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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-513

Pharmacology Review(s)

Note:

This will be the Standard CDER Coversheet

TABLE OF CONTENTS

EXECUTIVE SUMMARY	1
PHARMACOLOGY/TOXICOLOGY REVIEW	5
3.1 INTRODUCTION AND DRUG HISTORY.....	5
3.2 PHARMACOLOGY.....	9
3.2.1 Brief summary	9
3.2.2 Primary pharmacodynamics.....	9
3.2.3 Secondary pharmacodynamics.....	10
3.2.4 Safety pharmacology	11
3.2.5 Pharmacodynamic drug interactions.....	11
3.3 PHARMACOKINETICS/TOXICOKINETICS	16
3.3.1 Brief summary	16
3.3.3 Absorption	16
3.3.4 Distribution.....	16
3.3.5 Metabolism	16
3.3.6 Excretion.....	18
3.3.7 Pharmacokinetic drug interactions.....	18
3.3.10 Tables and figures to include comparative TK summary	18
3.4 TOXICOLOGY.....	20
3.4.1 Overall toxicology summary.....	20
3.4.2 Single-dose toxicity	21
3.4.3 Repeat-dose toxicity	22
3.4.4 Genetic toxicology.....	30
3.4.5 Carcinogenicity.....	30
3.4.6 Reproductive and developmental toxicology.....	38
3.4.7 Local tolerance.....	38
3.4.8 Special toxicology studies.....	37
3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS.....	42
3.7. APPENDIX/ATTACHMENTS	43

EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability: Approval is recommended from a Pharmacology/Toxicology perspective.

1.2 Recommendation for nonclinical studies: No new studies are recommended.

1.3 Recommendations on labeling:

Carcinogenesis, Mutagenesis, Impairment of Fertility

[

]

Pregnancy

Pregnancy: Pregnancy category C

[

]

2. Summary of nonclinical findings

2.1 Brief overview of nonclinical findings:

Cardiovascular effects:

Darifenacin competitively displaced [³H]-dofetilide from hERG receptors with an IC₅₀ value of 1.1 x 10⁻⁶ M (550 ng/ml) and an IC₁₀ of about 1 x 10⁻⁷ M (50 ng/ml). UK-088,862 (N=6) had no effect up to 4 x 10⁻⁷ M (112.2 ng/ml). UK-073,689 (n=6) had no effect up to 6 x 10⁻⁷ M (106.9 ng/ml). UK-148,993 had little or no effect on [³H]-dofetilide binding at concentrations up to 1 x 10⁻⁷ M (44.3 ng/ml). Effects on the hERG potassium current were also examined using the *in vitro* whole cell patch clamp technique. The parent UK-88,525-04 exhibited an IC₅₀ of 77 nM (39.1 ng/ml) (IC₃₀ at ~30 nM; IC₁₀ at ~10 nM). The metabolites, UK-148,993 (100 nM or 44.26 ng/ml), UK-88,862 (400 nM or 112.16 ng/ml) and UK-73,689 (600 nM or 106.92 ng/ml) had little or no effect on the hERG potassium current (12.7±3.2%, 2.4±3.5%, and 0.9±3.3%, respectively).

UK-088,525-04 had no significant effect on membrane potential, action potential amplitude or V_{max} at concentrations up to 100 nM when compared to time matched vehicle controls in dog isolated Purkinje fibers. No effect on action potential duration (APD₅₀ or APD₉₀) was observed up to 10 nM UK-088,525-04; however, at 30 nM and 100 nM, there was a concentration-related reduction in APD₅₀ with no effect on APD₉₀. No effects of UK-148,993 on any of the above mentioned parameters was observed at concentrations up to 50 nM. UK-088,525-04 had no effect up to 10000 nM on resting membrane potential when compared to time matched vehicle controls. UK-088,525-04 produced concentration-related reductions in both action potential amplitude and V_{max}, with significance reached (p < 0.01) at 10000 nM. UK-088,525-04 produced concentration related reductions in both APD₅₀ and APD₉₀ with significance at 1000 nM and 10000 nM. UK-088,862 up to 400 nM or 112.2 ng/ml and UK-073,689, up to 600 nM or 106.9 ng/ml had no effect on membrane potential, action potential amplitude, V_{max}, APD₅₀, or APD₉₀.

The hemodynamic and electrophysiological effects of UK-088,525-04 following cumulative 45 minute infusions of 1.27, 5.47, 18.07, and 60.07 µg/kg in the isoflurane anesthetized dog showed a no effect level at free plasma concentrations of 3.2 ng/ml (7.5 nM). At 10.7 ng/ml (25.1 nM), there was a 7% increase in QT interval along with a 10% increase in monophasic action potential, small increases in PR and QRS interval, and an increase in MAP during atrial pacing. At 43.2 ng/ml (101 nM), there was a 16% increase in QT interval. Effects on QT interval at higher doses are consistent with changes in the potassium I_{Kr} channels in hERG studies. Increases in ECG QRS and PR intervals at the highest dose, together with decreases in heart rate, diastolic blood pressure and cardiac contractility, suggest mixed ion channel antagonistic properties on sodium and calcium channels.

In a one-year toxicology study in dogs, no effect on QT interval was observed up to 6 mg/kg/day. There was some tendency for an increase in P width in high dose males and in P height in both high dose males and females 2 hours post dose. Heart rate was also increased in high dose males. No treatment related effects on blood pressure were observed. Blood concentrations up to 669 ng/ml UK-88,525 and 514 ng/ml UK-148,993 were reached.

A no-effect level for hemodynamic effects was 1 mg/kg in a conscious dog study. At 3 mg/kg increases in heart rate (20-30 beats/minute) and cardiac output (~0.5 L/min) were observed approximately 30 minutes following dosing; blood pressure was maintained by a reduction in systemic vascular resistance. These effects persisted up to 180 minutes following dosing.

Darifenacin did not inhibit ventricular fibrillation threshold and electroconversion to sinus rhythm in the anaesthetized dog with unbound plasma concentrations of Darifenacin at 5 minutes post-injection of 57.1 nM (approximately 190 times higher than the unbound steady state concentrations for humans of 0.3 nM).

General toxicology:

Rats were tested up to 30 mg/kg/day in a 6 month toxicology study, and dogs were tested up to 10 mg/kg/day in a 6 month study and 6 mg/kg/day in a one year study. Maximum tolerated doses were based on body weight and poor general condition in rats and on severe pharmacological effects in dogs. Effects were primarily due to the known pharmacology of the drug: mydriasis (and associated ocular effects), partially closed eyes, dry mouth, reduced intestinal motility (and associated dysphagia and emesis), hemodynamic effects, and hypersecretion of the Harderian gland. Increases in liver weight were observed, without histopathological correlate and with associated increases in metabolic enzymes.

Genetic toxicology:

Darifenacin was not mutagenic in the Ames test for bacterial mutation or in the Chinese hamster ovary assay. It was not clastogenic *in vitro* in the human lymphocyte assay or *in vivo* in the mouse bone marrow cytogenetics assay.

Carcinogenicity:

Darifenacin was tested at the maximally tolerated dose (based on body weight) in two year assays in male and female rats and mice. Statistical analysis determined that no increase in tumors was clearly related to the administration of darifenacin. The multiples of human exposure after dietary administration were as follows:

	Male rats	Female rats	Male mice	Female mice
Nominal dose (mg/kg/day)	15	15	100	100
Multiple of human 15 mg AUC	7 X	12 X	32 X	32 X

Reproductive toxicology:

	Rat Seg.I	Rat Seg.II	Rabbit Seg.II	Rat Seg. III
Nominal dose (mg/kg/day)	3, 10, 50	3, 10, 50	3, 10, 30	3, 10, 50
Multiple of human 15 mg AUC	4.6, 16, 78	3.5, 12, 59	2.8, 9, 28	5, 17, 87
Maternal no-effect level (mg/kg/day)	50 (fertility)	10	10	3
Offspring no-effect level (mg/kg/day)	10	10	3	3

In a segment I fertility study in rats, darifenacin was administered to males from 64 days ante-coitum and to females from days 14 days ante-coitum to 20 days post insemination or 21 days postpartum at 0, 3, 10, and 50 mg/kg/day. In the 50 mg/kg/day F0 group, 2 males and 2 females died and body weights were decreased. Exaggerated pharmacology was observed in the 10 and 50 mg/kg/day groups. Fertility was decreased in the 10 and 50 mg/kg/day groups but values were not statistically significant nor dose related and were still within the historical control values.. In the 50 mg/kg/day group, post implantation loss was increased, and the number of viable pups per dam, pup weight, and pup survival were decreased. Reduced ossification of the sacral and caudal vertebrae and urinary bladder dilatation of the offspring was observed.

In a segment II study in rats, darifenacin was administered to dams from days 6-17 days of gestation at 0, 3, 10, and 50 mg/kg/day. Maternal body weights were reduced in the 50 mg/kg/day group. At this dose, pup weights were reduced and delays in ossification of the sacral and caudal vertebrae of offspring were observed.

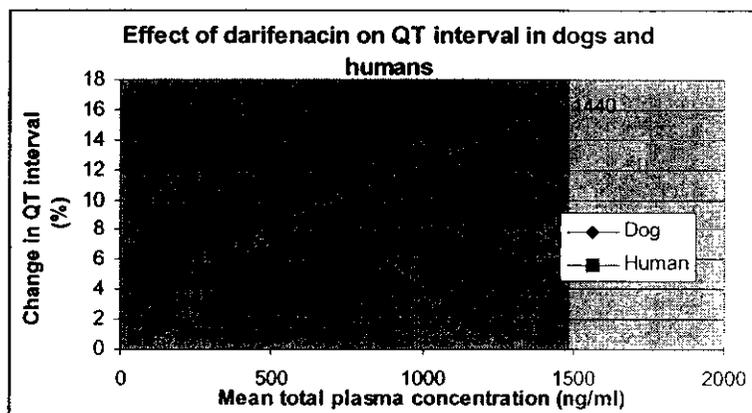
In a segment II study in rabbits, darifenacin was administered to dams from days 6 to 18 of gestation at 0, 3, 10, and 30 mg/kg/day. At 30 mg/kg/day, 2 dams aborted, and effects on body weight and food consumption were observed. At this dose, post implantation loss was increased and the number of viable fetuses per litter was decreased. In the offspring, dilated ureter and/or kidney pelvis was observed at 30

mg/kg/day and 1 case was observed at 10 mg/kg/day, along with urinary bladder dilation, consistent with pharmacological activity.

Two segment III developmental studies were performed in rats covering periods from 15 days post-insemination to day 20 postpartum and from day 6 postinsemination to day 21 postpartum, both at doses of 0, 3, 10, and 50 mg/kg/day. Maternal toxicity was observed at 50 mg/kg/day as evidenced by effects on body weight and food consumption and by dystocia, and prolonged gestation and parturition in both studies. Hypogalactia was also observed at 50 mg/kg/day, along with increased mortality of pups between days 1 and 4 postpartum, with an increased incidence of dilatation of the urinary bladder or renal pelvis. At this dose, decreased body weights of pups and delays in developmental landmarks (grasping reflex, appearance of incisors, eyelid opening, surface righting, preputial separation, and vaginal opening) were observed, although indices of learning and memory were not affected. At 10 mg/kg/day, dystocia (a potential pharmacological effect of muscarinic antagonism) was observed in one animal in the second study, while small decreases in pup weights and delays in development were observed in the first study. In the first study, developmental delays were observed at 3 mg/kg/day; however, in the second study which was better controlled for developmental age, to adjust for delays in parturition in some treated groups, no effects in pups were observed at 3 mg/kg/day.

2.2 Pharmacologic activity: Darifenacin is a selective M₃ muscarinic antagonist.

2.3 Nonclinical safety issues relevant to clinical use: The effects of darifenacin are primarily due to its known pharmacology. From preclinical data, darifenacin is expected to have minor effects on salivation and intestinal motility in the range of clinical doses; these effects have been quantitated clinically. Effects on QT prolongation are predicted by preclinical data to be minimal at about 5 times the average exposure of a 15 mg dose; however, effects have been observed clinically. The following chart includes two human data points (103 ng/ml mean total plasma concentration at 30 mg + ketoconazole, N = 16 and 45 ng/ml mean total plasma concentration at 60 mg) and three data points from isoflurane anesthetized dogs (107, 357, and 1440 ng/ml, N = 4).



The animal data therefore tends to confirm a change in QT (3.5 % or 12.1 msec in humans or 2.3% in dogs) at higher than the expected maximum clinical dose of 30 mg plus ketoconazole at mean total blood levels of 103 ng/ml and lack of a clinically significant effect at blood levels of 45 ng/ml or less.

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21513

Review number: 1

Sequence number/date/type of submission:

Information to sponsor: Yes (x) No ()

Sponsor (or agent): Pfizer Central Research, Eastern Point Road, Groton, CT 06340

Manufacturer for drug substance: Pfizer

Reviewer name: Laurie McLeod-Flynn

Division name: Division of Reproductive and Urologic Drug Products

HFD-580

Review completion date: 5 September 2003

Drug:

Code Name: UK-88,525-04

Generic Name: Darifenacin

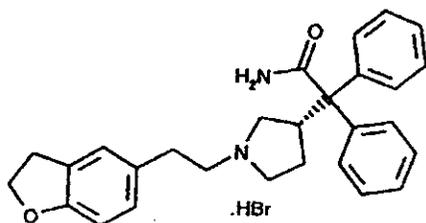
Trade Name:

Chemical Name: (S) -2- {1 - [2 - (2,3 - dihydrobenzofuran - 5- yl) ethyl] -3- pyrrolidinyl} -2,2-diphenylacetamide hydrobromide

CAS Registry Number:

Molecular Formula/ Molecular Weight: C₂₈H₃₀N₂O₂ • HBr / 507.5 for the hydrobromide salt, 426.6 for the base

Structure:



Relevant INDs/NDAs/DMFs:

Drug class: anti-muscarinic

Indication: treatment of the symptoms of overactive bladder

Clinical formulation:

Component	Quantity (g/Batch)	
	7.5 mg Tablets	15 mg Tablets
Darifenacin Hydrobromide	\	\
Dibasic Calcium Phosphate	\	\
Hydroxypropyl Methylcellulose	\	\
Magnesium Stearate	\	\
Total Weight	1,400,000	1,400,000

Route of administration: oral

Proposed use: starting dose of 7.5 mg once daily, and for patients requiring greater symptom relief, 15 mg once daily

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:**Pharmacology:**

The radioligand binding affinity of darifenacin (UK-88,525) for the human cloned muscarinic receptor subtypes M₁, M₂, M₃, M₄, and M₅ using [³H]-NMS.

Determination of the radioligand binding affinity of a range of muscarinic antagonists for the human cloned muscarinic receptor subtypes M₁, M₂, M₃, M₄, and M₅ using [³H]-NMS.

Further characterization of the radioligand binding affinity of darifenacin for the human recombinant M₃ receptor.

Radioligand binding screen of UK-84,149, UK-88,258, and UK-88,525.

Pharmacological effects of darifenacin on human isolated urinary bladder.

An evaluation of the effects of darifenacin (UK-88,525) and UK-148,993 on the isolated guinea-pig bladder preparation.

In vitro muscarinic receptor affinity profile of UK-88,525.

An investigation into the anti-muscarinic potency of darifenacin (UK-88,525) and its competitor compounds on M₁ receptors in the dog saphenous vein preparation.

An investigation of the potency of darifenacin (UK-88,525) and its competitor compounds at antagonizing M₂ receptors in the guinea pig atrial preparation.

The functional activity of darifenacin and competitor agents at human recombinant M₁, M₃, M₄, and M₅ receptors using microphysiology.

A comparison of the effects of darifenacin with oxybutynin, tolterodine and atropine on bladder contractility, salivary flow and heart rate in the anesthetized dog.

Comparison of darifenacin (UK-88,525) and atropine (UK-46,975) on bethanechol-induced smooth muscle responses in the anesthetized dog.

Oral efficacy of darifenacin against bethanechol-induced reduction in bladder capacity in the conscious

The effects of darifenacin (UK-88,525) on conscious rat cystometry and salivation.

The profiling of UK-148,993, UK-88,862-18, and UK-73,689 in various radioligand binding and enzyme assays.

A comparison of the effects of UK-148,993 and UK-200,881, the major active metabolites in man of darifenacin and oxybutynin, respectively, on bladder contractility, salivary flow and heart rate in the anesthetized dog.

The effects of darifenacin in comparison with UK-76,654 and atropine, on hemodynamics and CCK stimulated gastrointestinal motility in the anesthetized dog.

The effect of darifenacin on intestinal motility in the conscious dog.

The effect of oral darifenacin, zamifenacin and atropine on fasted motility in the conscious dog.

The effect of darifenacin (0.3, 1, and 3 mg/kg p.o.) on gastrointestinal propulsive activity in the conscious rat.

To determine the effects of orally administered UK-88,525 (darifenacin, 10, 30, and 100 mg/kg p.o.) on gastrointestinal propulsive activity in the rat.

A comparison of the effects of the anti-muscarinic agents, darifenacin, UK-76,654 and atropine on gastric emptying in the conscious dog.

The effect of darifenacin on gastric acid secretion in the conscious dog; a comparison with dicyclomine.

The effect of darifenacin on basal gastric acid secretion in the anesthetized rat.

A comparison of the functional antagonist potency of darifenacin (UK-88,525) and competitor agents in guinea pig isolated bladder and salivary gland.

Effect of darifenacin on methacholine-induced bronchoconstriction in the anesthetized guinea pig.

The effect of darifenacin on arterial blood pH, pCO₂ and pO₂ in the conscious rat.

The effect and selectivity of intravenously administered darifenacin and UK-148,993 on small bowel digestive motility in the conscious dog.

Safety Pharmacology:

General pharmacological properties of darifenacin

To determine any adverse effects upon activity and behavior induced by darifenacin in the rat over four days after a single oral dose.

The effects of darifenacin on alcohol and pentobarbitone sleeping times in the mouse.

The effect of darifenacin and atropine on pupil size and oxotremorine-induced salivation and tremor in the conscious mouse

Examination of the effect of darifenacin, UK-88,525-04, and the darifenacin metabolites, UK-88,862 and UK-73,689, on [³H]-dofetilide binding to the hERG potassium channel.

Summary report of the effect of the darifenacin metabolite, UK-148,993, on [³H]-dofetilide binding to the hERG potassium channel.

The effect of UK-88,525 and UK-148,993 on hERG potassium channels.

Effects of UK-88,525 and UK-148,993 on action potentials recorded from dog isolated Purkinje fibers *in vitro*.

Effects of UK-88,525-04 on action potentials recorded from dog isolated Purkinje fibers *in vitro*.

The hemodynamic and ECG effects of darifenacin (UK-88,525) following oral administration in the conscious dog.

The hemodynamic and electrophysiological effects of intravenously administered UK-88,525-04 in the isoflurane anesthetized dog.

The effects of darifenacin on ventricular fibrillation threshold and electroconversion to sinus rhythm in the anesthetized dog.

The effects of darifenacin on the excretion of fluid and electrolytes in the conscious rat.

The effects of darifenacin on the cardiovascular, autonomic and neuromuscular systems in the anesthetized cat.

Effect of darifenacin on electric eel acetylcholinesterase activity.

The effect of darifenacin on rat uterine smooth muscle. Effects of the darifenacin metabolites, UK-88,862 and UK-73,689 on action potentials recorded from dog isolated Purkinje fibers *in vitro*.

ADME:

Analysis of UK-88,525 and UK-148,993 in mouse plasma.

Analysis of UK-88,525 and UK-148,993 in mouse plasma by a specific HPLC-mass spectrometric method.

Analysis of UK-88,525 in rat plasma.

Analysis of UK-88,525 and UK-148,993 in rat plasma by a specific HPLC-mass spectrometric method.

Analysis of UK-88,525 in rabbit plasma.

Analysis of UK-88,525 in dog plasma.

Analysis of UK-88,525 and UK-148,993 in dog plasma by a specific HPLC-mass spectrometric method.

Pharmacokinetics of [¹⁴C]-UK-88,525 in the mouse following administration of a single intravenous (2 mg/kg) or oral (8 mg/kg) dose.

Pharmacokinetics of [¹⁴C]-UK-88,525 in the mouse following administration of a single oral (42 mg/kg) dose.

Pharmacokinetics of UK-88,525 in male and female rats following administration of a single intravenous (2.5 mg/kg) and oral (10 mg/kg) dose.

Pharmacokinetics of UK-88,525 in serial sampled male rats following a single intravenous (2.5 mg/kg) or oral (10 mg/kg) dose.

Pharmacokinetics of UK-88,525 in the dog following administration of a single intravenous (0.6 mg/kg) and oral (4 mg/kg) dose.

Pharmacokinetics of UK-88,525 in the rabbit after single oral administration (25 mg/kg).

Tissue distribution of [14 C]-UK-88,525 in male and female rats following administration of a single intravenous (4 mg/kg) dose.
Binding of [14 C]-UK-88,525 in the plasma of rabbit, rat, dog and man.
Binding of UK-88,525 and UK-148,993 in the plasma of mouse, rat, rabbit, dog, and man.
An investigation of the first pass metabolism of UK-88,525 in the dog following administration of single (0.6 mg/kg and 1.2 mg/kg) and multiple (0.6 mg/kg) portal doses.
Analysis of the UK-148,993 concentrations in dog plasma following administration of a single intravenous (0.5 mg/kg) and oral (4 mg/kg) UK-88,525 dose.
In vitro metabolism of UK-88,525.
Circulating concentrations of UK-73,689 and UK-88,862 following single oral administration of 86 mg/kg UK-88,525 to male mice.
Circulating concentrations of UK-73,689 and UK-88,862 following single oral administration of 10 mg/kg UK-88,525 to rat.
Circulating concentrations of UK-73,689 and UK-88,862 following single oral administration of 10 mg/kg UK-88,525 to dog.
The excretion and metabolism of [14 C]-UK-88,525 in male mice following administration of a single oral dose (50 mg/kg) dose.
The excretion and metabolism of [14 C]-UK-88,525 in the rat following administration of a single intravenous (2.5 mg/kg) or oral (10 mg/kg) dose.
The excretion and metabolism of [14 C]-UK-88,525 in male rats following administration of a single oral (10 mg/kg) dose.
The excretion and metabolism of [14 C]-UK-88,525 in female rabbits following administration of a single oral (10 mg/kg) dose.
The excretion and metabolism profile of [14 C]-UK-88,525 in the dog following single intravenous (0.6 mg/kg) and oral (4 mg/kg) doses.
The excretion and metabolism of [14 C]-UK-88,525 in two dogs following administration of a single oral (4 mg/kg) dose.
The secretion of radioactivity into rat milk following oral administration of [14 C]-UK-88,525 (10 mg/kg).

General toxicology:

Exploratory acute study in male and female mice.
Oral and intraperitoneal acute toxicity study in mice and rats.
14-Day oral range-finding in rats
14-day oral range-finding in dogs.
15-day oral range-finding in dogs.
A one-month gavage study in Sprague-Dawley rats.
1-month oral toxicity study in Sprague-Dawley rats.
Effects of 1-month dietary administration to rats of UK-88,525-04 on body weight, food consumption and the incidence of mydriasis.
3-month dietary preshrink toxicity in CD-1 mice.
3-month dietary preshrink toxicity in Sprague-Dawley rats.
6-month oral toxicity in Sprague-Dawley rats.
2-week intravenous toxicity study in Sprague-Dawley rats
A one month gavage study in Beagle dogs.
6-month oral toxicity in Beagle dogs
1 Year oral capsule study in Beagle dogs
2-week intravenous toxicity study in Beagle dogs.

Genotoxicity:

UK—88,525 Darifenacin hydrobromide microbial reverse mutation (Ames) assays I and II
Chinese hamster ovary/hypoxanthine-guanine phosphoribosyl transferase assay.
Human lymphocyte chromosomal aberration assay.
Mouse *in vivo* bone marrow metaphase analysis.

Carcinogenicity:

24-month in-diet toxicity and carcinogenicity study in CD-1 mice.
 24-month in-diet toxicity and carcinogenicity study in Sprague-Dawley rats.

Reproductive and developmental toxicity:

Preliminary reproduction study in Sprague-Dawley rats by the oral route.
 Fertility (segment I) study in rats by the oral route.
 Maternal toxicity study in rats by the oral route
 Maternal toxicity study in rabbits by the oral route.
 Teratology (segment II) study in Sprague-Dawley rats by the oral route.
 Teratology (segment II) study in New Zealand white rabbits by the oral route.
 Peri and postnatal development study (segment III) in Sprague-Dawley rats by the oral route.
 Oral pre- and postnatal development study of UK-88,525-04 in rats: assessment of learning and memory of F1 pups.

Local tolerance:

UK-88,525-04 primary dermal irritation study in the rabbit.
 UK-88,525 and UK-88,525-04 seven day dermal irritation study in the rabbit.
 Antigenicity study in guinea pigs.

3.2 PHARMACOLOGY

3.2.1 Brief summary: Pharmacology data is summarized below. In addition, see attached previous review in Appendix A.

3.2.2 Primary pharmacodynamics:

Mechanism of action: Darifenacin is a selective M₃ muscarinic receptor antagonist as demonstrated by binding affinity and receptor antagonist potency.

Human muscarinic receptor binding affinity (mean pK_i, N=4-8).

	M ₁	M ₂	M ₃	M ₄	M ₅
Darifenacin	8.15*	7.35*	9.12	7.35*	8.03*
<i>M3 selectivity^f</i>	<i>9</i>	<i>59</i>		<i>59</i>	<i>12</i>
Atropine	9.55	8.88*	9.56	8.94*	9.18*
<i>M3 selectivity^f</i>	<i>1</i>	<i>5</i>		<i>4</i>	<i>2</i>

* Comparative selectivity for the M₃ receptor over other receptor sub-types given in italics.
 * denotes pK_i at this receptor significantly different from that at M₂ receptor (P<0.05).
 Affinity data for other muscarinic receptor antagonists used in the treatment of OAB given in Pharmacology Written Summary (Module 2.6.2, Section 2.1.1)

Functional muscarinic antagonist potencies (pA₂) *in vitro* (N=4-8).

	M ₃			M ₁	M ₂
	Bladder ^b	Ileum ^c	Trachea ^c	Saphenous vein ^d	Atrium ^e
Darifenacin	8.9*	9.4	8.7	7.2	7.2
<i>M3 selectivity^f</i>				<i>50</i>	<i>50</i>
Atropine	9.2*	9.4	9.2	9.6	9.1
<i>M3 selectivity^f</i>				<i>0.4</i>	<i>1.3</i>

All tissues from guinea pig except dog saphenous vein. * - pK_a.
 * Comparative selectivity for the M₃ receptor in the bladder given in italics.
 CG003999 - ^b D1/004/89 - ^c CG003/01 - ^d CG002/01

Drug activity related to proposed indication:

Parameter	Effective Dose
Inhibition of neurogenic bladder contractions in the anaesthetised dog ^a .	ID ₅₀ 6.7µg/kg i.v.
Inhibition of bethanechol induced bladder contractions in the anaesthetised dog ^b .	ID ₅₀ 14.9µg/kg i.v.
Inhibition of bethanechol induced reductions in bladder capacity in the conscious dog ^c .	0.3 to 3mg/kg p.o.
Reduction in micturition pressure during cystometry in conscious rats ^d .	Minimum effective doses: 0.1mg/kg i.v. and 1mg/kg p.o.

a - DI/025/94. b - DI/005/92. c - DI/001/91. d - DI/020/94

3.2.3 Secondary pharmacodynamics

Comparative profiles of orally administered darifenacin and atropine in studies of gastrointestinal function in dogs and rodents

Effect	Darifenacin	Atropine
Inhibition of food-stimulated motility in the dog	MED 0.1mg/kg (DI/017/94)	ED ₅₀ 0.04mg/kg ^a
Prolongation of cycle length of fasted motility pattern in the dog (CG/001/98)	No effect at 0.2mg/kg	Prolonged at 0.1mg/kg
Gastric emptying in the dog (DI/004/90)	ID ₅₀ 2.5mg/kg	ID ₅₀ 0.3mg/kg
Gastric acid secretion in the dog (DI/006/95)	↓ at 3mg/kg	↓ at 0.01mg/kg
GI propulsive activity in rodents (DI/008/90, 95P-GPH-4 & CG/007/01)	↓7-23% over dose range of 0.3 to 100mg/kg	↓14-41% over dose range of 10 to 100mg/kg
Gastric emptying in the rat (CG/007/01)	↓ at ≥10mg/kg ^b	↓ at ≥10mg/kg ^b
Gastric acid secretion in the rat (95P-GPH-8, DB/007/90)	↓ at ≥1mg/kg	↓ at 1mg/kg
Bile secretion in the rat (95P-GPH-9)	No effect at <100mg/kg	n.d.

* Module 2.6.3 Tabulated Summary, Section 2.1. MED minimum effective dose. ↓ decreased.
^a Source: Wallis and Napier, 1999. ^b no effect dose was not determined
 Studies highlighted in bold show darifenacin to be weaker than atropine, suggesting that the effect is not mediated by muscarinic M₃ receptors

Effective doses of darifenacin as an inhibitor of salivation *in vivo*.

Parameter	Effective Dose
Inhibition of nerve-stimulated increases in salivary flow in anaesthetised dogs (DI/025/94) ^a	ID ₅₀ 62µg/kg, i.v.
Inhibition of basal salivation in conscious dogs (DI/017/94) ^b	Doses of ≥ 300µg/kg, p.o.
Inhibition of methacholine induced salivation in conscious rats (DI/020/94) ^a	Doses of ≥ 300µg/kg, i.v.

^a - Module 2.6.3 Tabulated Summary, Section 1.3. ^b - Module 2.6.3 Tabulated Summary, Section 2.1

Comparative muscarinic antagonist potencies of darifenacin, atropine, tolterodine and oxybutynin in guinea pig bladder and salivary gland *in vitro* (pK_B for inhibition of inositol phosphate generation (N=3-5)).

Compound	Bladder	Salivary gland	Bladder selectivity ratio
Darifenacin	9.15	8.23	8.4
Atropine	8.90	9.05	0.7
Tolterodine	8.77	7.99	6.0
Oxybutynin	8.07	7.27	6.3

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3.2.4 Safety pharmacology: Please see attached review in appendix A in addition to studies reviewed below.

Cardiovascular effects:

Examination of the effect of Darifenacin, UK-088,525-04 and the Darifenacin metabolites, UK-088,862 and UK-073,689 on [³H]-dofetilide binding to the hERG potassium channel (UK088525-04/IC/013/01). UK-088,525-04 (Darifenacin) (n=6) competitively displaced [³H]-dofetilide to hERG expressed in HEK293 cells to give an IC_{50} value of 1.1×10^{-6} M (550 ng/ml) and an IC_{10} of about 1×10^{-7} M (50 ng/ml). UK-088,862 (N=6) had no effect up to 4×10^{-7} M (112.2 ng/ml). UK-073,689 (n=6) had no effect up to 6×10^{-7} M (106.9 ng/ml). The standard compound dofetilide had an IC_{50} of 1.0×10^{-8} M.

Summary report of the effect of the Darifenacin metabolite, UK-148,993, on [³H]-dofetilide binding to the hERG potassium channel (UK999999/020/99). UK-148,993 had little or no effect on [³H]-dofetilide binding at concentrations up to 1×10^{-7} M (44.3 ng/ml).

The effect of UK-88,525 and UK 148, 993 on hERG potassium channels (UK088525 /IC/010/01). The effect of bath applied UK-88,525-04 and its circulating human metabolites, UK-148,993, UK-88,862 and UK-73,689 on hERG potassium channels expressed in HEK293 cells, were examined using the *in vitro* whole cell patch clamp technique. The parent UK-88,525-04 exhibited an IC_{50} of 77 nM (39.1 ng/ml) (IC_{30} at ~30 nM; IC_{10} at ~10 nM). The metabolites, UK-148,993 (100 nM or 44.26 ng/ml), UK-88,862 (400 nM or 112.16 ng/ml) and UK-73,689 (600 nM or 106.92 ng/ml) had little or no effect on the hERG potassium current ($12.7 \pm 3.2\%$, $2.4 \pm 3.5\%$, and $0.9 \pm 3.3\%$, respectively).

Effects of UK-088,525 and UK-148,993 on action potentials recorded from dog isolated Purkinje fibres *in vitro* (UK088525/IC/009/01). UK-088,525-04 (n=5) had no significant effect on membrane potential, action potential amplitude or Vmax at concentrations up to 100 nM when compared to time matched vehicle controls. No effect on action potential duration (APD₅₀ or APD₉₀) was observed up to 10 nM UK-088,525-04; however, at 30 nM and 100 nM, there was a concentration-related reduction in APD₅₀ with no effect on APD₉₀. No effects of UK-148,993 on any of the above mentioned parameters was observed at concentrations up to 50 nM. A large number of impurities were included in this analysis; concentrations were appropriately corrected. The data is suggestive of an effect on calcium channels.

Effects of UK-099,525-04 on action potentials recorded from dog isolated Purkinje fibres *in vitro* (UK088525-04/IC/012/01). UK-088,525-04 (n=5) had no statistically significant effect up to 10000 nM on resting membrane potential when compared to time matched vehicle controls. UK-088,525-04 produced concentration-related reductions in both action potential amplitude and Vmax, with significance reached (p < 0.01) at 10000 nM. UK-088,525-04 produced concentration related reductions in both APD₅₀ and APD₉₀ with significance at 1000 nM and 10000 nM. A possible mechanism involving sodium and calcium channels is suggested.

Effects of the Darifenacin metabolites, UK-088,862 and UK-073,689 on action potentials recorded from dog isolated Purkinje fibres *in vitro* (UK088862/IC/001/02). UK-088,862 (n=5), up to 400 nM or 112.2 ng/ml and UK-073,689, up to 600 nM or 106.9 ng/ml had no effect on membrane potential, action potential amplitude, Vmax, APD₅₀, or APD₉₀.

The hemodynamic and electrophysiological effects of intravenously administered UK-088525-04 in the isoflurane anaesthetized dog (UK088525/CG/017/01). The hemodynamic and electrophysiological effects of UK-088,525-04 following cumulative 45 minute infusions of 1.27, 5.47, 18.07, and 60.07 µg/kg were compared to vehicle infusions (5% DMSO, 15% hydroxypropyl β cyclodextrin in 0.9% saline) in isoflurane anesthetized dogs (N=4/group).

Sinus rhythm	Dog				Human				
	TBM formulation (mg)				7.5	15	60	15**	30**
Infusion dose (µg/kg)	1.27	5.47	17.07	60.07					
Mean total plasma conc. (ng/ml)	25.8	107	357	1440	1.94	5.86	45	67.6	103
Mean total plasma conc. (nM)	60.4	250	836	3370					
Free plasma concentrn. (ng/ml)	0.8	3.2	10.7	43.2					
Free plasma concentrn. (nM)	1.8	7.5	25.1	101	0.3	~2.3	~3.5	~5.3	
Diastolic blood pressure				-23%					
Heart rate				-19%					
QT interval (%)		+2.3	+7%	+16.2%					
Monophasic action potential**			+9.9%	+17.8%					
PR and QRS interval			↑	↑					

* 400 mg ketoconazole QD x 6 days

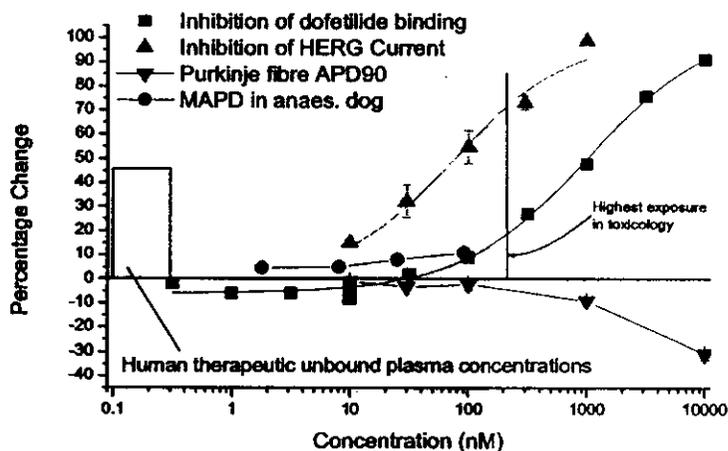
**from the endocardial surface of the right ventricle

Atrial pacing*	Dog			
Free plasma concentration (ng/ml)	0.8	3.2	10.7	43.2
MAP duration				
_ 150 bpm			+8.1%	+11%
_ 160 bpm				+8.2%
_ 170 bpm				+8.4%
_ 180 bpm				+9.4%
_ 190 bpm				+7.5%
AH interval slope				↑
HV interval slope			↑	

* compared to vehicle controls

Effects on QT interval at higher doses are consistent with changes in the potassium IKr channels in hERG studies . Increases in ECG QRS and PR intervals at the highest dose, together with decreases in heart rate, diastolic blood pressure and cardiac contractility, suggest mixed ion channel antagonistic properties on sodium and calcium channels.

Graphical summary of the effects of darifenacin in non-clinical assays examining the potential to prolong the electrocardiogram QT-interval in humans.



The assays shown are inhibition of [³H]dofetilide-binding (IC/013/01), inhibition of hERG current (IC/010/01), canine isolated Purkinje fibre APD90 (IC/009/01 & IC/012/01) and MAPD in the isoflurane anaesthetised dog (CG/017/01). Also shown are the mean unbound plasma concentrations associated with 7.5mg and 15mg p.o. treatment (left and right boundaries of the box), and the highest exposure in dog toxicology studies.

Pharmacological profile of darifenacin and its metabolites.

		Darifenacin	UK-148,993	UK-88,862	UK-73,689
Muscarinic receptor affinity (pK _i) ^a	M ₁	8.15	7.79	5.90	<6
	M ₂	7.35	7.13	<6	<6
	M ₃	9.12	8.54	5.83	<6
	M ₄	7.35	7.76	5.80	<6
	M ₅	8.03	8.19	6.19	<6
<i>in vitro</i> cardiac ion channel assays	Dofetilide binding ^b	IC ₅₀ 1.1µM	n.d.		
	HERG activity ^c	IC ₅₀ 77nM	No effects up to 100nM	No effects up to 400nM ^e	No effects up to 600nM ^e
	Purkinje fibre	↓APD ₅₀ ≥30nM ^d	No effects up to 50nM ^d		
Wide radioligand profile	No interactions up to 1µM ^f	Affinity for opioid and calcium binding sites at 10µM ^g	No interactions up to 10µM ^g	No interactions up to 10µM ^g	

n.d. not determined - ^a from studies DI/004/96 and CG/004/00 - ^b IC/013/01 - ^c IC/010/01
^d IC/009/01 - ^e Purkinje fibre data from IC/001/02 - ^f DB/009/89 - ^g CG/001/00

The effects of Darifenacin on ventricular fibrillation threshold and electroconversion to sinus rhythm in the anaesthetized dog (UK-88,525/D1/002/90). Darifenacin (1.0 mg base/kg, R1 batch, iv over 10-15 seconds) raised the fibrillation threshold and exhibited anti-arrhythmic activity by impairing the ability to induce ventricular fibrillation. The ability to defibrillate was facilitated. Heart rate and dP/dt max were decreased 10% and 15%, respectively, compared to control. Blood pressure and left ventricular pressure were depressed (30 % and 20 %, respectively) 30-60 seconds following administration, but returned to pre-dose in 2-10 minutes. The unbound plasma concentrations of Darifenacin at 5 minutes post-injection were 57.1 nM, approximately 190 times higher than the unbound steady state concentrations for humans (0.3 nM). Blood gases and electrolytes were not affected throughout the study.

The hemodynamic and ECG effects of Darifenacin (UK-88,525) following oral administration in the conscious dog (UK88525/DI/10/90). A no-effect level for hemodynamic effects was 1 mg/kg. At 3 mg/kg increases in heart rate (20-30 beats/minute) and cardiac output (~0.5 L/min) were observed approximately 30 minutes following dosing; blood pressure was maintained by a reduction in systemic vascular resistance. These effects persisted up to 180 minutes following dosing. No effects on ECG parameters were observed except for those caused by the changes in heart rate.

Pulmonary effects:

Effect of Darifenacin on methacholine-induced bronchoconstriction in the anaesthetized guinea pig (UK88525/DI/13/90). Bronchoconstriction, as monitored by an increase in lung resistance, reached a maximum in 30 seconds and recovered to baseline after 5 minutes post-methacholine administration. Guinea pigs (n=4) were given iv infusions to give a 45-150 % increase in lung resistance. Saliva was measured using a pre-weighed cotton ball left in the guinea pig's mouth for 10 minutes. Darifenacin hydrobromide (1, 3, 10, 30, 100, and 300 µg/kg in 6%PEG200/saline, 6 iv doses of 1 ml/kg) produced dose-related inhibition of methacholine-induced bronchoconstriction. The ID₅₀ for lung resistance was 8.4±0.92 µg/kg and for salivation was 57.1±5.4 µg/kg, with a lung to salivation selectivity ratio of about 6.8 to 1.

Gastrointestinal effects:

The effect and selectivity of intravenously administered darifenacin and UK-148,993 on small bowel digestive motility in the conscious dog (UK-88,525-04/DI/003/97 and UK-148,993/DI/001/97). Darifenacin (0.03 –1 ug/kg/min) and its hydroxylated metabolite UK-148,993 (0.1-3 ug/kg/min) caused dose related inhibition of jejunal motility which persisted for approximately 2 hours. No effects on pupil diameter or heart rate were observed under these conditions.

	UK-88,525	UK-148,993
Minimum dose with effect on motility (µg/kg/min)	0.1	0.3
Peak fall in motility after maximum dose (after 75 min.)	78%	77%
Minimum dose with effect on salivation (µg/kg/min)	0.3	1.0
Peak decrease in salivation after maximum dose	65%	65%

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3.3 PHARMACOKINETICS/TOXICOKINETICS

3.3.1 Brief summary: In addition to data summarized in this section, please see the attached review in Appendix A

3.3.3 Absorption: Bioavailability was determined to be about 16% in rats and 105% in dogs.

3.3.4 Distribution: The volumes of distribution of Darifenacin in mouse, rat, and dog were greater than 0.7 L/kg, indicating that in spite of its high plasma protein binding, it distributes beyond blood volume, probably due to its lipophilic nature. Darifenacin related radioactivity was extensively distributed to most tissues and most was eliminated within 24 hours. Foci of radioactivity were the liver and gut contents, and retina, skin, and substantia nigra (consistent with the lipophilic, basic nature of the drug) in which the half life was about 3 days. Darifenacin and its N-dealkylated metabolite UK-73,689 were poorly distributed to the brain, but the monohydroxylated metabolite UK-148,993 was observed at higher concentrations in the brain.

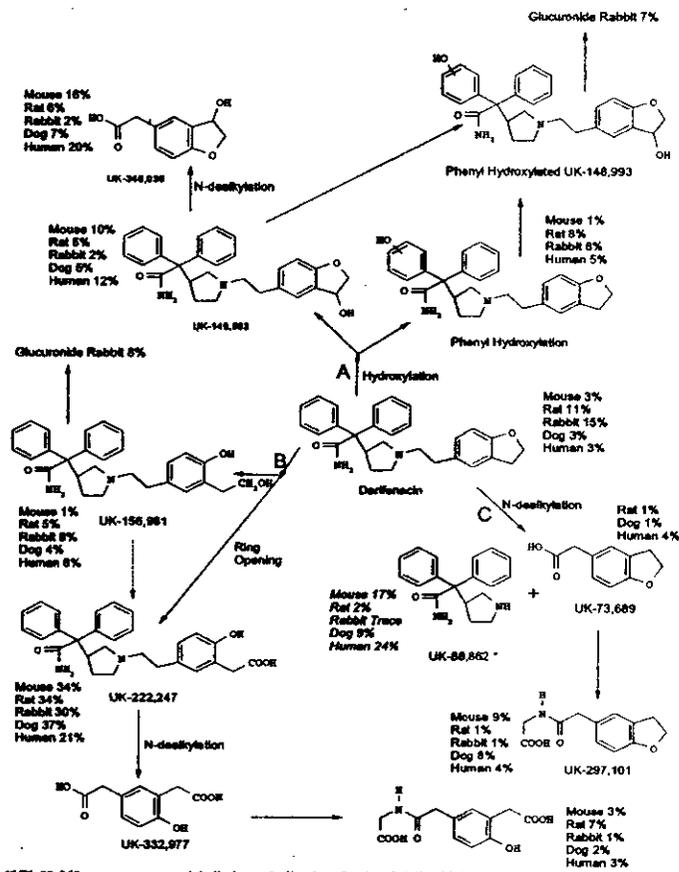
BLOOD TO PLASMA RATIOS OF [¹⁴C]-UK-88,525 AT A NOMINAL CONCENTRATION OF 16.8µg/ml (FREE BASE)

Species	Blood to plasma concentration ratio (mean n=3)
Mouse	0.99 ± 0.04
Rat	0.68 ± 0.02
Rabbit	1.60 ± 0.02
Dog	0.73 ± 0.04

APPEARS THIS WAY ON ORIGINAL

3.3.5 Metabolism

Proposed metabolites of darifenacin (% of the dose) following administration of [¹⁴C]-darifenacin to mouse, rat, rabbit, dog, and human.



*UK-88,862 represents an unlabelled metabolite (see Section 3.4.1) which was determined by specific assay of urine. All other identified metabolites contain the radiolabelled centre. In calculating the percentages of the dose, the total amount of radioactivity has been considered and thus an estimate of the dose (on a molar basis) excreted as UK-88,862 added. This leads to an overestimate of the total dose accounted for by these metabolites since effectively route C metabolites have been accounted for twice.

Circulating metabolites of darifenacin in toxicology species and humans (relative to parent).

Species (Study No.)	Dose	Relative molar concentrations at C _{max} (relative to darifenacin)			
		Darifenacin ¹	UK-148,993 ¹	UK-73,689	UK-88,862
Mouse (DM2A,DM19)	86mg/kg	1.0	1.83	6.30	0.83
Rat (DM3A, DM20)	10mg/kg	1.0	0.50	3.24	0.25
Dog (DM12, DM21)	10mg/kg	1.0	1.09	0.40	0.22
Human (DM22, DM11)	60mg	1.0	1.66	2.13	0.33

¹ Plasma concentrations extrapolated from 42mg/kg (mouse; DM2A); 4mg/kg (dog; DM12); 5mg (human; DM11). Rat was carried out at the same dose level in both studies 10mg/kg (DM3A).

3.3.6 Excretion: In the rat, rabbit and the dog, the predominant route of excretion was in the feces. In mouse and human, excretion was in both urine and feces.

