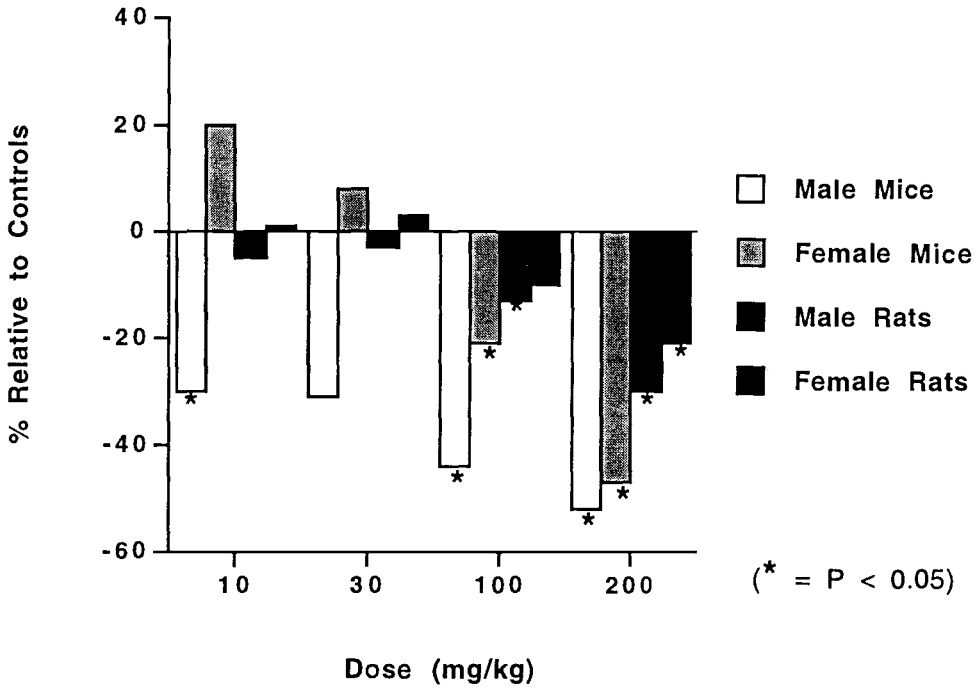


Figure 7A

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

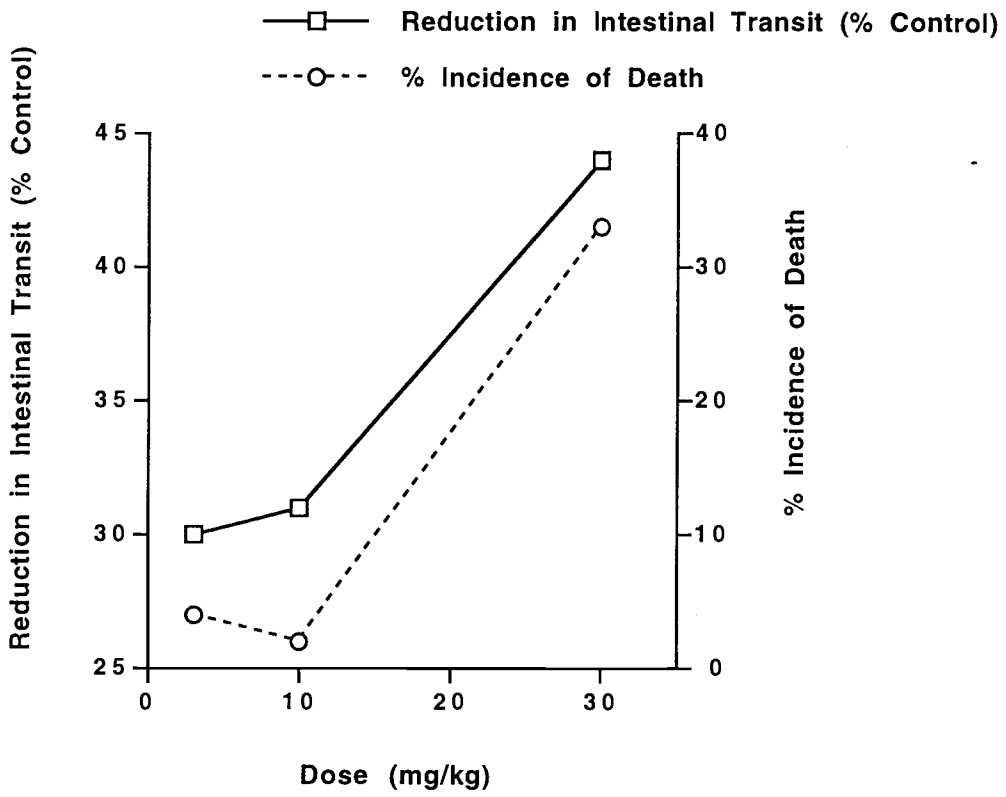
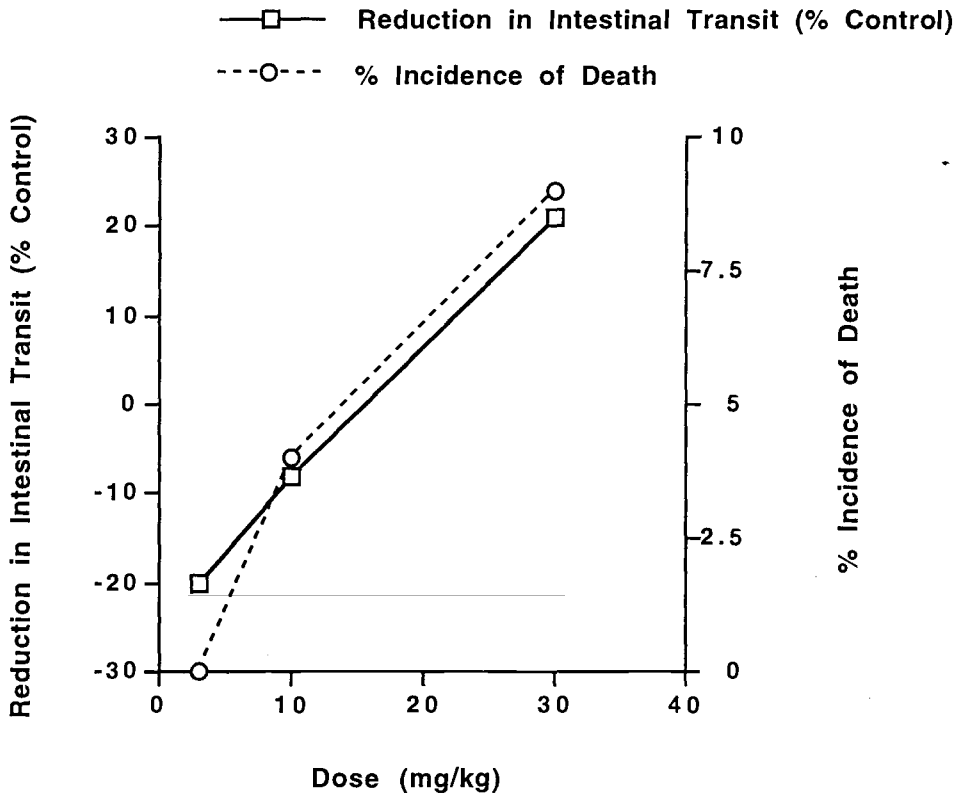


Figure 7B

Correlation Between Reduction in Intestinal Transit
and Death Due to Gastro-Intestinal 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 8A (males) and Figure 8B (females). As can be seen, exposure to UK-92,480 and UK-103,320 was dose-proportional in both sexes. As was the case in rats, male mice were exposed mostly to the metabolite UK-103,320, whereas female mice were exposed mostly to the parent drug UK-92,480.