3.3.10 Tables and figures, including comparative TK summary

Male mice (from Study No. 92046)

Dose (mg/kg/day, dietary)	Darifenacin AUC (nghr/ml)	UK-148, 993 AUC (nghr/ml)
25	574	768
50	874	1440
100	1548	3528

Rats (from Study No. 92051)

Dose (mg/kg/day, dietary)	Darifenacin AUC (nghr/ml)		UK-148, 993 AUC (nghr/ml)	
	Males	Females	Males	Females
25	1500	3128	716	1360
50	4168	5202	2174	2870
100	8534	15420	4446	7710

Humans

Dose (mg)	C _{max} (ng/ml)	AUC (nghr/ml)	Free AUC (nghr/ml)
7.5	1.94	29.7	
15	5.86	86.7	1.96
60 (30 mg x 2)	47.5	775	
7.5 + ketoconazole (400 mg QD, 6 days)	11.2	143	
15 + ketoconazole (400 mg QD, 6 days)	67.6	1110	
30 + ketoconazole (400 mg QD, 6 days)	130	2110	

Protein binding

Species	% Darifenacin bound to plasma protein		
	At 107 ng/ml	At 536 ng/ml	
Rabbit	78.9	77.3	76
Rat	95.4	94.1	98
Mouse			96
Dog	93.7	92.4	98
Human	94.2	94.3	98

Exposure multiples based on dose and plasma concentrations.

Darifenacin Toxicology Study	Reference dose (mg/kg)	Multiples over the 15mg controlled release formulation (0.3mg/kg) (Phase I subjects)				
		Dose	Total C _{max}	Free C _{max}	Total AUC _{24h}	Free AUC _{24h}
Species and Gender						
Mouse (M)	100	333	16	32	15.8	31.6
Rat (M + F)	10	33	14.4	14.4	8.9	8.7
Dog (M + F)	3	10	67.1	100.8	29	43.9

The mouse data are from an in-diet study. The other species were dosed by gavage. Studies used in the calculation of these multiples were mouse (92046, day 21), rat (92028, day 176) and dog (92029, day 177).

Free plasma darifenacin exposure at high doses in reproductive studies:

Species/ Study	Dose (mg/kg)	Free C _{max} (ng/ml)	Free AUC _{24h} (ng.h/ml)	Multiple of MRHD (mg/kg)	Multiple of MRHD (mg/m ²)
Rat Fertility (segment I)*	50	15.2	153	167	35
Rat teratology (segment II)	50	12.8	115	167	35
Rabbit teratology (segment II)	30	12.0	55	100	39
Pre and post- natal	50	15.4	170	167	35

Human data (Phase I data): Dose=0.3mg/kg, free C_{max}= 0.125ng/ml, free AUC= 1.96ng.h/ml.
 * data taken from 1-month rat study (94072)

Pharmacokinetic parameters for UK-88,525 following oral administration at a nominal dose of 25 mg base/kg:

Parameter	Rabbit 1	Rabbit 2	Rabbit 3	Mean±sd
Terminal half-life(h)	1			6.8±4.5
AUC _t (ng.h/ml)				189.1±43.4
C _{max} (ng/ml)				30.3±12.0
T _{max} (h)			1	4±3.6

CONCENTRATION OF TOTAL RADIOACTIVITY IN MILK AND PLASMA FOLLOWING SINGLE ORAL ADMINISTRATION OF [¹⁴C]-UK-88,525 AT A NOMINAL DOSE LEVEL OF 10mg/kg

Time (h)	Animal ID	Plasma (ng equiv/ml)	Milk (ng equiv/ml)	Ratio Milk:Plasma
2	1			0.88
	2			0.76
	3			0.79
	4			0.82
	5			1.39
	Mean (SD)	810 (191)	752 (253)	0.93 (0.26)
4	6			5.47
	7			1.20
	8			1.35
	Mean (SD)	528 (262)	1077 (535)	2.67 (2.42)
12	9			3.47
	10			4.91
	11			5.00
	Mean (SD)	66 (17)	289 (81)	4.46 (0.86)
24	12			0.88*
	13			0.95
	14			1.18
	Mean (SD)	22 (5)	21*(8.7)	1.00*(0.16)

* Results calculated from data less than 30dpm above background
 * Mean includes results calculated from data less than 30dpm above background

3.4 TOXICOLOGY

3.4.1 Overall toxicology summary

General toxicology:

Rats were tested up to 30 mg/kg/day in a 6 month toxicology study, and dogs were tested up to 10 mg/kg/day in a 6 month study and 6 mg/kg/day in a one year study. Maximum tolerated doses were based on body weight and poor general condition in rats and on severe pharmacological effects in dogs. Effects were primarily due to the known pharmacology of the drug: mydriasis (and associated ocular effects), partially closed eyes, dry mouth, reduced intestinal motility (and associated dysphagia and emesis), hemodynamic effects, and hypersecretion of the Harderian gland. Increases in liver weight were observed, without histopathological correlate and with associated increases in metabolic enzymes. While not clearly a pharmacological effect, effects on indices of QT prolongation similar to other anti-muscarinic drugs were observed (see details in the Safety Pharmacology section). Although measures of direct effects on potassium channels indicate little or no safety margin, a margin of about 5 times the average human exposure to a 15 mg dose of darifenacin is indicated by *in vivo* studies in dogs.

Genetic toxicology:

Darifenacin was not mutagenic in the Ames test for bacterial mutation or in the Chinese hamster ovary assay. It was not clastogenic *in vitro* in the human lymphocyte assay or *in vivo* in the mouse bone marrow cytogenetics assay.

Carcinogenicity:

Darifenacin was tested at the maximally tolerated dose (based on body weight) in two year assays in male and female rats and mice. Statistical analysis determined that no increase in tumors was clearly related to the administration of darifenacin. The multiples of human exposure after dietary administration were as follows:

	Male rats	Female rats	Male mice	Female mice
Nominal dose (mg/kg/day)	15	15	100	100
Multiple of human 15 mg AUC	7 X	12 X	32 X	32 X

Reproductive toxicology:

	Rat Seg.I	Rat Seg.II	Rabbit Seg.II	Rat Seg. III
Nominal dose (mg/kg/day)	3, 10, 50	3, 10, 50	3, 10, 30	3, 10, 50
Multiple of human 15 mg AUC	4.6, 16, 78	3.5, 12, 59	2.8, 9, 28	5, 17, 87
Maternal no-effect level (mg/kg/day)	50 (fertility)	10	10	3
Offspring no-effect level (mg/kg/day)	10	10	3	3

In a segment I fertility study in rats, darifenacin was administered to males from 64 days ante-coitum and to females from days 14 days ante-coitum to 20 days post insemination or 21 days postpartum at 0, 3, 10, and 50 mg/kg/day. In the 50 mg/kg/day F0 group, 2 males and 2 females died and body weights were decreased. Exaggerated pharmacology was observed in the 10 and 50 mg/kg/day groups. Fertility was decreased in the 10 and 50 mg/kg/day groups but values were not statistically significant nor dose related and were still within the historical control values. In the 50 mg/kg/day group, post implantation loss was increased, and the number of viable pups per dam, pup weight, and pup survival were decreased. Reduced ossification of the sacral and caudal vertebrae and urinary bladder dilatation of the offspring was observed.

In a segment II study in rats, darifenacin was administered to dams from days 6-17 days of gestation at 0, 3, 10, and 50 mg/kg/day. Maternal body weights were reduced in the 50 mg/kg/day group. At this dose, pup weights were reduced and delays in ossification of the sacral and caudal vertebrae of offspring were observed.

In a segment II study in rabbits, darifenacin was administered to dams from days 6 to 18 of gestation at 0, 3, 10, and 30 mg/kg/day. At 30 mg/kg/day, 2 dams aborted, and effects on body weight and food consumption were observed. At this dose, post implantation loss was increased and the number of viable fetuses per litter was increased. In the offspring, dilated ureter and/or kidney pelvis was observed at 30 mg/kg/day and 1 case was observed at 10 mg/kg/day, along with urinary bladder dilation, consistent with pharmacological activity.

Two segment III developmental studies were performed in rats covering periods from 15 days post-insemination to day 20 postpartum and from day 6 postinsemination to day 21 postpartum, both at doses of 0, 3, 10, and 50 mg/kg/day. Maternal toxicity was observed at 50 mg/kg/day as evidenced by effects on body weight and food consumption and by dystocia, and prolonged gestation and parturition in both studies. Hypogalactia was also observed at 50 mg/kg/day, along with increased mortality of pups between days 1 and 4 postpartum, with an increased incidence of dilatation of the urinary bladder or renal pelvis. At this dose, decreased body weights of pups and delays in developmental landmarks (grasping reflex, appearance of incisors, eyelid opening, surface righting, preputial separation, and vaginal opening) were observed, although indices of learning and memory were not affected. At 10 mg/kg/day, dystocia (a potential pharmacological effect of muscarinic antagonism) was observed in one animal in the second study, while small decreases in pup weights and delays in development were observed in the first study. In the first study, developmental delays were observed at 3 mg/kg/day; however, in the second study which was better controlled for developmental age, to adjust for delays in parturition in some treated groups, no effects in pups were observed at 3 mg/kg/day.

3.4.2 Single-dose toxicity: In addition, please see review in Appendix A.

Acute toxicity of darifenacin in mice and rats following oral or intraperitoneal administration.

Species	Route	Dose, mg/kg	Maximum nonlethal dose, mg/kg	Minimum lethal dose, mg/kg	Time to Death
Mice	Oral	100 (5/sex/dose) 200 (2/sex/dose)	N.D.	100	< 1 hr
Mice	IP	50 (5/sex/dose) 100 (2/sex/dose)	50	50 < dose <100	10-20 min
Rats	Oral	100 (5/sex/dose) 200 (2/sex/dose)	100	100 < dose <200	6 hr
Rats	IP	50 (5/sex/dose)	N.D.	50	5 min

3.4.3 Repeat-dose toxicity: In addition, please see review in Appendix A.

Study Title: UK-88,525-4 1 Year Oral Capsule Study in Beagle Dogs. Dose Levels: 0,1,3, and 6 mg/kg/day

Study No: 94-751-08

Volume # 9, and page #1

Conducting laboratory and location: Central Research Division, Pfizer Inc., Eastern Point Road, Groton, CT 06340

Date of study initiation: 11/28/94

GLP compliance: yes

QA- Report Yes (X) No ()

Dosing:

- species/strain: dogs, beagle
- #/sex/group or time point: 4/sex/dose
- age: 8 months
- weight: 10.0 – 14.0 kg for males, 7.9 – 11.4 kg for females
- satellite groups used for toxicokinetics or recovery:
- dosage groups in administered units: 0, 1, 3, and 6 mg/kg/day
- route, form, volume, and infusion rate: gelatin capsules, once daily in the morning

Drug, lot#, radiolabel, and % purity: Combinations of the following capsules to achieve dose:

Batch Number	Strength	Expiration Date
3039-187	Placebo	October 1999
3039-190	0.5 mg	October 1996
3039-191	1.0 mg	October 1996
3039-192	10.0 mg	October 1996
3039-193	3.0 mg	October 1996
3039-194	30.0 mg	October 1996

Formulation/vehicle: mixture of lactose regular, maize starch, colloidal anhydrous silica, and magnesium stearate

Observations and times:

- Ophthalmoscopy: pre-study and days 83-86, 166-169, 279-282, and 35-356.
- EKG: leads I, II, III, aVR, aVL, aVF, CV₆LL, and indirect systolic blood pressure recordings, twice pre-study and days 99, 184, 267, and 351 (pre-dose and ~2 hours post dose)
- Toxicokinetics: plasma concentrations of UK-88,525 and the active metabolite UK-148,993, 2,4,6,8 and 24 hours post-dose on days 177 & 366
- Heart rate: twice pre-study and dose days 99, 184, 267, and 351 (pre-dose and ~2 hours post dose)

Results:

- Clinical signs:

	1 mg/kg	3 mg/kg	6 mg/kg
Death	0	0	0
Dryness of mouth (mucous membranes)	1-7 hrs	1-7 hrs	1-7 hrs
Tremors (days 110, 295), difficulty maintaining balance (day 295)			1 male

- Body weights: No treatment related effects on body weights were observed.
- Food consumption: No treatment related effects on food consumption were observed.
- Ophthalmoscopy :

Incidence of Ocular Findings

OBSERVATION	1 mg/kg	3 mg/kg	6 mg/kg
Mydriasis	0/8	8/8	8/8
Blepharospasm/ conjunctival hyperemia (3+ months)	0/8	1/8	3/8
Ulcers of the cornea:			
__ with erosions (day 10 HD female)	0/8	0/8	1/8
__ with opacities (9 months HD male)	0/8	0/8	1/8
Neovascularization (3 months)	0/8	0/8	2/8
Pupillary light response	"slow"	diminished	"slight"

- Hematology:

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	1	3	6	0	1	3	6
Eosinophils (ct/mm³)								
__ day -20	223.0 +103.17 204.8	185.5 +29.44 165.5	243.5 +103.10 277.3	229.0 +83.55 184.3	178.8 +89.75 170.3	170.3 +52.10 327.5	166.0 +74.14 164.0	201.0 +102.82 218.8
__ day -13	+90.61 163.0	+44.43 238.3	+116.25 421.5	+39.37 361.0	+121.49 317.5	+261.33 304.8	+90.28 198.3	+48.02 317.0
__ day 40	+62.35 300.0	+115.96 341.3	+187.03* 361.8	+79.92 471.0	+89.15 411.0	+202.30 361.8	+79.28 306.0	+68.76 392.0
__ day 96	+63.58 318.5	+103.58 251.0	+83.99 410.8	+103.13* 399.0	+145.37 312.0	+160.08 286.0	+74.76 305.8	+96.32 418.8
__ day 181	+132.53 172.3	+63.59 179.3	+180.98 314.8	+103.23 368.5	+78.77 313.8	+100.35 369.5	+59.29 368.3	+79.74 346.0
__ day 279	+99.74 157.5	+35.25 174.5	+215.17 382.0	+155.49 398.5	+84.48 309.0	244.45 531.0	+141.33 308.0	+124.74 437.8
day 369	+65.04	+125.83	+214.58	+132.88	+129.87	+389.59	+214.65	+194.67
Hemoglobin (g/dl)								
__ day -20	16.33 +1.970	16.28 +1.237	17.03 +0.222	16.75 +0.592	16.73 +1.506	16.70 +0.757	16.88 +0.597	17.08 +1.135
__ day -13	+1.175 16.50	+0.846 16.13	+0.762 16.80	+0.827 17.05	+1.780 16.10	+1.010 16.30	+0.991 16.53	+1.204 16.35
__ day 40	+0.881 16.45	+1.315 16.23	+1.050 15.58	+1.663 15.50	+1.550 16.73	+0.759 16.78	+0.826 15.83	+0.750 15.23
__ day 96	+1.024 16.38	+1.348 15.55	+0.707 16.20	+0.926 16.05	+1.112 15.98	+0.618 16.42	+0.763 16.08	+1.879 15.43
__ day 181	+0.907 16.83	+0.866 15.98	+0.574 15.83	+1.176 15.73	+1.453 16.65	+0.309 16.33	+0.854 16.65	+1.150 15.73
__ day 279	+0.340 16.23	+0.818 15.95	+1.034 15.83	+1.398 15.70	+1.646 17.57	+0.762 16.30	+0.435 15.68	+1.266 15.93
__ day 369	+0.591 15.23	+1.014 15.88	+1.169 15.00	+1.147 14.25	+2.004 17.18	+0.608 16.03	+1.236 14.80	+1.170* 13.95
Hematocrit (%)								
__ day -20	48.85 +6.457	48.63 +3.806	51.60 +0.761	50.10 +1.892	49.80 +4.668	49.40 +3.422	49.50 +2.011	50.55 +3.676
__ day -13	+3.163 49.13	+2.532 47.13	+1.828 49.48	+2.660 50.25	+5.425 47.90	+3.012 48.50	+2.175 48.88	+3.301 47.70
__ day 40	+2.840 48.95	+4.131 48.18	+2.906 46.03	+5.333 45.90	+3.868 49.65	+2.392 49.88	+2.356 46.68	+2.282 44.90
__ day 96	3.051 47.83	+4.236 45.10	+2.265 47.73	+3.148 46.88	+3.703 47.10	+2.401 48.60	+2.834 47.28	+5.593 44.98
__ day 181	+1.965 49.98	+2.960 47.68	+1.986 46.80	+3.265 46.40	+4.832 49.28	+0.300 48.35	+3.125 49.03	+3.262 46.08
day 279	48.43	47.83	47.33	46.80	54.13	50.73	47.65	48.88

__day 369	+0.556 45.13 +1.870	+2.755 47.00 +2.915	+3.850 44.53 +4.126	+4.992 42.43 +3.480	+5.737 51.70 +6.869	+3.243 48.23 +2.354	+1.179 43.83 +3.871	+3.723 41.10 +3.744*
MCV (fl)								
__day -20	69.3 +0.96	68.0 +2.16	67.8 +1.50	67.0 +2.16	70.8 +0.96	69.3 +2.22	68.0 +1.83	68.3 +0.95
__day -13	68.8 +0.96	67.3 +1.71	67.0 +1.63	67.0 +1.41	69.8 +1.50	69.0 +2.16	68.0 +1.63	67.3 +0.96
__day 40	69.0 +0.82	67.3 +1.89	68.3 +2.06	67.0 +2.45	69.5 +1.29	68.3 +2.87	67.3 +1.71	66.5 +1.29
__day 96	67.8 +1.50	66.5 +2.08	67.5 +1.73	65.5 +1.73	69.3 +0.96	67.0 +2.16	66.0 +1.41*	66.3 +0.96*
__day 181	67.3 +0.96	66.8 +1.50	66.5 +1.91	64.8 +1.89	69.0 +0.82	67.8 +3.30	65.5 +2.08	65.5 +1.73
__day 279	68.0 +1.41	68.0 +1.41	67.0 +1.83	64.5 +2.08*	72.0 +1.63	70.5 +3.79	67.5 +2.65	67.0 +0.82*
__day 369	68.3 +0.50	68.5 +0.58	67.8 +2.06	65.8 +2.63	70.8 +2.06	69.8 +2.22	67.0 +1.41*	66.3 +1.50*
MCH (µg)								
__day -20	23.13 +0.608	22.75 +0.742	22.33 +0.395	22.43 +0.665	23.70 +0.365	23.38 +0.607	23.18 +0.465	23.08 +0.299
__day -13	23.05 +0.370	23.03 +0.579	22.80 +0.548	22.65 +0.719	23.48 +0.512	23.18 +0.492	22.93 +0.538	23.10 +0.316
__day 40	23.10 +0.566	22.60 +0.829	23.05 +0.580	22.48 +0.826	23.35 +0.580	22.93 +0.624	22.80 +0.560	22.58 +0.403
__day 96	23.15 +0.465	22.95 0.794	22.88 +0.741	22.43 +0.727	23.43 +0.660	22.68 +0.538	22.53 +0.532	22.73 +0.435
__day 181	22.60 +0.469	22.38 +0.450	22.48 +0.492	21.75 +0.526	23.33 +0.275	22.80 +0.868	22.28 +0.419*	22.23 +0.411*
__day 279	22.75 +0.252	22.73 +0.435	22.48 +0.299	22.63 +0.939*	23.35 +0.614	22.63 +0.745	22.18 +0.675	21.88 +0.386*
__day 369	22.98 +0.377	23.08 +0.411	22.73 +0.275	22.10 +0.848	23.53 +0.350	23.18 +0.877	2.58 +0.386	22.43 +0.579
Platelets (1000s/mm ³)								
__day -20	233.50 +46.429	263.00 +90.211	264.50 +35.464	257.67 +65.684	285.50 +47.339	315.33 +29.263	266.50 +49.548	278.50 +45.930
__day -13	222.75 +26.260	241.25 +42.343	242.75 +25.630	250.75 +26.813	317.50 +72.030	288.50 +72.012	261.75 +16.520	294.00 +71.540
__day 40	244.00 +49.578	268.00 +41.817	279.50 +46.148	310.50 +11.930	338.25 +179.59	292.75 +92.957	292.25 +28.756	362.50 +77.104
__day 96	247.00 +33.086	244.50 +49.020	290.50 +45.420	307.25 +62.564	277.33 +77.261	282.75 +64.267	303.00 +46.029	363.50 +96.504
__day 181	275.25 +31.309	258.75 +46.586	299.00 +26.596	303.50 +62.153	298.75 +32.816	317.00 +33.511	313.75 +52.880	399.00 +91.386
__day 279	260.00 +50.761	250.75 +28.135	294.50 +44.125	275.75 +42.003	279.50 +25.199	321.00 +93.199	309.50 +41.267	360.33 +45.786
__day 369	255.75 +42.688	247.75 +48.562	290.25 +66.920	277.75 +54.854	283.67 +36.005	304.50 +45.317	299.75 +60.852	368.50 +33.601

* Indicates that group mean is significantly different from control at p<0.05

** Indicates that group mean is significantly different from control at p<0.01

- Clinical chemistry:

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	1	3	6	0	1	3	6
Albumin (g/dl)								
__day -20	3.15 ±0.265	3.28 ±0.189	3.30 ±0.082	3.30 ±0.000	3.10 ±0.374	3.08 ±0.171	3.10 ±0.141	3.20 ±0.245
__day -13	3.13 ±0.171	3.33 ±0.299	3.30 ±0.000	3.33 ±0.050	3.15 ±0.387	3.13 ±0.096	3.18 ±0.250	3.15 ±0.129
__day 40	3.08 ±0.096	3.10 ±0.216	2.98 ±0.189	2.95 ±0.058	3.03 ±0.450	3.10 ±0.141	2.88 ±0.126	2.93 ±0.171
__day 96	3.13 ±0.096	3.18 ±0.150	3.10 ±0.082	3.00 ±0.082	3.18 0.275	3.10 ±0.141	3.05 0.208	3.03 ±0.150
__day 181	3.13 ±0.050	3.33 ±0.206	3.08 ±0.096	2.98 ±0.050	3.33 ±0.096	3.28 ±0.050	3.05 ±0.252	3.00 ±0.163*
__day 279	3.10 ±0.082	3.35 ±0.208	2.93 ±0.126	2.95 ±0.238	3.40 ±0.200	3.20 ±0.141	2.93 ±0.287*	3.00 ±0.216
__day 369	2.90 ±0.115	3.28 ±0.150**	2.85 ±0.129	2.80 ±0.141	3.38 ±0.171	3.18 0.050	2.78 ±0.263**	2.83 ±0.096**
A/G ratio								
__day -20	1.228 ±0.0727	1.350 ±0.0529	1.335 ±0.0835	1.348 ±0.0550	1.323 ±0.2439	1.325 ±0.1008	1.373 ±0.1431	1.263 ±0.1846
__day -13	1.193 ±0.0618	1.315 ±0.0759*	1.323 ±0.0450*	1.305 ±0.0436*	1.285 ±0.3022	1.310 ±0.1876	1.453 ±0.1837	1.273 ±0.0574
__day 40	1.220 ±0.0770	1.228 ±0.0900	1.240 ±0.1068	1.225 ±0.1212	1.263 ±0.3333	1.305 ±0.1597	1.206 ±0.1059	1.183 ±0.1725
__day 96	1.330 ±0.0804	1.343 ±0.1135	1.293 ±0.1702	1.258 ±0.0943	1.5553 ±0.1438	1.310 ±0.1192	1.430 ±0.1720	1.338 ±0.2924
__day 181	1.113 ±0.0818	1.263 ±0.1940	1.220 ±0.0622	1.133 ±0.0263	1.495 ±0.1793	1.318 ±0.1245	1.260 ±0.1445	1.123 ±0.2030*
__day 279	1.075 ±0.0926	1.228 ±0.1599	1.020 ±0.0337	1.098 ±0.0814	1.370 ±0.1192	1.090 ±0.0993*	1.063 ±0.0780*	1.028 ±0.1974**
__day 369	1.108 ±0.1464	1.268 ±0.0946	1.130 ±0.0683	1.115 ±0.1240	1.4440 ±0.1917	1.228 ±0.1156	1.215 ±0.1812	1.078 ±0.1471

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** Indicates that group mean is significantly different from control at p<0.01

- Urinalysis: No treatment related effects were observed. (One incidence of urinary sand occurred in a low dose male, with no clinical urinalysis correlates.)
- Electrocardiography: On days 184 and 192, a mid-dose animal demonstrated a possible ectopic beat ~2 hours post-dose. This finding was not observed over the remainder of the study. There was some tendency for an increase in P width in high dose males and in P height in both high dose males and females 2 hours post dose. Heart rate was also increased in high dose males. No effect on QT interval or other EKG parameters were observed.

P width, pre-dose	0 mg/kg/day		1 mg/kg/day		3 mg/kg/day		6 mg/kg/day	
	Males	Females	Males	Females	Males	Females	Males	Females
Pre-1	41+5	42+2	45+9	40+3	35+5	39+3	43+2	40+4
Pre-2	39+6	39+3	42+5	41+5	36+5	41+3	39+5	36+7
Day 99	44+1	44+1	50+8	45+6	39+2*	43+4	50+5	49+3
Day 184	44+3	44+2	48+4	48+6	41+2	41+1	45+3	45+3
Day 267	42+5	51+7	47+1	45+6	41+6	45+4	51+5*	47+4
Day 351	44+4	49+6	51+6	48+8	41+2	46+6	51+4	49+5

P width, 2 hours post-dose								
	0 mg/kg/day		1 mg/kg/day		3 mg/kg/day		6 mg/kg/day	
	Males	Females	Males	Females	Males	Females	Males	Females
Pre-1	41+5	42+2	45+9	40+3	35+5	39+3	43+2	40+4
Pre-2	39+6	39+3	42+5	41+5	36+5	41+3	39+5	36+7
Day 99	44+5	44+3	48+5	47+6	42+2	47+3	57+14	49+3
Day 184	44+2	52+7	52+6	46+4	41+2	42+6	61+12	50+3
Day 267	42+5	46+6	53+2**	46+4	42+2	44+4	53+6**	47+1
Day 351	43+2	49+8	51+5	45+6	42+3	48+9	54+6*	50+5

P height, pre-dose								
	0 mg/kg/day		1 mg/kg/day		3 mg/kg/day		6 mg/kg/day	
	Males	Females	Males	Females	Males	Females	Males	Females
Pre-1	0.20+0.06	0.21+0.05	0.19+0.06	0.27+0.09	0.16+0.08	0.18+0.05	0.21+0.05	0.21+0.03
Pre-2	0.17+0.07	0.13+0.06	0.19+0.08	0.17+0.04	0.17+0.08	0.18+0.05	0.21+0.06	0.26+0.06
Day 99	0.18+0.02	0.20+0.02	0.25+0.13	0.28+0.11	0.24+0.03	0.25+0.02*	0.27+0.05	0.32+0.05*
Day 184	0.20+0.04	0.23+0.03	0.23+0.06	0.26+0.06	0.25+0.03	0.27+0.04	0.28+0.11	0.32+0.03*
Day 267	0.21+0.05	0.23+0.03	0.23+0.03	0.29+0.06	0.23+0.05	0.25+0.07	0.31+0.09	0.29+0.03
Day 351	0.22+0.04	0.24+0.04	0.26+0.09	0.25+0.04	0.20+0.06	0.28+0.05	0.32+0.06	0.27+0.04

P height, 2 hours post-dose								
	0 mg/kg/day		1 mg/kg/day		3 mg/kg/day		6 mg/kg/day	
	Males	Females	Males	Females	Males	Females	Males	Females
Pre-1	0.20+.06	0.21+.05	0.19+.06	0.27+.09	0.16+.08	0.18+.05	0.21+.05	0.21+.03
Pre-2	0.17+.07	0.13+.06	0.19+.08	0.17+.04	0.17+.08	0.18+.05	0.21+.06	0.26+.06
Day 99	0.19+.06	0.20+.02	0.25+.01	0.29+.11	0.24+.03	0.25+.01*	0.33+.08**	0.34+.06*
Day 184	0.24+.04	0.23+.04	0.26+.05	0.28+.06	0.25+.03	0.28+.04	0.33+.06	0.33+.05*
Day 267	0.24+.04	0.22+.02	0.30+.05	0.29+.03	0.25+.05	0.28+.04	0.34+.07	0.34+.08*
Day 351	0.21+.04	0.26+.02	0.32+.09	0.22+.09	0.25+.07	0.31+.04	0.36+.07*	0.35+.11

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** Indicates that group mean is significantly different from control at p<0.01

- Blood pressure: No treatment related effects on blood pressure were observed.
- Heart rate:

Heart rate pre dose								
	0 mg/kg/day		1 mg/kg/day		3 mg/kg/day		6 mg/kg/day	
	Males	Females	Males	Females	Males	Females	Males	Females
Day Pre-1	75+38	94+28	89+37	135+9	83+8	98+24	80+6	112+14
Day Pre-2	76+26	89+11	105+42	114+15	86+9	80+29	91+14	145+60
Day 99	78+10	87+6	102+19	128+23*	113+43	110+21	96+17	113+16
Day 184	81+13	93+13	97+18	106+14	97+29	106+14	77+9	102+12
Day 267	73+21	84+9	93+20	113+15	83+32	95+25	76+15	94+9
Day 351	69+16	79+7	105+21	108+7*	75+29	87+18	77+9	98+16

Heart rate, 2 hours post dose (t _{max})								
	0 mg/kg/day		1 mg/kg/day		3 mg/kg/day		6 mg/kg/day	
	Males	Females	Males	Females	Males	Females	Males	Females
Day Pre-1	75+38	94+28	89+37	135+9	83+8	98+24	80+6	112+14
Day Pre-2	76+26	89+11	105+42	114+15	86+9	80+29	91+14	145+60
Day 99	78+12	85+9	109+15	125+24*	118+33*	109+18	132+15**	126+14*
Day 184	96+16	98+4	102+8	113+6	108+31	113+16	117+11	120+13*
Day 267	89+9	83+7	99+18	114+11*	102+34	104+26	123+13	124+8*
Day 351	76+10	90+17	107+19	116+14	103+27	96+10	121+6**	121+14*

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** Indicates that group mean is significantly different from control at p<0.01

- Organ Weights:

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	1	3	6	0	1	3	6
Liver (g)	328.03 +14.681	389.58 +30.695	354.75 +17.124	413.80 +66.491*	327.70 +29.067	297.28 +29.952	297.13 +27.589	339.88 +23.162
Liver (g%)	2.88 +0.417	2.78 +0.125	3.35 +0.364	3.41 +0.216	2.55 +0.090	2.36 +0.177	2.99 +0.471	3.26 +0.254*
Testes (g)	14.40 +2.176	16.90 +4.089	15.33 +3.103 (+6.5%)	17.75 +2.998 (+23%)				
Testes (g%)	0.13 +0.031	0.12 +0.032	0.14 +0.019 (+7.7%)	0.15 +0.016 (+15.4%)				
Ovaries (g)					1.273 +0.2061	1.278 +0.3508	1.250 +0.4044	1.533 +0.5004 (+20%)
Ovaries (g%)					0.010 +0.0020	0.010 +0.0030	0.013 +0.0055 (+33%)	0.015 +0.0040 (+50%)
Adrenals (g)	1.628 +0.0960	1.775 +0.4370 (+9%)	1.623 +0.3675 (-0%)	1.930 +0.1163 (+18.6%)	1.733 +0.2780	1.515 +0.2656 (-12.5%)	1.643 +0.1921 (-5%)	1.733 +0.1590 (-0%)
Adrenals (g%)	0.014 +0.0031	0.013 +0.0026 (-7%)	0.015 +0.0030 (+7%)	0.016 +0.0021 (+14%)	0.013 +0.0012	0.012 +0.0026 (-8%)	0.017 +0.0038 (+31%)	0.017 +0.0016 (+31%)
Kidneys (g)	55.10 +3.956	66.53 +8.534 (+21%)	54.88 +9.347 (-0%)	69.58 +12.303 (+26%)	46.88 +4.782	48.25 +6.668 (+3%)	44.88 +7.170 (-4%)	44.28 +2.957 (-6%)
Kidneys (g%)	0.48 +0.056	0.47 +0.034 (-2%)	0.51 +0.052 (+6%)	0.57 +0.081 (+19%)	0.36 +0.026	0.38 +0.048 (+6%)	0.45 +0.045* (+25%)	0.43 +0.049 (+19%)

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** Indicates that group mean is significantly different from control at p<0.01

- Gross pathology: There were "a few pin-point corneal opacities in the eyes of one mid-dose male and one high-dose male" (along with neovascularization).

- Histopathology

Dose (mg/kg/day)	Males (N=4)				Females (N=4)			
	0	1	3	6	0	1	3	6
Kidney								
Infiltration, mononuclear cells	0	0	0	1	0	0	0	0
Liver								
Infiltration, mononuclear cells	1	1	1	2	3	1	0	2
Trachea								
Infiltration, mononuclear cells	1	0	0	0	0	0	0	1
Thyroid								
Infiltration, mononuclear cells	0	0	0	1	0	0	0	0
Eye								
Neovascularization, cornea	0	0	1	1	0	0	0	0
Bone marrow								
Infiltration, mononuclear cells	0	0	0	0	0	0	0	1

- Toxicokinetics:

<u>Dose</u> <u>(mg/kg)</u>	COMPOUND	<u>Study</u> <u>Day</u>	<u>T_{max}</u> (hr)	<u>C_{max}</u> <u>(ug/mL)</u>	<u>AUC_{0-Tlast}</u> <u>(ug-hr/mL)</u>
1	UK-88,525 (parent)	177	nd	nd	nd
		366	nd	nd	nd
	UK-148,993 (metabolite)	177	3.0 +1.7	0.081 +0.053	nd
		366	4.7 +2.8	0.086 +0.019	0.502 +0.162
3	UK-88,525	177	3.2 +2.1	0.323 +0.094	1.567 +0.439
		366	2.0 +0.0	.249 +0.109	1.717 +0.916
	UK-148,993	177	7.2 +1.5	0.483 +0.178	nd
		366	4.2 +2.5	0.183 +0.047	1.758 +1.168
6	UK-88,525	177	2.2 +0.7	0.669 +0.295	5.120 +3.776
		366	3.5 +2.1	0.588 +0.213	3.612 +1.615
	UK-148,993	177	4.5 +2.1	0.572 +0.184	6.137 +4.139
		366	4.5 +0.9	0.514 +0.166	5.520 +3.371

nd not determined (insufficient data for calculation)

APPEARS THIS WAY
ON ORIGINAL

Histopathology inventory:

Study			
Species	Mouse 93043	Rat 93073	Dog 94-751-08
Adrenals	X	X	X
Aorta	X	X	X
Bone Marrow smear	X	X	X
Bone	X		X
Brain	X	X	X
Cecum			X
Cervix			X
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X	X	X
Esophagus	X	X	X
Eye	X	X	X
Gall bladder	X		X
Harderian gland	X	X	
Heart	X	X	X
Ileum	X	X	X
Jejunum			X
Kidneys	X	X	X
Liver	X	X	X
Lungs	X	X	X
Lymph nodes, cervical	X	X	X
Lymph nodes, mes.	X	X	X
Mammary Gland	X	X	X
Ovaries	X	X	X
Pancreas	X	X	X
Parathyroid	X	X	X
Pituitary	X	X	X
Prostate	X	X	X
Salivary gland	X	X	X
Sciatic nerve	X	X	X
Skeletal muscle	X	X	X
Skin	X	X	X
Spinal cord			X
Spleen	X	X	X
Sternum	X	X	X
Stomach	X	X	X
Testes	X	X	X
Thymus	X	X	X
Thyroid	X	X	X
Trachea	X	X	X
Urinary bladder	X	X	X
Uterus	X	X	X
Vagina	X	X	

3.4.4. Genetic toxicology (Please see attached review in Appendix A)

Summary: Darifenacin was not mutagenic in the Ames test for bacterial mutation or in the Chinese hamster ovary assay. It was not clastogenic *in vitro* in the human lymphocyte assay or *in vivo* in the mouse bone marrow cytogenetics assay.

3.4.5. Carcinogenicity (See CAC comments and conclusions, sponsor response, and CAC conclusions in Appendix B)

Study Title: 24-month in-diet toxicity and carcinogenicity study in CD-1 mice

Study Number: 93043

Test Facility: []

Study Date(s): 20 July 1993 – 21 July 1995

Date of Submission: 6 June 2000

GLP Compliance/Quality Assurance: yes

QA Report- Yes (x) No ()

Study Type: 2 year dietary

Species/strain: CD 1 mice []

Number of animals per group; age at start of study: 50/sex/group, age 7 weeks, mean body weight 30.1 g for males and 23.5 g for females.

Animal housing: individual

Drug Lot/Batch number(s): R9 (delivered 17 June and 17 August 1993, — active moiety) and R101 (delivered 3 August 1994, — active moiety)

Drug Purity / Stability / Homogeneity: UK-88,525-04, in concentrations ranging from 5 µg/g to 5000 µg/g, was shown to be stable for up to 4 weeks in open hoppers and trays. Drug concentration and homogeneity were assayed every month in the diet mixture.

Doses: 0, 3, 20, and 100 mg/kg/day

- Basis of Dose Selection: In a 3-month dietary study in mice at doses of 0, 25, 50, and 100 mg/kg/day, a 6 % decrease in body weight was observed at the high dose level. A dose related increase in mydriasis and partially closed eyes was observed at all dose levels. An increased accumulation of Harderian gland secretions showed a similar incidence at all treatment levels. An adaptive increase in liver weight and an induction of cytochrome P450 were also observed at the mid and high doses; there were no histopathological changes to the liver.
- Relation to Clinical Use: The exposure (AUC_{0-24hr}) of mice increased with dose level. At 100 mg/kg/day, the AUC_{0-24hr} and C_{max} were 1548 nghr/ml and 96 ng/ml, respectively. In humans, the maximal projected oral dose is 0.2 mg/kg t.i.d. with AUC_{0-24hr} and C_{max} of about 60 nghr/ml and 10 ng/ml, respectively.
- CAC Concurrence: yes
- Restriction Paradigm for Dietary Restriction Studies: no dietary restriction
- Route of Administration: oral, dietary
- Frequency of Drug Administration: continuous
- Dual Controls Employed: two identical control groups, analyzed separately for survival and histology, and combined for other parameters
- Interim Sacrifices: none
- Satellite PK or Special Study Group(s): none

- **Unscheduled Sacrifices or Deaths:**

	Males (mg/kg/day)					Females (mg/kg/day)				
	Cont. 1	Cont. 2	3	20	100	Cont. 1	Cont. 2	3	20	100
Number of animals	50	50	50	50	50	50	50	50	50	50
Found dead	18	17	13	14	19	18	18	15	15	9
Sacrificed as moribund	1	3	0	1	1	2	2	4	6	1
Accidental deaths	0	1	0	0	0	0	0	0	0	0
Total unscheduled deaths, no.	19	21	13	15	20	20	20	19	21	10
Total unscheduled deaths, %	38	42	26	30	40	40	40	38	42	20
Survivors at final sacrifice, no.	31	29	37	35	30	30	30	31	29	40
Survivors at final sacrifice, %	62	58	74	70	60	60	60	62	58	80

- Deviations from Original Study Protocol: none significant

Study Results and Frequency of Monitoring:

- **Clinical Observations:**

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	3	20	100	0	3	20	100
Mydriasis								
day 4	0/40	0/20	20/20	20/20	0/40	0/20	20/20	20/20
day 140	0/40	0/20	20/20	20/20	0/40	2/20	12/20	20/20
day 532	1/35	1/18	7/18	13/16	0/34	0/17	3/17	11/20
day 700	1/27	2/17	3/13	10/12	0/26	1/12	4/12	8/15

- **Mortality:** The treatment had no effects on the incidence of mortality, and no statistically significant differences in survival time were observed between control and treated groups.

- **Body Weights:**

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	3	20	100	0	3	20	100
Body weight, day 715 (g)	37.11 +2.87	38.03 +2.94	36.31 +3.06	33.26 +2.34 *	32.76 +3.54	32.99 +2.99	31.84 +2.93	29.35 +2.41 *
Change (%)	-----	+2 %	-2 %	-10 %	-----	+1 %	-3 %	-10 %

- **Food Consumption:**

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	3	20	100	0	3	20	100
Change (%), day 715	---	-3	-5	-4	---	+1	-3	-5

- **Ophthalmoscopy:** No treatment related effects were observed

- **Hematology:**

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	3	20	100	0	3	20	100
Hemoglobin (g/dl)	13.76 +0.97	13.63 +1.77	13.81 +1.33	14.26 +1.07*	12.48 +1.71	12.82 +1.12	12.40 +2.22	12.83 +1.00
Red cell distrib. width (%)	12.77 +0.89	13.11 +1.86	12.57 +0.96	12.46 +0.74	13.97 +3.05	13.08 +1.12	13.56 +2.05	12.69 +1.09**
Basophils (10 ³ /mm ³)	0.017 +0.016	0.013 +0.010	0.015 +0.010	0.014 +0.024	0.016 +0.016	0.013 +0.012	0.014 +0.016	0.009 +0.009*
Large unstained cells (10 ³ /mm ³)	0.035 +0.047	0.031 +0.027	0.036 +0.042	0.023 +0.023	0.060 +0.112	0.036 +0.057	0.089 +0.169	0.025 +0.026*

* Indicates group mean is significantly different from control at level p= .05.

** Indicates group mean is significantly different from control at level p=.01.

- Clinical Chemistry:

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	3	20	100	0	3	20	100
Chloride (mmol/l)	112.41 +6.27	112.22 +6.39	110.54 +5.43	108.63 +4.64*	112.65 +6.81	111.81 +6.93	112.62 +6.73	108.53 +5.69
Calcium (mg/l)	85.61 +3.77	85.95 +4.11	85.80 +3.50	85.53 +4.89	89.12 +4.37	88.52 +3.83	86.76 +4.95*	86.75 +3.36*

* Indicates group mean is significantly different from control at level p=.05.

** Indicates group mean is significantly different from control at level p=.01.

- Organ Weights:

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	3	20	100	0	3	20	100
Kidney (g)	0.833 +0.124	0.856 +0.130	0.801 +0.117	0.677 +0.123 ⁺	0.528 +0.088	0.523 +0.067	0.493 +0.066 [%]	0.459 +0.058 ^{\$}
Kidney (g/100g BW)	2.269 +0.278	2.273 +0.303	2.248 +0.292	2.050 +0.315 ⁺	1.635 +0.257	1.620 +0.219	1.603 +0.237	1.607 +0.183
Testes (g)	0.209 +0.034	0.204 +0.055	0.198 +0.051	0.227 +0.035 [%]				
Testes (g/100g BW)	0.573 +0.096	0.541 +0.141	0.557 +0.140	0.690 +0.104 ^{\$}				
Liver (g)	1.898 +0.408	1.862 +0.323	1.849 +0.311	1.975 +0.321	1.673 +0.482	1.527 +0.230	1.518 +0.300	1.560 +0.200
Liver (g/100g BW)	5.165 +1.025	4.940 +0.724	5.197 +0.866	5.986 +0.843 ⁺	5.167 +1.335	4.734 +0.759	4.916 +0.923	5.468 +0.729

*(+)= mean value of group was significantly different from control at p=0.05 (0.01), Dunnett's test of significance.

%(\$)= mean value of group was significantly different from control at p=0.05 (0.01), modified t test of significance.

- Gross Pathology:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	3	20	100	0	0	3	20	100
Harderian gland discoloration	3	4	4	14	17	8	10	10	17	20

- Histopathology:

Non-neoplastic:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	3	20	100	0	0	3	20	100
Harderian gland Hypersecretion	3/50	13/48	9/50	38/50***	39/50***	0/49	1/49	18/50	35/49***	43/50***
Liver Centrilobular hypertrophy	2/50	1/49	1/50	4/50	36/50***	1/50	0/50	0/50	0/50	3/50
Pigment	2/50	6/49	2/50	9/50(*)	13/50***	9/50	13/50	14/50	6/50	6/50

(*) = statistically significant at p=0.05 without Bonferroni adjustment

***= statistically significant at p=0.01 with Cochran-Armitage test

Neoplastic:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	3	20	100	0	0	3	20	100
Harderian gland B-adenoma	1/50	2/48	2/50	3/50	3/50	0/49	1/49	2/50	0/49	1/50
N- erythroleukemia	0	0	0	0	1	0	0	0	0	0

Overall Interpretation and Evaluation

- Adequacy of the carcinogenicity studies and appropriateness of the test model:

The doses were adequate based on a 10% decrease in body weight gain (in the absence of significant effects on food consumption) in both sexes at the high dose along with effects on the liver and eyes. Exposures were 25 times the expected human exposure (based on AUC of the parent drug). Protein binding was approximately equivalent for mice and humans. The major metabolite of Darifenacin, UK-148,993, is present in the plasma of both mouse and man, in similar concentrations as those of Darifenacin.

- Evaluation of Tumor Findings: tumorigenicity was negative.

Study Title: 24-Month In-diet Toxicity and Carcinogenicity Study in Sprague-Dawley Rats

Study Number: 93073

Test Facility: Pfizer Centre de Recherche, 37401 Amboise Cedex, France

Study Date(s): 7 October 1993 – 9 October 1995

Date of Submission: 6 June 2000

GLP Compliance/Quality Assurance: yes

QA Report- Yes (x) No ()

Study Type: 2-year, dietary

Species/strain: Sprague-Dawley albino rats, \square 1

Number of animals per group; age at start of study: 50/sex/group, aged 6 weeks

Animal housing: individual cages

Drug Lot/Batch number(s): R9 (delivered 17 June and 17 August 1993, \square active moiety) and R101 (delivered 3 August 1994, \square active moiety)

Drug Purity / Stability / Homogeneity: UK-88,525-04, in concentrations ranging from 5 $\mu\text{g/g}$ to 5000 $\mu\text{g/g}$, was shown to be stable for up to 4 weeks in open hoppers and trays. Drug concentration and homogeneity were assayed every month in the diet mixture.

Doses: 0, 1.5, 5, or 15 mg/kg

- Basis of Dose Selection: In a 3-month dietary toxicity study, rats exposed to 25, 50, or 100 mg/kg/day had reduced food intake and 10% decreases in body weight gain at all doses. Hepatic centrilobular hypertrophy was observed at 50 and 100 mg/kg/day. Mydriasis and partially closed eyes occurred, with increasing severity, at all doses. In a 1-month dietary study, rats exposed to 25 mg/kg/day showed a 10% decrease in body weight gain. Doses of 10 or 15 mg/kg/day induced 3 to 4 % decreases in body weight. Mydriasis was observed at 15 and 25 mg/kg/day.
- Relation to Clinical Use: At 15 mg/kg/day, the $\text{AUC}_{0-24\text{hr}}$ were 1190 (males) and 740 (females) nhr/ml, and C_{max} 10 - 72 ng/ml, respectively. In humans, the maximal projected oral dose is 0.2 mg/kg t.i.d. with $\text{AUC}_{0-24\text{hr}}$ and C_{max} of about 60 nhr/ml and 10 ng/ml, respectively.
- CAC Concurrence: no review of protocol
- Restriction Paradigm for Dietary Restriction Studies: no dietary restriction
- Route of Administration: oral, dietary
- Frequency of Drug Administration: continuous
- Dual Controls Employed: two identical control groups, 1 and 2
- Satellite PK or Special Study Group(s): 15 supplementary rats per sex and dose level

- **Unscheduled Sacrifices or Deaths:**

	Males (mg/kg/day)					Females (mg/kg/day)				
	Cont. 1	Cont. 2	1.5	5	15	Cont. 1	Cont. 2	1.5	5	15
Number of animals	50	50	50	50	50	50	50	50	50	50
Found dead	12	21	13	16	11	8	9	7	7	9
Sacrificed as moribund	19	13	14	17	10	23	19	26	25	19
Accidental deaths	0	0	0	0	1	0	0	0	0	0
Total unscheduled deaths, no.	31	34	27	33	22	31	28	33	32	28
Total unscheduled deaths, %	62	68	54	66	44	62	56	66	64	56
Survivors at final sacrifice, no.	19	16	23	17	28	19	22	17	18	22
Survivors at final sacrifice, %	38	32	46	34	56	38	44	34	36	44

- Deviations from Original Study Protocol: none significant

Study Results and Frequency of Monitoring:

- **Clinical Observations:** In both sexes, the Harderian gland showed a significant dose-dependent increase in hypersecretion with no no-effect level. Focal hyperplasia of the Harderian gland showed a tendency to increase in females which was not significant when the high dose was not included; it was increased in the high dose females when compared to control group 2 but not with control group 1. Discoloration of the Harderian gland was significantly increased in females in the mid and high dose groups and in the low dose group when compared to control 2 but not to control 1.
- **Mortality:** No treatment related effects on the incidence of mortality were observed, and no statistically significant differences in survival time were observed between control and treated groups.

- **Body Weight**

Reduction in mean body weight gain in the treated groups at the end of study day 722 (% change from control)		
Dose (mg/kg)	Males	Females
1.5	2	10
5	11*	11
15	22**	29**

- **Food Consumption:**

Reduction in mean food consumption in the treated groups at the end of study day 722 (% change from control)		
Dose (mg/kg)	Males	Females
1.5	3	4
5	3	(+2)
15	9*	5

- **Ophthalmoscopy:**

	Males (mg/kg/day)		Females (mg/kg/day)	
	0	15	0	15
Day 524				
Keratitis: sequelae	0/19	2/22	0/18	0/22
Day 714				
Cornea: diffuse opacification	0/8	2/13	0/11	1/12
Keratitis	0/8	2/13	1/11	0/12
Keratitis: sequelae	0/8	1/13	0/11	0/12

- Hematology:

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	1.5	5	15	0	1.5	5	15
White blood cell count (10 ³ /mm ³)	13.524 +4.245	14.117 +4.232	15.779 +4.912	16.054 +6.011	9.864 +3.423	17.556 +24.885	11.099 +4.491	11.750 +7.390
Lymphocytes (10 ³ /mm ³)	8.463 +2.801	8.732 +2.660	8.637 +1.733	10.628 +5.248	5.169 +1.431	9.180 +15.284	5.625 +1.715	6.594 +4.165
Monocytes (10 ³ /mm ³)	0.707 +0.298	0.779 +0.249	0.856 +0.418	1.143 +0.881 *	0.551 +0.236	0.692 +0.535	0.592 +0.208	0.812 +1.019
Basophils (10 ³ /mm ³)	0.057 +0.030	0.065 +0.035	0.069 +0.027	0.104 +0.089 *	0.34 +0.019	0.163 +0.469	0.041 +0.015	0.093 +0.219
Large unstained cells (10 ³ /mm ³)	0.426 +0.210	0.536 +0.424	0.558 +0.380	0.642 +0.509 *	0.229 +0.108	0.893 +2.445	0.281 +0.148	0.685 +1.991

- Clinical Chemistry:

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	1.5	5	15	0	1.5	5	15
Sodium (mmol/l)	142.77 +1.42	143.30 +2.74	142.65 +1.58	141.39 +1.85**	140.98 +2.31	140.59 +2.60	140.39 +1.42	140.36 +2.34
Chloride (mmol/l)	110.17 +3.20	109.26 +3.96	109.53 +3.87	113.29 +3.56**	109.32 +4.42	107.06 +4.59	108.44 +3.47	109.82 +4.56
Calcium (mg/l)	106.37 +7.50	109.13 +10.61	108.06 +11.03	102.25 +5.27*	103.98 +3.42	105.88 +5.70	102.67 +3.20	103.77 +4.50
Cholesterol (mg/dl)	155.26 +47.69	163.70 +48.38	166.76 +76.11	128.21 +30.92*	136.29 +33.32	147.94 +47.53	132.50 +28.66	123.23 +29.23
Protein (g/l)	69.66 +3.82	69.65 +5.24	69.65 +5.85	73.11 +7.91*	73.83 +5.73	75.53 +4.16	73.61 +5.73	75.95 +4.55
Urea (mg/dl)	35.37 +24.96	43.39 +31.32	47.12 +54.09	34.11 +17.59	27.29 +7.59	29.29 +15.88	33.22 +15.18	31.64 +7.93*
Creatinine (mg/dl)	0.78 +0.58	1.02 +0.82	1.05 +1.13	0.60 +0.14	0.58 +0.07	0.57 +0.08	0.63 +0.10	0.64 +0.12*

- Organ Weights:

	Males (mg/kg/day)			
	0	1.5	5	15
Testes (g)	3.655 +1.037	3.730 +0.523	3.439 +0.805	3.855 +0.877
Testes (g/100g BW)	0.471 +0.139	0.496 +0.077	0.509 +0.133	0.667 +0.215 ^s
	-----	(1.05X)	(1.08X)	(1.42X)

Sponsor's table of organ to brain weight ratio:

Groups	Terminal Body Weight (g)	Testes to Brain ratio (%)
Controls		
Mean	787.11	146.878
Standard Deviation	139.49	31.187
Number of animals observed	(35)	(35)
1.5 mg/kg		
Mean	761.78	147.791
Standard Deviation	116.02	22.735
Number of animals observed	(23)	(23)
5 mg/kg		
Mean	689.82*	156.119
Standard Deviation	114.69	50.949
Number of animals observed	(17)	(17)
15 mg/kg		
Mean	594.93+	155.067
Standard Deviation	91.54	37.114
Number of animals observed	(28)	(28)

* (+) = mean value of group was significantly different from control at P = 0.05 (0.01) with Dunnett's test of significance

- Gross Pathology:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	1.5	5	15	0	0	1.5	5	15
Harderian gland discoloration	6/50	5/50	8/50	12/50	7/50	2/50	0/50	3/50	12/50	11/50

- Histopathology:

Non-neoplastic:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	1.5	5	15	0	0	1.5	5	15
Adrenal hyperplasia, cortical, focal	29/50	29/50	36/49	31/50	34/50	27/50	38/50	31/50	35/50	41/50
Bone marrow hyperplasia, myeloid	3/50	4/49	4/50	4/50	6/50	4/50	9/50	6/50	7/50	13/49
Eye abscess, retrobulbar	0/50	0/50	0/50	0/50	2/50	0/50	0/50	0/50	0/50	0/50
Heart hyperplasia, mesothelial	1/50	1/50	1/50	1/50	3/50	2/50	0/50	2/50	0/50	1/50
Harderian gland hypersecretion	25/50	26/50	33/50	43/50	39/50	16/50	15/50	26/50	32/50	35/50
hyperplasia	19/50	15/50	22/50	21/50	15/50	12/50	9/50	9/50	15/50	17/50
Kidney infarct	1/50	1/50	2/50	3/50	3/50	0/50	0/50	1/50	0/50	0/50
hyperplasia	0/50	0/50	1/50	0/50	0/50	0/50	0/50	0/50	1/50	1/50
Liver hyperplasia, bile duct angiectasis	25/50 2/50	23/50 4/50	20/50 1/50	15/50 2/50	34/50 5/50	12/50 0/50	17/50 2/50	17/50 4/50	14/50 6/50	11/50 7/50
Pancreas eosinophilic change	1/50	0/50	2/50	3/50	6/50	1/50	0/50	1/50	3/50	5/50
Prostate prostatitis	27/50	29/50	29/50	27/50	35/50					
Skin and adnexa pyogranuloma	0/49	0/50	0/50	1/50	11/50	0/50	0/50	1/50	0/49	0/49

mam.gland lob.hyperplasia	3/49	4/50	2/50	1/50	7/50	13/50	10/50	2/50	7/49	14/49
Spleen										
fibrosis	0/50	0/50	0/50	0/50	3/50	0/50	0/50	0/50	0/50	0/50
lymphoid depletion	0/50	2/50	2/50	1/50	0/50	0/50	0/50	1/50	1/50	2/50
periarteritis	0/50	0/50	0/50	1/50	1/50	0/50	0/50	1/50	0/50	0/50
inflammation	0/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50	0/50
Thymus										
hyperplasia, epithelial	1/50	2/50	0/50	3/50	2/49	13/50	13/50	13/50	16/48	18/50
hyperplasia, lymphoid	1/50	0/50	4/50	1/50	0/49	1/50	1/50	2/50	2/48	3/50
Urinary bladder										
epithelial hyperplasia	1/50	0/50	3/50	5/50	1/50	0/50	0/49	0/50	0/50	1/50
Vagina										
dysplasia, squamous cell						0/45	0/48	0/47	0/48	1/49
mononuclear cell infiltration						1/45	1/48	2/47	1/48	8/49

Neoplastic:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	1.5	5	15	0	0	1.5	5	15
Adrenal										
B-cortical adenoma	1/50	3/50	5/49	7/50	3/50	5/50	1/50	3/50	1/50	8/50
Abdomen										
M-sarcoma, N.O.S.	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/50
Spleen										
lrge granular cell lymphoma	0/50	0/50	1/50	2/50	1/50	0/50	0/50	1/50	0/50	1/50
B-hemangioma	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/50
M-hemangiosarcoma	0/50	0/50	0/50	0/50	2/50	0/50	0/50	0/50	0/50	0/50
Kidney										
M-hemangiosarcoma	1/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50	0/50
Skin and adnexa										
B-hemangiopericytoma	0/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50	0/50
N-hemangiosarcoma	0/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50	0/50
Total hemangiosarcoma	1/50	0/50	0/50	0/50	4/50	0/50	0/50	0/50	0/50	0/50
Total hemangiosarcoma + hemangioma	1/50	0/50	0/50	0/50	4/50	0/50	0/50	0/50	0/50	1/50

- Toxicokinetics: The AUCs for the high dose represent 20 and 12 times the clinical dose for males and females, respectively.

Range of plasma concentrations on day 21 (µg/ml)						
	Males (mg/kg/day)			Females (mg/kg/day)		
	1.5	5	15	1.5	5	15
UK-88,525	BLD-0.010	BLD-0.018	0.010-0.059	BLD-0.011	BLD-0.024	0.017-0.072
Metabolite UK-148,993	BLD	BLD	BLD-0.035	BLD	BLD	BLD-0.039
AUC0-24h (µghr/ml)						
UK-88,525	-	-	1.19	-	-	0.74
Metabolite UK-148,993	-	-	-	-	-	-

Overall Interpretation and Evaluation

- Adequacy of the carcinogenicity studies and appropriateness of the test model: The doses were adequate based on a 22% and 29% decreases in body weight gain in males and females, respectively, at the high dose along with effects on the Harderian gland. A 2-year dietary study in Sprague-Dawley albino rats was employed using 7 and 12 times the expected human exposure in males and females, respectively.
- Evaluation of Tumor Findings: No clearly drug related increase in tumor incidence was observed.

Summary Conclusions and Recommendations

- Acceptability of Study(s) or Overall Testing Approach: acceptable
- Major Tumor Findings: None clearly related to darifenacin, after statistical analysis
- Non-neoplastic Findings: In both rats and mice, dose related effects on the Harderian glands were observed. In mice, dose related effects on the liver were also observed. In rats, there were possible effects on the adrenals, bone marrow, eyes, heart, kidney, liver, pancreas, prostate, skin, spleen, thymus, urinary bladder, and vagina.
- Potential Clinical Implications of Findings: None

3.4.6. Reproductive and developmental toxicology (In addition, please see attached review in Appendix A)

Prenatal and postnatal development

Study title: Oral pre- and postnatal development study of UK-88,525-04 in rats: assessment of learning and memory of F1 pups.

Key study findings: The No Observed Effect Levels were 3 mg/kg/day for the dams and 10 mg/kg/day for the pups. No effects on learning and memory of the pups were observed. Days post insemination were used as a measure of pup age to adjust for drug related effects on parturition.

Study no.: 00098/01063

Volume #26, and page #1

Conducting laboratory and location: Centre de Recherche, 37401 Amboise Cedex, France

Date of study initiation: 23 October 2000

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Darifenacin Hydrobromide, lot # 99135003M4, — pure

Methods

Doses: 0, 3, 10, and 50 mg/kg/day

Species/strain: Sprague-Dawley I— CD® (SD) IGS BR rats, approx. 249 g

Number/sex/group: 35 mated females/group

Route, formulation, volume, and infusion rate: oral in 10 ml/kg 0.5% (w/v) methylcellulose and 0.1% (w/v) Tween 80

Satellite groups used for toxicokinetics: 9-10/group

Study design: Dams were treated from day 6 postinsemination (implantation) to day 21 postinsemination (weaning)

Results

F₀ in-life:

	0 mg/kg/day	3 mg/kg/day	10 mg/kg/day	50 mg/kg/day
Mated females	35	35	35	35
Pregnant >1 implantation site	35	34	34	34
≥ viable pup on day 0	34	34	32	30
≥ viable pup on day 4	34	34	32	30
≥ viable pup on day 21	34	34	32	30

Dams, clinical signs	0 mg/kg/day	3 mg/kg/day	10 mg/kg/day	50 mg/kg/day
Found dead or sacrificed moribund	1		2	3
Dystocia			1	3
Abortion				1
Bloody vulval discharge				3
Lack of maternal care and cannibalism				2
Increased salivation	1		10	13
Eye discharge				6
Chromodacryorrhea	1			22

Dams, body weight and food consumption	0 mg/kg/day	3 mg/kg/day	10 mg/kg/day	50 mg/kg/day
Body weight (%), postinsemination				
__ day 6	--	0.3	0.0	-2.7
__ day 7	--	0.6	-0.5	-10.1***
__ day 8	--	0.7	-0.5	-12.5***
__ day 21	--	-0.9	-2.6	-19.0***
Body weight (%), postpartum				
__ day 1	--	1.3	-1.0	-17.0***
__ day 14	--	-0.1	-3.5**	-15.5
__ day 17	--	-1.5	-3.7*	-15.4***
__ day 21	--	-1.8	-3.3	-13.7
Body weight gain (%)				
__ day 1-6 postinsemination	--	7.7	-1.2	-2.4
__ day 6-21 postinsemination	--	-3.4	-8.2**	-53.3***
__ day 6-17 postpartum	--	-1.2	-8.0*	-66.2***
__ day 17-21 postpartum	--	-6.1	-8.6**	-38.1***
Food consumption (%)				
__ day 5-6 postinsemination	--	2.1	-0.4	-1.6
__ day 6-7 postinsemination	--	1.3	-7.8***	-69.1***
__ day 8-9 postinsemination	--	0.2	-4.5	-50.2***
__ day 9-10 postinsemination	--	7.2	-0.2	-41.6***
__ day 18-19 postinsemination	--	2.3	1.4	-26.1***
__ day 19-20 postinsemination	--	6.0	-0.2	-20.6***
__ day 20-21 postinsemination	--	-0.7	7.1	-38.2***

A number of pup and litter parameters (cold to touch, moribund, found dead) which were increased in the 10 and 50 mg/kg/day groups predominantly during lactation were considered to be due to increased maternal toxicity.

F₁ physical development:

Pup mean body weight (%)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	3	10	50	0	3	10	50
Day 1 postpartum	--	2.3	-3.2	-16.8***	--	1.4	-3.9	-18.1***
Day 14 postpartum	--	5.0	-4.7	-28.4***	--	5.6	-3.9	-28.2***
Day 28 postpartum	--	4.2	-2.4	-20.2***	--	4.1	-1.5	-19.4***
Day 56 postpartum	--	4.1	-1.7	-13.3***	--	3.8	2.8	-9.0***
Day 84 postpartum	--	4.2	-1.8	-11.7***	--	5.6	3.3	-7.2***

F₁ behavioral evaluation:

Developmental landmarks (in Days postinsemination)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	3	10	50	0	3	10	50
Surface righting reflex	25.13	25.07	25.07	25.17	25.37	25.39	25.71	26.16**
Incisors eruption	32.19	32.17	32.54	33.28***	32.08	31.97	32.46	33.35***
Eyelid opening	36.35	36.22	35.53	37.65***	36.26	36.03	36.44	37.42***
Vaginal opening	--	--	--	--	54.84	54.50	54.34	57.36**
Preputial separation	64.16	63.35	63.85	66.11**	--	--	--	--

There were no drug related effects in the Cincinnati water maze (effects on ability to swim, on learning latencies, or on learning ability) at any dose tested. There were no drug related effects on memory potential as assessed by the passive avoidance test.

Maternal and pup plasma concentrations on day 13 p.p.

UK-88,525	0 mg/kg/day	3 mg/kg/day	10 mg/kg/day	50 mg/kg/day
Range of concentration (µg/ml)	--	└		└
AUC ₀₋₂₄ (µghr/ml)	--	0.16	0.91	8.52
Tmax (hr)	--	1-3	1-3	1-3
Pup plasma concentration below limit of determination (└ 1 µg/ml) at all doses				

UK-148,993 was not detectable above the limit of determination (└ 1 µg/ml) in the low and mid dose groups except in one low dose sample at (└ 1 µg/ml). At 50 mg/kg/day, the highest maternal plasma concentrations occurred 1-6 hours following dosing and were between (└ 1) 1 µg/ml. The mean AUC₀₋₂₄ was 4.49 µghr/ml. At 5-7 hours following treatment of the dams, all plasma concentration of the pups were below the limit of determination of the assay.

Segment I fertility study historical controls:

Table 14: Pfizer and MARTA/MTA^a historical control values for pregnancy rate in Charles-River ED rats

	Number of studies	Period	Minimum (%)	Maximum (%)	Mean (%)
Pfizer Amboise, France	6**	1990 to 1995	80.0	100.0	94.08
Pfizer Groton, USA	9	1992 to 1996	68.0	100.0	91.0
MARTA/MTA	16	1990 to present	65.64	100.0	92.64

^a Middle-Atlantic Reproduction and Teratology Association (MARTA) and Midwest Teratology Association (MTA)

** Excluding darifenacin fertility study conducted in Amboise

Best Possible Copy

3.4.7 Local tolerance

Study Title: Primary Dermal Irritation Study in the Rabbit (Occluded Application)

Study No: 96052

Volume #, and page #:

Conducting laboratory and location: Pfizer Centre de Recherche, 37401 Amboise Cedex, France

Date of study initiation: 23 May 1996

GLP compliance: yes

QA- Reports Yes (x) No ():

Methods: 16 New Zealand White rabbits (2/sex/group)(2.5-3.5 kg, 3 months of age) were treated with a single dermal application of (0.5 ml/animal) test substance or vehicle under occlusive film. After 24 hours of exposure, any residual formulation or vehicle was removed by washing with warm water (about 30C).

Dosing: 0, 0.1, 0.5, and 1.0% (w/v)

Drug: UK-88,525-04 (Darifenacin Hydrobromide), batch R101, — active moiety

Formulation/vehicle: ethanol, glycerol, methyl laureate, glycerol monoleate and purified water (70.7, 28.1, 3.7, 3.8, and 30.0 g/150 ml)

Observations and times: Rabbits were examined immediately prior to application and at 25, 48, 72 and 96 hours after application. They were scored using OECD guidelines for acute dermal irritation/corrosion studies (No 404, 17 July 1992). Blood samples were taken from the three treated groups before and 10 and 25 hours after dermal application. All animals were observed daily for clinical signs.

Results: Plasma concentrations of UK-88,525 (Darifenacin) increased approximately with dose. However, there were no marked skin reactions at any dose. Erythema was observed in all treated and control groups beginning 48 hours after treatment. No dose relationship was seen, and no conclusion was drawn.

General Comments: The study was repeated at higher doses.

Study Title: Primary dermal Irritation Study in the Rabbit (Occluded Application)

Study No: 96066

Volume #, and page #:

Conducting laboratory and location: Pfizer Centre de Recherche, 37401 Amboise Cedex, France

Date of study initiation: 24 September 1996

GLP compliance: yes

QA- Reports Yes (x) No ():

Methods: 24 New Zealand White rabbits (4 females/group)(2.5-2.75 kg, 105 days of age) were treated with a single dermal application of (0.5 ml/animal) test substance or vehicle under occlusive film. After 24 hours of exposure, any residual formulation or vehicle was removed by washing with warm water (about 30C).

Dosing: 0, 0.1, 0.5, 1.0, 2.0, and 5.0% (w/v)

Drug :UK-88,525-04 (Darifenacin Hydrobromide), batch R101, active moiety

Formulation/vehicle: ethanol, glycerol, methyl laureate, glycerol monooleate and purified water (70.7, 28.1, 3.7, 3.8, and 30.0 g/150 ml)

Observations and times: Rabbits were examined immediately prior to application and at 25, 48, 72 and 96 hours after application. They were scored using OECD guidelines for acute dermal irritation/corrosion studies (No 404, 17 July 1992). Blood samples were taken from the treated groups before and 10 and 25 hours after dermal application. All animals were observed daily for clinical signs.

Results:

Incidence of erythema, 48 hours or greater after treatment:

Group (n=4)	No erythema	Barely perceptible erythema	Well-defined erythema	Moderate to severe erythema	Mean plasma C _{max} (range) (ng/ml)(t _{max} ~10 hrs)
Control	4	0	0	0	Not measured
0.1% (w/v)	3	1	0	0	-(below detect.-)
0.5% (w/v)	4	0	0	0	2.2
1.0% (w/v)	3	0 (1 at 25 hrs)	1	0	5.1
2.0% (w/v)	0	3 (3 at 25 hrs)	1	0	7.8
5.0% (w/v)	0	2 (2 at 25 hrs)	1	1 (with edema)	33.5

Summary:

Key finding(s): A no-effect level for dermal irritation by a single application of Darifenacin (UK-88,525-04) was 0.5% w/v. Plasma drug concentrations were increased in a dose-related manner after dermal (occluded) application of Darifenacin.

3.4.8 Special toxicology studies: NA

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Approval is recommended from a Pharmacology/Toxicology perspective.

Unresolved toxicology issues: none

Suggested labeling: see Executive Summary, p.1

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

3.7. APPENDIX/ATTACHMENTS

A. Previous review

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FEB - 6 1998

IND

REVIEW #1

Reviewer: Timothy W. Robison, Ph.D.
Pharmacologist, HFD-180

Sponsor: Pfizer Inc.
New York, NY

Date of Review: January 16, 1998

Date of Submission: February 27, 1996

Date of HFD-180 Receipt: February 29, 1996

Preliminary Safety Review: March 22, 1996 by Y.M. Chopra, M.D.,
Ph.D.

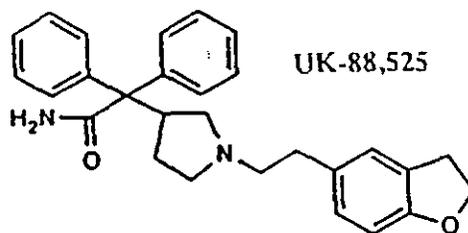
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

ORIGINAL SUMMARY

Drug: Darifenacin hydrobromide (UK-88,525-04); L

3 contain - 7.5, 15, L darifenacin.

Chemical Name and Structure: (S)-2-{1-[2-(2,3-dihydrobenzofuran-5-yl)ethyl]-3-pyrrolidinyl}-2,2-diphenylacetamide hydrobromide



Molecular formula: $C_{28}H_{30}N_2O_2 \cdot HBr$

Molecular weight: 507.5 for the hydrobromide salt.
426.6 for the base.

Category: L

1

Related Drugs/INDs/NDAs/MFs: IND — submitted by Pfizer Inc.
of New York, NY

3

Pertinent Memos/Correspondence: None.

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page(s) of trade secret.

and/or confidential

commercial information

(b4)

Preclinical Studies and Testing Laboratories:

- A. Laboratoires Pfizer
Centre de Recherche
37401 Amboise Cedex
France
- B. Drug Safety Evaluation Department
Pfizer Central Research
Pfizer Inc.
Groton, CT 06340
- C. [

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Study Type	Study Report #	Test Lab.	Drug Batch #	Review Page #
PHARMACOLOGY:				7-17
ABSORPTION:				
Pharmacokinetics in mice after oral and intravenous administration	UK-88,525/DM/35/92 & /DM/36/92			18-19
Pharmacokinetics in rats after oral and intravenous administration	UK-88,525/DM/2/92, /DM/3/92, & /DM/4/92			19-20
Pharmacokinetics in dogs after oral and intravenous administration	UK-88,525 DM/19/89 & DM/17/89			20-21
DISTRIBUTION:				
Plasma protein binding in rabbit, rat, dog, & man	UK-88,525/DM/33/92			21
Tissue Distribution in rats after intravenous administration	UK-88,525/DM/21/92			21-22

METABOLISM:				
In Vitro Metabolism	UK- 88,525/DM/ 027/92			22-23
Metabolism with the isolated, perfused rat liver and human microsomes	UK- 88,525/DM/9 /92 & /DM/11/92			23-24
Formation of UK-148,933 in dogs & its pharmacokinetics	UK- 88,525/DM/7 /93, /DM/10/93, & /DM/1/93			24-25
First pass metabolism in dogs	UK- 88,525/DM/1 8/89			25-26
EXCRETION AND METABOLISM:				
Excretion & metabolism in male mice after oral administration	UK- 88,525/DM/0 05/95 (PFZ 608/942850)			27-28
Excretion & metabolism in rats after intravenous administration	UK- 88,525/DM/ 13/92			29-30
Excretion & metabolism in female rabbits after oral administration	UK- 88,525/DM/2 9/9 (PFZ 609/943012)			31-33
Excretion & metabolism in dogs after intravenous and oral administration	UK- 88,525/DM/ 10/92 & /DM/12/92			34-36
ACUTE TOXICITY				
Mouse and Rat:				
Oral and Intraperitoneal Toxicity in mice and rats	90010, 90011, 90012, & 90013	A	R1	38-40

SUBACUTE TOXICITY:				
Mice:				
3 month dietary toxicity study in CD1 mice	92046	A	R7	40-44
Rat:				
2-week intravenous toxicity in rats	94029	A	R11	45-47
1 month gavage study in rats	89-751-03	B	R1	48-52
1 month dietary study in rats	93042	A	R7	52-53
1 month oral toxicity study in rats-new synthetic route	94072	A	R101	53-58
3 month dietary toxicity study in rats	92051	A	R7	59-65
Dog:				
2 week intravenous toxicity in beagle dogs	94028	A	R11	65-69
1 month gavage study in beagle dogs	89-751-02	B	R1	69-74
CHRONIC TOXICITY:				
Rats:				
6 month oral toxicity in rats	92028	A	R7	74-79
Dogs:				
6 month oral toxicity in dogs	92029	A	R7	79-85
REPRODUCTIVE TOXICITY:				
Rats:				
Segment I Fertility & Reproductive Performance study in rats	92101 and 93208	A		86-93
Segment II Teratology study in rats	91028 and 91029	A	R4	93-97
Segment III Peri- and Postnatal Development study in rats	93076	A	R9	98-102

Rabbits:				
Segment II Tetratology study in rabbits	91071 and 91072	A	R4	102-108
GENOTOXICITY:				
Ames Test	89-751-01 & 89-751-05	B	632-231-13	108-109
Chinese hamster ovary/hypoxanthine-guanine phosphoribosyl transferase assay	89-751-01 & 89-751-05	B	R1	109-110
Human lymphocyte chromosomal aberration assay	89-751-01 & 89-751-05	B	R1	111-112
Mouse bone marrow metaphase analysis	89-751-01 & 89-751-05	B	R1	112-113
SPECIAL TOXICITY:				
7 day dermal irritation study in rabbits	Not given	C	632-231-13	113-114

PHARMACOLOGY:

Darifenacin (UK-88,525) is a potent, competitive antagonist of the muscarinic M_3 receptor. Darifenacin displaced 3H -quinuclidinyl benzoate from the human muscarinic M_3 receptor subtype expressed in Chinese hamster ovary cells with a pK_i of 8.77. Its affinity for M_1 , M_2 , M_4 , and M_5 receptor subtypes was at least 1 to 2 orders of magnitude lower. Atropine did not differentiate between these human cloned muscarinic subtypes. With in vivo studies using dogs, darifenacin inhibited bethanechol, cholecystokinin, and food-induced increases of small intestinal motility. Its inhibition of stimulated increases in intestinal motility were more selective than atropine, which also had effects on salivation, pupil size, and heart rate in the same dose range. The hydroxylated metabolite of darifenacin, UK-148,933, is also a muscarinic M_3 receptor antagonist, and part of the in vivo activity of darifenacin is probably related to this compound. UK-148,993 is composed of two diastereoisomers, UK-154,954 and UK-186,865, which occur in plasma at a ratio of 4 to 1. In vitro, the more abundant diastereoisomer, UK-154,954, has a selectivity and potency similar to darifenacin. The pharmacology of darifenacin has been examined with regard to its affinity for M_3 muscarinic receptors as compared to other

receptor types, its affinity for muscarinic receptor subtypes versus atropine, its effects on gastrointestinal motility and propulsive activity, its effects on gastric emptying, and its pharmacodynamic actions as compared with atropine.

Primary Pharmacology

In Vitro Studies:

Muscarinic Receptor Affinity Profile In Vitro (Volume 4, Section 4.1.1, Page 8 26).

The ability of darifenacin, UK-148,993 (hydroxylated metabolite of darifenacin), and atropine to inhibit muscarinic agonist-induced responses of isolated tissues and ^3H -quinuclidinyl benzoate (^3H -QNB) binding to human muscarinic receptor subtypes was used to assess their affinity for distinct muscarinic receptor subtypes. Darifenacin was a potent, competitive antagonist of muscarinic M_3 receptor mediated contractions of smooth muscle preparation in vitro. Darifenacin was 5 times more active on the ileum than on the bladder and trachea. Slopes of Schild plots were close to unity indicating that antagonism was competitive in nature. Darifenacin was equipotent with atropine with regard to ileum and bladder. Compared with atropine, Darifenacin was 17 times weaker as an inhibitor of carbachol-induced (M_2 receptor mediated) slowing of the rate of beating of the spontaneously beating guinea pig isolated right atrium. The hydroxylated metabolite of Darifenacin, UK-148,933, was also a potent muscarinic receptor antagonist. Darifenacin displaced ^3H -QNB from the human muscarinic M_3 receptor subtype expressed in Chinese hamster ovary cells with a pK_i of 8.77. Its affinity for M_1 ($\text{pK}_i=7.07$), M_2 ($\text{pK}_i = 6.59$), M_4 ($\text{pK}_i=8.13$), and M_5 ($\text{pK}_i=8.09$) receptor subtypes was significantly lower. In contrast, atropine did not differentiate between these human cloned muscarinic subtypes (pK_i ranged from 8.57 to 9.27).

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In vitro muscarinic receptor affinity profile of Darifenacin, UK-148,993, and atropine (Adapted from Sponsor's Table 1).

Compound	pA ₂ /Schild plot	Guinea pig urinary bladder M ₃	Guinea pig ileum M ₃	Guinea pig trachea M ₃	Guinea pig atrium M ₂	Rabbit vas deferens M ₃
		Acetylcholine	Carbachol	Carbachol	Carbachol	McN-A-343
Darifenacin	pA ₂	8.70	9.44	8.70	7.48	7.90
	Schild	-0.74	-1.16	-1.08	-0.84	-0.94
UK-148,993	pA ₂	8.24			7.02	8.30
	Schild	-1.08			-1.20	-0.95
Atropine	pA ₂	9.01	9.44	9.20	8.72	9.58
	Schild	-0.86	-0.94	-1.18	-1.10	-0.90

In Vivo Studies:

Inhibition of Bethanechol-Induced Smooth Muscle Responses in Anesthetized Dogs (Volume 4, Section 4.1.2.1, Page 8 29).

The ability of darifenacin, administered by the intravenous route at 3 to 100 µg/kg, to antagonize bethanechol-induced urinary bladder contraction, salivation, and small intestinal motility was examined in an anesthetized dog model. Darifenacin inhibited bethanechol-induced urinary bladder contraction, salivation, and small intestinal motility in dose-dependent manner. The ED₅₀ for inhibition of salivation was higher than ED₅₀ values for inhibition of urinary bladder contraction or small intestinal motility. With doses ≤100 µg/kg, basal heart rate was unaffected. Atropine also inhibited these bethanechol-induced responses in a dose-dependent manner; however, a dose of 30 µg/kg elevated heart rate by 143.1%. Darifenacin was less potent than atropine; however, its effect appeared to be more selective for M₃-mediated receptor responses as compared with M₂-mediated responses (heart). Bethanechol-induced urinary bladder contraction, salivation, and small intestinal motility were similarly inhibited with intravenous infusion of darifenacin (0.1 to 30 µg/kg/min) and atropine (0.1 to 1 µg/kg/min).

The effects of darifenacin and atropine, administered by the intravenous route, on bethanechol-induced urinary bladder contraction, salivation, and small intestinal motility and on basal heart rate in the anesthetized dog (Adapted from Sponsor's Table 2).

Compound	ED ₅₀ (µg/kg)			ED _{143.1} (µg/kg)
	Urinary bladder contraction	Salivation	Small-intestinal motility	Heart Rate
Atropine	3.1	3.5	6.2	30
Darifenacin	14.9	26.1	19.3	> 100

The effects of darifenacin and atropine, administered by intravenous infusion, on bethanechol-induced urinary bladder contraction, salivation, and small intestinal motility and on basal heart rate in the anesthetized dog (Adapted from Sponsor's Table 3).

Compound	ED ₅₀ (µg/kg)			ED ₁₅₀ (µg/kg)
	Urinary bladder contraction	Salivation	Small-intestinal motility	Heart Rate
Atropine	0.21	0.23	0.27	0.46
Darifenacin	1.0	1.05	1.3	24

Inhibition of Pelvic Nerve Stimulation-Induced Bladder Contraction and Submandibular Gland Duct Stimulation-Induced Salivary Secretion in Anaesthetized Dogs (Volume 4, Section 4.1.2.2, Page 8 31).

The effect of darifenacin on urinary bladder contraction induced by electrical stimulation of the bladder branch of the pelvic nerve or salivary flow induced by electrical stimulation of the submandibular gland duct was examined in anesthetized dogs. Darifenacin, administered by the intravenous route, inhibited these responses in a dose-dependent manner as shown in the table below. A 50% increase in basal heart rate was not observed with doses ≤300 µg/kg. Atropine also inhibited these response in a dose-dependent manner; however, a 50% increase in basal heart rate was observed at 12.2 µg/kg. Darifenacin was less potent than atropine; however, its selectivity for M₃ receptor-mediated responses over M₂ receptor-mediated responses was greater. The hydroxylated metabolite of darifenacin, UK-148,993, also inhibited these responses in a dose-dependent manner similar to darifenacin. UK-148,993 produced a 50% increase in heart at 133.7 µg/kg; although, it appeared to retain a greater selectivity for M₃ receptor mediated response.

The effects of atropine, darifenacin, and UK-148,933, administered by the intravenous route, on urinary bladder contraction induced by electrical stimulation of the bladder branch of the pelvic nerve or salivary flow induced by electrical stimulation of the submandibular gland duct in anesthetized dogs (Adapted from Sponsor's Table 4).

Compound	ED ₃₅ (μg/kg)		ED ₁₅₀ (μg/kg)
	Urinary bladder contraction	Salivary secretion	Heart Rate
Atropine	5.2	5.1	12.2
Darifenacin	17.1	39.0	>300
UK-148,993	16.3	33.7	133.7

Effects on Bethanechol-Induced Reduction in Bladder Capacity in Conscious Dogs (Volume 4, Section 4.1.2.3, Page 8 34).

Darifenacin administered by the oral route at doses of 0.3 to 3 mg/kg inhibited the bethanechol-induced reduction of urinary bladder capacity with an ED₅₀ value of 0.6 mg/kg. Atropine was more potent with an oral ED₅₀ value of 0.03 mg/kg.

Effects on Cholecystokinin-Stimulated Small-Intestinal and Colonic (Large Bowel) Smooth Muscle in Anesthetized Dogs (Volume 4, Section 4.1.2.4, Page 8 34).

The effect of darifenacin on cholecystokinin octapeptide (CCK)-induced increases in the motility of the small intestine and colon were examined in an anesthetized dog model. CCK was intravenously infused at 75-100 ng/kg/min for 9 min. Darifenacin inhibited CCK-induced increases in the motility of the small intestine and colon with ED₅₀ values of 0.34 and 0.38 μg/kg/min, respectively. Infusion of darifenacin at doses ≤10 μg/kg/min had no effects on mean arterial blood pressure, heart rate, left ventricular systolic pressure, cardiac contractility, cardiac output, total peripheral resistance, PR interval, and electrocardiographic (ECG) parameters. However, infusion of 30 μg/kg/min produced an increase of heart rate (ED₁₅₀=26 μg/kg/min) and cardiac output (+42%) with a reflex fall in total peripheral resistance (-26%); however, mean arterial blood pressure, cardiac contractility, PR interval, and ECG parameters were unchanged. Intravenous infusion of atropine inhibited CCK-induced increases in motility of the small intestine and colon with ED₅₀ values of 0.63 and 0.8 μg/kg/min; however, changes in hemodynamic parameters were observed over this dose range (i.e., heart rate ED₁₅₀=0.68 μg/kg/min). Darifenacin was more potent than atropine, and displayed greater selectivity for M₃ receptor mediated responses.

Effects on Food-Stimulated Small Intestinal Motility in Conscious Dogs (Volume 4, Section 4.1.2.5, Page 8 39).

The effect of darifenacin on food-induced increases of intestinal motility were assessed in conscious dogs. Darifenacin administered by the oral route at doses of 0.03 to 0.3 mg/kg inhibited food-induced increases of small intestinal motility by 40 to 75%. Maximal activity occurred within 60-90 min. The oral ED₅₀ value was 0.1 mg/kg. Atropine, administered by the oral route at doses of 0.03 to 0.3 mg/kg, also inhibited increases of motility. Maximal activity occurred within 30 min. The oral ED₅₀ value was 0.04 mg/kg. With both darifenacin and atropine at 0.3 mg/kg, inhibition of the food stimulated response was still present at 3 hr. In a second set of experiments, darifenacin was administered by the oral route at 0.1 mg/kg/day for 10 days to assess its effects on food stimulated small intestinal motility on days 1, 4, 7, and 10. Food-stimulated increases of small intestinal motility were inhibited by 40-50% on each day. This suggests that there was no tolerance to or accumulation of its smooth muscle effect with repeated dosing. Total gastrointestinal transit time, as assessed by counting the appearance of radio-opaque markers in the feces, was unchanged.

Effects on Gastric Emptying in the Conscious Dog (Volume 4, Section 4.1.2.6, Page 8 42).

The effect of darifenacin on gastric emptying was assessed by measurement of the emptying rate of a liquid test meal given to conscious dogs. Atropine at oral doses of 0.1 and 0.3 mg/kg inhibited gastric emptying by 30 and 45%, respectively. The ED₅₀ was calculated as 0.26 mg/kg. Darifenacin at an oral dose of 3 mg/kg inhibited gastric emptying by 56%; however, there was no effect at 0.3 or 1 mg/kg. This dose is 30 times the oral ED₅₀ of 0.1 mg/kg for inhibition of the food-stimulated increase in small intestinal motility.

Gastrointestinal Propulsive Activity in Rats (Volume 4, Section 4.1.2.7, Page 8 42).

Darifenacin, administered by the oral route at doses of 0.3 to 3 mg/kg to rats, slightly reduced the transit of charcoal meal to 46.1-50.7% of small intestinal length as compared with 58.2% for the control. Morphine at an oral dose of 4 mg/kg significantly reduced the transit to 26.7% of the small intestinal length.

Activity at Other Muscarinic Sites:**Effects on Salivation (Volume 4, Section 4.2.1, Page 8 47).**

The effects of darifenacin on agonist-stimulated salivation in mice, guinea pigs, and dogs was examined. Darifenacin administered by the intravenous route at 0.1 to 3 mg/kg to mice caused a dose-dependent inhibition of oxotremorine-induced salivation with an ED₅₀ of 0.33 mg/kg. Darifenacin administered by the intravenous route to guinea pigs at 3 to 300 µg/kg caused a dose-dependent inhibition of methacholine-induced salivation with an ED₅₀ of 33.7 µg/kg. Darifenacin administered by the oral route at 0.3-3 mg/kg to conscious dogs caused a dose related inhibition of basal salivation with an ED₅₀ of 1 mg/kg. For all three species, atropine was more potent than Darifenacin.

Darifenacin and Atropine ED₅₀ values for inhibition of oxotremorine-induced salivation in mice, methacholine-induced salivation in guinea pigs, and basal salivation in dogs.

Mouse		Guinea pig		Dog	
Oxotremorine-induced salivation		Methacholine-induced salivation		Basal salivation	
IV Darifenacin	IV Atropine	IV Darifenacin	IV Atropine	Oral Darifenacin	Oral Atropine
ED ₅₀ = 0.33 mg/kg	ED ₅₀ = 0.03 mg/kg	ED ₅₀ = 6.8 µg/kg	ED ₅₀ = 33.7 µg/kg	ED ₅₀ = 1 mg/kg	ED ₅₀ = 0.06 mg/kg

Effects on Pupil Size in Mouse and Conscious Dog (Volume 4, Sections 4.2.2.1 and 4.2.2.2, Page 8 53).

The effects of darifenacin on pupil size in mice and conscious dogs were examined. Atropine administered by the intravenous route at 0.003-0.1 mg/kg caused a dose-dependant increase in pupil size in mice within 15 min. Darifenacin administered by the intravenous route to mice at 0.3-3 mg/kg caused a dose-dependent increase in pupil size; although, it was considerably less potent than atropine. Atropine administered by the oral route at 0.3 and 1 mg/kg in mice caused an increase in pupil size within 15 min. Darifenacin administered by the oral route at 20, 30, and 100 mg/kg to mice caused a dose-dependent increase in pupil size; although, its effect was considerably less potent than atropine. Conscious dogs received darifenacin by the oral route at 3 mg/kg and only 1 of 4 dogs was found with an increase in pupil size. In contrast, atropine produced mydriasis in all dogs after oral doses of 0.1 and 0.3 mg/kg.

Darifenacin ED₂₀₀ values for increased pupil size in mice following administration by the intravenous or oral route.

Intravenous Route		Oral Route	
Darifenacin	Atropine	Darifenacin	Atropine
0.46 mg/kg	0.003 mg/kg	14 mg/kg	0.24 mg/kg

Effects on Heart Rate and Hemodynamic Parameters:

Effects of Darifenacin on Hemodynamic Parameters in Conscious Dogs
(Volume 4, Section 4.2.3.3, Page 8 58).

In conscious dogs, darifenacin administered by the oral route at 1 or 3 mg/kg produced increases in heart rate (10-30 beats/min) and cardiac output (0.1-0.5 L/min) with a reflex decrease in total peripheral resistance. Maximum increases in heart rate and cardiac output occurred within 30 min and some degree of recovery had occurred within 280 min. Other hemodynamic parameters (i.e., stroke volume, left ventricular systolic pressure, left ventricular end diastolic pressure, and LV dp/dt max) and electrocardiogram parameters were unchanged. Tachycardia appeared to be the consequence of cardiac M₂ muscarinic receptor antagonism.

Tremors in Mice (Volume 4, Section 4.2.4, Page 8 59).

Darifenacin with an intravenous ED₅₀ value of 0.8 mg/kg blocked the oxotremorine-induced centrally mediated skeletal muscle tremor in mice. In contrast, atropine blocked this response with an intravenous ED₅₀ value of 0.1 mg/kg. This result suggested that the potential for central nervous system side effects with darifenacin may be considerably lower than that observed for atropine.

Affinity for Other Receptors and Enzymes (Volume 4, Section 4.3, Page 8 59).

The selectivity of darifenacin for muscarinic receptors was compared with its effects on acetylcholinesterase, and a number of physiological receptors and binding sites using standard radioligand binding techniques and appropriate isolated tissue preparations. Darifenacin at 1000 nM had no significant interactions in binding studies with α_1 , α_2 , and β -adrenoreceptors, adenosine (A1), dopamine (D2), 5-HT₂, histamine (H₁) and opioid receptors as well as dihydropyridine and benzodiazepine binding sites. The IC₅₀ of darifenacin in the muscarinic receptor assay was 103 nM. Darifenacin at concentrations ≤ 10 μ M had no effect on acetylcholinesterase activity using an electric eel preparation. Darifenacin had no effect on basal contractions of rat uterine smooth muscle; however, oxytocin-induced contraction were inhibited in a dose-dependent manner with an IC₅₀ value of 5.9 μ M. Darifenacin at concentrations ≤ 1 μ M had no effect on histamine-induced contractions of the guinea pig isolated ileum.

Darifenacin is a potent, competitive antagonist of the muscarinic M_3 receptor. Darifenacin displaced 3H -quinuclidinyl benzoate from the human muscarinic M_3 receptor subtype expressed in Chinese hamster ovary cells with a pK_i of 8.77; however, its affinity for M_1 , M_2 , M_4 , and M_5 receptor subtypes was at least 1-2 orders of magnitude lower. Atropine did not differentiate between these receptor subtypes. Darifenacin antagonized carbachol-induced contractions of a ileal smooth muscle preparation in vitro with a pA_2 of 9.44. The inhibitory effects of darifenacin on stimulated increases of intestinal motility were examined in several experiments with dogs. In general, its effects were more selective than those of atropine, which also had effects on salivation, pupil size, and cardiovascular parameters at the same doses. Darifenacin by the intravenous route inhibited bethanechol-induced increases of small intestinal motility ($ED_{50}=19 \mu\text{g}/\text{kg}$). Intravenous infusion of darifenacin inhibited cholecystokinin (CCK)-induced increases in the motility of the small intestine and colon with ED_{50} values of 0.34 and 0.38 $\mu\text{g}/\text{kg}/\text{min}$, respectively. Darifenacin at doses $\leq 10 \mu\text{g}/\text{kg}/\text{min}$ had no effects on cardiovascular parameters. Infusion of atropine inhibited CCK-induced increases in motility of the small intestine and colon with ED_{50} values of 0.63 and 0.8 $\mu\text{g}/\text{kg}/\text{min}$, respectively; however, changes in hemodynamic parameters occurred over this dose range. Darifenacin by the oral route inhibited food-induced increases of small intestinal motility ($ED_{50}=0.1 \text{ mg}/\text{kg}$). Further, darifenacin, at an oral dose of 0.1 $\text{mg}/\text{kg}/\text{day}$ for 10 days, reduced food-stimulated increases of small intestinal motility by 40-50%. Apparently, there was no tolerance to or accumulation of the effect of darifenacin on smooth muscle. Total gastrointestinal transit time was not significantly affected by Darifenacin. Darifenacin at an oral dose of 3 mg/kg to dogs, inhibited gastric emptying by 56%; however, this dose is 30-times the ED_{50} for inhibition of food stimulated increases of small intestinal motility. Darifenacin inhibited basal salivation with an ED_{50} of 1 mg/kg ; however, this dose was 10 times the ED_{50} for inhibition of food stimulated increases of small intestinal motility. Conscious dogs received darifenacin by the oral route at 3 mg/kg and only 1 of 4 dogs was found with an increase in pupil size. In contrast, atropine produced mydriasis in all dogs after oral doses of 0.1 and 0.3 mg/kg . In conscious dogs, darifenacin administered by the oral route at 1 or 3 mg/kg produced increases in heart rate (10-30 beats/min) and cardiac output (0.1-0.5 L/min) with a reflex decrease in total peripheral resistance. Tachycardia appeared to be the consequence of cardiac M_2 muscarinic receptor antagonism. Darifenacin and atropine blocked the oxotremorine-induced centrally mediated skeletal muscle tremor in mice with intravenous ED_{50} values of 0.8 and 0.1 mg/kg , respectively, suggesting the potential for central nervous system side effects may be lower with darifenacin.