Figure 8A

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

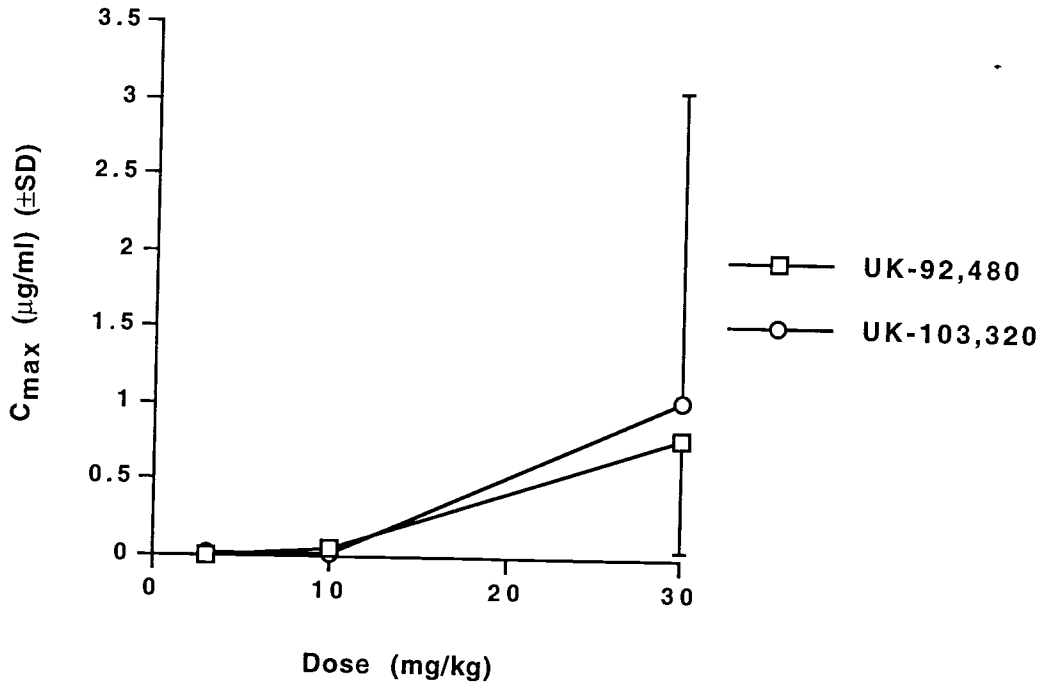
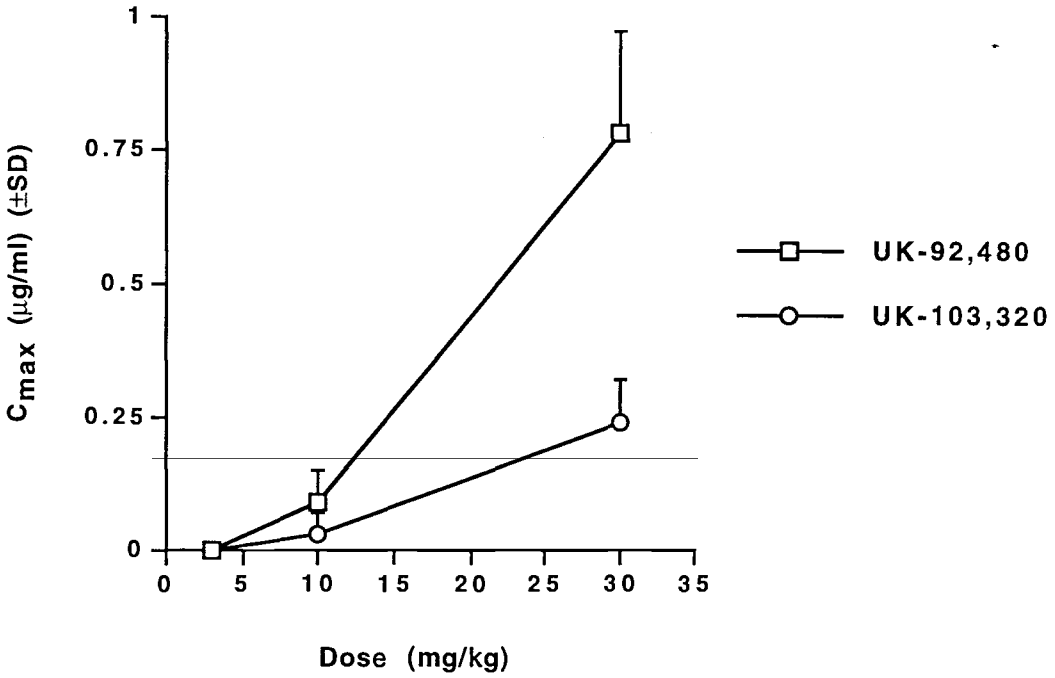


Figure 8B

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



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

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

Table 10

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

Species	Dose	UK-92,480 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 11.

Table 11

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

Table 12

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

Species	Dose (mg/kg)	Unbound UK-92,480 C_{max} (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 13). As shown, mice given the mid dose of 10 mg/kg/day for >18 months were exposed to 7.0X the MRHD of 1.43 mg/kg (= 100 mg/70 kg) when based on a mg/kg basis. However, when based on 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 13

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.

10/22/97

Thomas Papoian, Ph.D.
Pharmacologist

Concur: _____

10/22/97

cc:
Orig. NDA
HFD-110
HFD-110/T. Papoian

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

ADMINISTRATIVE DOCUMENTS

690

REQUEST FOR TRADEMARK REVIEW

TO: Labeling and Nomenclature Committee
Attention: Dan Boring HFD-530

FROM: Division of: Cardio-Renal Drug Products HFD-110
Attention: ~~Robert~~ Wolters Phone: 594-5376

DATE: October 31 1996

SUBJECT: Request for Assessment of a Trademark for a Proposed Drug Product

Proposed Trademark: Viagra NDA/ANDA IND 46,863

Company Name: Pfizer

Established name, including dosage form: Sildenafil Oral Tablets 50 and 100 mg

Other trademarks by the same firm for companion products:

Indications for Use (may be a summary if proposed statement is lengthy):
Oral therapy for male erectile dysfunction

Initial comments from the submitter: (concerns, observations, etc.)

Note: Meetings of the Committee are scheduled for the 4th Tuesday of the month. Please submit this form at least one week ahead of the meeting. Responses will be as timely as possible.