Safety Pharmacology

Effects on the Central and Peripheral Nervous System:

Acute Oral Symptomatology of Darifenacin in the Rat (Volume 4, Section 5.1.1, Page 8 65).

The effects of darifenacin on behavior, core temperature, mydriasis, and food and water consumption were examined in rats following oral administration of 3, 10, 30, and 100 mg/kg. Darifenacin at 3-100 mg/kg had no effect on behavior or core temperature. Darifenacin at 10 and 30 mg/kg produced mydriasis which persisted >8 hr; however, at 100 mg/kg, mydriasis lasted from 24-30 hr. Doses of 3 and 10 mg/kg had no effect on food and water intake. A dose of 30 mg/kg reduced water intake by 20% on the first night; however, it returned to control levels by the second night. A dose of 100 mg/kg reduced food and water intake by 22 and 55% on the first night, respectively; however, these parameters had returned to control levels by the second night. Body weight gain for rats treated with 100 mg/kg was reduced to 23 g as compared with 31 g in controls.

Effects of Darifenacin on Motor Coordination in the Mouse (Volume 4, Section 5.1.2, Page 8 66).

Darifenacin administered by the oral route at doses ≤ 3 mg/kg had no effect on motor coordination in mice at 1 and 3 hr after dosing using the rotarod test. The positive control, chlorpromazine, at oral doses of 2.5-10 mg/kg significantly reduced motor coordination.

Effects of Darifenacin on Alcohol- and Pentobarbital-Induced Sleeping Times in Mice (Volume 4, Section 5.1.3, Page 8 66).

Darifenacin at oral doses ≤ 3 mg/kg had no effect on alcohol- or pentobarbital-induced sleeping time in mice. The positive control, chlorpromazine at oral doses of 1.25-5 mg/kg significantly prolonged alcohol and pentobarbital sleeping times.

Effects on the Cardiovascular System, Autonomic Function, and Somatic Function in Anesthetized Cats:

Effects of Darifenacin on the Cardiovascular System in Cats (Volume 4, Section 5.2.1, Page 8 70).

Darifenacin administered by the intravenous route at 0.1, 0.3, and 1 mg/kg to anesthetized cats had no effects on mean arterial blood pressure, heart rate, and left ventricular systolic pressure over a 30 min period after dosing. Darifenacin at 1 mg/kg produced a small, but sustained decrease of cardiac contractility (LV dp/dt max); although, 0.1 and 0.3 mg/kg had no effect. The initial level

of cardiac contractility was 3514 mm Hg/sec, and Darifenacin at 0 and 30 min produced decreases of -37 ± 13 and -24 ± 11 , respectively. Effects on electrocardiogram conduction were not assessed.

Effects of Darifenacin on Autonomic Function in Cats (Volume 4, Section 5.2.2, Page 8 70).

Darifenacin administered by the intravenous route at 0.1, 0.3, or 1 mg/kg had no effect on resting or stimulated tension of the nictating membrane in cats. Nictating membrane tension was increased through electrical stimulation of the pre-ganglionic superior cervical sympathetic nerve. Darifenacin does not possess sympathomimetic or ganglion stimulating or blocking activity.

Darifenacin administered by the intravenous route at doses ≤ 1 mg/kg had neither marked nor dose-related effects on serotonin, isoprenaline, or phenylephrine-induced alterations of mean arterial blood pressure, left ventricular systolic pressure, or cardiac contractility. Darifenacin antagonized the cardiovascular effects of acetylcholine in a dose-dependent manner. Darifenacin potentiated the histamine-induced increase of cardiac contractility. Darifenacin appeared to possess no α - or β -adrenergic, histaminergic, or serotonergic blocking activity.

Effects of Darifenacin on Somatic Function in Cats (Volume 4, Section 5.2.3, Page 8 81).

Darifenacin administered by the intravenous route at doses ≤ 1 mg/kg had no effect on resting tension or the electrically-induced increase in tension of the gastrocnemius muscle in cats during a 30 min period after dosing.

Effects of Darifenacin on Gastric Acid Secretion in the Rats (Volume 4, Section 5.3, Page 8 81).

Darifenacin administered by the oral route at 0.3, 1, and 3 mg/kg had no effects on basal gastric acid secretion in rats. Citmedine at an oral dose of 25 mg/kg inhibited gastric acid secretion, while histamine given as a subcutaneous dose of 10 mg/kg stimulated secretion.

Effects of Darifenacin on the Excretion of Fluid and Electrolytes in Female Rats (Volume 4, Section 5.4, Page 8 81).

Darifenacin administered by the oral route to female rats at 0.3, 1, and 3 mg/kg had no effect on urinary pH or the urinary excretion of Na^+ , K^+ , Cl^- , and fluid. Furosemide at an oral dose of 20 mg/kg increased urinary excretion of fluid, Na^+ , and Cl^- and reduced urinary pH; although, excretion of K^+ was unaffected.

Effects of Darifenacin on the Respiratory System in Conscious Rats
(Volume 4, Section 5.5, Page 8 81).

The effect of Darifenacin on blood gas tensions and blood pH were examined in conscious rats for a period of 25 min after dosing. Darifenacin administered by the intravenous route at 1 mg/kg had no effect on arterial blood pH or pO_2 . The positive control, morphine at an intravenous dose of 4 mg/kg decreased arterial blood pH and pO_2 .

Effects of Darifenacin on Electroconversion in the Anesthetized Dog
(Volume 4, Section 5.6, Page 8 85).

Darifenacin administered by the intravenous route at 1 mg/kg did not effect the defibrillation of the heart (ventricular fibrillation achieved by high frequency electrical stimulation) in anesthetized dogs achieved by DC shock.

Darifenacin at oral doses of 3 to 100 mg/kg in rats had no effect on behavior or core temperature. Darifenacin at 10 and 30 mg/kg produced mydriasis which persisted >8 hr; however, at 100 mg/kg, mydriasis lasted from 24-30 hr. Darifenacin at oral doses ≤ 3 mg/kg had no effect on motor coordination in mice for 3 hr after dosing. Darifenacin at oral doses ≤ 3 mg/kg had no effect on alcohol- or pentobarbital-induced sleeping time in mice. Darifenacin at intravenous doses of 0.1, 0.3, and 1 mg/kg to anesthetized cats had no effects on mean arterial blood pressure, heart rate, and left ventricular systolic pressure for 30 min after dosing; however, at 1 mg/kg, it produced a small, but sustained decrease of cardiac contractility (LV dP/dt max). Darifenacin at intravenous doses of 0.1, 0.3, or 1 mg/kg had no effect on resting or stimulated tension of the nictating membrane in cats. Darifenacin at intravenous doses ≤ 1 mg/kg had neither marked nor dose-related effects on serotonin, isoprenaline, or phenylephrine-induced alterations of mean arterial blood pressure, left ventricular systolic pressure, or cardiac contractility. Darifenacin antagonized the cardiovascular effects of acetylcholine in a dose-dependent manner. Darifenacin potentiated the histamine-induced increase of cardiac contractility. Darifenacin at intravenous doses ≤ 1 mg/kg had no effect on resting tension or the electrically-induced increase in tension of the cat gastrocnemius muscle. Darifenacin at oral doses of 0.3-3 mg/kg had no effects on basal gastric acid secretion in rats. Darifenacin at oral doses of 0.3-3 mg/kg to female rats had no effect on urinary pH or the urinary excretion of Na^+ , K^+ , Cl^- , and fluid. Darifenacin at an intravenous dose of 1 mg/kg in rats had no effect on arterial blood pH or pO_2 . Darifenacin at an intravenous dose of 1 mg/kg did not effect the defibrillation of the heart in anesthetized dogs.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION:

Absorption:

Pharmacokinetics of [¹⁴C]-Darifenacin in the Mouse Following Single Oral and Intravenous Administration (Protocol numbers UK-88,525/DM/35/92 p.o. and UK-88,525/DM/36/92 i.v.; Volume 13, Section DM2, Page 8 3743).

Methods: The pharmacokinetics of darifenacin were determined in male CD1 mice following a single intravenous or oral administration of [¹⁴C]-darifenacin. The intravenous and oral doses were 1.9 and 7.9 mg/kg/day, respectively. Blood samples were collected at 0.1-48 hr following intravenous administration or at 0.25-48 hr after oral administration from 5 mice/time point. Total radioactivity in samples was determined by liquid scintillation counting. Plasma samples were also analyzed for Darifenacin and UK-148,993 by HPLC with either ultraviolet or fluorescence detection.

Results: Darifenacin was widely distributed beyond the blood volume owing to its lipophilic nature. Darifenacin was rapidly cleared from the plasma at a level that significantly exceeded hepatic plasma flow (~54 mL/min/kg) and the half-life was correspondingly short. A metabolic profile of the plasma found that only 8% of the radioactivity was darifenacin suggesting extensive metabolism of the parent compound. UK-148,993 was not found following intravenous administration. Using pooled plasma collected from 0-48 hr, the parent compound and 8 compounds were found following oral administration, while the parent compound and six metabolites were found following intravenous administration. The major metabolite following intravenous or oral administration was a dihydrobenzofuran ring opened acid that made up 37 and 24% of the radioactivity, respectively. Darifenacin made up 30 and 16% of the radioactivity following intravenous or oral administration, respectively.

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Page 19

Toxicokinetic parameters of plasma darifenacin and UK-148,993 following intravenous or oral administration of [¹⁴C]-darifenacin at a dose of 2 or 8 mg/kg, respectively.

Parameter	Intravenous administration of 2 mg/kg		Oral administration of 8 mg/kg	
	Darifenacin	UK-148,993	Darifenacin	UK-148,993
C _{max} (ng/mL)	-	Not detected	302	107
T _{max} (hr)	-	-	0.5	0.5
Elimination t _{1/2} (hr)	0.5	-	-	-
AUC _{0-1hr} (ng·hr/mL)	114	-	-	-
AUC _{0-∞} (ng·hr/mL)	148	-	-	-
Plasma clearance (mL/min/kg)	282	-	-	-
Volume of distribution (L/kg)	12	-	-	-

Toxicokinetic parameters for plasma radioactivity following intravenous or oral administration of [¹⁴C]-darifenacin at a dose of 2 or 8 mg/kg, respectively.

Parameter	Intravenous administration	Oral administration
Half life (hr)	14.3	18.6
AUC _{0-48hr} , ng·hr/mL	1811	3742
AUC _{0-∞} , ng·hr/mL	1893	3989
% Parent in plasma	8	-

Pharmacokinetics of Darifenacin in Male and Female Rats Following Single Intravenous (2.5 mg/kg) and Oral (10 mg/kg) Administration (Protocol numbers UK-88,525/DM/2/92 (IV), UK-88,525/DM/3/92 (PO), and UK-88,525/DM/4/92 (assay validation); Volume 13, Section DM3, Page 8 3782).

Methods: The pharmacokinetics (toxicokinetics) of darifenacin in rats were determined following a single intravenous or oral administration. Plasma samples were analyzed for darifenacin using a HPLC assay with ultraviolet detection.

Results: Following oral administration of darifenacin to rats, the C_{max} and T_{max} were 106 ng/mL and 2 hr, respectively. The oral bioavailability was 16%. Darifenacin was widely distributed beyond the blood volume owing to its lipophilic nature. Darifenacin was rapidly cleared from the plasma at a level that significantly exceeded hepatic plasma flow (~33 mL/min/kg) and the half-life was correspondingly short.

Toxicokinetic parameters of darifenacin following a single intravenous or oral dose in rats.

Parameter	Intravenous		Oral	
	Male	Female	Male	Female
Dose (mg/kg)	2.1	2.4	8.7	9.6
AUC _{0-24hr} ng.hr/mL	554	720	229	652
Elimination $t_{1/2}$ (hr)	2.2	0.9	3.6	3.1
Clearance mL/min/kg	53	55	-	-
Volume of dist. (L/kg)	9.9	4.4	-	-
Bioavail- ability, %	-	-	10	22

Pharmacokinetics of Darifenacin in the Dog After Intravenous and Oral Administration (Protocol number: UK-88,525/DM/19/89 (iv) and UK-88,525/DM/17/89 (oral); Volume 13, Section DM4, Page 8 3790).

Methods: The pharmacokinetics of darifenacin were determined in 4 dogs (2 males and 2 females) following either an intravenous dose of 0.6 mg/kg or an oral dose of 4 mg/kg. For intravenous administration, darifenacin was dissolved in a vehicle of PEG 200: saline: 1 M HCl (2:4:0.2) and delivered into the cephalic vein. For oral administration, darifenacin was dissolved in 0.5% methocell A4M 0.1% Tween 80. Blood samples were collected at time points ranging from 0.1-24 hr after intravenous administration or 0.25-24 hr after oral administration. Plasma levels of darifenacin were determined using a HPLC assay with ultraviolet detection.

Results: Following oral administration of darifenacin to dogs, bioavailability was 105%. Darifenacin was widely distributed beyond the blood volume owing to its lipophilic nature. Darifenacin was rapidly cleared from the plasma at a level that significantly exceeded hepatic plasma flow (~18.5 mL/min/kg) and the half-life was correspondingly short.

Parameter	Intravenous Administration	Oral Administration
C _{max} (ng/mL)	-	332
T _{max} (hr)	-	1.25
Half-life (hr)	1.6	4.3
AUC _{0-∞} , ng·hr/mL	347	2394
Clearance, mL/min/kg	29.8	-
V _d , L/kg	4.2	-
Bioavailability, %		105

Distribution:

Binding of [¹⁴C]-Darifenacin in the Plasma of Rabbit, Rat, Dog, and Man (Protocol number: UK-88,525/DM/33/92; Volume 13, Section DM8, Page 8 3718).

Methods: The binding of [¹⁴C]-darifenacin in plasma of rabbit, rat, dog, and human was examined at concentrations of 107 and 536 ng/mL using a membrane dialysis procedure.

Results: Plasma protein binding in rat, dog, and human exceeded 90% indicative of extensive binding. Binding was lower in rabbit plasma as compared to rat, dog, and man.

Species	% Darifenacin bound to plasma protein	
	107 ng/mL	536 ng/mL
Rabbit	78.9	77.3
Rat	95.4	94.1
Dog	93.7	92.4
Human	94.2	94.3

The Tissue Distribution of Radioactivity in Male and Female Rats After Single Intravenous Administration of [¹⁴C]-Darifenacin at a Dose Level of 5 mg/kg (Protocol number: Darifenacin/DM/21/92; Volume 13, DM7, Page 8 3760).

Methods: The tissue distribution of radioactivity was examined by whole body autoradiography at 0.08, 1, 24, and 72 hr following intravenous administration of 5 mg/kg [¹⁴C]darifenacin to 4 pigmented male rats and 1 female rat.

Results: At 0.08 and 1 hr, radioactivity was widely distributed throughout the body, consistent with the basic and lipophilic nature of the compound, and tissue concentrations exceeded plasma concentrations by several fold. By 24 hr, excretion mainly through the bile and into the feces was complete. Binding to melanin was observed, which was consistent with the basic and lipophilic nature of the drug. Radioactivity bound to melanin was slowly eliminated.

Concentrations of Radioactivity in Selected Tissues ($\mu\text{g/g}$) of Rat at 0.08, 1, 24, and 72 hr after intravenous administration of 5 mg/kg [^{14}C]-darifenacin (Adapted from Sponsor's Table 2).

Tissue	0.08 hr-male	1 hr-male	1 hr-female	24 hr-male	72 hr-male
Blood	0.68	0.53	0.67	0.05	blq
Liver	7.4	6.8	alq	1.2	0.68
Adrenal gland	12.8	5.3	6.1	0.11	0.10
Lung	8.5	6.2	alq	0.07	blq
Pituitary gland	7.8	6.4	6.0	0.56	blq
Spleen	5.5	4.3	alq	blq	blq
Pineal gland	6.2	2.3	4.8	blq	blq
Eye (retina)	5.0	2.8	3.3	1.09	0.7

blq = below limit of quantitation

Metabolism:

In Vitro Metabolism of Darifenacin (Protocol number: UK-88,525/DM/027/92; Volume 13, Section In Vitro, Page 8 3709).

Methods: In vitro metabolism of darifenacin with the liver microsomal fraction from rat, dog, and man was examined. The involvement of cytochrome P450, subfamily 2D6 (CYP2D6) in the metabolism of darifenacin to UK-148,993 was examined using human microsomes. Further, the metabolism of Darifenacin to UK-148,993 was assessed using the AHH-1 TK +/- cell line that expresses high levels of CYP2D6 at it only cytochrome P450. Human microsomes were prepared from livers having either low or high bufurololol 1'-hydroxylase (CYP2D6 probe substrate) activity. Quantities of darifenacin and UK-148,993 were determined by HPLC assay with detection by either ultraviolet absorbance or fluorescence.

Results: The half lives of Darifenacin ($1 \mu\text{M}$) with liver microsomes (cytochrome P-450 concentration of $0.5 \mu\text{M}$) from rat, dog, and man were 6, 15, and 9 min, respectively. The major metabolite formed by rat and human microsomes was a hydroxylated

product, designated UK-148,993, in which an oxygen atom is inserted on one of the saturated carbons of the dihydrobenzofuran ring. For the metabolism of Darifenacin to UK-148,993 using human microsomes with high bufurolol 1'-hydroxylase activity, kinetics were biphasic with a high affinity enzyme ($K_m = 6 \mu\text{M}$, $V_{max} = 80 \text{ pmol/min/mg protein}$) and a low affinity enzyme ($K_m = 128 \mu\text{M}$, $V_{max} = 51 \text{ pmol/min/mg protein}$). In human liver microsomes with low bufurolol 1'-hydroxylase activity, kinetics were monophasic with a single low affinity site ($K_m = 125 \mu\text{M}$, $V_{max} = 1362 \text{ pmol/min/mg protein}$). In human microsomes with high bufurolol 1'-hydroxylase activity, quinidine, a selective CYP2D6 inhibitor, blocked the conversion of Darifenacin to UK-148,993 with an IC_{50} of $0.004 \mu\text{M}$. The less potent, less selective, diastereomer, quinine, had an IC_{50} value of $22 \mu\text{M}$. In human microsomes with low bufurolol 1'-hydroxylase activity, quinidine and quinine blocked the conversion of Darifenacin to UK-148,993 with IC_{50} values of 0.25 and $100 \mu\text{M}$, respectively. The metabolism of Darifenacin to UK-148,993 by the AHH-1 TK +/- cell line was mediated by a single high affinity enzyme ($K_m = 3 \mu\text{M}$, $V_{max} = 44 \text{ pmol/min/mg protein}$). The high affinity component mediating the metabolism of Darifenacin to UK-148,993 is CYP2D6.

Disposition of [^{14}C]-Darifenacin in the Isolated Perfused Rat Liver and Metabolism by Rat and Human Liver Microsomes (Protocol numbers UK-88,525/DM/9/92 and UK-88,525/DM/11/92; Volume 13, Section DM13, Page 8 3722).

Methods: The metabolic fate of [^{14}C]-Darifenacin was compared in microsomes prepared from rat and human. Further, metabolic fate was examined in the isolated, perfused rat liver. The profile of radioactive components was analyzed by HPLC with a radiochemical detector. Structural determination was performed by [

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Results: In microsomes prepared from rat and human, the major metabolite was determined to have a molecular weight equivalent to the hydroxylated parent and the site of hydroxylation was on the dihydrobenzofuran ring. With the isolated, perfused rat liver, darifenacin was cleared with an elimination half-life of 0.2 hr due to a perfusate clearance of 10.5 mL/min . With a hepatic flow of 15 mL/min , the hepatic extraction of darifenacin was 0.7. The volume of distribution was 160 mL , compared with a perfusate volume of 150 mL that suggested minimal hepatic distribution of parent drug. Approximately, 14% of the initial radioactivity remained after 90 min. Forty-one percent of the administered dose was excreted into the bile over 90 min. One major component (75%) and two minor components (approximately 12% each) were detected in the bile. The major component has tentatively been identified as the carboxylic acid metabolite, formed by opening of the ring and addition of two oxygen atoms. The two minor components were tentatively identified as glucuronides, one formed from the

carboxylic acid metabolite and other formed from the mono-hydroxylated metabolite. The carboxylic acid metabolite had a high biliary clearance; however, the glucuronide metabolites and hydroxylated metabolite partitioned back into the circulation, indicative of a low biliary clearance.

Analysis of the Concentrations of UK-148,993 Formed in Dog Plasma Following Intravenous Administration of UK-148,993 and Intravenous or Oral Administration of Darifenacin (Protocol numbers: Darifenacin/DM/7/93 po, Darifenacin/DM/10/93 iv, and UK-148,993/DM/1/93; Volume 13, Section DM 12, Page 8 3823).

Methods: The pharmacokinetics of UK-148,993 were determined in two dogs following intravenous administration of UK-148,993 and intravenous or oral administration of darifenacin. UK-148,993 and Darifenacin were both administered by the intravenous route at a dose of 0.5 mg/kg. Darifenacin was administered by the oral route at a dose of 4 mg/kg. For intravenous administration of UK-148,993 and intravenous or oral administration of darifenacin, the vehicle was PEG 200: saline: 1 M HCl (1:3:0.1). Blood samples were collected at time points of 0.1-30 hr following intravenous administration and 0.25-30 hr following oral administration. Plasma levels of darifenacin and UK-148,993 were determined by [

]

Results: Following intravenous administration of UK-148,993, this metabolite is widely distributed ($V_d = 7.3$ L/kg) and clearance is similar to hepatic clearance (~ 18.5 mL/min/kg). Following intravenous administration of darifenacin, approximately 18% of the parent drug was converted to UK-148,993. A V_d of 118 L/kg for UK-148,993 indicates that the metabolite was extensively distributed or highly bound. Further, a clearance value of 297 mL/min/kg for UK-148,993 indicates that this metabolite is rapidly clearance at levels significantly exceeding hepatic or renal clearance. Following oral administration of Darifenacin, bioavailability was 143% for darifenacin. Levels of UK-148,993 were significantly higher following oral rather than intravenous administration, suggesting this metabolite is formed by first pass metabolism of darifenacin by the liver.

Pharmacokinetic parameters for UK-148,993 following intravenous administration of UK-148,993 to dogs (Adapted from Sponsor's Table 3).

Parameter	Mean
AUC _{0-11hr} , ng·hr/mL	360
AUC _{0-∞} , ng·hr/mL	405
Elimination half-life, hr	3.7
Clearance (mL/min/kg)	22
V_d , L/kg	7.3

IND

Page 25

Pharmacokinetic parameters for darifenacin and UK-148,993 following intravenous administration of 0.5 mg/kg darifenacin to dogs (Adapted from Sponsor's Table 4).

Parameter	Darifenacin	UK-148,993
Elimination half-life, hr	2.0	4.9
AUC ₀₋₁ , ng·hr/mL	210	45
AUC _{0-∞} , ng·hr/mL	216	57
Clearance, mL/min/kg	39	297
V _d , L/kg	6.8	118
T _{max} , hr	-	0.8
C _{max} , ng/mL	-	7.5
‡ Conversion to UK-148,993	18	-

Pharmacokinetic parameters for darifenacin and UK-148,993 following oral administration of 4 mg/kg darifenacin to dogs (Adapted from Sponsor's Table 4).

Parameter	Darifenacin	UK-148,993
Elimination half-life, hr	6.0	10.3
AUC ₀₋₃₀ , ng·hr/mL	2270	3913
AUC _{0-∞} , ng·hr/mL	2430	4700
Oral bioavailability	143	-
T _{max} , hr	-	4.3
C _{max} , ng/mL	152	227

An Investigation into the First Pass Metabolism of Darifenacin (Protocol number: Darifenacin/DM/18/89; Volume 13, Section DM6, Page 8 3836).

Methods: The first pass metabolism of darifenacin was examined in a surgically prepared, conscious dog with a portal vein cannula. In the first study, toxicokinetic parameters of darifenacin were compared between intraportal doses of 0.6 and 1.2 mg/kg. In a second study, toxicokinetic parameters were compared between a single intraportal dose of 0.6 mg/kg and 7 daily intraportal doses of 0.6 mg/kg. An intravenous dose of 0.6 mg/kg was used as a reference. Following intraportal administration of drug, blood

samples were collected at time points of 0.25-11 hr on days 1 and 7 and 0.25-30 hr on day 21. Following intravenous administration of drug, blood samples were collected at time points between 0.25-24 hr on days -5 and 8. Plasma levels of darifenacin were measured using HPLC with ultraviolet detection.

Results: Plasma AUC values for darifenacin following intraportal administration of darifenacin at doses of 0.6 and 1.2 mg/kg were 115 and 487 ng·hr/mL, respectively. The plasma AUC value increased by >4-fold with a doubling of the dose. Plasma clearance had declined from 87 mL/min/kg with a dose of 0.6 mg/kg to 41 mL/min/kg with 1.2 mg/kg. The V_d was also decreased by 45% with the doubling of the dose. The 4-fold increase of the AUC with only a doubling of the dose may be a consequence of saturated metabolism as well as a decrease of plasma clearance. Following 7 daily doses of Darifenacin at 0.6 mg/kg/day, the AUC was increased to 570 ng·hr/mL as compared to 115 ng·hr/mL with a single dose of 0.6 mg/kg/day. Plasma clearance had declined from 87 mL/min/kg on day 1 to 17.5 mL/min/day on day 7. Intravenous administration of darifenacin on days -5 and 8 confirmed the decline of plasma clearance. Further, with multiple dosing, the V_d had declined by 75%. There was no accumulation of darifenacin, as plasma levels at 24 hr were negligible. Decreased plasma clearance appears to be responsible for the 4.95-fold increase of AUC from single to multiple dosing.

Toxicokinetic parameters for darifenacin following: a single intraportal dose of 0.6 mg/kg on day 1, a 7 day multiple dose study with 0.6 mg/kg/day ending on day 7, a single intraportal dose of 1.2 mg/kg/day on day 21, and intravenous doses of 0.6 mg/kg on days -5 and 8.

Parameter	Intraportal Administration			Intravenous Administration	
	Day 1	Day 7 ^A	Day 21	Day -5 ^B	Day 8
Dose	0.6	0.6	1.2	0.6	0.6
Elimination half-life, hr	2.4	3.0	2.8	1.3	2.1
Plasma clearance, mL/min/kg	87	17.5	41	26	13
V_d , L/kg	18.1	4.5	9.9	2.9	2.4
AUC _{0-∞} , ng·hr/mL	115	570	487	384	753
Relative availability, %	33	76	70	-	-

A. Last day of 7 day multiple dose study with a dose of 0.6 mg/kg/day.

B. The sponsor had inadvertently listed day 6.

Excretion and Metabolism:

The Excretion and Metabolism of [¹⁴C]-Darifenacin in Male Mice Following Oral Solution Administration of 50 mg/kg (Protocol numbers: Darifenacin/DM/005/95 (PFZ 608/942850); Volume 13, Section DM15, Page 8 3897).

Methods: The excretion and metabolism of [¹⁴C]-darifenacin was examined in male CD1 mice following oral administration of a 50 mg/kg dose. Urine and feces were collected in 24 hr intervals for up to 120 hr after dosing. Radioactivity was determined in the urine, feces (combustion), carcasses (solubilized), and cage washes. A metabolic profile in urine was determined using a pooled urine sample prepared by mixing 1% of the 0-24 and 24-48 hr samples. Analysis was performed by HPLC with ultraviolet and radioactivity detection. A metabolic profile in feces was determined using a pooled fecal sample prepared by combining 1% of the 0-24 and 24-48 hr samples. Analysis was performed by HPLC with radioactivity detection. Structural identification was performed using L

1 Concentrations of UK-88,862, an unlabeled metabolite, in mouse urine were determined by HPLC with ultraviolet detection.

Results: Following oral administration of [¹⁴C]-darifenacin to male mice, 45.9 and 48.0% of the radioactivity were excreted into the urine and feces, respectively. Eighty-five percent of the dose was excreted within the first 24 hr. Darifenacin was metabolized by three routes: hydroxylation, dihydrobenzofuran ring opening, and N-dealkylation. UK-88,862, a product of N-dealkylation in which the ¹⁴C label has been lost, was found at 17.4% of the dose in the urine within 24 hr after dosing. A urinary metabolite constituting 15.7% of the dose has not been identified. UK-297,101, formed by N-dealkylation at the pyrrolidine nitrogen and subsequent glycine conjugation, was found at 9% of the dose. In the feces, UK-222,247 was the major metabolite found at 33% of the dose.

Source	Percent of Administered Dose
Urine (0-120)	45.9
Feces (0-120)	48.0
Cage wash	0.5
Carcass	0.33
Total Excreted	94.73

Major radioactive metabolites in pooled mouse urine or feces following oral administration of 50 mg/kg [¹⁴C]-Darifenacin.

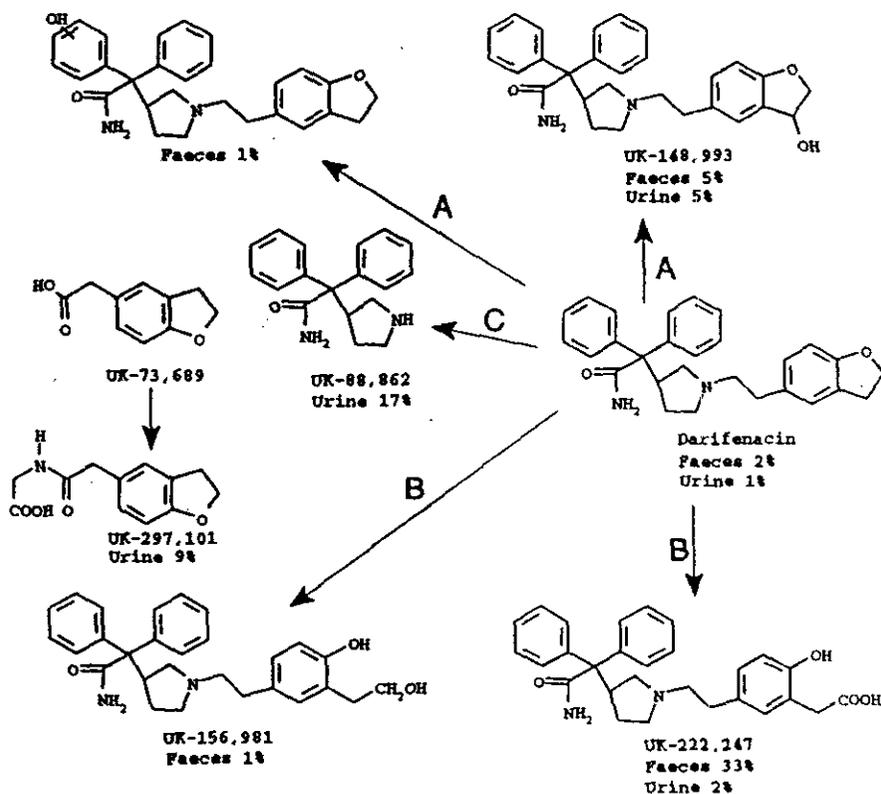
Compound	Urine		Feces	
	%Chrom.	%Dose	%Chrom.	%Dose
Unknown-1	35.0	15.7		
UK-297,101	20.1	9.0		
UK-222,247	3.7	1.7	67.8	32.8
UK-148,993	10.1	4.5	11.6 ^A	5.6 ^A
Darifenacin	1.1	0.5	37	1.8
UK-156,981			5.3	2.6

A. Two compounds were present in this peak, UK-148,933 (5% of the dose) and a head group hydroxylated version of 88,525 (1% of the dose).

Metabolism of Darifenacin in the Mouse (Sponsor's Figure 4; Volume 13, Page 8 3909).

METABOLISM OF UK-88,525 IN THE MOUSE

- Route A Monohydroxylation
- Route B Dihydrobenzofuran ring opening
- Route C N-dealkylation



The Excretion and Metabolism of [¹⁴C]-Darifenacin in the Rat Following Single Intravenous (2.5 mg/kg) and Oral (10 mg/kg) Administration (UK-88525/DM/13/92; Volume 13, DM9, Page 8 3769).

Methods: The excretion and metabolism of [¹⁴C]-darifenacin were examined in rats following a single intravenous (2.5 mg/kg) or oral (10 mg/kg) dose. For studies using the intravenous route, there was 1 rat/sex. For studies using the oral route, there were 2 rats/sex.

Results: Following either intravenous or oral administration of [¹⁴C]-darifenacin, the majority of the dose was excreted in the feces. The majority of radioactivity was eliminated within the first 48 hr after administration. Chromatographic analysis of fecal extracts, from male and female rats following intravenous administration of drug, found a single peak representing 95 and 52% of the dose, respectively. The peak was identified as the dihydrobenzofuran ring opened acid metabolite. Two minor metabolites were also found in fecal extracts from the female, a compound with a hydroxyl group in the diphenylcarboxamide head group (15% of the dose) and the dihydrobenzofuran ring opened alcohol metabolite (18% of the dose). Following oral administration, the dihydrobenzofuran ring opened acid metabolite was the major metabolite in fecal extracts. This compound was found at 44 and 31% of the dose in male and female rats, respectively. Three other compounds were also found in fecal extracts from females, the dihydrobenzofuran ring hydroxylated metabolite (28% of the dose), the dihydrobenzofuran ring opened alcohol (9% of the dose), and a dihydroxylated metabolite in the diphenylcarboxamide and dihydrobenzofuran ring (11% of the dose). Chromatographic analysis of urinary extracts following either intravenous or oral administration found several small peaks (all <5% of the dose).

Excretion of radioactivity in the urine and feces in rats following intravenous administration of 2.5 mg/kg [¹⁴C]-Darifenacin.

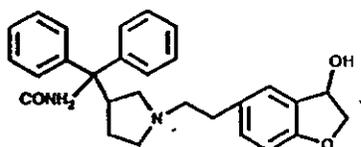
Sampling Time (hr)	Urine		Feces	
	Male	Female	Male	Female
0-24	8.9	8.0	93.0	18.7
24-48	0.3	0.5	2.0	65.5
Total (0-120)	9.5	8.7	97.6	87.2

Excretion of radioactivity in the urine and feces in rats following oral administration of 10 mg/kg [^{14}C]-Darifenacin.

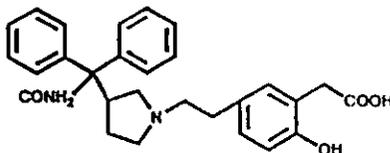
Sampling Time (hr)	Urine		Feces	
	Male	Female	Male	Female
0-24	18.95	8.7	49.4	31.85
24-48	1.7	0.95	13.35	42.4
Total (0-120)	21.25	10.25	64.3	80.15

Structures of the metabolites of Darifenacin in the Rat (Sponsor's Figure 6; Volume 13, Page 8 3781).

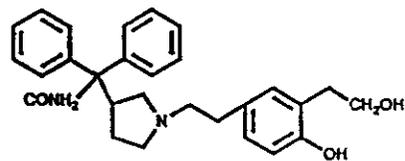
STRUCTURES OF THE METABOLITES OF UK-88,525



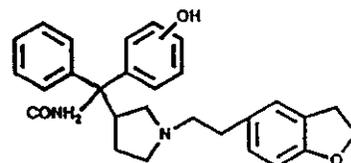
UK-148,993 (Benzylic Hydroxylation)



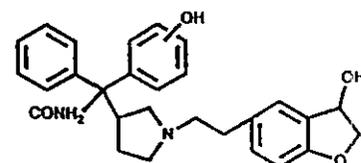
UK-222,247 Dihydrobenzofuran ring opened acid



Dihydrobenzofuran ring opened alcohol



Hydroxylation in diphenylcarboxamide



Hydroxylation in diphenylcarboxamide and benzylic position

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Page 31

The Excretion and Metabolism of [¹⁴C]-Darifenacin in Female Rabbits Following Oral Solution Administration of 10 mg/kg (Protocol numbers: Darifenacin/DM/29/9 (PFZ 609/943012); Volume 13, Section DM14, Page 8 3879).

Methods: The excretion, metabolism, and pharmacokinetics of [¹⁴C]-darifenacin were examined in female rabbits following oral administration of a 10.1-10.5 mg/kg dose. The vehicle was PEG200/saline/1 M HCl (1/3/0.1, v/v/v). Blood samples were collected for analysis of darifenacin and its metabolism. Animals (n=3) were placed in metabolism cages for collection of urine and feces in 24 hr intervals up to 120 hr after dosing. Radioactivity in plasma, urine, cage washes, and feces (combustion) were measured by liquid scintillation counting. Plasma levels of darifenacin and UK-148,993 were determined by HPLC with ultraviolet detection. The radioactive profile of circulating metabolites was determined with a pooled plasma sample, taking 1 mL from each plasma sample collected. The metabolic profile in feces was determined using a 0-48 hr pooled fecal sample prepared by mixing 0.1% of the 0-24 and 24-48 hr samples. The metabolic profile in urine was determined using a 0-48 hr pooled urine sample prepared by mixing 1% of the total volume of urine for the 0-24 and 24-48 hr samples. Samples were enzymatically analyzed for the presence of glucuronide and sulfate conjugates. Metabolites in plasma, feces, and urine were quantified by HPLC with ultraviolet or radioactive detection. Structural identification of metabolites in pooled fecal and urine samples were performed using []

Results: Following oral administration of 10 mg/kg [¹⁴C]-Darifenacin to rabbits, radioactivity was primarily eliminated in the feces. Maximal plasma concentrations of darifenacin were 10 and 1000 times lower than maximal levels of UK-148,993 and total radioactivity, respectively. Metabolism of darifenacin occurred by three principal routes: hydroxylation, dihydrobenzofuran ring opening, and N-dealkylation. The major circulating metabolites in plasma were the glucuronide of UK-156,981 and the glucuronide of dihydroxylated darifenacin. Metabolites in urine were similar to those found in the plasma. Major metabolites in the feces were UK-222,247 and darifenacin (unabsorbed drug). UK-222,247 was found at 29.9% of the dose in urine and feces.

Radioactivity excreted in urine and feces following oral administration of 10 mg/kg [¹⁴C]-Darifenacin.

Collection time (hr)	Urine	Feces
0-24	21.8	46.4
24-48	1.7	17.2
Total (0-120)	24.0	70.9

C_{max} values of Darifenacin, UK-148,993, and Total Radioactivity in Plasma from rabbits following oral administration of 10 mg/kg [¹⁴C]-Darifenacin.

Compound	C _{max} , ng equivalents/mL plasma	T _{max} , hr
Darifenacin	2.9	1
UK-148,993	29.7	0.5
Total Radioactivity	2755	1

Radioactive metabolites in rabbit plasma, urine, and feces following oral administration of 10 mg/kg [¹⁴C]-Darifenacin.

Compound	Plasma		Urine		Feces	
	%Chrom	%Dose	%Chrom	%Dose	%Chrom	%Dose
Darifenacin	-	-			23.7	15.1 ^A
Glucuronide of dihydroxylated Darifenacin	38.1	-	7.7	2.0	-	-
Glucuronide of dihydroxylated Darifenacin	-	-	19.7	5.0	-	-
Glucuronide of UK-222,247	5.2	-	-	-	-	-
Glucuronide of UK-156,981	40.4	-	32.9	8.3	-	-
Glucuronide of phenyl hydroxylated Darifenacin	7.1	-	8.2	2.1	-	-
UK-222,247	2.6	-	11.0	2.8	42.5	27.1
UK-156,981	-	-	-	-	12.5	8.0
UK-148,993	-	-	-	-	15.9	10.1

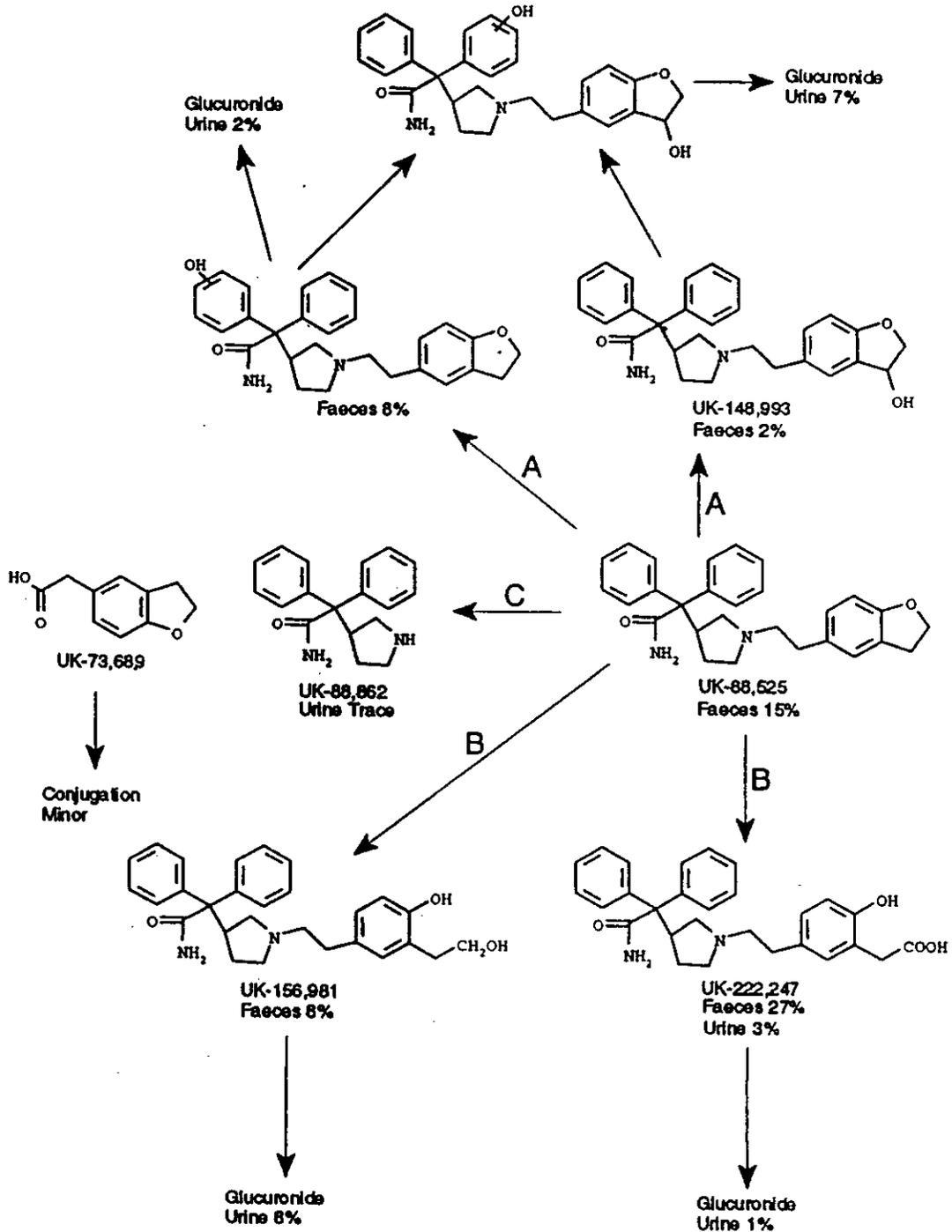
A. Unabsorbed drug.

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ON ORIGINAL

Metabolism of Darifenacin in the Rabbit (Sponsor's Figure 6; Volume 13, Page 8 3896).

METABOLISM OF UK-88,525 IN THE RABBIT

- Route A Monohydroxylation
- Route B Dihydrobenzofuran ring opening
- Route C N-dealkylation



The Excretion and Metabolism Profile of [¹⁴C]-Darifenacin in the Dog After Single Intravenous (0.6 mg/kg) and Oral (4 mg/kg) Doses (Protocol numbers: Darifenacin/DM/10/92 (iv) and Darifenacin/DM/12/92 (po); Volume 13, DM10, Page 8 3799).

Methods: The excretion and metabolism of [¹⁴C]-darifenacin were examined in one male and one female dog following either an intravenous dose of 0.6 mg/kg or an oral dose of 4 mg/kg. For intravenous or oral administration, darifenacin was dissolved in a mixture of PEG 200, saline, and 1 M HCl (5:10:0.2). Actual intravenous doses were 0.54 and 0.56 mg/kg. Actual oral doses were 4.1 and 4.0 mg/kg. Blood was collected at time points ranging from 0.1-72 hr after intravenous administration or 0.25-72 hr after oral administration. Urine and feces were collected separately on a daily basis. Radioactivity in plasma, urine, feces (after combustion) and red blood cells (after combustion) were determined by liquid scintillation counting. Plasma levels of darifenacin were determined by HPLC with ultraviolet detection. Metabolic profiles in pooled fecal or urine samples were also analyzed by HPLC with ultraviolet detection.

Results: Following intravenous or oral administration of [¹⁴C]-darifenacin, the majority of radioactivity was eliminated in the feces, which may be indicative of extensive biliary excretion. Radioactivity was primarily associated with plasma rather than red blood cells after either intravenous or oral administration. AUC values for total radioactivity with either intravenous or oral administration were 9.6-12.8 times AUC values for darifenacin, indicating the presence of high levels of circulating metabolites. Plasma clearance values exceed liver plasma flow (~18.5 mL/min/kg), which may be indicative of a high metabolic clearance. Chromatographic analysis of plasma extracts identified unchanged darifenacin and several polar metabolites. Analysis of fecal extracts found that a mixture of two compounds, the dihydrobenzofuran ring-opened acid metabolite (major) and the ring-opened alcohol (minor), constituted 70.8% of the dose following intravenous administration. These the ring opened-acid and alcohol metabolites constituted 40.6 and 8.1% of the dose, respectively, following oral administration. Also with oral administration, a compound with a single hydroxylation of the dihydrobenzofuran ring and unchanged darifenacin were found at 8.7 and 5.0% of the dose, respectively. Chromatographic analysis of urinary extracts following intravenous administration identified two peaks. The major peak was a mixture of the ring opened-acid and alcohol metabolites constituting 14.4% of the dose and the minor peak was unchanged darifenacin constituting 1.6% of the dose. Analysis of urinary extracts following oral administration identified 2 major and 2 minor compounds. Compounds identified were as follows: the ring opened-acid (9.5% of the administered dose), the dihydrobenzofuran ring hydroxylated metabolite (8.2% of the administered dose), an unidentified compound (7.3% of the administered dose) and unchanged Darifenacin (2.2% of the administered dose).

Excretion of Radioactivity in the Urine and Feces of Dogs after Intravenous Administration of 0.6 mg/kg [¹⁴C]-Darifenacin (Adapted from Sponsor's Table 1).

Sampling time after dosing (hr)	Urine		Feces	
	Male	Female	Male	Female
0-24	12.5	17.9	60.1	65.5
24-48	0.6	1.1	9.6	6.3
Total (0-120)	13.4	19.7	72.4	75.8

Excretion of Radioactivity in the Urine and Feces of Dogs after Oral Administration of 4 mg/kg [¹⁴C]-Darifenacin (Adapted from Sponsor's Table 2).

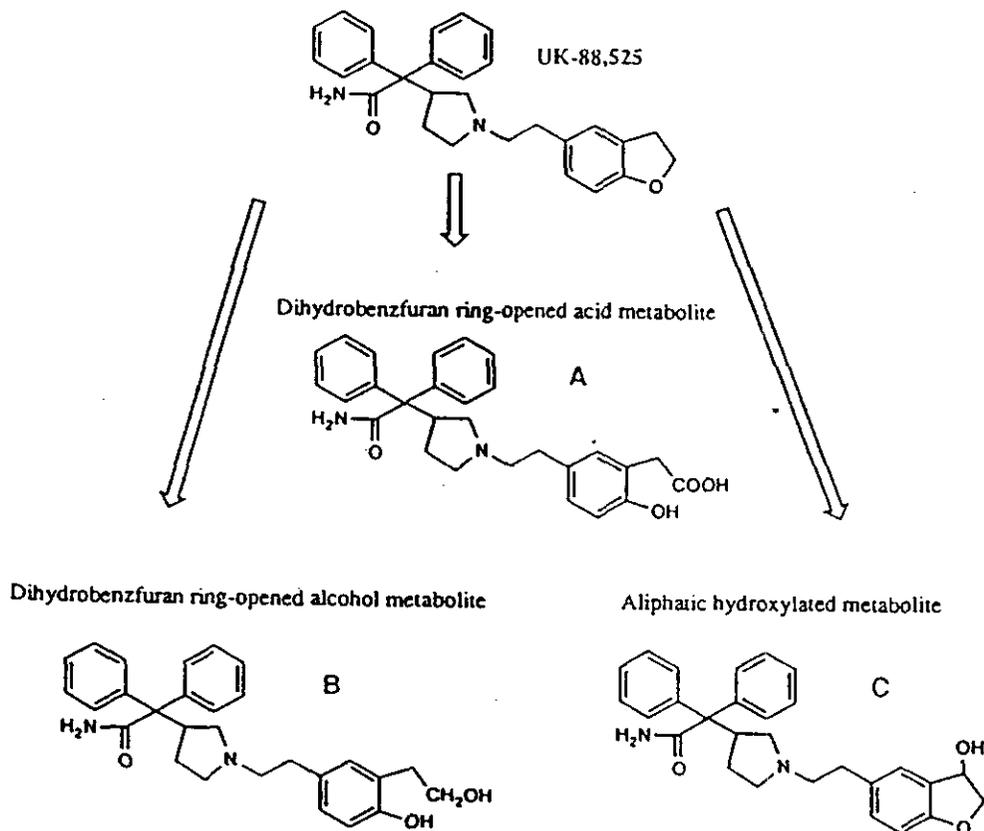
Sampling time after dosing (hr)	Urine		Feces	
	Male	Female	Male	Female
0-24	14.4	26.0	20.9	0.1
24-48	3.1	10.9	47.0	51.1
Total (0-120)	18.3	38.3	74.2	58.1

Pharmacokinetic parameters for Darifenacin in Dogs after intravenous and oral administration (Adapted from Sponsor's Table 5).

Parameter	Intravenous		Oral	
	Male	Female	Male	Female
Term. plasma half-life for Darifenacin (hr)	1.2	1.3	9.9	5.0
Term. plasma half-life for radioactivity (hr)	31.0	9.7	20.0	23.9
Plasma clearance mL/min/kg	58.1	46.8	47.3	37.4
V _d , L/kg	16.3	4	7	6
AUC _{0-∞} for Darifenacin, ng.hr/mL	172	214	1445	1783
AUC _{0-∞} for [¹⁴ C] ng equiv. hr/mL	1644	2118	16462	22840

Metabolism of Darifenacin in the Dog (Sponsor's Figure 9; Volume 13, Page 8 3822).

STRUCTURE OF PROPOSED METABOLITES OF UK-88,525 IN DOG



The pharmacokinetics of darifenacin were investigated in mice, rats, and dogs and are presented in the summary table below. Information for human volunteers is also included in the table. The oral bioavailability in rats and dogs were determined to be 16 and 105%, respectively. Darifenacin was widely distributed beyond the blood volume in all three species, which would be expected with the lipophilic characteristics of the compound. Plasma clearance of darifenacin in all three species was rapid and exceeded hepatic clearance. It appears that darifenacin was rapidly removed by a metabolic clearance and half-lives of <4 hr were observed in all species. Autoradiography studies in rats confirmed the extensive tissue distribution of darifenacin in rats and its subsequent rapid elimination mainly through the bile into the feces. Plasma protein binding of darifenacin exceeded 90% in rats, dogs, and man.

Metabolism of darifenacin in mice, rats, rabbits, and dogs occurred principally by 3 routes: hydroxylation, dihydrobenzofuran ring opening, and N-dealkylation. With rat and human liver microsomes, the major metabolite was the hydroxylated metabolite of darifenacin, UK-148,993. In studies with human microsomes, it was determined that UK-148,993 was derived from cytochrome P450 subfamily 2D6. In studies using the isolated, perfused rat liver, the hepatic extraction of darifenacin was found to be 0.7. The major metabolite was a carboxylic acid metabolite, formed by opening of the ring and addition of two oxygen atoms, which had a high biliary clearance. However, the glucuronide conjugates of the carboxylic acid metabolites and a mono-hydroxylated metabolite had low biliary clearances. In dogs, UK-148,993 was widely distributed beyond the blood volume and rapidly cleared from the plasma at a level similar to hepatic plasma flow. Following intravenous administration of darifenacin to dogs, 18% was converted to UK-148,993; however, plasma levels of UK-148,993 were significantly higher following oral administration of darifenacin suggesting the importance of first pass metabolism. In mice, the hydroxylated metabolite of darifenacin, UK-148,993, was not found following intravenous administration of darifenacin and appeared to be a product of first pass metabolism by the liver following oral administration. In excretion studies with rats, rabbits, and dogs using radiolabeled darifenacin, it was determined that radioactivity was primarily eliminated in the feces. In studies with male mice, radioactivity was found to be eliminated equally between the feces and urine. The metabolic profiles in fecal and urinary extracts from mice, rats, rabbits, and dogs were examined. Analysis of fecal extracts from mice found that UK-222,247 was the major metabolite. Analysis of urinary extracts found that an unknown metabolite and UK-297,101, formed by N-dealkylation at the pyrrolidine nitrogen, were the predominant metabolites. Analysis of fecal extracts from male and female rats following intravenous or oral administration of darifenacin found that the dihydrobenzofuran ring opened acid metabolite was the major metabolite. Other metabolites identified for female rats included a compound with a hydroxyl group in the diphenylcarboxamide head group, the dihydrobenzofuran ring opened alcohol metabolite, and a dihydroxylated metabolite in the diphenylcarboxamide and dihydrobenzofuran ring. Analysis of urinary extracts from rats found several metabolites, all at <5% of the dose. For rabbits, the major circulating metabolites in plasma were the glucuronide of UK-156,981 and the glucuronide of dihydroxylated Darifenacin. Metabolites in urine were similar to those found in the plasma. Major metabolites in the feces were UK-222,247 and darifenacin (unabsorbed drug). UK-222,247 was found at 29.9% of the dose in urine and feces combined. For dogs, chromatographic analysis of plasma extracts identified unchanged darifenacin and several polar metabolites. Analysis of fecal extracts found that a mixture of two compounds, the dihydrobenzofuran ring-opened acid metabolite (major) and the ring-opened alcohol (minor), were the major

metabolites following intravenous or oral administration. Chromatographic analysis of urinary extracts following intravenous and oral administration identified the ring opened-acid and alcohol metabolites, unchanged darifenacin, the dihydrobenzofuran ring hydroxylated metabolite, and an unidentified compound.

Single Dose Pharmacokinetics of Darifenacin in Mice, Rats, and Dogs (Adapted from Sponsor's Table 32).

Species	Dose, mg/kg & route	C _{max} ng/mL	AUC ng·hr/mL	Cl mL/min/kg	V _d L/kg	t _{1/2} hr	Oral bioavailability
Mouse	2.0 IV	228	148	282	12	0.5	
	8.0 oral	302	N.D.			N.D.	N.D.
Rat	2.3 IV	536	637	54	7	1.5	
	9.2 oral	106	440			3.3	16%
Dog	0.6 IV	192	347	30	4.2	1.6	
	4.0 oral	332	2394			4.3	105%
Human	0.2 oral	10	34.8				
	0.4 Oral	20.5	75.7			2.3	

TOXICOLOGY:

Acute Toxicity

Oral and Intraperitoneal Acute Toxicity Study in Mice and Rats (Study numbers 90010, 90011, 90012, and 90013; Volume 5, Page 8 508).

Testing Laboratory: Laboratoires Pfizer
Centre de Recherche
37401 Amboise Cedex
France

Study Started: December 26, 1989

Study Completed: July 19, 1990

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Sprague Dawley rats [CD(SD)BR] and CD1 mice [CD1(ICR)BR] were used in this study. Male rats for the oral and intraperitoneal dosing studies had mean weights of 185.7 and 227.0 g, respectively. Female rats for the oral and intraperitoneal dosing studies had mean weights of 140.7 and 168.6 g, respectively. Male mice for the oral and intraperitoneal dosing studies had mean weights of 29.1 and 31.1 g, respectively. Female mice for the oral and intraperitoneal dosing studies had mean weights of 26.9 and 27.8 g, respectively.

Drug Batch: Darifenacin-04, Batch R1.

Methods: The acute toxicity of darifenacin was examined in mice and rats following administration by the oral and intraperitoneal routes. Darifenacin was suspended in a 0.5% w/v methylcellulose 4000cps solution containing 0.1% Tween 80. Animals were monitored for clinical signs of toxicity and weighed weekly. Fourteen days after treatment, animals were sacrificed and subjected to a gross examination.

Results: For mice that received oral doses of 100 and 200 mg/kg and rats that received an oral dose of 200 mg/kg, observed effects included mydriasis, eyes partially closed, tremor, and depression within 2-3 hr following dosing. These anticholinergic symptoms were also observed following intraperitoneal doses of 50 and 100 mg/kg to mice and 50 mg/kg to rats. For rats that received an oral dose of 100 mg/kg, observed effects included mydriasis and eyes partially closed. Mice, that died following oral administration of 200 mg/kg, were observed with convulsions within 5-15 min after dosing. Mydriasis generally persisted for at least 24 hr; however, no other clinical signs were observed on subsequent days. Gross examination did not reveal any significant lesions.

Acute toxicity of darifenacin in mice and rats following oral or intraperitoneal administration.

Species	Route	Dose, mg/kg	Maximum nonlethal dose, mg/kg	Minimum lethal dose, mg/kg	Time to Death
Mice	Oral	100 (5/sex/dose) 200 (2/sex/dose)	N.D.	100	< 1 hr
Mice	IP	50 (5/sex/dose) 100 (2/sex/dose)	50	50 < dose <100	10-20 min
Rats	Oral	100 (5/sex/dose) 200 (2/sex/dose)	100	100 < dose <200	6 hr
Rats	IP	50 (5/sex/dose)	N.D.	50	5 min

The acute toxicity of darifenacin was examined in mice and rats following administration by the oral and intraperitoneal routes. Minimum lethal oral doses were 100 mg/kg for mice and between 100 and 200 mg/kg for rats. Minimum lethal intraperitoneal doses were between 100 and 200 mg/kg for mice and 50 mg/kg for rats. The anticholinergic signs of mydriasis and eyes partially closed were observed in both mice and rats. Mice were observed with convulsion following an oral dose of 200 mg/kg. Rats were observed with tremor, ataxia, dyspnea, and depression following an oral dose of 200 mg/kg.

Subacute Toxicology:

Mice

3-Month Dietary Prechronic Toxicity in CD-1 Mice (Study number 92046; Volume 5, Page 8 778).

Testing Laboratory: Laboratoires Pfizer
Centre de Recherche
37401 Amboise Cedex
France

Study Started: May 5, 1992

Study Completed: May 12, 1993

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: CD1 mice [CD1(CR)BR], approximately 6 weeks of age, were used in this study. Mean body weights were 30.43 g for males and 21.94 g for females.

Drug Batch: Darifenacin-04 Batch number R7.

Methods: This study was intended as a dose range finding study to assist in the identification of doses for a subsequent dietary carcinogenicity study in mice. Mice received a diet supplemented with darifenacin (hydrobromide salt) at levels equivalent to 25, 50, or 100 mg/kg/day for a period of 3 months (94 or 95 days). The compound was mixed with the diet at concentrations that yielded desired dose levels. Concentrations were adjusted weekly. Controls received the unsupplemented diet over the same period. There were 10 mice/sex/ group. Animals were observed daily for clinical signs of toxicity except for mydriasis and partially closed eyes. Mydriasis was examined using a diagnostic lamp [] on day 1, prior to treatment, and on days 8, 42, and 77 at 8.00 and 14.00 hr. Observations of partially closed eyes were performed on day 2 at 14.00 hr and on days 2-8, 42, and 77 at 8.00

and 14.00 hr. Body weight was measured weekly. Food consumption was measured over periods of 7 days and water consumption was determined over a 24 hr period once weekly. Blood for hematology and clinical chemistry was collected at the end of treatment. Twenty-four hr after the last dose, animals were killed and subjected to gross examination. The heart, kidneys, and liver were weighed. The eyes (with Harderian glands), heart, kidneys, liver, lung, thyroid gland, and any tissues with gross abnormalities were examined microscopically. Livers from control and 100 mg/kg/day groups (5 mice/sex/group) were examined by electron microscopy. Hepatic microsomal cytochrome P450 content was determined for 7 mice/sex/group. Using an additional 15 males/treatment group, blood was collected on day 21 at 8.00, 12.00, 16.00, 20.00, and 24.00 hr for determination of plasma levels of darifenacin and UK-148,993. Plasma was extracted with diethyloxide and drug levels were measured using HPLC with UV absorbance.

Results:

1. Achieved Doses: The mean achieved intakes of darifenacin, based upon weekly mean food consumption and mean body weights at the start and end of the respective week, for the male 25, 50, and 100 mg/kg/day treatment groups were 24.48 ± 0.85 , 48.77 ± 2.33 , and 98.57 ± 4.55 mg/kg/day, respectively. Mean achieved intakes for the female 25, 50, and 100 mg/kg/day treatment groups were 24.49 ± 1.18 , 49.40 ± 3.54 , and 98.13 ± 5.07 mg/kg/day, respectively. Drug intakes were within 2% or less of intended dosages.

2. Observed Effects: Mydriasis occurred in a dose-dependent manner for male and female mice receiving darifenacin. There were no findings for the control group. The highest incidences of mydriasis were as follows: 25 mg/kg/day, 3/10 males and 3/10 females; 50 mg/kg/day, 7/10 males and 7/10 females; and 100 mg/kg/day, 10/10 males and 10/10 females. There was an increased incidence of partially closed eyes for animals receiving darifenacin; although, the dose response relationship was relatively flat. The incidence of partially closed eyes was as follows: controls, 1/10 males and 1/10 females; 25 mg/kg/day, 5/10 males and 2/10 females; 50 mg/kg/day, 5/10 males and 3/10 females; and 100 mg/kg/day, 6/10 males and 5/10 females. Mydriasis and partially closed eyes are more than likely related to the anticholinergic properties of the darifenacin.

3. Mortality: One female control died on day 56. This animal was found to have multiple abscesses on the skin and lungs containing gram positive cocci, hepatic sinusoidal leukocytosis, and gastric hyperkeratosis. Death was attributed to a systemically disseminated chronic bacterial infection occurring secondary to a subcutaneous abscess on the lower jaw. One female of the 50 mg/kg/day group died on day 95 due to anaesthesia used for blood sampling.

4. Body Weight and Food Consumption: Body weight gain was impaired for male 100 mg/kg/day group and the female 50 and 100 mg/kg/day groups. Body weights for the male controls on days 1 and 91 were 30.71 and 37.57 g, respectively. Body weight gains for the male 25, 50, and 100 mg/kg/day groups were 102.5, 94.6, and 63.9% of the control, respectively. Body weights for the female controls on days 1 and 91 were 22.09 and 25.54 g, respectively. Body weight gains for the female 25, 50, and 100 mg/kg/day groups were 98.2, 78.7, and 78.0% of the control, respectively. There were no treatment-related changes of food consumption. Water consumption for male treatment groups was generally decreased to 75-87% of the control throughout most of the treatment period; however, there was no consistent dose response relationship. Further, water consumption was unchanged between female control and treatment groups.

4. Hematology: Red blood cell counts were increased and mean corpuscular volume was decreased for the male 50 and 100 mg/kg/day groups and hemoglobin levels were decreased for the female 25 and 100 mg/kg/day groups; however, these changes were small (<6%) and have little biological significance.

5. Blood Chemistry and Urinalysis: No treatment-related changes.

6. Vital Signs and Physical Examination: No measurements were performed.

7. Organ Weight: Relative liver weight was increased for the male 50 and 100 mg/kg/day groups and the female 100 mg/kg/day group. Relative liver weights for male 50 and 100 mg/kg/day groups were increased to 110.1 and 113.7% of the control (5.175%), respectively. Relative liver weight for the female 100 mg/kg/day group was increased to 111.6% of the control (5.885%), respectively. The relative left kidney weight for the male 100 mg/kg/day group was decreased to 89.9% of the control (0.965%). The relative right kidney weight for the male 100 mg/kg/day group was decreased to 93.4% of the control (0.981%).

8. Gross Pathology: A greyish discoloration of the Harderian glands was observed for darifenacin treatment groups; although, there was no response relationship. The incidence was as follows: 25 mg/kg/day, 1 male and 1 female; 50 mg/kg/day, 3 males and 2 females; and 100 mg/kg/day, 1 male.

9. Histopathology: There was an increased incidence of Harderian hypersecretion in darifenacin treatment groups; however, a dose response relationship was not present. Hypersecretion was characterized by the intraluminal accumulation of pigmented secretions. The scoring system for changes of the Harderian gland was as follows: Grade 1, small accumulations of brown amorphous material in several scattered alveoli; Grade 2, intraluminal

accumulation of secretions and/or dilation of more than several, but fewer than 20% of alveoli; Grade 3, 20-50% of alveoli affected; and Grade 4, greater than 50% of alveoli affected. The incidence for the control with a grade ≥ 2 was 1/4. For the 25, 50, and 100 mg/kg/day groups, the incidence of animals with grade ≥ 2 was 12/18, 17/18, 9/14, respectively. One female of the 100 mg/kg/day group was observed with a follicular adenoma of the thyroid gland, which is unusual for an animal of this age. Electron microscopic examination of liver from the control and 100 mg/kg/day groups (5 mice/sex/group) found that the liver from one female of the 100 mg/kg/day group had evidence of minimal proliferation of the smooth endoplasmic reticulum.

Histopathological changes for mice receiving darifenacin in the diet at 0, 25, 50, or 100 mg/kg/day for 3 months.

Histopathological Change	0		25		50		100	
	M	F	M	F	M	F	M	F
Harderian gland								
-hypersecretion	0	4	10	8	9	9	7	7
-inflammation, chronic focal	0	0	0	1	0	0	0	3
Kidney								
-hyperplasia, tubular, focal	0	0	0	2	0	0	0	2
-hyperplasia, tubular, cystic	2	2	0	1	0	2	3	0
-vacuolation, tubular	0	0	0	1	1	0	0	0
Liver								
-vacuolation, centrilobular	1	1	1	1	0	2	0	0
Thyroid								
-cyst	4	0	3	3	2	2	2	5
-adenoma, follicular	0	0	0	0	0	0	0	1

10. **Plasma Drug Levels:** Plasma darifenacin and UK-148,993 AUC values were approximately proportional to dose for male mice. Plasma drug levels for human volunteers following single oral doses of 0.2 or 0.4 mg/kg were 34.8 and 75.7 ng.hr/mL. Human plasma AUC values ranged from 19 to 8.9 times lower than the average level observed for rats at 25 mg/kg, respectively; however, the respective dose differential was 125 and 62.5-fold.

Plasma darifenacin AUC values for male mice that received darifenacin in the diet at 25, 50, and 100 mg/kg/day.

Dose mg/kg/day	Darifenacin AUC ng/hr/mL	UK-148,993 AUC ^A ng/hr/mL
25	574	768
50	874	1440
100	1548	3528

A. There was insufficient plasma available for analysis of UK-148,994 and one pool of residue samples per dose level was constituted and analyzed. Plasma concentrations were found to be 32, 60, and 147 ng/mL at the 25, 50, and 100 mg/kg/day dose levels, respectively. The corresponding AUC ($C_{av} \times 24$) values were calculated.

11. **Liver Biochemistry:** The specific content of hepatic microsomal cytochrome P450 for the 50 and 100 mg/kg/day groups was increased to 140.7 and 165.2% of the control, respectively.

Effect of darifenacin on hepatic microsomal cytochrome P450 content in mice that received darifenacin in the diet at dose levels of 25, 50, or 100 mg/kg/day. Each represents the P450 content of a pool of 7 livers/sex/group (Adapted from Sponsor's table on page 791/781).

Dose mg/kg/day	Cytochrome P450 content nmoles P450/mg microsomal protein	
	Males	Females
0	1.05	1.11
25	1.24	1.18
50	1.48	1.56
100	1.69	1.88

In a dose range finding study for the mouse carcinogenicity study, mice received darifenacin in the diet at dose levels of 25, 50, and 100 mg/kg/day for 3 months. The no effect level was 25 mg/kg/day on the basis of impairment of body weight gain at doses of 50 and 100 mg/kg/day. The maximum tolerated dose was 50 mg/kg/day for male mice and 25 mg/kg/day for female mice. There was no treatment-related mortality. Body weight gain was impaired >10% for the male 100 mg/kg/day group and the female 50 and 100 mg/kg/day groups. The target organs of toxicity was the Harderian glands. There was an increased incidence of Harderian gland hypersecretion for darifenacin treatment groups; although, the dose response relationship was flat. Hepatic cytochrome P450 levels were increased in the 50 and 100 mg/kg/day groups; although, histopathology and electron microscopy revealed no significant changes. One female of the 100 mg/kg/day group was observed with a follicular adenoma of the thyroid gland, which is unusual for an animal of this age. Plasma AUC values for darifenacin and UK-148,993 levels were approximately proportional to dose for male mice.

Rats

2-Week Intravenous Toxicity Study in Sprague Dawley Rats (Study No. 94029; Volume 10, Page 8 2730).

Testing Laboratory: Laboratoires Pfizer
Centre de Recherche
37401 Amboise Cedex
France

Study Started: March 17, 1994

Study Completed: September 30, 1994

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Sprague Dawley rats [CD(SD)BR], with an approximate age of 7 weeks, were used in this study. Mean body weights at the start of treatment were 276.5 g for males and 203.5 g for females.

Drug Batch: Darifenacin, Lot number R11.

Methods: Rats received darifenacin by the intravenous route at 0, 1.25, and 5.0 mg/kg/day for 14 consecutive days. There were 10 rats/sex/group. The vehicle was a 5% aqueous mannitol solution, pH 6.3. The dosing volume was 10 mL/kg. Animals were observed daily for mortality. On days 1, 2, 7, and 14, rats were observed for clinical signs of toxicity before treatment and 1 hr after dosing. Body weight was measured on days -3, 1, 7, and 14. Food consumption was measured over periods of 7 days, at days 7 and 14. Water consumption was measured over 24 hr periods, at days 6 and 13. An ophthalmic exam was performed on the control and 5 mg/kg/day groups prior to the start of treatment and at the end of the treatment period. Blood was collected at the end of the study for measurement of hematological and clinical chemistry parameters. A urine sample was collected overnight (16.5 hr) at the end of treatment for urinalysis. Using 5 supplementary rats/sex/treatment group, blood was collected on day 14 at 0.5, 1, and 5 hr after dosing for analysis of plasma levels of darifenacin and UK-148,993. Quantities of darifenacin and UK-148,993 were determined by HPLC using ultraviolet detection. At the treatment, animals were sacrificed and subjected to a gross examination. Absolute and relative organ weights for the adrenal gland, brain, heart, kidneys, liver, spleen, and testes were measured. Sections for histopathological analysis were collected from any tissues with macroscopic abnormalities plus samples from major organs/tissues (adrenal glands, aorta, brain, caudal vein, cervical lymph node, colon, duodenum, epididymis, eyes/Harderian glands, heart, ileum, kidneys, liver, lung, mammary glands/skin, mesenteric lymph node,

esophagus, ovaries, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, spleen, sternum with marrow, stomach, striated muscle thigh, testes, thymus, thyroid gland, trachea, urinary bladder, and uterus).

Results:

1. **Observed Effects:** Mydriasis was observed for animals in both the 1.25 and 5 mg/kg/day group from day 1 through the end of treatment.

2. **Mortality:** One male from the 5 mg/kg/day group died on day 10, immediately following drug administration. Histopathological analysis of the lung for this animal found a perivascular eosinophilic infiltration, edema, and congestion. The cervical lymph nodes were also found with edema and congestion. The sponsor attributed the death to trauma from handling and unrelated to treatment, as there were no clinical signs to prior to death. Histopathological findings in the lung appear to be consistent with trauma. For the 5 mg/kg/day group, the only significant histopathological findings were confined to the Harderian glands.

3. **Body Weight and Food Consumption:** There were no treatment-related effects on body weight gain and food and water consumption. Mean body weights for male controls on days 1 and 14 were 276.58 and 358.54 g, respectively. Body weight gains for the male 1.25 and 5 mg/kg/day group were 111.04 and 102.59% of the control, respectively. Mean body weights for female controls on days 1 and 14 were 205.18 and 227.67 g, respectively. Body weight gains for the female 1.25 and 5 mg/kg/day group were 133.71 and 125.5% of the control, respectively.

4. **Hematology:** There were no treatment-related changes of hematological parameters.

5. **Blood Chemistry:** There were no treatment-related changes of blood chemistry or urinalysis parameters.

6. **Vital Signs and Physical Examination:** The ophthalmic examination found no treatment-related changes with regard to ocular, corneal, or posterior segment abnormalities.

7. **Organ Weight:** There were no treatment-related changes of absolute or relative organ weights.

8. **Gross Pathology:** A dark or brown discoloration of the Harderian glands was found for 2 males and 1 female of the 5 mg/kg/day group.

9. **Histopathology:** A treatment-related Harderian gland hypersecretion was found for 6 males and 1 female of the 5 mg/kg/day group. This histopathological change was characterized by a minimal dilation of the acinar lumina by a brown, loose and flocculent material. One female of the 5 mg/kg/day group was found to be missing the right ovary and right uterine horn (i.e., congenital anomaly); although, this anomaly has no relationship to treatment.

10. **Plasma Drug Levels:** Plasma AUC values for darifenacin were proportional to dose. Plasma levels of the hydroxylated metabolite, UK-148,993, were undetectable with a darifenacin dose of 1.25 mg/kg/day; although, the metabolite was detected at a dose of 5 mg/kg/day. There were no differences in AUC values for darifenacin and UK-148,993 between male and females rats.

Plasma C_{max} , T_{max} , and AUC values for darifenacin with rats that received darifenacin by the intravenous route at 1.25 or 5 mg/kg/day.

Dose mg/kg/day	C_{max} , $\mu\text{g/mL}$		T_{max} , hr		$\text{AUC}_{0.5-5\text{hr}}$, $\mu\text{g}\cdot\text{hr/mL}$	
	M	F	M	F	M	F
1.25	0.13	0.126	0.5	0.5	0.226	0.22
5	0.608	0.598	0.5	0.5	1.114	1.008

Plasma C_{max} , T_{max} , and AUC values for UK-148,993 with rats that received darifenacin by the intravenous route at 1.25 or 5 mg/kg/day.

Dose mg/kg/day	C_{max} , $\mu\text{g/mL}$		T_{max} , hr		$\text{AUC}_{0.5-5\text{hr}}$, $\mu\text{g}\cdot\text{hr/mL}$	
	M	F	M	F	M	F
1.25	BLD ^A	BLD	-	-	-	-
5	0.082	0.072	0.6	0.6	0.216	0.186

A. Plasma UK-148,993 levels were below the limit of detection for rats treated with a dose of 1.25 mg/kg/day.

Rats received darifenacin by the intravenous route at doses of 0, 1.25, or 5 mg/kg/day for 14 days. The no effect dose was 5 mg/kg/day. The target organ of toxicity was the Harderian glands. Harderian gland hypersecretion was observed for 6 of 10 males and 1 of 10 females in the 5 mg/kg/day group.

A One-Month Gavage Study in Sprague Dawley Rats (Study number 89-751-03; Volume 6, Page 8 927).

Testing Laboratory: Drug Safety Evaluation Department
Pfizer Central Research
Pfizer Inc.
Groton, CT 06340

Study Started: October 30, 1989

Study Completed: July 27, 1990

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Male and female Sprague Dawley rats CD BR] were used in this study. Animals were 8 weeks old at the start of the study and mean body weights for male and female control rats were 329.5 and 210.9 g, respectively.

Drug Batch: Darifenacin-04 Bulk Lot Number R1:

Methods: Rats received darifenacin by oral gavage at dose levels of 0, 3, 10, and 50 mg/kg/day for 1 month (36-39 days). Dose selection was based upon a 14 day dose range finding study (Study number 89040) in which rats received darifenacin by oral gavage at doses of 0, 10, 30, or 100 mg/kg/day for 14 days. The no effect dose was 10 mg/kg/day. Mortality occurred for 3 females in the 100 mg/kg/day, apparently due to the anticholinergic properties of the drug. The target organ of toxicity was the Harderian glands, in which dilations of the glandular acini and ducts with a brown-pigmented hypersecretion were found to varying degrees in all treatment groups. Dysphagia occurred in the 30 and 100 mg/kg/day groups, which led to the presence of food within the esophagus. Harderian gland hypersecretion and dysphagia may be related to the anticholinergic properties of the drug. In the present study, there were 10 rats/sex/group. The vehicle was 0.5% methylcellulose (4000 cps) containing 0.1% polysorbate 80 (Tween 80). The 0.3 and 1.0 mg/mL concentrations were solutions and the 5.0 mg/mL concentration was a suspension. The dose volume was 10 mL/kg. All animals were observed at least twice daily for mortality and clinical signs of toxicity. Body weights and food consumption were determined prior to the initiation of treatment and at weekly intervals during the study. Ophthalmic examinations were performed prior to the start of treatment and at the end of the study. Animals were examined for pupillary dilation several times daily using a hand held indirect ophthalmoscope. Blood for hematology and serum chemistry determinations and urine for urinalysis were collected prior to treatment and on days 15-18 and 37-40. Blood was collected on days 1, 19, and 30 at 1 and 5 hr after dosing for determination of plasma drug concentrations. Drug quantities were

determined by HPLC with UV detection. On days 37-40, after overnight fasting, animals were sacrificed and subjected to a gross examination. Organ weights for the kidneys, liver, and right and left testes were determined. Sections for histopathological analysis were collected from any tissues with macroscopic abnormalities plus samples from major organs/tissues (adrenal glands, aorta, brain, caudal vein, cervical lymph node, cervix, colon, duodenum, epididymis, esophagus, eyes/Harderian glands, heart, ileum, kidneys, liver, lung, mammary glands/skin, mesenteric lymph node, esophagus, ovaries, pancreas, pituitary gland, prostate, salivary glands, seminal vesicles, sciatic nerve, spinal cord, spleen, sternum with marrow, stomach, striated muscle thigh, testes, thymus, thyroid gland, trachea, urinary bladder, and uterus).

Results:

1. **Observed Effects:** Pupillary dilation occurred in all treatment groups in a dose-dependent manner following daily dosing with darifenacin. The degree of dilation for each treatment group was as follows: 3 mg/kg/day, slight to moderate; 10 mg/kg/day, mostly moderate; and 50 mg/kg/day, moderate to marked. No pupillary dilation was observed in control rats. Salivation was observed within 2 min after dosing in the 50 mg/kg/day group on day 11 and in the 10 mg/kg/day group on day 12 and continued until the end of the study. No salivation was observed in the control and 3 mg/kg/day groups. Females in the 50 mg/kg/day group on days 5-9 were observed with reddish pigment staining around the orbit of each eye, most likely chromodacryorrhea. Staining did not recur.

2. **Mortality:** There were no treatment-related mortalities. A control female died on day 37 of the study. This animal was injured on day 18 as a result of a dosing accident, which produced a fluid filled sack in the right axillary region. The sack developed into an abscess-like lesion, which ruptured on day 29; however, this was reported to have no detrimental effect on the animal.

3. **Body Weight and Food Consumption:** Body weight gain for male and female rats treated with darifenacin at 50 mg/kg/day was impaired by >10%. Mean body weights for control males on days 1 and 36 were 329.5 and 436.1 g, respectively. Body weight gains for male 3, 10, and 50 mg/kg/day groups were 127.6, 108.2, and 81.8% of the control, respectively. Mean body weights for female controls on days 1 and 36 were 210.9 and 259.3 g, respectively. Body weight gains for female 3, 10, and 50 mg/kg/day groups were 93.8, 91.7, and 47.2% of the control, respectively. Food consumption for the male 50 mg/kg/day group on day 29 was reduced to 84.8% of the control (184.3 grams/week). Food consumption for the female 50 mg/kg/day group on days 8 and 29 was reduced to 80.2 and 87.7% of the control (123.4 and 135.4 gm/week), respectively.

4. Hematology: There were no treatment-related changes for white and red blood cell counts, hemoglobin, hematocrit, and platelet counts. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin content (MCHC) for the female 50 mg/kg/day group on day 17 were decreased to 96.8 and 98.2% of control values (19.01, units not specified, and 31.43%), respectively.

5. Blood Chemistry and Urinalysis: Triglyceride levels for the female 50 mg/kg/day group at 15 and 37 days were elevated to 223.3 and 264.5% of controls (36.4 and 36.78, units were not specified), respectively. Cholesterol levels for the female 50 mg/kg/day group at days 15 and 37 were elevated to 135.8 and 139.3% of controls (63.1 and 65.33, units were not specified), respectively. 5-Nucleotidase activities for the female 50 mg/kg/day group on days 15 and 37 were increased to 141.7 and 135% of the controls (54.2 and 60.33, units were not specified), respectively. BUN levels for the male 10 and 50 mg/kg/day groups were elevated above controls prior to the initiation of treatment and continued to be elevated above controls at days 15 and 37; however, these changes were not statistically significant and appear to have no relationship to treatment.

In the urinalysis report, no treatment-related effects were reported for urinary volume, pH, or specific gravity. Line listing for protein, glucose, ketones, bilirubin, blood, and urobilinogen were scattered over several pages and difficult to follow in an intelligent manner.

6. Ophthalmic Examination: An ophthalmic examination on day 33 found no treatment-related changes.

7. Organ Weight: Absolute and relative liver weights were increased for the male and female 10 and 50 mg/kg/day groups; however, there were no corresponding histological changes. Absolute liver weights for the male 3, 10, and 50 mg/kg/day groups were increased to 107.5, 113.8, and 111.8% of the control (10.92 g), respectively. Relative liver weights for the male 10 and 50 mg/kg/day groups were increased to 110.9 and 113.9% of the control (2.66%), respectively. Absolute liver weights for the female 10 and 50 mg/kg/day groups were increased to 105.9 and 109.9% of the control (6.29 g), respectively. Relative liver weights for the female 10 and 50 mg/kg/day groups were increased to 110 and 120.4% of the control (2.60%), respectively.

8. Gross Pathology: There were no treatment-related macroscopic lesions.

9. **Histopathology:** Acinar luminal dilations of the Harderian glands were reported for control and treatment groups; however, the incidence and severity was significantly greater for males and females of the 50 mg/kg/day group. Changes of the Harderian glands were characterized by decreased cytoplasmic volume of the acinar epithelial cells and increased luminal space (dilation), which often contained yellowish brown, granular pigmented secretory material.

Histopathological changes for the Harderian glands in rats receiving darifenacin by oral gavage at 0, 3, 10, or 50 mg/kg/day for 1 month.

Tissue/Organ	0		3		10		50	
	M	F	M	F	M	F	M	F
Harderian gland -luminal dilation, acini	5	3	3	1	2	7	9	9

10. **Plasma Drug Levels:** Plasma drug levels were determined on days 1, 19, and 30. For the 3 mg/kg/day group, plasma drug levels were below the limit of detection. Plasma drug concentrations for male and female rats treated with darifenacin at 10 and 50 mg/kg/day appeared to be approximately proportional to dose. There were no differences in drug concentrations on days 1, 19, and 30. Plasma drug concentrations appeared to be slightly higher in females than males.

Plasma darifenacin concentrations ($\mu\text{g/mL}$) on days 1, 19, and 30 in rats receiving darifenacin by oral gavage at 10 and 50 mg/kg/day.

Day	Time after dosing	10 mg/kg/day		50 mg/kg/day	
		M	F	M	F
1	1	0.04	0.062	0.256	0.320
	5	0.13	0.052	0.202	0.436
19	1	0.01	0.04	0.328	0.335
	5	0.326	0	0.18	0.376
30	1	0.012	0.038	0.23	0.354
	5	0.026	0.113	0.304	0.502

Rats received darifenacin by oral gavage at doses of 0, 3, 10, and 50 mg/kg/day for 1 month (36-39 days). The no effect level was 10 mg/kg/day. There was no treatment-related mortality. The target organ of toxicity was the Harderian glands, where an

increased incidence of acinar luminal dilation was found in the 50 mg/kg/day treatment group. Small increases (<15%) of absolute and relative liver weights were found for the 10 and 50 mg/kg/day groups; however, there were no corresponding histological changes. For several parameters under blood chemistry and urinalysis, the sponsor did not provide means and standard deviations. Further, the individual line listings were scattered and difficult to follow.

Effect of a 1 Month Dietary Administration to Rats of Darifenacin-04 on Body Weight, Food Consumption, and the Incidence of Mydriasis (Study No. 93042; Volume 11, Page 8 3110).

Testing Laboratory: Pfizer
Centre de Recherche
Laboratoire Pfizer
37400 Amboise
France

Study Started: May 25, 1993

Study Completed: July 12, 1994

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Sprague Dawley rats CD(SD)BR], with an age of 6 weeks, were used in the study. Mean body weights were 206.2 g for males and 174.1 g for females.

Drug Batch: Darifenacin-04, Batch number R7.

Methods: This dietary dose range finding study was used to assist in the identification of doses for the rat carcinogenicity study. Rats received a diet supplemented with darifenacin to result in an average daily intake of 0, 10, 15, or 25 mg/kg/day for 27 days. There were 10 rats/sex/group. Body weight and food consumption were measured each week. Pupil size was examined prior to start of treatment and after 1 and 4 weeks of treatment. Animals were sacrificed without necropsy on day 28.

Results:

1. **Achieved Doses:** Achieved doses were calculated based upon weekly food consumption and mean body weights at the start and end of each week. Achieved doses for male treatment groups were 9.19 ± 0.97 , 13.48 ± 1.78 , and 22.89 ± 2.39 mg/kg/day, respectively. Achieved doses for female treatment groups were 9.10 ± 0.56 , 13.49 ± 0.74 , and 22.58 ± 1.59 mg/kg/day, respectively.

IND

Page 53

2. **Observed Effects:** Mydriasis was observed on day 8 for 2 of 10 males and 5 of 10 females of the 15 mg/kg/day group and 4 of 10 males and 8 of 10 females of the 25 mg/kg/day group. On day 28, mydriasis was observed for only 3 of 10 females of the 25 mg/kg/day group.

3. **Mortality:** None.

4. **Body Weight and Food Consumption:** Body weight gains for the male 25 mg/kg/day group and the female 15 and 25 mg/kg/day groups were impaired by >10% as compared to controls. Body weights for the male controls on days 1 and 28 were 204.5 and 411.65 g, respectively. Body weight gains for the male 10, 15, and 25 mg/kg/day groups were decreased to 85.4, 92.1, and 76.4% of the control, respectively. Body weights for female controls on days 1 and 28 were 172.99 and 256.69 g, respectively. Body weight gains for the female 10, 15, and 25 mg/kg/day groups were 91.0, 85.9, and 72.5% of the control, respectively. Food consumption for the male 15 and 25 mg/kg/day groups was generally decreased by <10% during the treatment period.

This dietary dose range finding study was used to assist in the identification of doses for the rat carcinogenicity study. Rats received a diet supplemented with darifenacin to result in an average daily intake of 0, 10, 15, or 25 mg/kg/day for 27 days. Body weight gains for the male 25 mg/kg/day group and the female 15 and 25 mg/kg/day groups were impaired by >10%. Animals were sacrificed without necropsy on day 28.

1-Month Oral Toxicity Study in Sprague-Dawley Rats: Repeat Study with Bulk Produced by a New Synthetic Route (Study No. 94072; Volume 11, Page 8 3146).

Testing Laboratory: Pfizer
Centre de Recherche
37401 Amboise Cedex
France

Study Started: August 10, 1994

Study Completed: June 20, 1995

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Sprague-Dawley rats (SD)BR], with an age of 7 weeks at the start of treatment, were used in this study. Mean body weights were 264.3 g for males and 199.5 g for females.

Drug Batch: UK 88,525-04, Batch number R101.

Methods: Rats received darifenacin by oral gavage at doses of 0, 3, and 50 mg/kg/day for 1 month (29 or 30 consecutive days). There were 10 rats/sex/group. The vehicle was 0.5% aqueous methylcellulose, 4000 cps, containing 0.1% Tween 80. The dosing volume was 10 mL/kg. Rats were observed daily in their cages for mortality. Animals were observed for clinical signs of toxicity once prior to the start of treatment (day -2) and then on days 1, 2, 3, 8, 15, 22, and 29 prior to dosing and at approximately 1 and 3 hr after dosing. Body weights were measured prior to treatment (day -2) and prior to dosing on days 1, 8, 15, 22, and 29. Food consumption was measured over 7 day intervals (i.e., days 1-8, 8-15, 15-22, and 22-29). Water consumption was measured over periods of 24 hr (i.e., days 6-7, 9-10, 16-17, and 23-24). An ophthalmic examination was performed on the control and 50 mg/kg/day groups prior to treatment (day -2) and on day 28. Blood was collected for determination of hematological and clinical chemistry parameters at the end of the study. Urine was collected at the end of the study for urinalysis by placing animals in metabolism cages overnight (16 hr). A supplementary group of 5 rats/sex/group received darifenacin by oral gavage at 50 mg/kg/day for determination of plasma levels of darifenacin and its hydroxylated metabolite, UK-148,993. Blood was collected on days 1 and 22 at 1 and 5 hr after dosing for determination of plasma levels of darifenacin and UK-148,993. Plasma samples were extracted and quantities of darifenacin and UK-148,993 were determined by HPLC with ultraviolet detection. Area under plasma concentrations versus time curves for both compounds were determined by the trapezoidal rule using data obtained at the 1 and 5 hr time points. These supplementary animals were sacrificed on day 22 after blood collection without gross examination. Animals from the main study group were sacrificed and subjected to a gross examination. Absolute and relative organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, spleen, and testes. Sections were collected from tissues with macroscopic abnormalities plus sections from the following tissues: adrenal glands, aorta, brain, cervical lymph node, colon, duodenum, epididymis, eyes/Harderian glands, heart, ileum, kidneys, liver, lung, mammary glands/skin, mesenteric lymph node, esophagus, ovaries, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, spleen, sternum with marrow, stomach, striated muscle thigh, testes, thymus, thyroid gland, trachea, urinary bladder, and uterus. Tissues from the control and 50 mg/kg/day groups were subjected to microscopic analysis. The Harderian glands were also examined in the 3 mg/kg/day group.

Results:

1. **Observed Effects:** Mydriasis was observed in both the 3 and 50 mg/kg/day groups. Onset occurred within 1 to 3 hr after the start of treatment. Mydriasis was observed at 24 hr after dosing for the 50 mg/kg/day group. The incidence for the 3 mg/kg/day

group ranged from 4/10 to 8/10 for males and 7/10 to 10/10 for females. All male and female rats of the 50 mg/kg/day group were observed with mydriasis. Chromodacryorrhea was observed beginning on day 8 in the 50 mg/kg/day group with an incidence ranging from 3/9 to 5/9 for males and 5/10 to 6/10 (5/9) for females. Chromodacryorrhea correlated with histopathological findings of Harderian gland hypersecretion. Partially-closed eyes were observed on days 1 and 2 in the 50 mg/kg/day group with an incidence of 1/10 to 2/10 for males and 4/10 to 5/10 for females.

2. Mortality: Two animals in the 50 mg/kg/day group died during the treatment period. One male (M201) in the 50 mg/kg/day group died on day 5, approximately 10 min after dosing. The death of this animal was attributed to gavage error. One female (F701) of the 50 mg/kg/day group died on day 23, about 24 hr after dosing. Gross examination found discoloration of the lungs and Harderian glands and compacted food within the esophagus. Histopathological examination found the following: mild, multifocal hemorrhage in the lung; hemorrhage in the thymus; Harderian gland hypersecretion; and dilation of the esophagus, as the lumen was expanded by ingesta. The death of this animals was considered treatment-related and may have been caused by dysphagia.

3. Body Weight, Food Consumption, and Water Consumption: Body weight gains for male and female rats of the 50 mg/kg/day group were impaired by >10%. Food consumption was decreased during the first week of treatment for the 50 mg/kg/day group. Water consumption for the 50 mg/kg/day group was increased throughout the treatment period. Body weights for the male controls on days 1 and 29 were 259.21 and 429.55 g, respectively. Body weight gains for the male 3 and 50 mg/kg/day groups were 97.6 and 68.8% of the control, respectively. Body weights for the female controls on days 1 and 29 were 199.55 and 265.69 g, respectively. Body weight gains for the female 3 and 50 mg/kg/day groups were 108.4 and 73.7% of the control, respectively. During the first week of treatment, food consumption for male and female rats of the 50 mg/kg/day group was decreased to 89.3 and 82.7% of the control (31.56 and 22.90 g/animal/day). Water consumption for male rats of the 50 mg/kg/day group measured on days 7, 17, and 24 was increased to 150.8, 128.2, and 145.2% of the control (30.79-47.20 g/animal/day). Water consumption for female rats of the 50 mg/kg/day group measured on days 7, 10, 17, and 24 was increased to 161.9, 139.3, 139.2, and 177.4% of the control (16.21-33.35 g/animal/day), respectively.

4. Hematology: For the male 50 mg/kg/day group, red blood cell counts, hemoglobin levels, and the hematocrit were increased to 107.4, 106.0, and 106.6% of control values (RBC, $8.582 \times 10^6/\text{mm}^3$; Hgb, 15.81 g/dL; and Hct, 47.12%), respectively. These changes appear to be related to dehydration with subsequent hemoconcentration. White blood cell counts for the female 50 mg/kg/day group were increased to 112.7% of the control ($10.180 \times 10^3/\text{mm}^3$). This change appeared to be the result of an increase in monocyte counts to 153.8% of the control ($0.156 \times 10^3/\text{mm}^3$).