Rev. Dec.95

Consult #690 (HFD-110)

VIAGRA sildenafil oral tablets, 50 and 100 mg

There were no look-alike/sound-alike conflicts or misleading aspects noted with the proposed proprietary name. However, the Committee believes the non-proprietary name, sildenafil, has not been formally adopted yet by the United States Adopted Names Council. The Committee can only provide provisional acceptance of the proposed proprietary name pending final acceptance of the USAN name.

The Committee has no reason to find the proposed proprietary name unacceptable, pending final adoption of the non-proprietary name by the USAN Council.

P Uhering 11/18/96, Chair
CDER Labeling and Nomenclature Committee

Note sildenafil approve as USAN name 8-96.

Central Research Division
Pfizer Inc.
Eastern Point Road
Groton, CT 06340

Clinical Research

DATE: **February 10, 1998**

TO: **Mr. Gary Buehler**

FROM: **Dr. Sandra Croak-Brossman**

SUBJECT: **VIAGRA™, sildenafil citrate CAS name**

Question

The IUPAC name given by Pfizer for sildenafil citrate does not appear to be correct. The name given by Pfizer is:

1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-d]pyrimidin-5-yl)phenylsulphonyl]-4-methylpiperazine citrate

We suggest that the CAS name is correct. The CAS name is given below:

1-{[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl}-4-methylpiperazine citrate

Response

At the time of filing, Pfizer believed that the name that we submitted was correct utilizing IUPAC guidelines. After further consideration of the IUPAC and the CAS guidelines, we now consider that the CAS name is preferable.

Therefore, we agree that the chemical name for sildenafil citrate is:

1-{[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl}-4-methylpiperazine citrate

and we will change the name in the product labeling to reflect this.

Question

What is the CAS number for sildenafil citrate?

Response

The CAS number for sildenafil citrate is 171599-83-0.

Question

Is Sildenafil Citrate the USAN name? If so, when was it adopted?

Response

Sildenafil citrate is the USAN and it was adopted by the USAN Council Aug. 1996. A copy of the Output from the USAN Electronic Database is given in Appendix 1.

Appendix 1

Output from the USAN Electronic Database

L1 ANSWER 1 OF 1 USAN COPYRIGHT 1998 USPC

Accession Number (AN): 1998:7242 USAN
Publication Year (PY): 1997
Generic Name (CN): ***Sildenafil Citrate***
OTHER NAMES:
Chemical Name (CN): ***(Sildenafil is INN and BAN)***
Chemical Name (CN): Piperazine, 1-((3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo(4,3-d)pyrimidin-5-yl)-4-ethoxyphenyl)sulfonyl)-4-methyl-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1)
Chemical Name (CN): 1-((3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo(4,3- d)pyrimidin-5-yl)-4-ethoxyphenyl)sulfonyl)-4-methylpiperazine citrate (1:1)
Trade Name (CN): Viagra (Pfizer)
Code Designation (CN): UK-92,480-10
CAS Registry No. (RN): 171599-83-0; 139755-83-2 (sildenafil)
Lin. Str. Formula (LSF): C22 H30 N6 O4 S . C6 H8 O7
Molecular Weight (MW): 666.71
Classification (CC): Impotence therapy

USAN

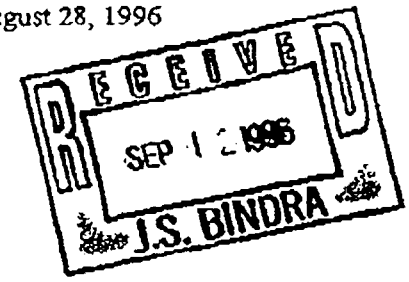
UNITED STATES ADOPTED NAMES COUNCIL

American Medical Association
515 North State Street
Chicago, Illinois 60610

Telefax: 312-464-4184
Telex: 280248 AMA CGO

SOPHIA V. FUERST, Assistant Secretary
(312) 464-5352

August 28, 1996



II-97

Pfizer Inc.
Central Research Division
Eastern Point Road
Groton, CT 06340

Attn: Jasjit S. Bindra, PhD
Senior Science Adviser

Dear Dr. Bindra:

It is my pleasure to inform you that the USAN Council adopted **sildenafil citrate** as the United States Adopted Name for UK-92,480-10; Viagra™, Pfizer Inc.'s selective inhibitor of cyclic GMP specific phosphodiesterase (Type V) with vasodilator action, used in the treatment of male erectile dysfunction.

Enclosed is a copy of the Statement of Adoption on **sildenafil citrate**. I plan to schedule publication of this information in the journal of Clinical Pharmacology and Therapeutics unless you request a delay within the next thirty days. Please use the enclosed statement to provide comments or additions. If this information is accurate, and may be published, please initial the statement and return it to me.