5. Blood Chemistry and Urinalysis: Chloride levels for the male 3 and 50 mg/kg/day groups were increased to 102 and 107.3% of the control (107.4 mmol/L), respectively. Chloride levels for the female 50 mg/kg/day group were increased to 105% of the control (110.80 mmol/L). The sponsor attributed these elevations of chloride levels to interference by bromide in the salt with measurement using an ion selective electrode. Glucose levels for the male 3 and 50 mg/kg/day groups were increased to 110.7 and 111.2% of the control (125.10 mg/dL), respectively. Albumin levels for the male 50 mg/kg/day group were increased to 104.1% of the control (34.8 g/L). Calcium levels for the female 50 mg/kg/day group were increased to 103% of the control (104.4 mg/L). Phosphate levels for the female 50 mg/kg/day group were increased to 110.5% of the control (59.2 mg/L). Creatinine levels for the female 3 and 50 mg/kg/day groups were decreased to 86.9 and 78.7% of the control (0.61 mg/dL). Cholesterol levels for the female 50 mg/kg/day group were increased to 126.7% of the control (73.4 mg/dL). Triglyceride levels for the female 50 mg/kg/day group were increased to 140.3% of the control (34.7 mg/dL). For the female 3 and 50 mg/kg/day groups, urinalysis found that urinary volumes were increased and corresponding densities were decreased. Urinary volumes for the female 3 and 50 mg/kg/day groups were increased to 148.8 and 236.8% of the control (13.3 mL), respectively. Urinary densities for the female 3 and 50 mg/kg/day groups were slightly decreased to 99.1 and 98.9% of the control (1.0225 g/mL).

6. Vital Signs and Physical Examination: An ophthalmic examination on day 28 for the 50 mg/kg/day group found incomplete pupillary dilation for 6/9 males and 9/9 females. This finding appeared to be a consequence of mydriasis.

7. Organ Weight: Changes in absolute or relative weights were observed for several organ; however, there were no histopathological correlations.

Brain: Relative brain weight for the male and female 50 mg/kg/day groups were increased to 113.8 and 107.6% of the control (0.530 and 0.828%), respectively.

Liver: Relative liver weight for the female 50 mg/kg/day group was increased to 109.1% of the control (4.128%).

Adrenal: Relative adrenal gland weight for the female 50 mg/kg/day group was increased to 127% of the control (0.0404%).

Spleen: Absolute spleen weight for the male 50 mg/kg/day group was decreased to 82.5% of the control (0.7995 g).

8. **Gross Pathology:** A dark discoloration of the Harderian glands was observed in the 50 mg/kg/day group for 10/10 males and 5/10 females. A dark discoloration of the periocular skin was observed in the 50 mg/kg/day group for 1/10 males and 5/10 females.

9. **Histopathology:** Tissues from the control and 50 mg/kg/day groups were subjected to microscopic analysis. The Harderian glands were also examined in the 3 mg/kg/day group. The target organ of toxicity was the Harderian glands, which showed minimal to moderate hypersecretion in all animals of the 50 mg/kg/day group. Hypersecretion was characterized by dilation of acini and ducts by a granular to reticular, pigmented (golden-brown) material. The cervical lymph node may also have been a target organ of toxicity as plasmacytosis was observed for animals receiving 50 mg/kg/day. An increased incidence of lung hemorrhage was observed for animals receiving 50 mg/kg/day.

Histopathological analysis of organs and tissues from rats that received darifenacin by oral gavage at doses of 0 or 50 mg/kg/day for 1 month. The Harderian glands were also examined in the 3 mg/kg/day group.

Organs/Tissues	0 mg/kg/day		3 mg/kg/day		50 mg/kg/day	
	Male	Female	Male	Female	Male	Female
Harderian gland -hypersecretion	0	0	0	0	10	10
Cervical node -plasmacytosis	0	0	-	-	2	3
Lungs -hemorrhage	2	1	-	-	5	2
Urinary bladder -infiltration of mononuclear cells	0	0	-	-	2	0
Epididymis -inflammation	0	-	-	-	2	-
Liver -necrosis	0	0	-	-	1	0
Colon -dilation	0	0	-	-	1	1
Esophagus -dilation	0	0	-	-	0	1*

*The female (F701) that died during the treatment period was found to have a dilated esophagus, as the lumen was expanded by compacted food.

10. Plasma Drug Levels: For male rats that received darifenacin at 50 mg/kg/day, plasma AUC values for darifenacin and UK-148,993 were similar on days 1 and 22. For female rats that received darifenacin at 50 mg/kg/day, plasma AUC values for darifenacin and UK-148,993 were higher on day 22 than on day 1. There were no differences in plasma AUC values for darifenacin between male and female rats on day 1; however, on day 22, values were higher for females than males.

Plasma C_{max} , T_{max} , and AUC values for darifenacin in rats that received darifenacin by oral gavage at a dose of 50 mg/kg/day for 1 month.

Day	C_{max} , $\mu\text{g/mL}$		T_{max} , hr		$\text{AUC}_{1.5\text{hr}}$, $\mu\text{g}\cdot\text{hr/mL}$	
	Male	Female	Male	Female	Male	Female
1	0.508	0.506	4.2	3.4	1.824	1.728
22	0.556	0.96	2.6	1	1.832	3.104

Plasma C_{max} , T_{max} , and AUC values for UK-148,993 in rats that received darifenacin by oral gavage at a dose of 50 mg/kg/day for 1 month.

Day	C_{max} , $\mu\text{g/mL}$		T_{max} , hr		$\text{AUC}_{1.5\text{hr}}$, $\mu\text{g}\cdot\text{hr/mL}$	
	Male	Female	Male	Female	Male	Female
1	0.276	0.282	2.6	2.6	0.988	0.972
22	0.232	0.362	3.4	1	0.752	1.208

Rats received darifenacin by oral gavage at doses of 0, 3, and 50 mg/kg/day for 1 month. The no effect dose was 3 mg/kg/day. One female of the 50 mg/kg/day group died on day 23, possibly due to dysphagia. Histopathological examination found dilation of the esophagus, as the lumen was expanded by ingesta. Body weight gains for male and female rats of the 50 mg/kg/day group were impaired by >10%. The target organ of toxicity was the Harderian glands, which showed minimal to moderate hypersecretion in all animals of the 50 mg/kg/day group. Dysphagia may have been related to the anticholinergic properties of darifenacin.

IND

Page 59

Three Month Dietary Subchronic Toxicity in Sprague Dawley Rats
(Study number 92051; Volume 6, Page 8 1067).

Testing Laboratory: Pfizer
Centre de Recherche
37401 Amboise Cedex
France

Study Started: July 7, 1992

Study Completed: September 9, 1993

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Sprague Dawley rats [CD(SD)BR], approximately 6 weeks of age at the start of the study, were used in this study. Mean body weights were 212.6 g for males and 171.1 g for females.

Drug Batch: UK 88,525-04 Batch number R7.

Methods: This study was intended as a dose range finding study to assist in the identification of doses for a subsequent dietary carcinogenicity study in rats. Rats were fed a diet supplemented with the hydrobromide salt of darifenacin for a three month period (93 or 94 days). Average daily intake was set at 0, 25, 50, or 100 mg/kg/day. There were 10 rats/sex/group. Rats were observed daily for mortality and clinical signs of toxicity. The presence or absence of mydriasis was determined on day 1, prior to the initiation of treatment, and on days 4, 42, and 84. Examinations for partially closed eyes and chromodacryorrhea were performed on days 1 through 9 and on days 42 and 84. Body weights were measured weekly. Animals were also examined for the presence of palpable masses during weighing. Food consumption was measured over periods of 7 days and water consumption was measured over periods of 24 hr once weekly. On day 94, each rat was placed in a metabolism cage with food and an overnight sample of urine (16-16.5 hr) was collected. At the end of the treatment period, blood samples were collected for determination of hematological and clinical chemistry parameters, and animals were sacrificed and subjected to a gross examination. Absolute and relative heart, kidney, and liver weights were determined. Tissues displaying any macroscopic abnormalities along with sections of the heart, kidneys, liver, lung, thyroid gland, and eyes (including Harderian glands) were collected for microscopic analysis. Additional sections of liver were collected from 7 rats/sex/group for determination of cytochrome P450 content and 5 rats/sex/group for electron microscopy. On day 21 of the study, blood samples were collected at 08.00, 12.00, 16.00, 20.00, and 24.00 hr from an additional 15 rats/sex/treatment group for determination of plasma levels of darifenacin and UK-148,993. Plasma samples were extracted and quantities of darifenacin and UK-148,993 were measured by HPLC with UV detection.

Results:

1. **Achieved Doses:** Achieved doses were calculated based upon weekly food consumption and mean body weights at the start and end of each week. Achieved doses for male treatment groups were 25.18 ± 1.75 , 50.85 ± 4.12 , and 103.40 ± 8.77 mg/kg/day, respectively. Achieved doses for female treatment groups were 24.66 ± 1.80 , 50.37 ± 4.85 , and 100.91 ± 13.24 mg/kg/day, respectively. The achieved dose for the female 100 mg/kg/day group is questionable. In the report, food consumption data for the female 100 mg/kg/day group was only included for weeks 1-5. The sponsor reported that due a marked incidence of spillage in this group, calculation of mean food intake was unreliable from day 35 to the end of treatment.

2. **Observed Effects:** Treatment-related clinical signs consisted of mydriasis, chromodacryorrhea, and partially closed eyes. Mydriasis occurred with a dose-related incidence from day 4 to the end of the treatment. The incidences for the 25, 50, and 100 mg/kg/day groups were 2-3/20, 5-12/20, and 17-20/20, respectively. Partially closed eyes were observed beginning on day 42 in the 25 mg/kg/day group, day 7 in the 50 mg/kg/day group, and day 5 in the 100 mg/kg/day group. The incidences for the 25, 50, and 100 mg/kg/day groups were 1-4/20, 1-4/20, and 3-10/20, respectively. Chromodacryorrhea was observed beginning on day 3 or 4 and occurred with a similar incidence in all treatment groups. The highest incidences for the 25, 50, and 100 mg/kg/day groups were 13/20, 12/20, and 12/20, respectively.

3. **Mortality:** One female from the 50 mg/kg/day group was found dead on day 40 of the study. During the week preceding death, this animal was observed with a hunched posture, dyspnea, and significant reductions of food and water consumption and body weight. The relationship of the death to treatment with darifenacin is unclear due to the lack of mortality at 100 mg/kg/day.

4. **Body Weight, Food Consumption, and Water Consumption:** Body weight gain for all male and female treatment groups was impaired by >10%. Further, food and water consumption were reduced in all male and female treatment groups. Body weights for male controls on days 1 and 91 were 208.48 and 575.95 g, respectively. Body weight gains for the male 25, 50, and 100 mg/kg/day groups were reduced to 73.9, 65.1, and 50.3% of the control, respectively. Body weights for the female controls on days 1 and 91 were 172.55 and 324.87 g, respectively. Body weight gains for female 25, 50, and 100 mg/kg/day groups were reduced to 64.3, 51, and 33.9% of the control, respectively. Food consumption for all male and female treatment groups was consistently reduced throughout the treatment period as compared to controls. The average weekly food consumption throughout the treatment period for the male 25, 50, and 100 mg/kg/day groups were reduced to 88.5, 86.7, and 82.8% of

the control (29.485 g/animal/day), respectively. The average weekly food consumption throughout the treatment period for the female 25 and 50 mg/kg/day groups were reduced to 78.0, and 83.4% of the control (22.344 g/animal/day), respectively. Food consumption for the female 100 mg/kg/day group was only provided from weeks 1 through 5. Water consumption was for all male and female treatment groups was consistently reduced throughout the treatment period as compared to controls. The average weekly water consumption throughout the treatment period for the male 25, 50, and 100 mg/kg/day groups were reduced to 90.6, 93.2, and 71.9% of the control (33.73 g/animal/day), respectively. The average weekly water consumption throughout the treatment period for the female 25, 50, and 100 mg/kg/day groups were reduced to 83.8, 79.5, and 66.4% of the control (29.327 g/animal/day), respectively.

5. Hematology: Red blood cell counts, hemoglobin levels, and packed cell volume were mildly elevated in male treatment groups due to apparent dehydration associated with decreased water intake; although, no corresponding changes were observed for female treatment groups. Red blood cell counts for the male 50 and 100 mg/kg/day groups were increased to 104.4, and 104.7% of the control (9.360×10^6 cells/mm³), respectively. Hemoglobin levels for the male 25, 50, and 100 mg/kg/day groups were increased to 104.9, 106.1, and 107.3% of the control (15.83 g/dL), respectively. Packed cell volume for the male 25, 50, and 100 mg/kg/day groups were increased to 104.5, 104.7, and 107.1% of the control (45.87%), respectively. Mean corpuscular volume for the female 25, 50, and 100 mg/kg/day groups were decreased to 97.3, 96.7, and 93.5% of the control (53.04 fL), respectively. Mean corpuscular hemoglobin for the female 25, 50, and 100 mg/kg/day groups were decreased to 96.8, 96.2, and 93.9% of the control (18.56 pg), respectively. Red blood cell distribution width for the female 100 mg/kg/day group was increased to 121.1% of the control (12.36%), respectively.

6. Blood Chemistry and Urinalysis: Chloride levels for male and female treatment groups were elevated, and sponsor has speculated that bromide administered with darifenacin may have interfered with the assay for chloride.

Males: There was no evidence of renal damage that might led to elevated Cl⁻ levels. Chloride levels for the male 50 and 100 mg/kg/day groups were increased to 113.8 and 132.8% of the control (118.4 mmol/L), respectively. Alanine aminotransferase activities for the male 25, 50, and 100 mg/kg/day groups were increased to 121.2, 115.9, and 129.6% of the control (22.6 IU/L), respectively; although, only values for the 25 and 100 mg/kg/day groups were significantly different. Calcium levels for the male 50 and 100 mg/kg/day groups were increased to 103 and 103.2% of the control (100.8 mg/L), respectively. Protein levels for the male 25, 50, and 100 mg/kg/day groups were increased to 104.6, 106.3, and 104.5% of the control (66.7 mg/mL), respectively. Urinalysis found no significant changes in male treatment groups.

Females: Chloride levels for the female 50 and 100 mg/kg/day were increased to 111.5 and 131% of the control (117.5 mmol/L), respectively. Aspartate aminotransferase activities for the 25, 50, and 100 mg/kg/day were increased to 121.2, 121.5, and 120.7% of the control (38.6 IU/L), respectively. Alanine aminotransferase and alkaline phosphatase activities for the female 100 mg/kg/day group were increased to 132.5 and 149% of the control (20.0 and 83.7 IU/L), respectively. Phosphate levels for female 100 mg/kg/day group were increased to 125.3% of the control (59.0 mg/L). Glucose levels for the female 25, 50, and 100 mg/kg/day groups were decreased to 91.7, 86.4, and 91.5% of the control (129.7 mg/dL), respectively. Albumin levels for the female 25, 50, and 100 mg/kg/day groups were decreased to 94.7, 96.8, and 91.0% of the control (37.8 mg/mL), respectively; although, the value for the 50 mg/kg/day group was not significantly different. The urinary pH level for the female 100 mg/kg/day groups was increased to 110.3% of the control (pH 7.15).

7. Hepatic Cytochrome P450 Content: The hepatic cytochrome P450 content was increased in the male 25 and 100 mg/kg/day groups to 127 and 153% of the control. The increase for the male 25 mg/kg/day group was regarded as not biologically significant due to contamination of microsomes with haem from lysed red blood cells. The hepatic cytochrome P450 content for the female 100 mg/kg/day group was increased to 132.6% of the control.

Hepatic cytochrome P-450 content in rats that received darifenacin at doses of 0, 25, 50, or 100 mg/kg/day for 3 months Seven rats/sex/group were examined (Adapted from sponsor's table).

Dose, mg/kg/day	Male	Female
0	0.77	0.46
25	0.98*	0.48
50	0.83	0.55
100	1.18*	0.61

* p < 0.05.

8. Organ Weight: Changes in relative heart, liver, and kidney weights appear to be related to the significant impairment of weight gain that occurred in all treatment groups.

Heart: Absolute heart weights for the male 50 and 100 mg/kg/day groups were decreased to 86.2 and 76.2% of the control (1.91 g), respectively. Relative heart weights for the male 25, 50, and 100 mg/kg/day groups were increased to 108.9, 110.9, and 111.7% of the control (0.350%), respectively. Absolute heart weights for the female 25, 50, and 100 mg/kg/day groups were decreased to 86.5, 82.3, and 77.6% of the control (1.261 g), respectively.

Liver: Absolute liver weights for the male 25, 50, and 100 mg/kg/day groups were decreased to 85.5, 86.3, and 81.7% of the control (18.226 g), respectively. Relative liver weights for male 50 and 100 mg/kg/day groups were increased to 110.6 and 119.3% of the control (3.347%), respectively. Absolute liver weights for the female 25, 50, and 100 mg/kg/day groups were decreased to 86.8, 83.4, and 85.9% of the control (10.274 g), respectively. Relative liver weights for female 50 and 100 mg/kg/day groups were increased to 110.2 and 124% of the control (3.454%), respectively.

Left Kidney: Absolute left kidney weight for the male 25, 50, and 100 mg/kg/day groups were decreased to 91.1, 87.5, and 77.6% of the control (2.005 g), respectively. Relative left kidney weights for the male 25, 50, and 100 mg/kg/day groups were increased to 108.1, 111.9, and 113% of the control (0.370%), respectively. Absolute left kidney weights for the female 25, 50, and 100 mg/kg/day groups were decreased to 84.6, 79.2, and 79.2% of the control (1.218 g), respectively. Relative left kidney weight for the female 100 mg/kg/day group was increased to 114.4% of the control (0.410%).

Right Kidney: Absolute right kidney weights for the male 25, 50, and 100 mg/kg/day groups were decreased to 88.9, 85.1, and 76.1% of the control (2.090 g), respectively. Relative right kidney weights for the male 50 and 100 mg/kg/day groups were increased to 109.1 and 110.9% of the control (0.385%), respectively. Absolute right kidney weights for the female 25, 50, and 100 mg/kg/day groups were decreased to 84.7, 81.2, and 80.7% of the control (1.216 g), respectively. The relative right kidney weight for the female 100 mg/kg/day group was increased to 116.3% of the control (0.410%).

9. Gross Pathology: Grey or dark bilateral discoloration of the Harderian glands was observed for 2 males of the 50 mg/kg/day group and 2 males and 1 female of the 100 mg/kg/day group.

10. Histopathology: Treatment-related histopathological changes were principally found in the liver and Harderian glands. Microscopic analysis of the liver found centrilobular hypertrophy in the 50 and 100 mg/kg/day groups, which were classified as minimal (Grade 1). Electron microscopic analysis of livers from the control and 100 mg/kg/day group found minimal proliferation of the smooth endoplasmic reticulum in the centrilobular hepatocytes of 2 of 5 males and 2 of 5 females in the 100 mg/kg/day group. At doses of 50 and 100 mg/kg/day, generally >30-70% of alveoli in the Harderian glands were affected.

Histopathological changes for rats that received darifenacin at doses of 0, 25, 50, or 100 mg/kg/day for 3 months.

Organ/Tissue	0		25		50		100	
	M	F	M	F	M	F	M	F
Liver								
-centrilobular hypertrophy	0	0	0	0	2	0	6	5
-centrilobular vacuolation	0	0	0	0	1	2	2	1
Harderian Gland								
-hypersecretion	1	0	2	2	7	8	9	10
Thyroid								
-Inflammation, chronic, focal	0	0	0	0	0	0	2	1
-ectopic thymus	0	0	0	0	0	2	2	0
Lung								
-Osseous metaplasia	0	1	0	2	0	0	1	0
-foam cell foci	0	0	1	0	0	0	4	0
Eye								
-Retinal atrophy	0	0	0	0	0	0	1	1

11. Plasma Drug Levels: Plasma C_{max} and AUC values for darifenacin and UK-148,993 were approximately proportional to dose for both males and females. Plasma C_{max} and AUC values for darifenacin and UK-148,993 were greater in females than males. There no evidence of saturation in the metabolic conversion of darifenacin to its hydroxylated metabolite, UK-148,993. Plasma drug levels for human volunteers following single oral doses of 0.2 or 0.4 mg/kg were 34.8 and 75.7 ng.hr/mL. Human plasma AUC values ranged from 66 to 39 times lower than the average level observed for rats at 25 mg/kg, respectively; however, the respective dose differential was 125 and 62.5-fold.

Plasma C_{max} and AUC values for darifenacin in rats that received darifenacin at doses of 25, 50, and 100 mg/kg/day for 3 months.

Dose mg/kg/day	C_{max} , ng/mL		AUC _{24 hr} , ngh/mL	
	Male	Female	Male	Female
25	80	209	1500	3128
50	231	236	4168	5202
100	416	688	8534	15420

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Page 65

Plasma C_{max} and AUC values for UK-148,993 in rats that received darifenacin at doses of 25, 50, and 100 mg/kg/day for 3 months.

Dose mg/kg/day	C_{max} , ng/mL		AUC _{24 hr} , ngh/mL	
	Male	Female	Male	Female
25	63	101	716	1360
50	128	165	2174	2870
100	243	427	4446	7710

Rats received darifenacin in the diet at doses of 0, 25, 50, or 100 mg/kg/day for 3 months. Mortality occurred for 1 female in the 50 mg/kg/day group; although, its relationship to treatment was unclear. Based upon impairment of weight gain, a no effect dose was not established. Body weight gain was impaired by >10% in all treatment groups; although, decreased weight gain appeared to correlate with decreased food and water consumption. Organ toxicity was minimal at all doses. The target organs of toxicity were the liver and Harderian glands. Hepatic centrilobular hypertrophy was found in rats that received darifenacin at doses of 50 and 100 mg/kg/day. An increased incidence of Harderian gland hypersecretion was found in all treatment groups. Plasma C_{max} and AUC values for darifenacin and UK-148,993 were approximately proportional to dose for both males and females. Plasma C_{max} and AUC values for darifenacin and UK-148,993 were greater in females than males. Based upon the correlation of impaired weight gain with decreased food consumption, the maximum tolerated dose was 100 mg/kg/day for both male and female rats.

Dogs

2-Week Intravenous Toxicity Study in Beagle Dogs (Study No. 94028; Volume 10, Page 8 2563).

Testing Laboratory: Laboratoires Pfizer
Centre de Recherche
37401 Amboise Cedex
France

Study Started: March 24, 1994

Study Completed: September 27, 1994

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Beagle dogs, approximately 10 months old, were used in this study. On the first day of treatment, mean body weights for male and female dogs were 12.2 and 10.0 kg, respectively.

Drug Batch: Darifenacin, Lot number R11.

Methods: Beagle dogs received darifenacin by the intravenous route at doses of 0, 0.5, and 2.5 mg/kg/day for 2 weeks (14 or 15 days). There were 3 dogs/sex/group. The vehicle was a 5% aqueous mannitol solution, pH 6.3. The dosing volume was 5 mL/kg. During the treatment period, animals were observed daily for mortality and clinical signs of toxicity, once prior to dosing and approximately 1 hr after dosing. Body weights were measured prior to treatment and on treatment days 1, 7, and 13. An ophthalmic examination was performed for animals of the control and 2.5 mg/kg/day groups prior to treatment and on day 8. Cardiovascular parameters, including heart rate, systolic blood pressure, and electrocardiogram, were measured prior to treatment and on day 5 or 6 (a baseline reading was taken 24 hr after the previous dose and a test reading was taken 1 hr after dosing). The electrocardiogram was recorded while the dog was in a normal standing position using three standard bipolar limb leads I, II, and III, and three augmented unipolar limb leads, aVR, aVL, and aVF. The P and QRS durations, PQ and QT intervals, and P amplitude were recorded. Blood was collected for determination of hematological and clinical chemistry parameters prior to treatment on days -31, -14, and -1 and on treatment day 1. Urine was collected overnight (16.5 hr) for urinalysis prior to treatment on day -2 and on treatment day 14. Blood was collected on day 9 at 0.5, 1, and 5 hr after dosing for determination of plasma levels of darifenacin and UK-148,993. Plasma was extracted and quantities of darifenacin and UK-148,993 were determined by HPLC using ultraviolet detection. Animals were sacrificed and subjected to a gross examination. Organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, ovaries, spleen, and testes. Microscopic analysis was performed with tissues displaying macroscopic abnormalities plus sections of the following organs/tissues (adrenal glands, aorta, brain, cephalic vein, cervical lymph node, colon, duodenum, epididymis, esophagus, gall bladder, heart, ileum, kidneys, liver, lung, mammary glands/skin, mesenteric lymph node, esophagus, ovaries, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, spinal cord, spleen, sternum with marrow, stomach, striated muscle, testes, thymus, thyroid gland, trachea, urinary bladder, and uterus).

Results:

1. **Observed Effects:** Treatment-related observed effects included mydriasis, and dryness of the mouth and/or nose. The incidences of mydriasis for the male 0, 0.5, and 2.5 mg/kg/day groups were 0 of 3, 0 to 2 of 3, and 0 to 3 of 3, respectively. The incidences of mydriasis for the female 0, 0.5, and 2.5 mg/kg/day groups were 0 of 3, 0 of 3, and 0 to 3 of 3, respectively. Other ophthalmic changes are discussed below. The incidences of dry mouth and/or dry nose for the male 0, 0.5, and 2.5 mg/kg/day groups were 0 of 3, 0 to 3

of 3, and 0 to 3 of 3, respectively. The incidences of dry mouth and/or dry nose for the male 0, 0.5, and 2.5 mg/kg/day groups were 0 of 3, 0 to 2 of 3, and 0 to 3 of 3, respectively. These observed effects were consistent with the anticholinergic properties of darifenacin.

2. Mortality: None.

3. Body Weight and Food Consumption: Body weights were decreased for the male and female 0.5 and 2.5 mg/kg/day groups. Body weights for the male controls on days 1 and 13 were 12.07 and 12.10 kg, respectively, which yielded a net change of 0.03 kg or a 0.25% increase of initial weight. For the male 0.5 and 2.5 mg/kg/day groups, net changes were -0.03 (0.24% loss of initial weight) and -0.70 kg (5.70% loss of initial weight), respectively. Body weights for the female controls on days 1 and 13 were 10.07 and 10.00 kg, respectively, which yielded a net change of -0.07 kg or a 0.7% loss of initial weight. For the female 0.5 and 2.5 mg/kg/day groups, net changes were -0.73 (7.35% loss of initial weight) and -0.33 kg (3.32% loss of initial weight), respectively. Food intake was reduced for the male and female 0.5 and 2.5 mg/kg/day groups.

4. Hematology: Fibrinogen levels for the male 2.5 mg/kg/day group on day 15 were increased to 121.2% of the control (2.077 g/L). Monocyte counts for the female 0.5 and 2.5 mg/kg/day groups on day 15 were increased to 146.8 and 159.2% of the control (0.427×10^3 cells/mm³); although, this difference was only significant for the 2.5 mg/kg/day group.

5. Blood Chemistry and Urinalysis: K⁺ levels for the male 2.5 mg/kg/day group on day 15 were increased to 111.3% of the control (3.80 mmol/L). Alanine aminotransferase activities for the male 0.5 and 2.5 mg/kg/day groups on day 15 were increased to 128.2 and 143.6% of the control (26.00 IU/L), respectively, although, this difference was only significant for the 2.5 mg/kg/day group. Urea levels for the female 2.5 mg/kg/day group on day 15 were increased to 125.8% of the control (31.00 mg/dL). Bilirubin levels for the female 2.5 mg/kg/day group on day 15 were increased to 150% of the control (0.080 mg/dL). There were no treatment-related changes found with urinalysis.

6. Vital Signs and Physical Examination: An ophthalmic examination on day 8 found that the pupillary reflex for male and female dogs that received darifenacin at 2.5 mg/kg/day was absent. The pupillary reflex was normal for control animals. One male of the 2.5 mg/kg/day group had bulbar conjunctiva. Heart rate for the 2.5 mg/kg/day group on day 5, 1 hr after dosing, was mildly increased to 109.6% of the control (112.7 bpm). There were no treatment-related changes of systolic blood pressure or electrocardiographic parameters (i.e., the P and QRS durations, PQ and QT intervals, and P amplitude).

7. **Organ Weight:** There were no treatment-related changes of absolute and relative organ weights. Relative liver weights were increased for the male and female 2.5 mg/kg/day groups; although, these changes were not statistically significant.

8. **Gross Pathology:** There were no treatment-related gross pathological findings.

9. **Histopathology:** Microscopic changes in the stomach were found for two males (M21 and M23) of the 2.5 mg/kg/day group, which consisted on a chronic, multifocal gastric inflammation located in the pyloric zone. For one male (M21), these changes were associated with chronic, multifocal vasculitis in the same region. Inflammation was also found in the ileum for this animal. For the other male (M23), the gastric inflammation occurred concomitantly with bacterial overgrowth.

Histopathological changes for dogs that received darifenacin by the intravenous route at doses of 0, 0.5, and 2.5 mg/kg/day for 2 weeks.

Organ/Tissue	0		0.5		2.5	
	M	F	M	F	M	F
Stomach						
-inflammation	0	0	0	1	2	0
-hemorrhage	0	0	0	0	1	0
-vasculitis	0	0	0	0	1	0
Ileum						
-Inflammation	0	0	0	0	1	0
Duodenum						
-inflammation	0	0	0	0	0	1

10. **Plasma Drug Levels:** Plasma AUC values for darifenacin and its hydroxylated metabolite, UK-148,993, were approximately proportional to dose. There were no significant differences in AUC values between male and female dogs. The T_{max} values for UK-148,993 at doses of 0.5 and 2.5 mg/kg/day were 1 and 5 hr, respectively, which, as expected, lag behind the T_{max} for the parent compound, darifenacin.

Plasma T_{max} , C_{max} , and AUC values for darifenacin in dogs that received darifenacin by the intravenous route at doses of 0.5 and 2.5 mg/kg/day for 2 weeks.

Dose mg/kg/day	T_{max} , hr		C_{max} , $\mu\text{g/mL}$		$\text{AUC}_{0.5-5\text{hr}}$, $\mu\text{g}\cdot\text{hr/mL}$	
	Male	Female	Male	Female	Male	Female
0.5	0.5	0.5	0.17	0.14	0.41	0.34
2.5	0.5	0.5	0.88	1.11	2.73	3.06

Plasma T_{max} , C_{max} , and AUC values for UK-148,993 in dogs that received darifenacin by the intravenous route at doses of 0.5 and 2.5 mg/kg/day for 2 weeks.

Dose mg/kg/day	T_{max} , hr		C_{max} , $\mu\text{g/mL}$		$AUC_{0.5-5hr}$, $\mu\text{g}\cdot\text{hr/mL}$	
	Male	Female	Male	Female	Male	Female
0.5	1 hr	1 hr	0.087	0.077	0.36	0.30
2.5	5 hr	5 hr	0.25	0.33	0.96	1.35

Dogs received darifenacin by the intravenous route at doses of 0, 0.5, and 2.5 mg/kg/day for 2 weeks. The no effect dose was 0.5 mg/kg/day. There was no mortality. The target organ of toxicity was the stomach. Two males of the 2.5 mg/kg/day group were observed with a chronic, multifocal gastric inflammation located in the pyloric zone.

A One Month Gavage Study in Beagle Dogs-Dose Levels: 0, 1, 4, or 16 mg active moiety/kg/day (Protocol No. 89-751-02; Volume 7, Page 8 1614).

Testing Laboratory: Drug Safety Evaluation Department
Pfizer Central Research
Pfizer Inc.
Groton, CT 06340

Study Started: October 31, 1989

Study Completed: July 30, 1990

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Male and female purebred beagle dogs, approximately 9-10 months old, were used in this study. Mean body weights for male and female controls at the start of treatment were 10.7 and 9.2 kg, respectively.

Drug Batch: Darifenacin-04 Bulk lot number R1.

Methods: Beagle dogs received darifenacin by oral gavage at doses of 0, 1, 4, and 16 mg base/kg/day for 1 month (36 consecutive days). Dose selection was based upon a 2 week range finding study (Study number 89039) in which beagle dogs received darifenacin by the oral route at 0, 8, 15, or 30 mg/kg/day. The no effect level was 8 mg/kg/day. Mortality occurred for 1 male treated with 30 mg/kg/day. The pharynx for this animal was partially blocked with chewed food pellets. Histopathological analysis of the lung found a multifocal, suppurative broncho-pneumonia consistent with

inhalation pneumonia related to regurgitation and subsequent inhalation of food. Regurgitation of chewed and undigested food pellets occurred in all treatment groups; however, there was only 1 episode for the 8 mg/kg/day group, while several episodes occurred for 2 or more animals treated with 15 or 30 mg/kg/day. Regurgitation of food and the presence of food within the esophagus was attributed to dysphagia, which may be related to anticholinergic properties of darifenacin. In subsequent studies, food was moistened before presentation to animals to minimize problems related to dysphagia. In the present study, there were 3 dogs/sex/group. The vehicle was 0.5% methylcellulose (4000 cps) containing 0.1% polysorbate 80 (Tween 80). The 1 and 4 mg/mL concentrations were solutions and the 16 mg/mL concentration was a suspension. The dosing volume was 1 mL/kg. Dosing began over a 4 day period with the 16 mg/kg/day group starting first, followed by the 4 mg/kg/day, 1 mg/kg/day, and control groups. Dogs were observed at least twice daily for clinical signs of toxicity. Body weights were measured prior to the start of treatment, on days 1 and 8, and at weekly intervals thereafter. Food consumption was monitored daily and a notation was made if the animal consumed <75% of its ration. Electrocardiographic tracings (leads I, II, III, aVR, aVL, aVF, and CV₆LL) and indirect systolic blood pressure recordings were obtained from conscious animals twice prior to the start of treatment and at the end of treatment. Vital signs (heart rate, respiratory rate, and rectal temperature) were obtained twice prior to start of treatment and on days 1, 15, and 36 (prior to dosing and at 2 hr after dosing). Ophthalmic exams were performed once prior to the start of treatment and at the end of treatment. Pupillary dilation was monitored daily using a flashlight. Hematology, serum chemistry, and urinalysis parameters were measured twice prior to the start of treatment and on days 12-16 and 27-31. For measurement of plasma drug concentrations, blood was collected on days 1, 15, and 30 at 1, 3, 5, 7, and 24 hr after dosing. Drug quantities were determined using HPLC with ultraviolet detection. The assay limit of detection ranged from [] $\mu\text{g/mL}$. Animals were sacrificed on day 37 and subjected to a gross examination. Absolute organ weights were determined for both kidneys, liver, and testes. Microscopic analysis was performed with tissues displaying macroscopic abnormalities plus sections of the following organs/tissues (adrenal glands, aorta, brain, colon, duodenum, epididymis, esophagus, eye, gall bladder, heart, ileum, jejunum, kidneys, liver, lung, mammary glands/skin, mesenteric lymph node, ovaries, pancreas, pituitary gland, prostate, salivary glands, spinal cord, spleen, sternum with marrow, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, and uterus).

Results:

1. **Observed Effects:** Dogs from all treatment groups were observed with a dose-related pupillary dilation throughout the treatment period. Sporadic episodes of emesis were observed for 2 dogs of the 1 mg/kg/day group (1 episode/dog during the treatment period), 2 dogs of the 4 mg/kg/day group (1 episode/dog during the treatment period), and all 6 dogs of the 16 mg/kg/day group (1-3 episodes/dog during the treatment period). No feces were observed for any animals of the 16 mg/kg/day group during the first 4-6 days of treatment; the first stool passed for 3 of these animals were dry. Difficulty swallowing was observed for two females of the 16 mg/kg/day group on day 9 and one female of the 4 mg/kg/day group on day 10. Difficulty inserting the gavage tube was observed for one of these females of the 16 mg/kg/day group on day 10. Excessive salivation was observed on days 7-12 for 5 of 6 animals of the 16 mg/kg/day group and on day 36 for 3 of 6 of these animals. On day 27, 1 female of the 16 mg/kg/day group was observed to be dehydrated and taking shallow, rapid breaths. This animal was given extra water and by day 29, hydration and breathing returned to normal; however, by day 36, this animal was again taking shallow rapid breaths.

2. **Mortality:** None.

3. **Body Weight and Food Consumption:** There were no treatment-related effects on body weight gain. Initial and final mean body weights for the male controls were 10.7 and 10.2 kg, respectively, yielding a net change of -0.5 kg or a 4.7% loss of initial body weight. Net change in body weights for the male 1, 4, and 16 mg/kg/day groups were 0, -0.6 (6.12% loss), and -0.2 (2.13% loss) kg, respectively. Initial and final mean body weights for the female controls were both 9.2 kg, yielding a net change of 0 kg. Net change in body weights for the female 1, 4, and 16 mg/kg/day groups were 0.5 (6.17% increase), -0.3 (3.26% loss), and -0.3 (4.11% loss) kg, respectively.

4. **Hematology:** White blood cell differential counts, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin content were determined; however, the sponsor did not provide mean or standard deviation values for this data. Further, the data was scattered over several pages making any sort of review impossible. A number of small changes in hematological parameters were observed for the male 16 mg/kg/day group. White blood cell counts for the male 16 mg/kg/day group on day 27 were increased to 133.3% of the control (8.10). Red blood cell counts, hemoglobin levels, and hematocrit for the male 16 mg/kg/day group on day 12 were decreased to 88.6, 92.1, and 90.1% of the control (7.60, 16.83, and 51.50%), respectively.

5. Blood Chemistry and Urinalysis: Globulin levels and the albumin to globulin ratio were determined and urinalysis was performed; however, the sponsor did not provide mean or standard deviation values for this data. Further, the data was scattered over several pages making any sort of review impossible. A number of small changes in serum chemistry parameters were observed for the male and female 16 mg/kg/day groups.

Males: Total protein levels for the male 16 mg/kg/day group on days 12 and 27 was decreased to 89.8 and 92.6% of the control (6.57 and 6.23), respectively. Sorbitol dehydrogenase activity for the male 16 mg/kg/day group on day 12 was decreased to 76.7% of the control (10.00).

Females: Alanine aminotransferase and aspartate aminotransferase activities for the female 16 mg/kg/day group were increased to 153.5 and 159.5% of control values (28.67 and 29.67), respectively; however, these changes were not statistically significant. Na^+ levels for the female 16 mg/kg/day group on day 27 were decreased to 96.9% of the control (150.67). Ca^{2+} levels for the female 16 mg/kg/day group on day 27 were decreased to 96.1% of the control (11.13).

6. Vital Signs, Physical Examination, and Ophthalmic Examination:

Vital Signs: Body temperature was determined; however, the sponsor did not provide mean or standard deviation values for this data. Further, the data was scattered over several pages making any sort of review impossible.

Electrocardiographic Examination: Heart rate, the RR interval, and systolic blood pressure were determined; however, the sponsor did not provide mean or standard deviation values for this data. Further, the data was scattered over several pages making any sort of review impossible. On day 1, two hr after drug administration, heart rate for the male and female 16 mg/kg/day groups were increased to 132.6 and 144.2% of the controls (118.2 and 114.7 beats/min). On day 36, two hr after drug administration, heart rate for the male 4 and 16 mg/kg/day groups were increased to 142.4 and 127.4% of the control (106.7 beats/min).

Ophthalmic Exam: From the 4 mg/kg/day (1 male and 1 female) and the 16 mg/kg/day (1 male and 1 female) groups, a white to yellow ocular discharge was observed. The discharge persisted for 2 days for the 2 animals of the 4 mg/kg/day group and for 4 days for the 1 male of the 16 mg/kg/day. For the female of the 16 mg/kg/day group, the discharge began on day 8 and persisted throughout the treatment period. Both eyes of this female of the 16 mg/kg/day group as well as the right eye of the male from the same group, had a sunken appearance and surface vessels on the sclera were

ruptured. Final ophthalmic examinations for these two animals of the 16 mg/kg/day group found mild corneal edema and a moderate accumulation of mucoid material. The two animals of the 4 mg/kg/day group had mild to moderate accumulations of mucoid material.

7. Organ Weight: Relative liver weight for the male and female 16 mg/kg/day groups were increased to 119.5 and 137.8% of respective controls (29.86 and 28.54%).

8. Gross Pathology: There were no treatment-related gross pathological lesions.

9. Histopathology: There were no treatment-related histopathological lesions.

10. Plasma Drug Levels: Plasma drug levels for dogs that received a dose of 1 mg/kg/day were undetectable on days 1, 15, and 30. On days 1, 15, and 30, plasma AUC values for dogs treated with 4 mg/kg/day were similar. On days 1 and 15, plasma AUC values for dogs treated with 4 and 16 mg/kg/day were approximately proportional to dose. On day 30, plasma AUC values for male and female dogs treated with 16 mg/kg/day were 7.45 and 6.73 times corresponding values at 4 mg/kg/day, respectively.

Plasma darifenacin T_{max} , C_{max} , and AUC values for male dogs that received darifenacin by the oral route at 4 and 16 mg/kg/day for 1 month.

Day	4 mg/kg/day			16 mg/kg/day		
	T_{max} hr	C_{max} $\mu\text{g}/\text{mL}$	AUC (0-7) $\mu\text{g}\cdot\text{hr}/\text{mL}$	T_{max} hr	C_{max} $\mu\text{g}/\text{mL}$	AUC (0-7) $\mu\text{g}\cdot\text{hr}/\text{mL}$
1	1.67	0.6	2.2	1	2.0	9.6
15	1.67	0.7	3.3	5	1.9	9.8
30	1	0.6	2.4	1	3.7	17.9

Plasma darifenacin T_{max} , C_{max} , and AUC values for female dogs that received darifenacin by the oral route at 4 and 16 mg/kg/day for 1 month.

Day	4 mg/kg/day			16 mg/kg/day		
	T_{max} hr	C_{max} $\mu\text{g}/\text{mL}$	AUC (0-7) $\mu\text{g}\cdot\text{hr}/\text{mL}$	T_{max} hr	C_{max} $\mu\text{g}/\text{mL}$	AUC (0-7) $\mu\text{g}\cdot\text{hr}/\text{mL}$
1	1.67	0.2	0.9	1	1.8	9.3
15	1.67	0.5	2.2	3.67	1.3	6.7
30	1.67	0.3	1.5	1	2.5	10.1

Dogs received darifenacin by oral gavage at doses of 0, 1, 4, and 16 mg/kg/day for 1 month. The no effect dose was 4 mg/kg/day. There was no treatment-related mortality. A target organ of toxicity was not identified. Relative liver weights were increased for the male and female 16 mg/kg/day groups; although, there were no corresponding histological changes. No feces were observed for any animals of the 16 mg/kg/day group during the first 4-6 days of treatment. Difficulty swallowing (i.e., dysphagia) was observed for 2 females of the 16 mg/kg/day group. Final ophthalmic examinations for two animals of the 16 mg/kg/day group found mild corneal edema and a moderate accumulation of mucoid material.

Chronic Toxicity:

Rats

6 Month Oral Toxicity in Sprague Dawley Rats (Study number 92028; Volume 7, Page 8 1245).

Testing Laboratory: Laboratoires Pfizer
Centre de Recherche
37401 Amboise Cedex
France

Study Started: March 19, 1992

Study Completed: March 30, 1993

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Sprague Dawley rats VAF-CD(SD)BR], approximately 7 weeks of age at the start of the study, were used. Mean body weights were 267 g for males and 203.2 g for females.

Drug Batch: Darifenacin-04 Batch number R7.

Methods: Rats received darifenacin by oral gavage at dose levels of 0, 3, 10, or 30 mg/kg/day for 6 months (181-183 days). There were 20 rats/sex/group. The vehicle was methylcellulose, 4000 cps, as a 0.5% (w/v) aqueous solution containing 0.1% (w/v) Tween 80. The dose volume was 5 mL/kg. Animals were observed daily for mortality or clinical signs of toxicity. The first 10 animals of each group were selected for observation of pupil size (absence or presence of mydriasis) at 1 and 5 hr after dosing on days 1 and 2, weekly during the first month, and then monthly. These same 10 animals/group were observed for chromodacryorrhea, salivation, and partially closed eyes. From days 1-5, these observations were performed at 0.5, 1, 3, 5, and 7 hr after administration. From day 9, these observations were performed weekly during the first month, and monthly thereafter. For chromodacryorrhea and partially closed

eyes, observations from day 9 to the end of the study were performed at 1 and 5 hr after dosing. For salivation, observations were performed immediately and at 5 min after dosing. If an animal died within this group of 10, it was replaced. Body weight was recorded weekly. Food consumption was measured over periods of one week except when animals were subjected to blood collection. Water consumption was measured monthly over periods of 24 hr. An ophthalmic examination was performed on control and 30 mg/kg/day groups prior to the initiation of treatment and at the end of the study (days 173-174). Blood for hematology and clinical chemistry determinations and urine for urinalysis were collected at days 63, 126, and 182. Twenty-four hr after the last dose, animals were sacrificed and subjected to a gross examination. Organ weights for the adrenal glands, brain, heart, kidneys, liver, spleen, and testes were reported. Sections were collected from tissues with macroscopic abnormalities plus sections from the following tissues: adrenal glands, aorta, brain, cervical lymph node, colon, duodenum, epididymis, esophagus, eyes/Harderian glands, heart, ileum, kidneys, liver, lung, mammary glands/skin, mesenteric lymph node, esophagus, ovaries, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, spleen, sternum with marrow, stomach, striated muscle thigh, testes, thymus, thyroid gland, trachea, urinary bladder, and uterus. Five additional rats/sex/group were used for determination of plasma levels of darifenacin and the hydroxylated metabolite of darifenacin, UK-148,993. On day 176, blood was collected at 1, 3, 5, and 24 hr after dosing. Plasma was extracted with diethyl ether and quantities of darifenacin and UK-148,993 were determined by HPLC with UV absorbance.

Results:

1. Observed Effects: Treatment-related clinical signs consisted of mydriasis, chromodacryorrhea, partially closed eyes, and salivation. These signs occurred in a dose-dependent manner and were generally not found in control animals. Mydriasis appeared in all treatment groups within 5 hr after dosing on day 1. Chromodacryorrhea and partially closed eyes appeared after day 5 in the 3 and 10 mg/kg/day groups and after day 4 in the 30 mg/kg/day group. Salivation appeared after day 5 in the control and 3 mg/kg/day groups and after day 2 in the 10 and 30 mg/kg/day groups. The highest incidence of mydriasis for each treatment group was as follows: 3 mg/kg/day, 2/10 males and 4/10 females; 10 mg/kg/day, 10/10 males and 10/10 females; and 30 mg/kg/day, 10/10 males and 10/10 females. The highest incidence of chromodacryorrhea for each treatment group was as follows: 3 mg/kg/day, 4/10 males and 2/10 females; 10 mg/kg/day, 4/10 males and 3/10 females; and 30 mg/kg/day, 10/10 males and 6/10 females. The highest incidence of partially closed eyes for each treatment group was as follows: 3 mg/kg/day, 2/10 males and 1/10 females; 10 mg/kg/day, 5/10 males and 1/10 females, and 30 mg/kg/day, 5/10 males and 2/10 females.

The highest incidence of salivation for each group at 5 min after dosing was as follows: Control, 1/10 males and 0/10 females, 3 mg/kg/day, 5/10 males and 2/10 females; 10 mg/kg/day, 9/10 males and 6/10 females; and 30 mg/kg/day, 9/10 males and 9/10 females.

2. **Mortality:** Mortality occurred in the 30 mg/kg/day group for 1 male (day 66) and 4 females (days 31, 44, 72, and 150). The cause of death was unknown; although, it was definitely related to darifenacin treatment. All animals were observed with pulmonary congestion; although, this is a relatively common finding in dead animals.