Sincerely yours,

Sophia V. Fuerst

Sophia V. Fuerst
Associate Secretary
USAN Council

SF

Enclosure: N96; 55

N96
55

August 28, 1996

STATEMENT ON A NONPROPRIETARY NAME ADOPTED BY THE USAN COUNCIL:

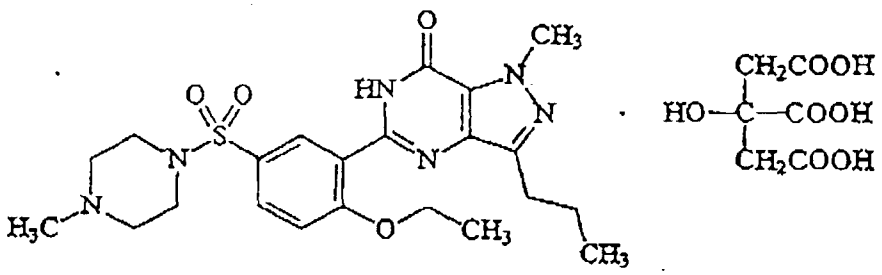
USAN (II-97)	SILDENAFIL CITRATE
PRONUNCIATION	sil de' na' fil
THERAPEUTIC CLAIM	treatment of male erectile dysfunction (selective inhibitor of cyclic GMP specific phosphodiesterase (Type V) with vasodilator action)

CHEMICAL NAMES

(1) 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine, 2-hydroxy-1,2,3-propanetricarboxylate (1:1)

(2) 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine citrate (1:1)

STRUCTURAL FORMULA



MOLECULAR FORMULA	$C_{22}H_{30}N_6O_4S \cdot C_6H_8O_7$ or $C_{28}H_{38}N_6O_{11}S$
MOLECULAR WEIGHT	666.79
TRADEMARK	Viagra
MANUFACTURER	Pfizer Inc.
CODE DESIGNATION	UK-92,480-10
CAS REGISTRY NUMBER	171599-83-0
WHO NUMBER	7374
SF	

USAN Council

List No. 397

New Names

The following nonproprietary names for the compounds described have been adopted by the United States Adopted Names (USAN) Council, which is sponsored by the American Medical Association, the American Pharmaceutical Association, and the US Pharmacopeial Convention, Inc., in cooperation with the respective pharmaceutical manufacturers. The designation USAN (United States Adopted Name) distinguishes these formally adopted nonproprietary names from other names. The information given under the category Therapeutic Claim is based on the manufacturer's claims at the time of publication. Adoption of USAN does not imply endorsement of the claims or products by the AMA, the US Pharmacopeia, or the APhA.

In some instances, the drugs listed are available only from the manufacturer to properly qualified investigators for clinical study.

Reprints of the *USAN Council New Names Column* are available at no charge by contacting: United States Adopted Names Council, American Medical Association, 515 North State Street, Chicago, IL 60610, (312) 464-4056.

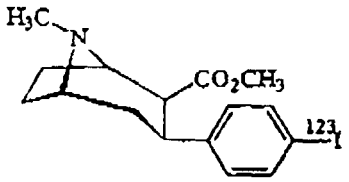
USAN	IOMETOPANE I 123
Pronunciation	eye oh me toh' pane
Therapeutic claim	diagnostic aid (in vivo diagnostic imaging of dopamine and serotonin transporter sites)

Chemical names

(1) methyl [1*R*-(*exo*,*exo*)]-3-[4-iodo-^{123}I)phenyl]-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate

(2) methyl 3β-(*p*-[^{123}I]iodophenyl)-1αH,5αH-tropane-2β-carboxylate

Structural formula



Molecular formula	$C_{16}H_{20}{}^{123}INO_2$
Molecular weight	385.2
Trademark	Dopascan™ Injection
Manufacturer	Guilford Pharmaceuticals Inc.
Code designation	GPI-200
CAS registry No.	136794-86-0
WHO No.	7569

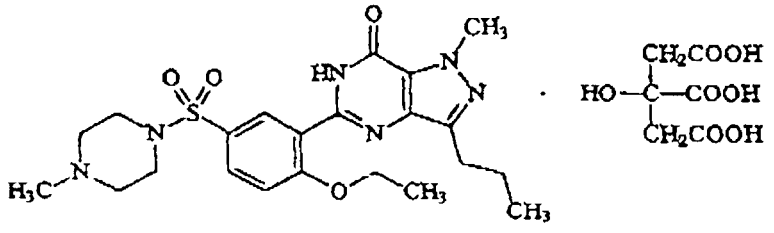
USAN	SILDENAFIL CITRATE
Pronunciation	sil de' na fil
Therapeutic claim	treatment of male erectile dysfunction (selective inhibitor of cyclic GMP specific phosphodiesterase (Type V) with vasodilator action)

Chemical names

(1) 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine, 2-hydroxy-1,2,3-propanetricarboxylate (1:1)

(2) 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazone citrate (1:1)

Structural formula



Molecular formula	$C_{22}H_{30}N_6O_4S \cdot C_6H_8O_7$ or $C_{28}H_{38}N_6O_{11}S$
Molecular weight	666.79
Trademark	Viagra
Manufacturer	Pfizer Inc.
Code designation	UK-92,480-10
CAS registry No.	171599-83-0
WHO No.	7374

[redacted]

DRUG STUDIES IN PEDIATRIC PATIENTS
(To be completed for all NME's recommended for approval)

NDA # 20-895 Trade (generic) names Viagra (sildenafil) Tablets

Check any of the following that apply and explain, as necessary, on the next page:

___ 1. A proposed claim in the draft labeling is directed toward a specific pediatric illness. The application contains adequate and well-controlled studies in pediatric patients to support that claim.

___ 2. The draft labeling includes pediatric dosing information that is not based on adequate and well-controlled studies in children. The application contains a request under 21 CFR 210.58 or 314.126(c) for waiver of the requirement at 21 CFR 201.57(f) for A&WC studies in children.

___ a. The application contains data showing that the course of the disease and the effects of the drug are sufficiently similar in adults and children to permit extrapolation of the data from adults to children. The waiver request should be granted and a statement to that effect is included in the action letter.

___ b. The information included in the application does not adequately support the waiver request. The request should not be granted and a statement to that effect is included in the action letter. (Complete #3 or #4 below as appropriate.)

___ 3. Pediatric studies (e.g., dose-finding, pharmacokinetic, adverse reaction, adequate and well-controlled for safety and efficacy) should be done after approval. The drug product has some potential for use in children, but there is no reason to expect early widespread pediatric use (because, for example, alternative drugs are available or the condition is uncommon in children).

___ a. The applicant has committed to doing such studies as will be required.

___ (1) Studies are ongoing.
___ (2) Protocols have been submitted and approved.
___ (3) Protocols have been submitted and are under review.
___ (4) If no protocol has been submitted, on the next page explain the status of discussions.

___ b. If the sponsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.

X 4. Pediatric studies do not need to be encouraged because the drug product has little potential for use in children.

____ 5. If none of the above apply, explain.

Explain, as necessary, the foregoing items: _____

1/30/98

Signature of Preparer Date

cc: Orig NDA
HFD-___/Div File
NDA Action Package

13. PATENT AND EXCLUSIVITY INFORMATION FOR VIAGRA (SILDENAFIL CITRATE)

1.	Active Ingredient:	1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-d]pyrimidin-5-yl)phenylsulfonyl]-4-methylpiperazine citrate
2.	Strengths:	25, 50 and 100 mg
3.	Trade Name:	VIAGRA
4.	Dosage Form / Route of Administration:	Tablets / Oral
5.	Application Firm Name:	Pfizer Pharmaceuticals Production Corporation Ringaskiddy County Cork, Ireland
6.	NDA Number:	20-895
7.	Exclusivity Period:	5 years from the date of the approval of this application, as provided in FDCA Section 505 (j) (4) (D) (ii)
8.	Applicable Patent Numbers and Expiration Dates:	5,250,534 June 18, 2011

14. PATENT CERTIFICATION

With respect to the drug, VIAGRA™, which is the subject of this Application (NDA-20-895) and the U.S. patent which is listed in Section 13 of this Application, Pfizer certifies that the drug, VIAGRA™, and formulations thereof are claimed by U.S. Patent No. 5,250,534.

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

CORRESPONDENCE

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

Food and Drug Administration
Rockville MD 20857

G. Buehler

NDA 20-895

OCT 8 1997

Central Research Division
Pfizer Inc.
Attention: Sandra J. Croak-Brossman, Ph.D.
Eastern Point Road
Groton, CT 06340

Dear Dr. Croak-Brossman:

We have received your new drug application (NDA) submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for the following:

Name of Drug Product: Viagra (sildenafil citrate) Tablets

Therapeutic Classification: P

Date of Application: September 29, 1997

Date of Receipt: September 29, 1997

Our Reference Number: NDA 20-895

Unless we notify you within 60 days of the receipt date that the application is not sufficiently complete to permit a substantive review, this application will be filed under section 505(b) of the Act on November 28, 1997 in accordance with 21 CFR 314.101(a).