3. **Body Weight and Food and Water Consumption:** Body weight gains for the male 30 mg/kg/day group and the female 10 and 30 mg/kg/day groups were by >10%. Water consumption for the 10 and 30 mg/kg/day groups was generally increased over control levels. Mean body weights for the male controls on days 1 and 181 were 265.85 and 642.01 g, respectively. Body weight gains for the male 3, 10, and 30 mg/kg/day groups were 110, 100.15, and 85.3% of the control, respectively. Mean body weights for the female controls on days 1 and 181 were 201.68 and 361.35 g, respectively. Body weight gains for the female 3, 10, and 30 mg/kg/day groups were 102.15, 89.6, and 79.2% of the control, respectively. There were no treatment-related changes of food consumption for male or female treatment groups. Water consumption for the male 10 mg/kg/day group on days 113-114 and 169-170 and the male 30 mg/kg/day group on days 57-58, 113-114, and 169-170 was increased to 147.5-177.4% of the control (22.78-31.75 mL). Water consumption for the female 10 mg/kg/day group on days 85-86 and the female 30 mg/kg/day group on days 5-6, 57-58, 85-86, 113-114, and 169-170 was increased to 121.7-178.9% of the control (23.36-36.67 mL).

4. **Hematology:** White blood cell counts for the female 30 mg/kg/day on days 126 and 182 were increased to 119.7 and 125.6% of the control (8.601 and $9.012 \times 10^3/\text{mm}^3$). Neutrophil counts for the female 30 mg/kg/day group on day 182 were increased to 150.6% of the control ($1.5389 \times 10^5/\text{mm}^3$). Monocyte counts for the female 30 mg/kg/day group on days 126 and 182 were increased to 123 and 161% of the control (0.364 - $0.403 \times 10^3/\text{mm}^3$), respectively. Hemoglobin and red blood cell counts were slightly increased (<5%) for the female 30 mg/kg/day group on days 126 and 182 (HGB, 15.19-15.32 g/dL and RBC, 8.355 - $8.361 \times 10^6/\text{mm}^3$).

5. **Blood Chemistry and Urinalysis:**

Males: Chloride levels for the male 30 mg/kg/day on days 63, 126, and 182 were increased to 105.5, 116.5, and 106.9% of the control (110.5-112.8 mmol/L), respectively. Chloride levels for the male 10 mg/kg/day group on day 126 were increased to 104.7% of the control (110.5 mmol/L). Glucose levels on days 126 and 182 were decreased to 89.9 and 88.9% of the control (153.1-163.3 mg/dL), respectively.

Urinalysis revealed only small and sporadic changes that did not appear to have any relationship to darifenacin treatment.

Females: Chloride levels for the female 30 mg/kg/day on days 63, 126, and 182 were increased to 103.3, 112.7, and 103.2 of the control (112.1-115.8 mmol/L), respectively. Chloride levels for female 10 mg/kg/day group on day 126 were increased to 103.5% of the control (112.1 mmol/L). Phosphate levels for the female 30 mg/kg/day group on days 63, 126, and 182 were increased to 108.9, 115.2, and 109.1% of the control (49.3-57.6 mg/L), respectively. Potassium levels for the 30 mg/kg/day group on days 126 and 182 were increased to 106.8 and 107% of the control (3.92-3.97 mmol/L), respectively. Potassium levels for the 10 mg/kg/day group on day 126 were increased to 105% of the control. Alkaline phosphatase levels for the 30 mg/kg/day group on days 126 and 182 were increased to 141.7 and 136.1% of the control (49.6-57.7 IU/L), respectively. Alkaline phosphatase levels for the 10 mg/kg/day group on day 126 were increased to 125.3% of the control (57.7 IU/L). Aspartate aminotransferase (ASAT) activity for the female 30 mg/kg/day group on days 126 and 182 were decreased to 64.6 and 60.9% of the control (63.0-70.3 IU/L), respectively. ASAT activity for the 10 mg/kg/day group on days 126 and 182 was decreased to 71.7 and 62.5% of the control, respectively; although, the change at day 182 was not significant. ASAT activity for the 3 mg/kg/day group was decreased to 65.55 and 80.9% of the control; although, the change at day 182 was not significant.

Urinalysis revealed only small and sporadic changes that did not appear to have any relationship to darifenacin treatment.

6. **Ophthalmic Examination:** There were no treatment-related ophthalmic changes.

7. **Organ Weight:** Several small changes in absolute and relative organ weights were found in the 30 mg/kg/day group; however, there were no corresponding histological changes. For the male 30 mg/kg/day group, there were small increases (<10%) in relative weights for liver, brain, and testes, that appeared to be related to decreased body weight gain. Similarly, small increases (<10%) of relative liver weights for the female 10 and 30 mg/kg/day groups appeared to be related to decreased body weight gain. For the male 30 mg/kg/day group, there were small decreases (10-15%) in absolute weight for the spleen and right adrenal gland.

8. **Gross Pathology:** A dark or reddish discoloration of the Harderian glands was found for a few animals of each treatment group as follows: 3 mg/kg/day (1 male and 1 female); 10 mg/kg/day (2 males and 2 females); and 30 mg/kg/day (2 males and 3 females).

9. Histopathology: Bacterial overgrowth in the non-glandular stomach, characterized by a granular, blue layer adherent to the superficial keratin was observed in 2 males and 11 females from the 30 mg/kg/day group. This change may be related to decreased gastric motility and decreased acid secretion produced by darifenacin. An increased incidence of Harderian gland hypersecretion, characterized by dilation of the glandular lumen and a brown-speckled secretion sometimes containing cellular debris, was found in the 10 and 30 mg/kg/day groups. The incidence of Harderian gland hypersecretion with a grade ≥ 2 ($\geq 30-70\%$ of acini affected) was as follows: Control, 0; 3 mg/kg/day, 1; 10 mg/kg/day, 17; and 30 mg/kg/day, 33. Tumors found in the study were as follows: a pituitary adenoma in a female of the 10 mg/kg/day group, an astrocytoma in a control male, and mammary adenocarcinomas in a control female and a female of the 10 mg/kg/day group. These tumors do not appear to have any relationship to darifenacin treatment.

Incidence of histopathological changes for rats that received darifenacin by oral gavage at dose levels of 0, 3, 10, or 30 mg/kg/day for 6 months. There were 20 rats/sex/group.

Organ/Tissue	0		3		10		30	
	M	F	M	F	M	F	M	F
Stomach -bacterial growth	0	0	0	0	0	0	2	11
Harderian gland -hypersecretion	2	1	1	2	14	9	18	20
Ovary -cyst	0	1	0	2	0	2	0	3
Uterus -dilation	0	1	0	1	0	5	0	4
Prostate -inflammation	2	0	0	0	2	0	4	0
Mammary gland -adenocarcinoma	0	1	0	0	0	1	0	0
Pituitary gland -cholesterol cleft -adenoma	2 0	0 0	0 0	0 0	2 0	0 1	4 0	0 0
Brain -degen. of optic nerve -astrocytoma	0 1	2 0	0 0	1 0	2 0	1 0	0 0	1 0

10. Plasma Drug Levels: Plasma AUC values for darifenacin and UK-148,993 in male treatment groups were approximately proportional to dose. For female treatment groups, the AUC values for darifenacin and UK-148,993 at 30 mg/kg/day were 20-24 and 6.7-8.2 times values observed at 3 and 10 mg/kg/day, respectively. AUC values for male treatment groups relative to corresponding female groups varied with dose.

Plasma AUC values for darifenacin and UK-148,993 in male and female rats that received darifenacin by oral gavage at dose levels of 3, 10, or 30 mg/kg/day for 6 months.

AUC _{1-5h} µg.hr/mL	3 mg/kg/day		10 mg/kg/day		30 mg/kg/day	
	M	F	M	F	M	F
Darifenacin	0.112	0.082	0.326	0.246	0.958	1.65
UK-148,993	0.04	0.044	0.216	0.13	0.786	1.07

Rats were treated with darifenacin by oral gavage at doses of 0, 3, 10, or 30 mg/kg/day for 6 months. The no effect dose was 10 mg/kg/day. Mortality occurred at 30 mg/kg/day for 1 male and 4 females; however, the cause of death was unknown. The target organs of toxicity were the stomach and the Harderian glands. Bacterial overgrowth in the non-glandular stomach was observed in 2 males and 11 females from the 30 mg/kg/day group. An increased incidence of Harderian gland hypersecretion was found in the 10 and 30 mg/kg/day groups.

Dogs

Six Month Oral Toxicity in Beagle Dogs (Study No. 92029; Volume 8, Page 8 1715).

Testing Laboratory: Pfizer
Centre de Recherche
37401 Amboise Cedex
France

Study Started: April 15, 1992

Study Completed: August 3, 1993

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Male and female beagle dogs, 8 to 11 months old, were used in this study. Mean body weights for male and female dogs were 9.9 and 7.8 kg, respectively.

Drug Batch: Darifenacin-04 R7.

Methods: Beagle dogs received darifenacin by the oral route of administration in gelatin capsules at doses of 0, 1, 3, or 10 mg/kg/day for 6 months (183 or 184 days). Animals were observed twice daily for general appearance, consistency of stools, behavior, posture, vomiting, estrus in females, and treatment-related effects. Pupil size and pupillary reflex were regularly examined with a diagnostic Starlite lamp. A full clinical examination was performed prior to the start of treatment and at the end of treatment. Rectal temperature was measured prior to the start of treatment and weekly from week 13 to the end of the study. Body weight was measured weekly. Food consumption was estimated daily. An ophthalmic examination was performed prior to the start of treatments and days 57-59 and 181-183. Cardiovascular parameters (i.e., electrocardiogram, systolic blood pressure, and heart rate) were examined twice prior to the start of treatment and during treatment weeks 5, 13, and 25 at both 24 hr after the previous dose and 2 hr after dosing. Electrocardiograms were recorded using six leads, standard bipolar limb leads, I, II, and III, and augmented unipolar limb leads, aVR, aVL, and aVF. Blood was collected for determination of hematological and clinical chemistry parameters at three times prior to treatment and on days 62, 84, 125, and 184. Urine was collected over a 16.5 hr period for urinalysis. Blood for measurement of plasma concentrations of darifenacin and its hydroxylated metabolite, UK-148,993 was collected on days 15 and 177 at 1, 3, 5, 7, 11, and 24 hr after dosing. Quantities of darifenacin and UK-148,993 were measured in separate HPLC assays using ultraviolet detection. Animals were sacrificed 24 hr after the last dose and subjected to a gross examination. Organ weights for the adrenal glands, brain, heart, kidneys, liver, ovaries, spleen, and testes were measured. Sections were collected tissues showing any macroscopic abnormalities plus samples from major organs/tissues (adrenal glands, aorta, brain, cervical lymph node, colon, duodenum, tail of epididymis, eyes, gall bladder, heart, ileum, kidneys, liver, lung, mammary gland/skin, mesenteric lymph node, esophagus, ovaries, pancreas, pituitary, prostate, salivary gland, sciatic nerve, spleen, stomach, sternum with muscle, striated muscle, testes, thymus, thyroid gland, trachea, urinary bladder, and uterus.

Deviations from the protocol included the following: a male in the 1 mg/kg/day group (M13) was not dosed on days 8 and 9 due to surgery for reduction of paraphymosis, and a male in the 10 mg/kg/day group (M31) was not dosed on days 16-19 due to marked body weight loss.

Results:

1. Observed Effects: Observed effects included mydriasis, dryness of the mouth, emesis, regurgitation, and changes to the conjunctiva and cornea. Ocular effects are discussed below. Dryness of the mouth was observed for all animals of the 10 mg/kg/day group from days 14 to 182. The incidence of dryness of mouth in the 1 mg/kg/day group ranged from 0 to 4 animals, and in the 3 mg/kg/day group ranged from 0 to 8 (n = 8 for both groups). During the first 12 days of treatment, an increased incidence of emesis, regurgitation, and reduced food intake were observed in the 3 and 10 mg/kg/day groups. The incidence of emesis during days 1-12 for the 3 mg/kg/day group ranged between 1 and 5 animals, and for the 10 mg/kg/day group ranged between 2 and 7 animals. The incidence of regurgitation during days 1-12 for the 3 mg/kg/day group ranged from 3 to 8 animals, and for the 10 mg/kg/day group ranged from 4 to 7 animals. Emesis, regurgitation, and reduced food intake appear to be related to dysphagia induced by darifenacin. The incidence and frequency of emesis and regurgitation were lowered after day 12 when food was moistened before distribution.

2. Mortality: One male of the 10 mg/kg/day group died on day 83 of the study. A few days before death, the animal was depressed and prostrate with clinical signs of dyspnea, titubation, blood discharges from the anus, hypothermia, and dehydration. Histopathological analysis of the lungs found a necro-hemorrhagic bronchopneumonia with numerous colonies of bacteria. For the intestines, predominantly the caecum, there was an acute superficial necrotizing enteritis accompanied by lymphoid atrophy. Death appeared to be related to an inhalation pneumonia, a severe enteritis, and a prerenal uremic syndrome. The sponsor considered the death to be unrelated to treatment; however, the death may have been caused by treatment-related difficulties in swallowing food (i.e., dysphagia) and subsequent aspiration of food into the lungs. Kidney damage was indicated by elevated plasma levels of urea (309 mg/dL), creatinine (2.4 mg/dL), and phosphate (139 mg/L) and decreased levels of Na⁺ (124 mmol/L) and Cl⁻ (75 mmol/L). Historical control values were as follows: urea, 33.7 mg/dL; creatinine, 0.75 mg/dL; phosphate, 52.8 mg/L; Na⁺, 147.2 mmol/L; and Cl⁻, 110.8 mmol/L.

3. Body Weight and Food Consumption: Body weight gain for the 3 and 10 mg/kg/day groups was impaired by >10%. The sponsor attributed impaired body weight gain to difficulties swallowing food (i.e., dysphagia) from days 1-12; however, body weight gain did not recover for these two groups by the end of the 6 month treatment period. Food intake during the first 12 days was reduced for 1-2 animals of the 3 mg/kg/day group and 3-5 animals of the 10 mg/kg/day group. Mean body weights for the male controls on days 1 and 182 were 10.09 and 11.86 kg, respectively. Body weight gains for the male 1, 3, and 10 mg/kg/day groups were 89.8, 43.5,

and 63.0% of the control, respectively. Mean body weights for the female controls on days 1 and 182 were 7.66 and 8.75 kg, respectively. Body weight gains for the female 1, 3, and 10 mg/kg/day groups were 93.1, 48.7, and 38.0% of the control, respectively.

4. Hematology: A number of small changes (<10%) were noted for hematological parameters, principally in the 10 mg/kg/day group; however, changes were sporadic and not evident at the end of treatment.

5. Blood Chemistry and Urinalysis: A number of small changes (<10%) were noted in blood chemistry parameters, principally for the 10 mg/kg/day group; however, changes were sporadic and not evident at the end of treatment. Phosphate levels for the female 10 mg/kg/day group from days 62-184 were increased to 115-143% of the control (39.0-44.5 mg/L); however, the change was only significant at 184 days. No treatment-related changes were found with urinalysis.

6. Vital Signs, Physical Examination, and Ophthalmic Examination:

Ophthalmic Examination: Mydriasis was observed in all animals of the 10 mg/kg/day group beginning on day 1 from 0.5-5 hr after dosing. Beyond day 2, this sign persisted for 24 hr. Mydriasis was observed occasionally in the 3 mg/kg/day group for 1 of 8 animals and occurred from 1-5 hr after dosing. The pupillary reflex was diminished in 4 of 8 animals from the 3 mg/kg/day group and abolished in all animals of the 10 mg/kg/day groups. Conjunctival redness was observed for 1 of 8 animals in the control group at the end of study, 4 of 8 animals in both the 1 and 3 mg/kg/day groups after 3 months and persisted to the end of treatment, and 7 of 8 animals in the 10 mg/kg/day group after 3-56 days and persisted to the end of treatment. Redness was accompanied by a muco-purulent discharge for 1 male and 4 females of the 10 mg/kg/day group. At the end of treatment, corneal edema was observed in the 3 mg/kg/day group for 1 of 4 females, and in the 10 mg/kg/day for 1 of 4 males and 4 of 4 females. Keratitis (i.e., vascular infiltration in the cornea) was observed for 3 of 4 females in the 10 mg/kg/day group. Photophobia (i.e., half-closed eyes and resistance to ophthalmic examination) was observed for 2 of 4 females in the 10 mg/kg/day group.

Cardiovascular Examination: There were no treatment-related effects on heart rate, systolic blood pressure, and electrocardiographic tracings. For the control group, 1 male was observed with ventricular premature complexes during weeks 5 and 25, and one female was observed with marked sinus arrhythmia and sinus pause at weeks -1 and 13. For the 1 mg/kg/day group, 1 male was observed with marked sinus arrhythmia and sinus pause during week 13, and 1 female was observed with second degree atrioventricular block prior to dosing during both weeks 5 and 13.

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Page 83

This female of the 1 mg/kg/day group was found to also have marked sinus arrhythmia and sinus pause before dosing during week 5.

Rectal Temperature: There were no treatment-related effects on rectal temperature.

7. Organ Weight: Changes in liver and spleen weights were observed for the 10 mg/kg/day group.

Liver: Absolute liver weight for the male and female 10 mg/kg/day groups were increased to 127.4 and 117.2% of the control (287.7 and 247.2 g), respectively. Relative liver weight for the male and female 10 mg/kg/day groups were increased to 141.9 and 125.3% of the control (2.498 and 2.889%), respectively.

Spleen: Absolute and relative spleen weight for the female 10 mg/kg/day group was decreased to 64.4 and 68.3% of the control (74.2 g and 0.868%).

Heart: Absolute heart weight for the female 10 mg/kg/day group was decreased to 84.6% of the control (76.4 g); however, no change was noted for the relative weight, suggesting the decreased absolute weight was related to impaired body weight gain.

8. Gross Pathology: No treatment-related changes were reported.

9. Histopathology: Treatment-related changes were observed for the eye. The conjunctivae was observed with hyperplasia of the epithelium and infiltration of lymphocytes and plasma cells into the subepithelium. Corneal changes consisted of hyperplastic epithelium with infiltration of lymphocytes and the presence of small neovessels in the stroma. These effects appeared to be due to an inhibition of lacrimal gland secretion by this anticholinergic agent. Esophageal inflammation may be related to difficulties in swallowing as well as emesis and regurgitation. For the 50 mg/kg/day group, the following histopathological changes were observed with an incidence of one: spleen atrophy, pituitary inflammation, and colon atrophy. Enteritis was found in the ileum and caecum for the male of the 10 mg/kg/day group that died.

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Histopathological changes for dogs treated with darifenacin at doses of 0, 1, 3, and 10 mg/kg/day for 6 months. Changes denoted with an asterisk are for a male of the 10 mg/kg/day group that died on day 83.

Organ/Tissue	0		1		3		10	
	M	F	M	F	M	F	M	F
Eye -keratoconjunctivitis -conjunctivitis					1		1	3 1
Skin -fibrosis, focal							1	1
Esophagus -inflammation, chronic, focal	0	0	1	1	1	1	0	2
Lung -bronchopneumonia	0	0	0	0	0	1	1*	0
Heart inflammation, chronic, focal	0	1	3	2	2	1	2	1
-myocarditis	0	0	0	0	0	0	1	0
-myocardial fibrosis	0	0	0	0	0	0	0	1
-epicarditis, focal	0	0	0	0	0	2	0	1
-endocardiosis	0	0	1	1	0	1	0	0

10. Plasma Drug Levels: Plasma concentrations of darifenacin and its hydroxylated metabolite, UK-148,993, were determined on days 15 and 177. On days 15 and 177, plasma AUC and C_{max} values for darifenacin were proportional to dose. Plasma AUC and C_{max} values for darifenacin on day 177 were greater than those observed on day 15. On days 15 and 177, plasma AUC and C_{max} values for UK-148,993 were proportional to dose. Plasma AUC and C_{max} values for UK-148,993 on day 177 were slightly greater than those observed on day 15. There were no significant differences in plasma AUC and C_{max} values between male and female dogs for either darifenacin or UK-148,993 on days 15 or 177.

Plasma T_{max} , C_{max} , and AUC values for darifenacin on day 15 for dogs receiving darifenacin at doses of 1, 3, or 10 mg/kg/day.

Dose mg/kg/day	T_{max} , hr		C_{max} , $\mu\text{g/mL}$		AUC _{0-11 hr} , $\mu\text{g hr/mL}$	
	Male	Female	Male	Female	Male	Female
1	3	4.5	0.04	0.08	0.21	0.31
3	4	5	0.20	0.17	1.31	1.13
10	4	7	0.73	0.60	5.97	4.51

Plasma T_{max} , C_{max} , and AUC values for darifenacin on day 177 for dogs receiving darifenacin at doses of 1, 3, or 10 mg/kg/day.

Dose mg/kg/day	T_{max} , hr		C_{max} , $\mu\text{g/mL}$		$AUC_{0-11\text{hr}}$, $\mu\text{g}\cdot\text{hr/mL}$	
	Male	Female	Male	Female	Male	Female
1	3	1.5	0.15	0.10	0.83	0.47
3	2	1	0.29	0.55	2.01	2.28
10	3	3	0.88	1.19	7.22	9.24

Plasma T_{max} , C_{max} , and AUC values for UK-148,993 on day 15 for dogs receiving darifenacin at doses of 1, 3, or 10 mg/kg/day.

Dose mg/kg/day	T_{max} , hr		C_{max} , $\mu\text{g/mL}$		$AUC_{0-11\text{hr}}$, $\mu\text{g}\cdot\text{hr/mL}$
	Male	Female	Male	Female	Male + Female
1	7	7	0.05	0.05	0.27
3	3	4.5	0.24	0.19	1.55
10	6.5	6.5	0.62	0.51	4.72

Plasma T_{max} , C_{max} , and AUC values for UK-148,993 on day 177 for dogs receiving darifenacin at doses of 1, 3, or 10 mg/kg/day.

Dose mg/kg/day	T_{max} , hr		C_{max} , $\mu\text{g/mL}$		$AUC_{0-11\text{hr}}$, $\mu\text{g}\cdot\text{hr/mL}$	
	Male	Female	Male	Female	Male	Female
1	2	2	0.11	0.09	0.84	0.58
3	3.5	2	0.22	0.33	1.84	2.24
10	3.67	4	0.65	0.76	5.72	6.96

Beagle dogs received darifenacin by the oral route of administration in gelatin capsules at doses of 0, 1, 3, and 10 mg/kg/day for 6 months. The no effect dose was 3 mg/kg/day. One male of the 10 mg/kg/day group was found dead on day 83. Death appeared to be related to an inhalation pneumonia, a severe enteritis, and a prerenal uremic syndrome. The sponsor considered the death to be unrelated to treatment; however, the death may have been caused by treatment-related difficulties in swallowing food (i.e., dysphagia) and subsequent aspiration of food into the lungs. The target organ of toxicity was the eyes. The conjunctivae was observed with hyperplasia of the epithelium and infiltration of lymphocytes and plasma cells into the subepithelium. Corneal changes consisted of hyperplastic epithelium with infiltration of lymphocytes and the presence of small neovessels in the stroma. These effects might be due to an inhibition of lacrimal gland secretion by this anticholinergic agent.

Reproductive Toxicology:

Rats

Fertility (Segment I) Study in Rats by the Oral Route (Study Nos. 92101 and 93028; Volume 12, Page 8 3415).

Testing Laboratory: Pfizer
Centre de Recherche
37401 Amboise Cedex
France

Study Started: November 16, 1992

Study Completed: August 8, 1994

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Sprague-Dawley rats [CD(SD)BR] were used in this study. On the first day of treatment, mean ages were 56 days for males and 63 days for females. Mean body weights were 291.00 g for males and 231.18 g for females.

Drug Batch: Darifenacin-04.

Methods: This Segment I study examined the fertility of male and female adult rats treated with darifenacin as well as the viability, development, and behavior of their offspring. The fertility of the F₁ offspring was also assessed. In a preliminary Segment I study (Study No. 92057), F₀ male and female rats were treated with darifenacin by oral gavage at doses of 0, 3, 10, and 50 mg/kg/day for 2 weeks prior to mating, through the mating and gestation periods, and up to day 4 post parturition. Mating and fertility were unaffected by treatment with darifenacin at doses of 10 and 30 mg/kg/day. For the 50 mg/kg/day group, 2 females died! The mating index and pregnancy rate for the 50 mg/kg/day group were slightly reduced as compared to controls; these effects may have been related to the anticholinergic properties of the drug. The dead in-utero rate for treatment groups was increased as compared to controls. The mean litter size for the 50 mg/kg/day group was reduced at 24 hr and 4 days to 85.5 and 65.8% of the control (15.9 and 15.8 pups per dam), respectively. Pup survival rates for the 50 mg/kg/day group at 24 hr and 4 days were significantly reduced as compared to controls. In the present study, darifenacin was administered by oral gavage at doses of 0, 3, 10, and 50 mg/kg/day. There were 28 rats/sex/group. The vehicle was 0.5% aqueous methylcellulose, 4000 cps, containing 0.1% Tween 80. Males received darifenacin for 64 days prior to mating, during the mating period, and until the end of the littering period for a total of 96 days. Females received darifenacin for 14 days prior to mating,

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throughout the mating period, and during gestation. One-half of the females were sacrificed on day 20 of gestation to assess the effects of darifenacin with regard to fertility, embryotoxicity, and teratogenicity. Remaining females were treated with darifenacin during the lactation period (days 1 to 20 post-partum). The duration of treatment for females sacrificed on day 20 of gestation was 36 days and for females allowed to litter was 58 days. Animals of the F_0 generation were examined for clinical signs of toxicity twice a day during the treatment period. Body weights of F_0 males were measured at weekly intervals. Body weights of F_0 females were measured at weekly intervals prior to mating and on days 1, 3, 6, 9, 12, 16, 18, and 20 of gestation. Following parturition, body weight was measured on days 1, 7, 14, and 21 post-partum. Males (F_0) were mated with females (F_0) of the same dose group at a ratio of 1 to 1 over a period of 6 days. Vaginal smears were taken each morning to check for the presence of spermatozoa. The day of a positive finding was designated day 0 of gestation and the female was housed singly. For females sacrificed on day 20 of gestation, the uteri and ovaries were removed and the number of corpora lutea and the number, type, and position of implantation sites were recorded. Fetuses were examined for external and buccal malformations, weighed, and sex was determined. Alternate fetuses were prepared for a skeletal examination and examined for the degree of ossification and presence of anomalies. Sternebral ossification and the number of ribs were determined. Remaining fetuses were examined for visceral abnormalities and anatomical variations. For females allowed to litter, the length of gestation was determined. A qualitative estimation of lactation was performed by examining the teats, the stomachs of neonates, and growth of offspring. The number of F_1 pups (alive and dead) was recorded, any external abnormalities were noted, and viable pups were counted and weighed on days 1, 4, and 21. Post natal development was determined with the following tests: surface righting reflex (day 6), grasping reflex (day 6), appearance of incisors (day 11), and opening of palpebral fissures (day 15). At weaning, 5 pups/sex/group were necropsied. After weaning, ophthalmic and auditory examinations were performed on 1 male and 1 female F_1 pup/litter. Spontaneous activity of all pups was assessed using an open field test on days 66-69. At 3 months of age, 1 male and 1 female F_1 pup/litter were allowed to mate to produce the F_2 generation; care was taken so that male and female rats from the same litter were not placed together. F_1 males and females were weighed at weekly intervals. F_1 females were also weighed on days 1, 3, 6, 9, 12, 15, 18, and 20 of gestation and days 1 and 4 post-partum. The number of F_2 pups were counted, any external abnormalities were noted, and weighed and sex checked on days 1 and 4 post-partum.

Results:

1. **Observed Effects:** Salivation was observed for male and female rats in the 10 and 50 mg/kg/day groups. Chromodacryorrhea was observed for 2 males in the 10 mg/kg/day group and 10 males and 15 females in the 50 mg/kg/day. The incidence of this clinical sign was highest shortly after the initiation of treatment.

2. **Mortality:** Mortality occurred for 4 animals in the 50 mg/kg/day group. Two males, M326 and M305, died on days 45 and 65 of treatment; however, there were no findings at necropsy. One female, F813, died on day 12 of treatment (study day 61) with no findings at necropsy. Another female, F820, died on day 17 post partum with necropsy findings of the stomach and intestines inflated with air.

3. **Body Weight:** Body weight gain for the male 50 mg/kg/day group after 92 days of treatment was decreased to 76.6% of the control. Body weight gain for the female 50 mg/kg/day group from days 1-15 of treatment prior to mating was decreased to 56% of the control. Body weight gain for the female 50 mg/kg/day group from days 1-20 of gestation was decreased to 84.1% of the control. During the day 1-21 post partum, body weight gain for the female 50 mg/kg/day group was increased to 128.4% of the control; however, for the 3 and 10 mg/kg/day groups, weight gains were decreased to 39.6 and 22.0% of the control, respectively.

4. **Reproductive Parameters for the F₀ Generation:** The mating and fertility indexes were not unaffected by darifenacin treatment. For females sacrificed on day 20 of gestation, the number of corpora lutea/dam, implantation sites/dam, viable fetuses/dam, and the implantation rate were not unaffected by darifenacin treatment. The sex ratio for fetuses and fetal body weight were not different between control and treatment groups. Embryomortality for the 50 mg/kg/day group was slightly increased to 11.4% as compared to 7.5% for the control group. Gross examination found 1 female dam of the 3 mg/kg/day group with pyometer. One female from each of the 10 and 50 mg/kg/day groups were observed with hydrometer of both uterine horns. For females allowed to deliver their offspring, gestational length and labor and delivery were similar between control and treatment groups. The post-implantation loss was increased to 10% for the 50 mg/kg/day group as compared to a control value of 4.3%. The in utero death rate for the 50 mg/kg/day group was increased to 4.8% as compared to 0.57% for controls. The number of viable pups/dam was reduced to 12.0 for the 50 mg/kg/day group as compared to 15.9 for controls. No macroscopic abnormalities were found in dams sacrificed after weaning of litters. T.M.H.

5. Development of the F₁ Generation: No major external malformations were observed in the F₁ generation. For fetuses obtained on day 20 of gestation, ossification of the sacral and caudal vertebrae was slightly delayed for 46.15% of fetuses in the 50 mg/kg/day group as compared to 29.3% for controls. Historical control values for slight delay in ossification of the sacral and caudal vertebrae range from 0-24.6%. For visceral anomalies, urinary bladder dilatation was found for 58.2% of fetuses in the 50 mg/kg/day group (39 fetuses in 8 litters). A historical control value was not provided for this change. The incidence of dilatation of the ureter was increased to 4.2 and 6.0% in the 10 and 50 mg/kg/day groups as compared to 2.1% for controls; although, historical value was 17.3%. For dams allowed to deliver, offspring body weight and survival for the 50 mg/kg/day group on days 1, 4, and 21 were reduced as compared with control. With regard to development, the grasping reflex for F₁ pups of the 50 mg/kg/day group was reduced to 60.9% as compared to a control value of 82.1%. No treatment-related differences were found for righting reflex, appearance of incisors, or opening of palpebral fissures. Ophthalmic and auditory examinations found no treatment-related differences.

6. Reproductive Parameters of the F₁ Generation: The mating index for F₁ male and female rats of the 10 and 50 mg/kg/day groups were reduced to 72.7 and 77.8% as compared to 100% for controls. However the fertility index and gestational length were not different between control and treatment groups. The number of implantation sites/dam was decreased to 15.1 for the 50 mg/kg/day group as compared to 16.8 for controls. The number of viable pups/dam was slightly reduced to 14.3 as compared to 16.1 for controls. Body weight gains over an 8 week period for F₁ males and a 4 week period for F₁ females were unaffected by treatment of the F₀ generation with darifenacin. Body weight gains for F₁ females during days 1 to 20 of gestation and days 1 to 4 post-partum were unaffected.

7. Body Weight and Survival of the F₂ Generation: Body weight and survival for F₂ pups on days 1 and 4 post-partum were not different between control and treatment groups (F₀ generation treated with darifenacin).

Mating and Fertility Indexes for F₀ females sacrificed on day of gestation or allowed to litter.

Dose, mg/kg/day	0	3	10	50
Mating Index	24/28 (85.7)	26/28 (92.9)	28/28 (100.0)	24/27 (88.9)
Fertility Index	23/24 (95.8)	25/26 (96.2)	22/28 (78.6)	20/24 (83.3)

Numbers in parentheses represent the percentage.

Reproductive parameters for F₀ females sacrificed on day 20 of gestation.

Dose, mg/kg/day	0	3	10	50
Pregnancy rate (%)	12/12 (100)	12/13 (92)	11/14 (79)	10/12 (83)
Viable litters (%)	12/12 (100)	12/12 (100)	10/11 (91)	10/10 (100)
Corpora lutea/dam	17.8	17.8	16.0	15.8
Implantation sites/dam	16.7	16.7	14.3	14.9
Viable fetuses/dam	15.4 (185/12)	16.0 (192/12)	13.9 (139/10)	13.2 (132/10)
Implantation rate	200/213 (93.9)	200/214 (93.5)	143/160 (89.4)	149/158 (94.3)
Embryomortality	15/200 (7.5)	8/200 (4.0)	4/143 (2.8)	17/149 (11.4)
Sex Ratio (M/F)	94/91 (103)	97/95 (102)	60/79 (76)	59/73 (81)
Fetal weight				
-males	3.68	3.68	3.56	3.52
-females	3.53	3.56	3.41	3.35

Numbers in parentheses represent the percentage.

Degree of Bone Ossification for Fetuses derived from F₀ Females Sacrificed on Day 20 of Gestation.

Dose, mg/kg/day	0	3	10	50	Hist. Control
No. of fetuses examined	92	99	68	65	
Sternebrae					
-total	1456	1599	1078	1021	
-mean	15.83	16.15	15.85	15.71	
Sacral and caudal vertebrae					
-normal	65	89	55	34	
-slight delay	27 (29.3)	10 (10.1)	13 (19.1)	30 (46.15)	(0-24.6%)
-marked delay	0	0	0	1	
Skull					
-normal	90	93			
-slight delay	1 (1.1)	4 (4.04)	0	3 (4.6)	(6.7%)
-marked delay	1	2	3	1	
Pubic, Right					
-normal	90	97			
-rudimentary	2 (2.17)	2 (2.02)	4 (5.88)	3 (4.6)	(0-4.8)
-absent	0	0	0	0	
Pubic, Left					
-normal	89	97			
-rudimentary	3 (3.26)	2 (2.02)	3 (4.5)	4 (6.15)	(0.8-2.9)
-absent	0	0	1	0	

Numbers in parentheses represent the percentage.

Visceral Anomalies and Anatomical Variants for Fetuses derived from F₀ Females Sacrificed on Day 20 of Gestation.

Dose (mg/kg/day)	0	3	10	50	Historical control
-number of fetuses	93	93	93	87	6053
-number of litters	12	12	10	10	
Dilatation of ureter(s)					
-number of fetuses	2 (2.1)	2 (2.1)	3 (4.2)	4 (6.0)	1047 (17.3)
-number of litters	2	2	3	2	
Dilatation of renal pelvis					
-number of fetuses				1 (1.5)	1047 (17.3)
-number of litters				1	
Hydroureter					
-number of fetuses				1 (1.5)	101 (1.7)
-number of litters				1	
Hydroureter(s) and renal pelvis					
-number of fetuses	1 (1.1)				
-number of litters	1				
Dilatation of urinary bladder					
-number of fetuses				39 (58)	
-number of litters				8	

Numbers in parentheses represent the percentage.

Reproductive Parameters for F₀ Females Allowed to Litter.

Dose, mg/kg/day	0	3	10	50
Pregnancy rate (%)	11/12 (91.6)	13/13 (100)	11/14 (78.6)	10/12 (83.3)
Gestational length (days)	21.1	21.5	21.1	21.5
Implantation sites/dam (day 21 post-partum)	16.7	17.5	16.5	14.0
Post-implantation loss	8/184 (4.3)	9/228 (3.9)	4/181 (2.2)	14/140 (10)*
Viable pups/dam at birth	15.9 =175/11	16.6 =216/13	16.0 =176/11	12.0* =120/10
In utero death (%)	0.57% =1/176	1.4% =3/219	0.56% =1/177	4.8%* =6/126

Numbers in parentheses represent the percentage.

* p < 0.05

Body Weight and Survival Indices of F₁ Pups on days 1, 4, and 21.

Dose, mg/kg/day	0	3	10	50
Day 1 -mean body weight (M/F) -survival indices	6.23/5.93 100% (175/175)	6.36/5.99 100% (216/216)	6.08/5.83 100% (176/176)	5.72/5.38 98.3%* (118/120)
Day 4 -mean body weight (M/F) -survival indices	8.86/8.50 98.9% (173/175)	8.89/8.40 99.1% (214/216)	8.41/8.20 99.4% (175/176)	8.00/7.51 91.7%* (110/120)
Day 21 -mean body weight (M/F) -survival indices	36.34/35.20 95.4% (167/175)	36.05/33.98 94.9% (205/216)	32.77/33.68 96.0% (169/176)	36.97/35.59 88.6%* (93/95 ^A)

A. Dam F820 died on day 17 post-partum and 10 pups were excluded from the calculation.

Reproductive Parameters for F₁ Females Allowed to Litter.

Dose, mg/kg/day	0	3	10	50
Mating Index	11/11 (100)	12/13 (92.3)	8/11 (72.7)	7/9 (77.8)
Fertility Index	11/11 (100)	12/12 (100)	6/8 (75)	7/7 (100)
Gestational length (days)	21.4	21.5	21.5	21.6
Implantation sites/dam	16.8	17.1	16.2	15.1*
Post-implantation loss	3.8% (7/185)	4.4% (9/205)	3.1% (3/97)	5.7% (6/106)
Viable pups/dam	16.1 (177/11)	16.3 (195/12)	15.5 (93/6)	14.3 (100/7)

Body Weight and Survival Indices of F₁ Pups on days 1 and 4.

Dose, mg/kg/day	0	3	10	50
Day 1 -mean body weight (M/F) -survival indices	6.60/6.18 100 (177/177)	6.35/5.90 100 (195/195)	6.51/6.14 100 (93/93)	6.68/6.30 100 (100/100)
Day 4 -mean body weight (M/F) -survival indices	9.12/8.57 88.7 (157/177)	7.98/7.29 76.9 (150/195)	8.72/8.47 83.9 (78/93)	8.81/8.84 87 (87/100)

In a Segment I study, the effects of darifenacin, administered to F₀ male and female rats at doses of 0, 3, 10, and 50 mg/kg/day, were examined on mating and fertility as well as the viability, development, and behavior of their offspring. The fertility of the F₁ offspring was also assessed. Mortality occurred for 4 animals in the 50 mg/kg/day group. ~~Body weight gains for male and female rats receiving darifenacin at 50 mg/kg/day were impaired.~~ The mating index for the F₀ generation was not unaffected by darifenacin treatment; however, the fertility index for the 10 and 50 mg/kg/day groups were reduced to 78.6 and 83.3%, respectively, as compared to 95.8% for the control. ~~Maternal toxicity was evident at a dose of 50 mg/kg/day.~~ The number of viable pups/dam was reduced to 12.0 for the 50 mg/kg/day group as compared to 15.9 for controls. For visceral anomalies, ~~urinary bladder dilatation,~~ was found for 58.2% of fetuses in the 50 mg/kg/day group. For dams allowed to deliver, ~~offspring body weight and survival for the 50 mg/kg/day group on days 1, 4, and 21 were reduced as compared with control.~~

Teratology (Segment II) Study in Sprague-Dawley Rats by the Oral Route (Study Nos. 91028/29; Volume 9, Page 8 2122).

Testing Laboratory: Pfizer
Centre de Recherche
37401 Amboise Cedex
France

Study Started: June 25, 1991

Study Completed: April 9, 1992

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Pregnant, female Sprague Dawley rats CD(SD)BR], with an age of 69-72 days, were used in this study. On day 1 of gestation, mean body weights for the main study and toxicokinetics groups were 243 and 245 g, respectively. Each female rat was placed with one male rat overnight, and the following day, vaginal smears were collected to determine the presence of spermatozoa. The presence of spermatozoa was designated as day 0.

Drug Batch: Darifenacin-04 Batch number R4.

Methods: Pregnant, female rats received darifenacin by oral gavage at doses of 0, 3, 10, and 50 mg/kg/day from days 6 to 17 of gestation (12 consecutive days). In preliminary Segment II study (Protocol No. 90153), pregnant, female rats received darifenacin by oral gavage at doses of 0, 10, 30, and 50 mg/kg/day from days 6 to 15 of gestation. Chromodacryorrhea and piloerection were evident

in females treated with 30 and 50 mg/kg/day. Body weight gain was impaired for the 50 mg/kg/day group was impaired during the treatment period. Mean number of corpora lutea, implantation sites, and viable fetuses were not significantly different between control and treatment groups. Female fetal body weight was significantly reduced. In the present study, there were 20 pregnant, female rats per group. A group of 5 pregnant, female rats was designated for toxicokinetic analysis of darifenacin levels and received a dose of 50 mg/kg/day from days 6 to 17 of gestation. The vehicle was 0.5% methylcellulose containing 0.1% Tween 80. The dosing volume was 10 mL/kg. Animals were examined for clinical signs of toxicity twice daily during the treatment period and once daily outside the treatment period. Body weights were recorded on days 1, 3, 6, 9, 12, 15, 18, and 20 of gestation. On day 20, animals were sacrificed. The ovaries were removed and the number of corpora lutea, and the number, type, and position of implantation sites were determined. All fetuses were examined for external and buccal malformations and weighed. Fetuses were numbered and sexed prior to sacrifice. Skeletal examinations were performed on alternate fetuses to determine the degree of ossification and the presence of anomalies. Remaining fetuses were examined for visceral abnormalities or anatomical variations. Blood was collected from the toxicokinetic group on day 17 at 1, 3, and 6 hr after dosing. Females were sacrificed after the 6 hr blood collection, and the amniotic fluid and fetuses were collected. Darifenacin concentrations were determined in maternal plasma, amniotic fluid, and fetal tissue using an HPLC assay with ultraviolet detection.

Results: One female of the 50 mg/kg/day group was observed with chromodacryorrhea. Body weight gain for the 50 mg/kg/day group was reduced to 60.3% of the control (106.14 g) during the treatment period. Three females were not pregnant, 2 at 30 mg/kg/day and 1 at 50 mg/kg/day. For the 10 mg/kg/day group, 1 female was pregnant with only late resorptions and uterine horns were found to be hemorrhagic. Mean number of corpora lutea, implantation sites, and viable fetuses were not significantly different between control and treatment groups. Fetal body weights were not significantly different between control and treatment groups. There were no treatment-related external, skeletal, or visceral abnormalities. For the 10 mg/kg/day group, the number of fetuses with more than 13 ribs was increased as compared with controls. However, no change in rib number was evident between the control and the 3 and 50 mg/kg/day treatment groups suggesting the change at 10 mg/kg/day was unrelated to treatment. For the 50 mg/kg/day group, the incidence of a slight delay in ossification of the sacral and caudal vertebrae was increased as compared to controls. Darifenacin concentrations in maternal plasma and fetal tissue at 6 hr after dosing were similar; however, levels in amniotic fluid were significantly lower. Darifenacin has a low affinity for hydrophilic compartments as shown by low levels in amniotic fluid.

Reproductive variables and fetal development for pregnant, female rats that received darifenacin by oral gavage at doses of 0, 3, 10, and 50 mg/kg/day from days 6 to 17 of gestation (Sponsor's table on page 8-2136-2125).

Reproductive variables for sacrificed females	0	3	10	50
Pregnancy rate (%)	20/20 (100)	18/20 (90)	20/20 (100)	19/20 (95)
Viable litters, day 20	20/20 (100)	18/20 (90)	20/20 (100)	19/20 (95)
Corpora lutea/dam	17.5	17.0	17.8	17.3
Implantation sites/dam	15.8	15.9	16.2	16.2
No. fetuses/dam	14.6	14.9	14.9	15.3
Implantation rate (%)	316/351 (90.0)	286/306 (93.5)	308/338 (91.1)	307/329 (93.3)
Embryomortality rate (%)	25/316 (7.9)	18/286 (6.3)	24/308 (7.8)	17/307 (5.5)
Fetal Development				
Sex ratio (M/F)	140/151 (93)	140/128 (109)	143/141 (101)	149/141 (106)
Male fetal body weight	3.86	3.84	3.96	3.67
Female fetal body weight	3.65	3.65	3.65	3.51

External Examination of Fetuses: Major and minor anomalies (Sponsor's table on Page 8-2184-2172). Incidence designated as number of fetuses/number of litters.

Dose (mg/kg/day)	0	3	10	50
Hematoma on left ear	1/1			
Pale fetus		1/1		
Agnathia, microglossia, microtomia		1/1		
Hematoma on interior jaw				1/1
Hematoma on left part of head				1/1

Skeletal Examination of Fetuses: Variations in Rib Number
(Sponsor's Table 5 on Page 8-2140-2129).

Dose (mg/kg/day)	0	3	10	50
No. of fetuses examined	146	134	143	145
13 rib pairs	134 (91.8)	120 (89.6)	116 (81.1)	134 (92.4)
14 rib pairs	1 (0.7)	0	0	0
14 th pair rudimentary	9 (6.2)	7 (5.2)	16 (11.2)	8 (5.5)
14 th right rib only	1 (0.7)	1 (0.8)	3 (2.1)	0
14 th left rib only	1 (0.7)	6 (4.5)	8 (5.6)	3 (2.1)

Numbers in parentheses represent the percentage.

Skeletal Examination of Fetuses: Degree of Bone Ossification
(Sponsor's Table 6 on Page 8-2141-2130).

Dose (mg/kg/day)	0	3	10	50
No. of fetuses examined	146	134	143	145
Sternebrae				
-Total	2129	1909	2121	2107
-Mean	14.58	14.25	14.83	14.53
Sacral and caudal vertebrae				
-normal	119	98	118	103
-slight delay	26	36	23	42
-marked delay	1	0	2	0

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Visceral Examination of Fetuses: Visceral Anomalies and Anatomical Variants (Sponsor's Table 6 on Page 8-2141-2130). The incidence is designed as follows: # fetus(es) (Percentage)/# litters.

Dose (mg/kg/day)	0	3	10	50
No. of fetuses examined	145	134	14	145
No. of litters	20	18	19	19
Interventricular septal defect			1 (0.7%) (1 lit.)	
Dilatation of cerebral ventricles		1 (0.7%) (1 lit.)		
Dilatation of ureter(s)	34 (23.4%) (14 lit.)	19 (14.2%) (11 lit.)	18 (12.8%) (9 lit.)	13 (9.0%) (8 lit.)
Dilatation of ureter(s) and renal pelvis(es)	4 (2.8%) (3 lit.)	1 (0.7%) (1 lit.)	1 (0.7%) (1 lit.)	2 (1.4%) (2 lit.)
Hydroureter(s) and dilatation of renal pelvis	3 (2.1%) (2 lit.)	1 (0.7%) (1 lit.)	1 (0.7%) (1 lit.)	
Hydroureter and hydronephrosis	1 (0.7%) (1 lit.)			
Bilateral ectopic testes			1/74 males (1.4%) (1 lit.)	