Under 21 CFR 314.102(c) of the new drug regulations you may request an informal conference with this Division (to be held approximately 90 days from the above receipt date) for a brief report on the status of the review but not on the application's ultimate approvability. Alternatively, you may choose to receive such a report by telephone. Should you wish a conference, a telephone report, or if you have any questions concerning this NDA, please contact:

Mr. Gary Buehler
Regulatory Health Project Manager
(301) 594-5332

Please cite the NDA number listed above at the top of the first page of any communications concerning this application.

Sincerely yours,

Natalia A. Morgenstern
Chief, Project Management Staff
Division of Cardio-Renal Drug Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

cc
Orig. NDA
HFD-110
DISTRICT OFFICE
HFD-110/GBuehler;10/1/97
sb/10/1/97;10/7/97
R/D: NMorgenstern/10/1/97

ACKNOWLEDGEMENT - AC

GB

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

OCT 31 1997

Pfizer Pharmaceutical Production Corporation
Attention: Mr. Daniel P. Cronin
Ringaskiddy
County Cork
Ireland

Dear Mr. Cronin:

We acknowledge receipt of your correspondence notifying the Food and Drug Administration of a change of sponsorship of:

Name of Drug: Viagra (sildenafil) Tablets

IND Number: 46,863

Date of Submission: September 18, 1997

Date of Receipt: October 1, 1997

Name of Former Sponsor: Pfizer Central Research

Change in ownership of this IND will require you as the new sponsor to provide:

1. A commitment to amend the IND within 60 days to cover all changes in the IND resulting from new ownership and to provide for subsequent changes by amendments in accord with the IND regulations.

2. A commitment to inform all active investigators of the change and obtain an updated Form FDA 1572 and commitments to you.

3. A list of all active investigators or a statement that they are the same as currently listed in the IND, if this is the case.

4. Any changes in the investigators' curriculum vitae, study protocols or other study parameters.

All future communications regarding this IND should be forwarded in triplicate, identified with the IND number 46,863 and addressed as follows:

Food and Drug Administration
Center for Drug Evaluation and Research, HFD-110
Attention: DOCUMENT CONTROL ROOM
5600 Fishers Lane
Rockville, MD 20857

Should you have any questions, please contact:

Mr. Gary Buehler
Regulatory Health Project Manager
Telephone: (301) 594-5332

Sincerely yours,

Natalia A. Morgenstern
Chief, Project Management Staff
Division of Cardio-Renal Drug Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

cc: Pfizer Central Research
Attention: Sandra Croak-Brossman, Ph.D.
Eastern Point Road
Groton, CT 06304-5146

cc:
Original IND
HFD-110
HFD-110/GBuehler;10/23/97
sb/10/17/97;10/24/97
R/D: JAdvani/10/23/97
EBarry/10/23/97
ADeFelice/10/23/97
NStockbridge/10/24/97
NMorgenstern/10/24/97

INFORMATION REQUEST

G. Buehler

JAN 23 1998

NDA 20-895

Pfizer Pharmaceuticals Production Corporation
c/o Pfizer Central Research Division
Attention: Sandra J. Croak-Brossman, Ph.D.
Eastern Point Road
Groton, CT 06340

Dear Dr. Croak-Brossman:

Please refer to your new drug application (NDA) for Viagra (sildenafil citrate) 25, 50 and 100 mg Tablets.

In reviewing your submission of September 29, 1997, our Medical Officer, Statistician and Biopharmaceuticist raised a number of questions that require your attention. Our comments on your submission are detailed as part of this correspondence (see enclosure).

Sincerely yours,

Natalia A. Morgenstern
Chief, Project Management Staff
Division of Cardio-Renal Drug Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Enclosure
Joint Review - Drs. Stockbridge, Marroum and Mahjoob

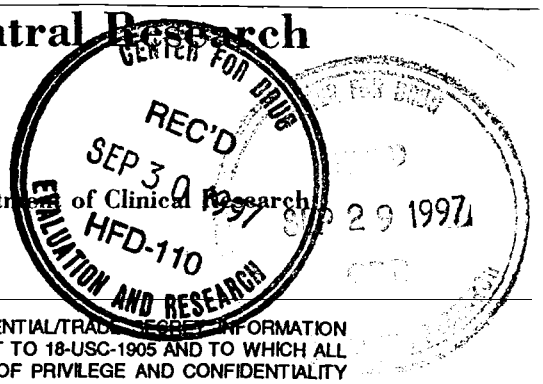
cc:
Original NDA
HFD-110
HFD-110/GBuehler/1/23/98
sb/1/23/98

GENERAL CORRESPONDENCE

Pfizer Inc
Eastern Point Road
Groton, CT 06340
Tel 860 441 4100

Central Research

Department of Clinical Research



September 29, 1997

Raymond J. Lipicky, M.D., Director
Division of Cardio-Renal Drug Products
Center for Drug Evaluation and
Research HFD #110
ATT: DOCUMENT CONTROL ROOM #16B-30
5600 Fishers Lane
Rockville, MD 20857

Dear Doctor Lipicky:

RE: New Drug Application # 20-895 - VIAGRA ™ (sildenafil citrate) Tablets
IND - # 46,863 / User Fee ID # 3303

Pursuant to Paragraph 505(b) of the Federal Food, Drug and Cosmetic Act, and 21 CFR 314.1, we are submitting herewith a New Drug Application (#20-895) for VIAGRA™ (sildenafil citrate) Tablets. Pfizer, Inc. is filing this NDA on behalf of Pfizer Pharmaceuticals Production Corporation, Ringaskiddy, County Cork, Ireland.

The chemistry, preclinical, and clinical data obtained during the investigation of VIAGRA™ under IND- have been organized in this Application in accord with the requirements as currently set forth under 21 CFR 314.50. This Application is also provided in electronic format as discussed and agreed with the Division on May 28, 1997. Text and image information supplied electronically is identical to that provided by paper. Please note, however, that case report forms (CRFs) and case report form tabulations are only being supplied electronically, in accordance with a waiver granted by CDER (reference letter of Dr. Janet Woodcock dated April 24, 1997, attached).

The location of the various sections of this Application, number of volumes being submitted, and other explanatory notes are listed in Attachment 1 of this letter.

The manufacturing site identified in this Application is located in Brooklyn, NY. As such, the Sponsor hereby certifies that a field copy of portions of this Application has been provided to the FDA district office in Brooklyn, NY, and that it is an exact copy of the Chemistry, Manufacturing and Controls section, FDA Form 3439 and the Application Summary contained in the archival and review copies of this NDA.

VIAGRA™ is a selective inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type 5 (PDE5) and is indicated for the treatment of erectile dysfunction. The physiological mechanism responsible for erection of the penis involves the release of nitric oxide (NO) in the corpus cavernosum in response to sexual stimulation. Nitric oxide activates the enzyme guanylate cyclase, which results in locally increased levels of cGMP, thereby producing smooth muscle relaxation in the corpus cavernosum and allowing inflow of blood. By inhibiting PDE5, VIAGRA™ enhances the normal physiological action of nitric oxide/cGMP, thereby allowing patients with erectile dysfunction to obtain erections adequate for sexual intercourse.

Dr. Lipicky, M.D., Director
NDA-20-895

This NDA contains safety data from a total of 4213 subjects enrolled in Phase II/III clinical trials and an additional 576 subjects treated with sildenafil in Phase I clinical trials. Separate safety summaries are provided for 194 subjects participating in studies conducted in Japan and 281 subjects treated in Phase II/III studies with study designs which did not allow them to be incorporated into other groupings. Thus a total of 4526 subjects have received sildenafil and 2091 have received placebo in the clinical development program. The safety data base includes 559 subjects treated for at least one year.

As previously agreed upon with the Agency, the primary endpoints of the pivotal studies are questions # 3 and # 4 of the International Index of Erectile Function (IIEF) questionnaire. Data in this NDA establish the safety and efficacy of VIAGRA™ in the treatment of erectile dysfunction. Four randomized, double-blind, placebo-controlled, multi-centered trials provide substantial evidence of efficacy. In addition, two key supportive studies clearly provide proof of efficacy in patients with diabetes mellitus and patients with spinal cord injury.

Sildenafil, a new chemical entity, is the first oral treatment for erectile dysfunction and thus offers a substantial advantage over the currently available treatments for erectile dysfunction. Accordingly, and as previously discussed and agreed with the Agency, we are requesting a Priority Review for this Application.

Currently, sildenafil is not marketed in any country. Marketing Applications for sildenafil will be filed in Canada, the European Medicinal Evaluation Agency (EMEA) utilizing the centralized procedure, and eventually other countries.

In accordance with the requirement of the Generic Drug Enforcement Act of 1992, and in connection with this Application, to the best of its knowledge, Pfizer Inc did not use in any capacity the services of any person debarred under Section 306 of the Federal Food, Drug, and Cosmetic Act.

Please be advised that the applicable user fee for this submission has been remitted in accordance with the Prescription Drug User Fee Act of 1992. We believe NDA-20-895 to be complete for review by the Division and look forward to working closely with the Division.

Should you have any questions regarding the organization or content of this Application, please contact Dr. Sandra J. Croak-Brossman at (860) 441-1903 (phone) or (860) 441-0870 (fax).

Sincerely yours,

Steven W. Ryder, M.D.
Vice President
U.S. Clinical Operations

Sandra J. Croak-Brossman, Ph.D.
Associate Director
Regulatory Strategy & Registration

enclosures
Serial No. 000