Darifenacin Concentrations in Maternal Plasma, Amniotic fluid, and Fetal Homogenates (Sponsor's Table 11 on Page 8-2146-2135). Pregnant, female rats received darifenacin at an oral dose of 50 mg/kg/day from days 6 to 17 of gestation. Blood was collected on day 17 at 1, 3, and 6 hr after dosing.

C _{max} (µg/mL)	T _{max} (hr)	Maternal plasma at 6 hr (µg/mL)	Amniotic fluid at 6 hr (µg/mL)	Fetal tissue (µg/g)
0.64	1.4	0.35	0.09	0.35

Pregnant, female rats received darifenacin by oral gavage at doses of 0, 3, 10, and 50 mg/kg/day from days 6 to 17 of gestation. Darifenacin was not teratogenic or embryotoxic. There were no treatment-related external, skeletal, or visceral abnormalities. Darifenacin concentrations in maternal plasma and fetal tissue at 6 hr after dosing were similar; however, levels in amniotic fluid were significantly lower.

Peri and Postnatal Development Study (Segment III) in Sprague Dawley Rats by the Oral Route (Study No. 93076; Volume 11, Page 8 2938).

Testing Laboratory: Pfizer
Centre de Recherche
37401 Amboise Cedex
France

Study Started: August 17, 1993

Study Completed: November 26, 1994

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Female rats [CD(SD)BR] were used in this study. Each female was caged with 1 stock male rat. Vaginal smears were taken to check for the presence of spermatozoa (designated day 0 post-insemination). On day 1 post-insemination, rats were 9-14 weeks of age, with a mean body weight of 270 g.

Drug Batch: Darifenacin-18 Lot number R9.

Methods: This Segment III study was designed to examine the effects of darifenacin on dams during the pre-natal period and on parturition as well as during the post-natal development period for offspring. Pregnant, female rats received darifenacin by oral gavage at doses of 0, 3, 10, or 50 mg/kg/day from day 15 of gestation until parturition and throughout the whole lactation period until day 20 post-partum (27-29 consecutive days). There were 24 female rats per group. The vehicle was a 0.5% aqueous methylcellulose solution, 4000 cps, containing 0.1% Tween 80. The dosing volume was 10 mL/kg. F₀ female rats were examined for clinical signs of toxicity twice daily during the treatment period and once daily outside the treatment period. Body weights were measured on days 1, 6, 15, and 18 of gestation and on days 1, 4, 7, 10, 13, 16, 19, and 21 post-partum. The length of gestation was measured from day 0 post insemination until the end of parturition. Lactation was qualitatively examined by examining teats, stomachs of neonates, and growth of offspring. The number of implantation sites was determined in dead females, non-littering females at sacrifice, and littering females at sacrifice. At weaning of pups, dams were sacrificed and a gross examination was performed. The number of pups (dead and alive) and any external abnormalities were recorded. Viable pups were counted, sexed, and weighed at 24 hr, 4, 7, 14, 21, and 28 days. For the first few days following birth, suckling was observed to detect signs of agalactia or cleft palates. Dead pups were subjected to a gross examination when possible. The following tests were used to assess post-natal development: surface righting reflex on day 6 post partum,

grasping reflex on day 6 post partum, appearance of incisors on day 11 post partum, and opening of the palpebral fissure (bilateral) on day 15 post partum. After weaning, 1 pup/sex/litter were selected for an ophthalmic examination and auditory testing (Preyer's reflex). The spontaneous activity of 2 pups/sex/group was assessed in an open field test between days 68 and 70 post partum. Five weaned pups/sex/group were subjected to a gross examination.

Results: Salivation was observed in all females treated with 50 mg/kg/day from day 18 post-insemination (pi) until the end of treatment. Six of 24 females from the 10 mg/kg/day group were observed with salivation from day 21 until the end of treatment. Other clinical signs observed for the 50 mg/kg/day group included chromodacryorrhea, piloerection, dyspnea, and prostration that began at day 17 or 18 of gestation. Body weight gain for the 50 mg/kg/day from days 15 to 20 of gestation was reduced to 2.2% of the control. Several pregnant dams treated with 50 mg/kg/day had difficulties in littering (i.e., dystocia). Gestational length for the 50 mg/kg/day group was increased as the number of dams with a duration >21 days was 12 of 16 (75%) as compared to 10 of 21 (47.6%) for the control. Two females of the 50 mg/kg/day group (F802, F818) had a poor clinical status at the end of gestation. Three females of the 50 mg/kg/day group (F809, F816, F819) had prolonged labor, resulting in sacrifice on day 24 pi. The death in utero rates for the 10 and 50 mg/kg/day groups were increased to 3.7 and 12.9% as compared to a control value of 1.2%. Fetal body weights for the male 3, 10, and 50 mg/kg/day groups and the female 10 and 50 mg/kg/day groups were lower than corresponding control levels. No external abnormalities were reported for fetuses from any treatment group. Many littering females of the 50 mg/kg/day group (9 of 16) lost their litters before day 4 post partum (pp). Pup viability for the 50 mg/kg/day group declined from 87.1% (210/241) to 31.4% (66/210) at day 4 pp. Several females of the 50 mg/kg/day group (8 of 9) were observed with hypogalactia as evidenced by little or no milk in mammary glands at necropsy. From days 4 to 21 pp, there was no further decline of pup viability for the 50 mg/kg/day group. From days 1 to 21 of lactation, body weight gains for F₀ dams from all treatment groups were increased to 125.6-252.2% of the control. Through day 28 post partum, pup body weights for the 10 and 50 mg/kg/day groups were depressed below control values; however, body weights for the 3 mg/kg/day group were not different from controls. The grasping reflex was delayed for the 10 and 50 mg/kg/day groups. The appearance of incisors and opening of palpebrae fissures was delayed for all treatment groups. Spontaneous activity for pups, as assessed by an open field test, were unchanged between control treatment groups. However, for male and female pups of the 50 mg/kg/day group, the number of vertical movements inside and outside of the 10-90th percentiles were significantly lower than those found for the control group. Ophthalmic and auditory examinations found no

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Page 100

treatment-related changes. At the scheduled sacrifice for F₀ dams, no macroscopic abnormalities were observed. Examination of pups that died before day 21 pp, when possible, found the presence of hydroureter(s), sometimes accompanied by dilatation of renal pelvis(es) or dilatation of urinary bladder, for 1 pup of the control group, 1 pup of the 3 mg/kg/day group, and 15 pups from 5 litters of the 50 mg/kg/day group.

Unscheduled Deaths of F₀ female rats (Sponsor's table on pages 2946 and 2947).

Dose mg/kg/day	Dam No.	Day of Death	Mode of Death	Reason for sacrifice
0	501	24 pi	Sacrifice (S)	Abortion
	507	24 pi	S	Non-pregnant
	508	24 pi	S	Vaginal atresia
3	614	2 pp	S	No pups alive
	621	24 pi	S	No littering (consisted of 1 male and 1 female fetuses)
50	801	19 pi	D	-
	802	21 pi	S	Dystocia
	803	2 pp	S	No pups alive
	804	3 pp	D	-
	805	2 pp	S	No pups alive
	807	2 pp	S	No pups alive
	809	24 pi	S	Dystocia
	810	2 pp	S	No pups alive
	813	3 pp	S	No pups alive
	814	2 pp	S	No pups alive
	816	24 pi	S	Dystocia
	817	17 pi	D	-
	818	22 pi	S	Dystocia
	819	24 pi	S	Dystocia
	820	2 pp	S	No pups alive
	821	24 pi	S	Non-pregnant
	822	3 pp	S	No pups alive
	824	2 pp	S	No pups alive

Gestational length, number of implantation sites, and number of pups (viable and dead) at birth for F₀ dams that received darifenacin by oral gavage at 0, 3, 10, or 50 mg/kg/day.

Dose, mg/kg/day	0	3	10	50
Number of littering dams	21	23	24	16
Length of gestation (days)	21.5	21.2	21.3	21.8
Implantation sites/dam	16.2 (340/21)	15.7 (360/23)	16.4 (393/24)	17.2 (275/16)
Viable pups at birth	320/324 (98.8%)	320/327 (97.9%)	368/382 (96.3%)	210/241 (87.1%)
Dead pups at birth	4/324 (1.2%)	7/327 (2.1%)	14/382 (3.7%)*	31/241 (12.9%)*
Viable pups/dam at birth	15.2 (320/21)	13.9 (320/23)	15.3 (368/24)	13.1 (210/16)

* p < 0.05

Viable pups/dam at birth, 24 hr, 4 days, and 21 days (Sponsor's tables on pages 3014-3017).

Dose, mg/kg/day	0	3	10	50
At Birth	15.2 (320/21)	13.9 (320/23)	15.3 (368/24)	13.1 (210/16)
24 hr	15.2 (320/21)	13.9 (320/23)	15.3 (368/24)	12.8* (205/16)
4 days post partum	15.1 (318/21)	13.7 (314/23)	15.0 (361/24)	4.4* (66/15)
21 days post partum	15.0 (314/21)	13.5 (311/23)	14.8 (354/24)	4.3* (65/15)

* p < 0.05

Pup Weight at days 1, 4, 7, and 28.

Day	0		3		10		50	
	M	F	M	F	M	F	M	F
1	6.93	6.35	6.37*	6.12	6.13*	5.79*	4.60*	4.39*
4	9.93	9.20	9.30	9.07	8.59*	8.11*	6.51*	6.48*
7	14.64	13.53	13.74	13.48	12.58*	11.99*	9.71*	9.68*
28	77.67	71.38	76.64	71.96	68.77*	65.08*	62.38*	58.40*

* p < 0.05

Pup Development

Test	0	3	10	50
Surface Righting reflex (Day 6)	60.9%	56.7%	54.4%	58.5%
Grasping reflex (Day 6)	72.2%	69.2%	55.0%*	49.2%*
Appearance of incisors (Day 11)	57.5%	46.9%*	38.5%*	40.0%*
Opening of palpebral fissures (Day 15)	95.2%	84.9%*	61.4%*	87.7%*

* p < 0.05

Pregnant, female rats received darifenacin by oral gavage at doses of 0, 3, 10, and 50 mg/kg/day from day 15 of gestation until day 20 post-parturition. Maternal toxicity was evident at 50 mg/kg/day. Minor developmental delays were observed for pups at all dose levels. The gestational length for the 50 mg/kg/day group was increased. The death in utero rates for the 10 and 50 mg/kg/day groups were increased to 3.7 and 12.9% as compared to a control value of 1.2%. Fetal body weights for the male 3, 10, and 50 mg/kg/day groups and the female 10 and 50 mg/kg/day groups at birth were lower than corresponding control levels. Pup viability for the 50 mg/kg/day group declined from 87.1% at day 1 pp to 31.4% at day 4 pp. Several females of the 50 mg/kg/day group (8 of 9) were observed with hypogalactia as evidenced by little or no milk in mammary glands at necropsy. Through day 28 pp, pup body weights for the 10 and 50 mg/kg/day groups were depressed below control values; however, body weights for the 3 mg/kg/day group were not different from controls. The grasping reflex was delayed for the 10 and 50 mg/kg/day groups. The appearance of incisors and opening of palpebrae fissures were delayed slightly for all treatment groups. An increased incidence of hydroureter(s), sometimes accompanied by dilatation of renal pelvis(es) or dilatation of urinary bladder, was found for pups of the 50 mg/kg/day group that died between days 1 and 4 pp.

Rabbits

Teratology (Segment II) Study in New Zealand White Rabbits by the Oral Route (Study nos. 91071 and 91072; Volume 8 pg. 8 2320).

Testing Laboratory: Pfizer
Centre de Recherche
37401 Amboise Cedex
France

Study Started: November 19, 1991

IND

Page 103

Study Completed: September 18, 1992

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Female New Zealand white rabbits, with an age range of 19-25 weeks, were used in this study. Mean body weights for the Segment II and toxicokinetic studies were 3.03 and 2.95 kg, respectively. Female rabbits were artificially inseminated.

Drug Batch: Darifenacin-04 Batch number R4.

Methods: This study examined the maternal toxicity, fetotoxicity, and teratogenicity of darifenacin in pregnant, female rabbits. In a preliminary Segment II study (Protocol No. 90130), pregnant, female rabbits received darifenacin by oral intubation at doses of 0, 10, 30, and 100 mg/kg/day from days 7 to 18 of gestation. Body weight gains for the 10, 30, and 50 mg/kg/day groups during the treatment period were impaired by >10%. For the 10, 30, and 50 mg/kg/day treatment groups, the number of implantation sites/dam was decreased and the embryomortality rate was increased as compared to the control; however, these changes did not occur in a dose-related manner and were only statistically significant for the 10 mg/kg/day group. In the present study, pregnant, female rabbits received darifenacin by the oral route using esophageal intubation at doses of 0, 3, 10, and 30 mg/kg/day from days 6 to 18 of gestation (13 consecutive days). There were 20 inseminated rabbits per group. A toxicokinetics group consisting of 4 rabbits was treated with darifenacin at a dose of 30 mg/kg/day for the same treatment period. The vehicle was 0.5% methylcellulose 4000 cps containing 0.1% Tween 80. The dosing volume was 1 mL/kg. Animals were monitored for clinical signs of toxicity twice daily during the treatment period and once daily outside the treatment period. Body weights were measured on days 1, 3, 6, 9, 12, 15, 19, 21, 24, and 28. Animals were sacrificed on day 28 and gross examination of all organs was performed. Uteri and ovaries were removed and the number of corpora lutea and the number, type, and position of implantation sites were recorded. All fetuses were examined for external and buccal malformations and weighed. Alternate fetuses were prepared for skeletal examinations and the degree of ossification and presence of anomalies were determined. Total and mean values of sternebral ossification were calculated for each dose. Remaining fetuses were examined for visceral abnormalities or anatomical variations. Blood was collected from animals in the toxicokinetics group on day 18 at 1, 3, and 6 hr after dosing. After the 6 hr blood collection, rabbits were sacrificed and amniotic fluid and fetuses were collected for determination of drug levels. Drug quantities in maternal plasma, amniotic fluid, and fetal tissue were determined with an HPLC assay using ultraviolet detection.

Results: Two females, F811 and F801, of the 30 mg/kg/day group spontaneously aborted on days 22 and 24, respectively. One of these females (F811) was observed with blood at the vulva, on the muzzle, and under the cage on day 21, and darkened urine on day 21. Another female (F805) of the 30 mg/kg/day group was found to have no feces on day 19. During the treatment period, the 30 mg/kg/day lost 2.5% of their initial body weight, and food consumption for this group was decreased. Gross examination found that one female of the 30 mg/kg/day group had reddish lungs and white spots on the right lobe. The number of fetuses/dam for the 30 mg/kg/day group was reduced to 73.5% of the control. In a corresponding manner, embryomortality for the 30 mg/kg/day group was increased to 21.7% as compared to 8.1% for the control. The implantation rate for the 3 and 30 mg/kg/day groups were reduced to 73.4 and 78.3%, respectively, as compared to 88.8% of the control. External examination found that 2 fetuses (3.1%) from the 30 mg/kg/day group had flexed hindpaws as compared to no findings for concurrent controls and a 0.4% incidence for historical controls. For the 10 and 30 mg/kg/day groups, the incidences of fetuses with 13 pairs of ribs were 37.3 and 37.5%, respectively, as compared to a control value of 26.9%. There was a slight delay of skull ossification for 3 animals (9.4%) in the 30 mg/kg/day group as compared to normal ossification for all controls. There was rudimentary ossification of the pubic bone for 5 (8.5%) and 5 (15.6%) fetuses in the 10 and 30 mg/kg/day groups, respectively, as compared to 3 (5.8%) for the control. Visceral examination found several minor and major abnormalities as noted in the table below. The incidences for diaphragmatic hernia (major anomaly) at doses of 3 and 30 mg/kg/day exceeded the historical control; although, there was not a dose response relationship. The incidence of kidney/ureter agenesis (major anomaly) at doses of 10 and 30 mg/kg/day exceeded the historical control. Right microphthalmia, plication of the right retina, and left lens agenesis found at 3.0% for the 30 mg/kg/day group exceeded the historical control incidence of 0.1%. Post caval lung lobe (agenesis) found at 2.2 and 3.0% in the 3 and 30 mg/kg/day groups, respectively, exceeded the historical control incidence of 0.6%; however, a dose response relationship was not found. A divided gall bladder found at 3.0% in the 30 mg/kg/day group exceeded the historical control incidence of 0.1%. Toxicokinetic analysis found that concentrations of darifenacin in maternal plasma and fetal tissue were similar; however, the concentration in amniotic fluid was significantly lower.

IND

Page 105

Reproductive variables and fetal development for pregnant, female rabbits that received darifenacin by oral intubation at doses of 0, 3, 10, and 30 mg/kg/day from days 6 to 18 of gestation (Sponsor's table on page 2340).

Dose (mg/kg/day)	0	3	10	30
Rabbits/group	20	20	20	20
No. pregnant ^A	15	17	20	16
Viable litters, day 28	15	16	18	13
Corpora lutea/dam	8.3	8.9	8.6	8.2
Implantation sites/dam	7.4	6.6	7.4	6.4
Fetuses/dam	6.8 ± 2.08	5.8 ± 2.27	6.8 ± 1.63	5.0 ± 1.41 ^B
Implantation rate (%)	111/125 (88.8)	105/13 (73.4) ^B	134/154 (87.0)	83/106 (78.3) ^B
Embryomortality rate (%)	9/111 (8.1)	13/105 (12.4)	12/134 (9.0)	18/83 (21.7) ^B
Fetal weight	31.03	33.15	31.63	30.73

A. Several female rabbits did not become pregnant: 5 in the control, 3 in the 3 mg/kg/day group, and 4 in the 30 mg/kg/day group.

B. p < 0.05

External Examination of Fetuses (Sponsor's table on page 2330).

Dose (mg/kg/day)	0	3	10	30	Historical Controls
No. of fetuses examined	102	92	122	65	1336
No. of litters	15	16	18	13	-
Absence of claw(s)	5 (4.9%) (4 lit.)	5 (5.4%) (4 lit.)	6 (4.9%) (4 lit.)	3 (4.6%) (3 lit.)	0.5% (0-1.9%)
Flexed hindpaws	0	0	0	2 (3.1) (2 lit.)	0.4% 0-0.5%

Skeletal Examination of Fetuses: Variations in Rib Number
(Sponsor's Table 6 on page 2345).

Dose (mg/kg/day)	0	3	10	30	Historical Control
No. of fetuses examined	52	47	59	32	627
12 pairs	17 (32.7%)	23 (48.9%)	19 (32.2%)	14 (43.8%)	19.1% (14.6-25.2%)
13 pairs	14 (26.9%)	10 (21.3%)	22 (37.3%)	12 (37.5%)	
13 th pair rudimentary	14 (26.9%)	8 (17.0%)	12 (20.3%)	4 (12.5%)	
13 th right rib only	0	3 (6.4%)	3 (5.1%)	1 (3.1%)	
13 th left rib only	7 (13.5%)	3 (6.4%)	3 (5.1%)	1 (3.1%)	

Examination of Fetuses: Degree of Bone Ossification (Sponsor's Table 7 on page 2346).

Dose (mg/kg/day)	0	3	10	30	Historical Control
No. of fetuses examined	52	47	59	32	
Sternebrae					
-Total	933	845	1055	567	
-Mean	17.94	17.98	17.88	17.72	
Skull					
-normal	52	47	59	29	
-slight delay	0	0	0	3	
-marked delay	0	0	0	0	
Pubic					
-normal	48	46	53	27	
-rudimentary	3	1	5	5	
-absent	1	0	1	0	

Visceral Examination of Fetuses: Visceral Anomalies and Anatomical Variants (Sponsor's Table 8 on page 2347). The incidence is designed as follows: # fetus(es) (Percentage)/# litters.

Dose (mg/kg/day)	0	3	10	30	Historical Control
No. of fetuses examined	50	45	63	33	668
No. of litters	15	16	18	13	-
Plication of right retina & microphthalmia, left lens agenesis				1 (3.0%) (1 lit.)	0.1% (0-0.5%)
Post caval lung lobe (agenesis)		1 (2.2%) (1 lit.)		1 (3.0%) (1 lit.)	0.6% (0-1.1%)
Diaphragmatic hernia		1 (2.2%) (1 lit.)		1 (3.0%) (1 lit.)	0.4% (0-0.7%)
Divided gall bladder				1 (3.0%) (1 lit.)	0.1% (0-0.5%)
Kidney and ureter agenesis			1 (1.6%) 1 lit.	1 (3.0%) (1 lit.)	0.3% (0-0.8%)
Persistence of left cardinal vein	6 (12.0%) (4 lit.)	6 (13.3%) (5 lit.)	6 (9.5%) (6 lit.)	6 (18.2%) (3 lit.)	13.2% (9-19.0%)

Darifenacin Concentrations in Maternal Plasma, Amniotic fluid, and Fetal Homogenates (Sponsor's Table 13 on Page 2353). Pregnant, female rabbits received darifenacin at an oral dose of 30 mg/kg/day from days 6 to 18 of gestation. Blood was collected on day 18 at 1, 3, and 6 hr after dosing.

C _{max} (ng/mL)	T _{max} (hr)	Maternal plasma at 6 hr (ng/mL)	Amniotic fluid at 6 hr (ng/mL)	Fetal tissue (ng/g)	
				Right horn	Left horn
50	2.75	41.3	2	46	62

Pregnant females received darifenacin by oral intubation at 0, 3, 10, and 30 mg/kg/day from days 6 to 18 of gestation. Darifenacin was embryotoxic at 30 mg/kg/day. The number of fetuses/dam for the 30 mg/kg/day group was reduced and embryomortality was increased. However, darifenacin did not appear to be teratogenic. External and visceral anomalies found principally at the 30 mg/kg/day dose occurred with a low incidence of not more than 1 fetus effected, displayed little or no evidence

IND

Page 108

of a dose response relationship, and may have been related to maternal toxicity. External examination found that 2 fetuses (3.1%) from the 30 mg/kg/day group had flexed hindpaws as compared to no findings for concurrent controls and a 0.4% incidence for historical controls. The incidences of kidney/ureter agenesis (major anomaly) at doses of 10 and 30 mg/kg/day were 1.6 and 3.0% and exceeded the historical control incidence of 0.3%. Right microphthalmia, plication of the right retina, and left lens agenesis found at 3.0% for the 30 mg/kg/day group exceeded the historical control incidence of 0.1%. A divided gall bladder found at 3.0% in the 30 mg/kg/day group exceeded the historical control incidence of 0.1%. The number of fetuses/dam for the 30 mg/kg/day group was reduced and embryomortality was increased. Evidence of maternal toxicity at 30 mg/kg/day included spontaneous abortions for two females and weight loss during the treatment period.

Genotoxicity:

Microbial Reverse Mutation Assays (Ames Test) (Protocol No. 89-751-01 and 89-751-05; Volume 8, Page 8 2412).

Testing Laboratory: Department of Safety Evaluation
Pfizer Central Research
Groton, CT

Study Started: March 1989

Study Completed: May 3, 1991.

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Drug Batch: Darifenacin-18 Lot number 632-231-13.

Methods: Darifenacin was tested for induction of reverse mutation in Salmonella typhimurium strains, TA 1535, TA 1537, TA 98, and TA 100 with and without metabolic activation. The plate incorporation technique was used for these studies. Darifenacin was tested at concentrations ranging from 0.005 to 10 mg/plate without metabolic activation and 0.001 to 0.5 mg/plate with metabolic activation. The vehicle was dimethylsulfoxide. The S9 fraction was prepared from livers of male rats CD(SD)BR] or mice CD-1 (ICR)BR], which were treated with a single intraperitoneal injection of 500 mg/kg Aroclor 1254, 5 days prior to sacrifice. Positive controls in the absence of metabolic activation were sodium nitrite (TA 1535 and TA 100), ICR-191 (TA 1537 and TA 98), 2-nitrofluorene (TA 1537 and TA 98), and nitrofurantoin (TA 1535 and TA 100). Positive controls with metabolic activation were sodium nitrite (TA 1535 and TA 100), nitrofurantoin (TA 1535 and TA 100), ICR-191 (TA 1537 and TA 98), 2-nitrofluorene (TA 1537 and TA 98), and 2-anthramine (TA 1535,

TA 1537, TA 98, and TA 100). Plates were incubated for 60 hr at 37°C and the number of revertants were determined. A positive response was considered to be a dose-related, reproducible three-fold increase over the control value.

Results: No insoluble compound was observed at any darifenacin concentration tested. Inhibition of cell growth was found at darifenacin concentrations ≥ 2.0 mg/plate. Darifenacin did not produce an increase in the number of revertants for any tester strain. Darifenacin was not mutagenic in the Ames test. This assay does not meet ICH guidelines. Tester strains, TA 1535, TA 1537, TA 98, and TA 100, detect changes at G-C (Guanine-cytosine) sites with target histidine genes. Some mutagenic carcinogens also modify A-T (adenine-thymine) base pairs, and it is necessary to include either the strain TA 102 or Escherichia coli WP2 uvrA, which detect mutations at A-T sites.

Darifenacin was not mutagenic with tester strains TA 1535, TA 1537, TA 100, and TA 98; however, the assay was incomplete. It is necessary to include either the strain TA 102 or Escherichia coli WP2 uvrA, which detect mutations at A-T sites.

Mammalian Cell Gene Mutation Assay: CHO/HGPRT (Protocol No. 89-751-01 and 89-751-05; Volume 8, Page 8 2435).

Testing Laboratory: Department of Safety Evaluation
Pfizer Central Research
Groton, CT

Study Started: February 1990

Study Completed: May 3, 1991

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Drug Batch: Darifenacin-04 Lot number R1.

Methods: Darifenacin was tested for mutational activity in the Chinese hamster ovary/hypoxanthine-guanine phosphoribosyl transferase (CHO/HGPRT) assay in the presence and absence of metabolic activation. The HGPRT^r subclone [] was obtained from either the [] or the []

[] The S9 fraction was prepared from livers of male rats [CD(SD)BR], which were treated with a single intraperitoneal injection of 500 mg/kg Aroclor 1254, 5 days prior to sacrifice. Positive controls were 3-methylcholanthrene, which requires metabolic activation, and ethylmethane-sulfonate, which acts directly without activation. [] monolayers, in F12

medium containing 3% serum with or without the S9 liver fraction, were exposed to darifenacin for 5 hr. After the 5 hr treatment with darifenacin, cells were replated for cytotoxicity determination and expression of phenotypic mutation. For two experiments, monolayers were washed following darifenacin exposure and incubated for an additional 19 hr prior to replating for cytotoxicity determination and expression of phenotypic mutation. For selection of the 6-thioguanine (6TG)-resistant phenotype, cells were subcultured on days 7-9 in medium containing 10 μ M 6TG. After 6-8 days of incubation, colonies are fixed, stained and counted for both mutant selection and cloning efficiency. An assay was considered acceptable for evaluation of results, if the following criteria were met: the average absolute cloning efficiency of the solvent controls was 70-120%; the background mutant frequency for activation and nonactivation assays was 0-40 $\times 10^{-6}$; positive controls must produce a characteristic response; the range of test article concentrations in the assay must include those that reduce clonal survival to 10-20% (or, if not possible, reach twice the solubility limit); the mutant frequency must be calculated with a minimum of 4 dishes for mutant colony count and two dishes for viable colony count; and mutant frequencies are determined with a minimum of 3 concentration levels. The exact criteria for determination of a positive response were not included.

Results: A 5 hr exposure to darifenacin at concentrations from 14 to 250 μ g/mL in the absence of metabolic activation produced a relative cloning efficiency, a measure of cytotoxicity, of 0 to 126% of the DMSO solvent control; concentrations ≥ 126 μ g/mL generally had a 0% relative cloning efficiency. The absolute cloning efficiency for DMSO was 67-91% and the mutant frequency per 10^6 survivors ranged from 4 to 20. The mutant frequency per 10^6 survivors for darifenacin-treated cultures in the absence of metabolic activation ranged from 0 to 37. A 5 hr exposure to darifenacin at concentrations of 56 to 200 μ g/mL produced a relative cloning efficiency of 0 to 86% of DMSO; concentrations ≥ 138 μ g/mL generally had a 0% relative cloning efficiency. The absolute cloning efficiency of DMSO was 81-104% and the mutant frequency per 10^6 survivors ranged from 0 to 17. The mutant frequency per 10^6 survivors for darifenacin-treated cultures in the presence of metabolic activation ranged from 0 to 13. Darifenacin was not mutagenic in the CHO/HGPRT assay in the presence or absence of metabolic activation.

Darifenacin was not mutagenic in the CHO/HGPRT assay in the presence or absence of metabolic activation. The exact criteria for determination of a positive response were not included in the protocol.

IND
Page 111

In Vitro Cytogenetics Studies: Human Lymphocyte Chromosomal Aberration Assay (Protocol No. 89-751-01 and 89-751-05; Volume 8, Page 8 2532).

Testing Laboratory: Department of Safety Evaluation
Pfizer Central Research
Groton, CT

Study Started: December 1989

Study Completed: May 3, 1991

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Drug Batch: Darifenacin-04 Lot number R1.

Methods: Darifenacin was examined for in vitro clastogenic activity in human lymphocyte cultures with and without exogenous metabolic activation. Dose range finding cytotoxicity studies were performed using the mouse lymphoma L5178Y cell line with darifenacin concentrations of 0.62 to 100.0 µg/mL and an incubation period of 24 hr at 37°C. Darifenacin was found to be cytotoxic with L5178Y cells at concentrations of 5.0 to 12.5 µg/mL. Human lymphocytes were treated with phytohemagglutinin at 1% and cultured for 48 hr. Darifenacin was assayed for clastogenic activity with human lymphocytes using a concentration range of 0.1 to 20.0 µg/mL in the absence of metabolic activation and 5.0 to 50.0 µg/mL in the presence of metabolic activation. For the assay without metabolic activation, cells were incubated with darifenacin for 24 hr at 37°C. Colcemid was present for the final 3 hr. The positive control was mitomycin C, a direct acting mutagen. For assays with metabolic activation, cells were exposed to darifenacin plus S9 for 3 hr. Medium was then replaced and incubation continued for another 21 hr. Colcemid was present for the final 3 hr. The positive control was cyclophosphamide, which requires metabolic activation. The liver S9 fraction was prepared from male rats [CD(SD)Br] treated with 0.1% sodium pentobarbital in drinking water for 10 days. Negative controls for assays with and without activation were treated with DMSO. Cells were harvested and the frequency of mitosis was determined. At least 100 metaphase figures were analyzed for chromosomal aberrations from each culture. Aberrations were classified as chromatid or chromosome breaks and rearrangements. Cells with pulverized chromosomes or ≥7 aberrations were recorded along with abnormal cells. Gaps were recorded but not included with the aberration total. The criteria for a positive response was a statistically significant, reproducible and dose-related increase in the number of abnormal cells as compared to test solvent controls and negative historical controls.

Results: The incidence of abnormal cells (contains structural chromosome damage) with darifenacin concentrations of 2.5 to 7.5 $\mu\text{g}/\text{mL}$ in the absence of metabolic activation were not significantly different from the control; although, the mitotic index was suppressed by 31 to 43%. Concentrations ≥ 10 $\mu\text{g}/\text{mL}$ had insufficient mitoses for analysis. The incidence of abnormal cells with darifenacin concentrations of 30.0-40.0 $\mu\text{g}/\text{mL}$ in the presence of metabolic activation were not significantly different from the control; although, the mitotic index was suppressed by 10 to 34%. Concentrations ≥ 45 $\mu\text{g}/\text{mL}$ had insufficient mitoses for analysis. Darifenacin did not produce chromosomal aberrations in human lymphocytes in vitro with or without metabolic activation.

Darifenacin did not produce chromosomal aberrations in human lymphocytes in vitro with or without metabolic activation.

In Vivo Cytogenetic Assays; Mouse Bone Marrow Metaphase Analysis
(Protocol No. 89-751-01 and 89-751-05; Volume 8, Page 8 2553).

Testing Laboratory: Department of Safety Evaluation
Pfizer Central Research
Groton, CT

Study Started: February 27, 1990

Study Completed: May 3, 1991

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Drug Batch: Darifenacin-04 Lot number R1.

Methods: CD-1 mice [CD-1(ICR)BR] received darifenacin by oral gavage at a dose of 125 mg/kg. Dose selection was based on acute toxicity studies in which died following treatment with 250 mg/kg, but there were no deaths at 125 mg/kg. Animals were sacrificed at 6, 24, and 48 hr after treatment with a single dose. There were 5 mice/sex/group. The dose volume was 10 mL/kg. Darifenacin at 12.5 mg/mL formed a milky suspension, which would precipitate if not continuously mixed. Positive controls were treated with 3 mg/kg mitomycin C i.p. Controls received distilled water. One hr prior to sacrifice, each animal received colchicine at 2 mg/kg i.p. Bone marrow was flushed from femurs and prepared for analysis. At least 50 metaphase figures from each mouse were examined for chromosomal damage. Aberrations are classified as chromatid or chromosome breaks (i.e., displacement and rearrangements). Two additional categories included were multiple breaks (≥ 7 aberrations per cell) and pulverized chromosomes (small fragments). Gaps were recorded but not included in the aberration total.

IND

Page 113

Results: Darifenacin at 125 mg/kg did not produce chromosomal aberrations in mice at 6, 24, and 48 hr after exposure. The study performed by the sponsor appears to be inadequate based upon guidelines published in Mutation Research 189:157-165, 1987. A minimum of 3 doses is recommended to obtain dose-response information. In order to obtain a measure of induced aberration frequency, cells in their first metaphase after treatment have to be analyzed. It is possible that cells containing aberrations may be lost from the analyzed population because of failure to divide, acentric fragments can be lost from daughter cells, and chromatid-type aberrations can segregate to give normal and aberrant daughter cells. A series of 3 fixation times at 6, 12, and 24 hr after treatment will meet sampling time requirements. The 24 and 48 hr time points used by the sponsor are too late. The 24 hr sample will include many second divisions in the absence of substantial cell cycle delays and the 48 hr sample will include second, third, and subsequent divisions.

Darifenacin at 125 mg/kg did not produce chromosomal aberrations in mice at 6, 24, and 48 hr after exposure. However, the study performed by the sponsor appears to be inadequate based upon guidelines published in Mutation Research 189:157-165, 1987 and should be repeated.

Special Toxicity:

Seven Day Dermal Irritation Study in the Rabbit (Volume 12, Page 8 3384).

Testing Laboratory: [

↑

Study Started: August 24, 1994

Study Completed: May 4, 1995

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: New Zealand white rabbits :NZW], approximately 10-12 weeks of age and with a weight range of 2.0-2.4 kg, were used in this study.

Drug Batch: Darifenacin-18 Lot number 632-231-13.

Methods: This study was designed to assess the dermal irritation in the rabbit following 7 consecutive days of topical application of darifenacin or its hydrobromide salt, darifenacin-04. Darifenacin or darifenacin-04 were administered by occluded dermal application at doses of 0.4, 4, or 40 mg base/kg/day. There were 2 rabbits/sex/group. The vehicle was ethanol/water (75/25). The dose volume was 1 mL/kg/day. A common control group consisting of 2 rabbits/sex received the vehicle. The selection of the high dose was based upon the maximum achievable concentration of darifenacin or darifenacin-04 in the vehicle. The treatment area was a shaven area of skin measuring 12 x 8 cm in the dorsal region and approximately 10% of the total body surface area. The skin sites were not abraded and clipping was repeated as necessary during the treatment period. An appropriate volume of test substance was spread evenly over the prepared skin and the treatment site was occluded by covering with a bandage for 6 hr each day. After the treatment period each day, the skin was washed. Animals were observed three times each day for mortality and signs of toxicity. Local irritation was recorded immediately prior to start of treatment and on a daily basis during the treatment period. Reactions (i.e., erythema and edema) were assessed using the Draize scoring system. Body weights were recorded on days 1 and 8. Food consumption was measured over the treatment period. On day 8, animals were sacrificed and subjected to a gross examination of the abdominal and thoracic cavities.

Results: No erythema or edema was observed with rabbits that received darifenacin at 0.4 mg/kg/day. Slight to well-defined erythema and edema were observed with animals that received darifenacin at doses of 4 and 40 mg/kg/day. Slight to well-defined erythema and edema were observed at all dose levels for darifenacin-04. Body weight gain was impaired for animal that received darifenacin at doses of 4 and 40 mg/kg/day and darifenacin-04 at doses of 0.4, 4, and 40 mg/kg/day.

No erythema or edema was observed with rabbits that received darifenacin at 0.4 mg/kg/day.

SUMMARY AND EVALUATION:

Darifenacin is a potent, competitive antagonist of the muscarinic M_3 receptor. Darifenacin is a potent antagonist of human muscarinic M_3 receptor subtype expressed in Chinese hamster ovary cells. Further, its affinity for M_1 , M_2 , M_4 , and M_5 receptor subtypes was at least 1-2 orders of magnitude lower. Atropine did not differentiate between these receptor subtypes. The inhibitory effects of darifenacin and atropine were compared on stimulated increases of intestinal motility in dogs, and the effects of darifenacin were more selective than those of atropine, which also had effects on salivation, pupil size, and cardiovascular

IND

Page 115

parameters at the same doses. Darifenacin by the intravenous route inhibited bethanechol-induced increases of small intestinal motility ($ED_{50}=19 \mu\text{g}/\text{kg}$). Intravenous infusion of darifenacin inhibited cholecystokinin (CCK)-induced increases in the motility of the small intestine and colon with ED_{50} values of 0.34 and 0.38 $\mu\text{g}/\text{kg}/\text{min}$, respectively. Darifenacin at doses $\leq 10 \mu\text{g}/\text{kg}/\text{min}$ had no effects on cardiovascular parameters. Infusion of atropine inhibited CCK-induced increases in motility of the small intestine and colon with ED_{50} values of 0.63 and 0.8 $\mu\text{g}/\text{kg}/\text{min}$, respectively; however, changes in hemodynamic parameters occurred over this dose range. Darifenacin by the oral route inhibited food-induced increases of small intestinal motility ($ED_{50}=0.1 \text{ mg}/\text{kg}$). Further, darifenacin, at an oral dose of 0.1 $\text{mg}/\text{kg}/\text{day}$ for 10 days, reduced food-stimulated increases of small intestinal motility by 40-50%. Apparently, there was no tolerance to or accumulation of the effect of darifenacin on smooth muscle. Total gastrointestinal transit time was not significantly affected by darifenacin.

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the sponsor has submitted preclinical reports on the following: pharmacology; absorption, distribution, metabolism, and excretion studies in mice, rats, rabbits, and dogs; acute toxicity studies in mice and rats; two week intravenous toxicity studies in rats and dogs; two week oral dose range finding studies in rats and dogs; 1 month oral toxicity studies in rats and dogs; 3 month dietary dose range finding studies in mice and rats; 6 month oral toxicity studies in rats and dogs; reproductive toxicity studies that included a Segment I fertility and reproductive performance study in rats, Segment II, teratology studies in rats and rabbits, and a Segment III peri- and postnatal development study in rats; genotoxicity studies that included the Ames Test, Chinese hamster ovary/hypoxanthine-guanine phosphoribosyl transferase assay, human lymphocyte chromosomal aberration assay, and mouse bone marrow metaphase analysis; and a special toxicity study of dermal irritation with rabbits.

The absorption, distribution, metabolism, and excretion of darifenacin were investigated in mice, rats, and dogs. The oral bioavailability in rats and dogs were determined to be 16 and 105%, respectively. Darifenacin was widely distributed beyond the blood volume in all three species, which would be expected with the lipophilic characteristics of the compound. Plasma clearance of darifenacin in all three species was rapid and exceeded hepatic clearance suggestive of a rapid metabolic clearance. It appears that darifenacin was rapidly removed by a metabolic clearance and the half-life was <4 hr in all species. Autoradiography studies in rats confirmed the extensive tissue distribution of darifenacin in rats and its subsequent rapid elimination mainly through the bile into the feces. Plasma protein binding of darifenacin exceeded 90% in rats, dogs, and man. Metabolism of darifenacin in mice, rats, rabbits, and dogs occurred principally by 3 routes: hydroxylation, dihydrobenzofuran ring opening, and N-dealkylation. With rat and human liver microsomes, the major metabolite was the pharmacologically-active, hydroxylated metabolite of darifenacin, UK-148,993. In studies with human microsomes, it was determined that UK-148,993 was derived from cytochrome P450 subfamily 2D6. In studies using the isolated, perfused rat liver, the hepatic extraction of darifenacin was found to be 0.7. Further, the major metabolite was a dihydrobenzofuran ring opened acid metabolite (UK-222,247), which had a high biliary clearance. However, glucuronide conjugates of the ring opened acid metabolite and a mono-hydroxylated metabolite had low biliary clearances. In dogs, UK-148,993 was widely distributed beyond the blood volume and rapidly cleared from the plasma at a level similar to hepatic plasma flow. Following intravenous administration of darifenacin to dogs, 18% was converted to UK-148,993; however, plasma levels of UK-148,993 were significantly higher following oral administration of darifenacin suggesting the importance of first pass metabolism. In mice, UK-148,993 was not found following intravenous administration of darifenacin, further supporting first pass metabolism following oral administration. In excretion studies with rats, rabbits, and dogs using radiolabeled darifenacin, it was determined that radioactivity was primarily eliminated in the feces. In studies with male mice, radioactivity was found to be eliminated equally between the feces and urine. The metabolic profiles in fecal and urinary extracts from mice, rats, rabbits, and dogs were examined. Analysis of fecal extracts from mice found that UK-222,247 was the major metabolite. Analysis of urinary extracts found that an unknown metabolite and UK-297,101 (product formed by N-dealkylation at the pyrrolidine nitrogen) were the predominant metabolites. Analysis of fecal extracts from male and female rats following intravenous or oral administration of darifenacin found that UK-222,247 was the major metabolite. Other metabolites identified for female rats included a compound with a hydroxyl group in the diphenylcarboxamide head group, the dihydrobenzofuran ring opened alcohol metabolite, and a metabolite with hydroxyl groups on both the diphenylcarboxamide and

dihydrobenzofuran rings. Analysis of urinary extracts from rats found several metabolites, all at <5% of the dose. For rabbits, the major circulating metabolites in plasma were the glucuronide of UK-156,981 and the glucuronide of dihydroxylated darifenacin. Metabolites in urine were similar to those found in the plasma. Major metabolites in the feces were UK-222,247 and darifenacin (unabsorbed drug). UK-222,247 was found at 29.9% of the dose in urine and feces combined. For dogs, chromatographic analysis of plasma extracts identified unchanged darifenacin and several polar metabolites. Analysis of fecal extracts found that a mixture of two compounds, the dihydrobenzofuran ring-opened acid metabolite (major) and the ring-opened alcohol (minor), were the major metabolites following intravenous or oral administration. Chromatographic analysis of urinary extracts following intravenous and oral administration identified the ring opened-acid and alcohol metabolites, unchanged darifenacin, the dihydrobenzofuran ring hydroxylated metabolite, and an unidentified compound. Excretion and metabolism of darifenacin was examined in human volunteers, who were either extensive or poor metabolizers of dextromethorphan, that received 5 mg T.I.D. for 5 days. Concentrations of radioactivity in plasma were 17 times higher than that of darifenacin, indicative of extensive metabolism. Fifty-eight percent of the drug was excreted in the urine. Concentrations of radioactivity were higher in the plasma than whole blood. Metabolites identified in plasma included the dihydrobenzofuran ring opened acid metabolite (UK-222,247), unchanged darifenacin, and the dihydrobenzofuran hydroxylated metabolite (UK-148,993). Metabolites in urine were similar to those found in plasma.

The acute toxicity of darifenacin was examined in mice and rats following administration by the oral and intraperitoneal routes. Minimum lethal oral doses were 100 mg/kg for mice and between 100 and 200 mg/kg for rats. Minimum lethal intraperitoneal doses were between 100 and 200 mg/kg for mice and 50 mg/kg for rats. The anticholinergic signs of mydriasis and eyes partially closed were observed in both mice and rats. Mice were observed with convulsion following an oral dose of 200 mg/kg. Rats were observed with tremor, ataxia, dyspnea, and depression following an oral dose of 200 mg/kg.

In a 14 day study, rats received darifenacin by the intravenous route at doses of 0, 1.25, or 5 mg/kg/day. The no effect dose was 5 mg/kg/day. The target organ of toxicity was the Harderian glands. Harderian gland hypersecretion was observed for 6 of 10 males and 1 of 10 females in the 5 mg/kg/day group. It is known that cholinergic systems regulate the secretion of rat Harderian gland cells which have muscarinic receptors. Carbamylcholine, a cholinergic secretagogue, stimulates secretion, which is inhibited by atropine, a muscarinic antagonist. Harderian

gland hypersecretion found after treatment with darifenacin, a M₃ muscarinic antagonist, appears somewhat paradoxical and may have no relationship to the pharmacological properties of this agent. Plasma AUC values for darifenacin were proportional to dose.

In a 1 month toxicity study, rats received darifenacin by oral gavage at doses of 0, 3, 10, and 50 mg/kg/day. Dose selection was based upon the 14 day range finding study. The no effect level was 10 mg/kg/day. There was no treatment-related mortality. The target organ of toxicity was the Harderian glands, where an increased incidence of acinar luminal dilation was found in the 50 mg/kg/day treatment group. Small increases (<15%) of absolute and relative liver weights were found for the 10 and 50 mg/kg/day groups; however, there were no corresponding histological changes. Plasma darifenacin concentrations on days 1, 19, and 30 were proportional to dose.

In a 1 month toxicity study, rats received a diet supplemented with darifenacin to result in an average daily intake of 0, 10, 15, or 25 mg/kg/day. This dietary dose range finding study was used to assist in the identification of doses for the rat carcinogenicity study. Body weight gains for the male 25 mg/kg/day group and the female 15 and 25 mg/kg/day groups were impaired by >10%. Animals were sacrificed without necropsy on day 28.

In a 1 month toxicity study, rats received darifenacin by oral gavage at doses of 0, 3, and 50 mg/kg/day. Darifenacin was synthesized using a new procedure. The no effect dose was 3 mg/kg/day. One female of the 50 mg/kg/day group died on day 23, possibly due to dysphagia. Histopathological examination found dilation of the esophagus, as the lumen was expanded by ingesta. Body weight gains for male and female rats of the 50 mg/kg/day group were impaired by >10%. The target organ of toxicity was the Harderian glands, which showed minimal to moderate hypersecretion in all animals of the 50 mg/kg/day group. Dysphagia may have been related to the anticholinergic properties of darifenacin. Plasma AUC values for darifenacin and UK-148,993 for females treated with 50 mg/kg/day were higher on day 22 than day 1; however, there were no differences between male and female rats. In the earlier 1 month study, the Harderian glands were also the target organ of toxicity; however, there was no mortality with a dose of 50 mg/kg/day and dysphagia was not observed.

In a 3 month dose range finding study for the rat carcinogenicity assay, rats received darifenacin in the diet at doses of 0, 25, 50, or 100 mg/kg/day. A no effect dose was not established based upon decreased body weight gain at all doses. Mortality occurred for 1 female in the 50 mg/kg/day group; although, its relationship to treatment was unclear. Body weight gain was impaired by >10% in all treatment groups. Food and water consumption were reduced for all treatment groups. The target

organ of toxicity was the Harderian glands. Hepatic centrilobular hypertrophy was found in rats that received darifenacin at doses of 50 and 100 mg/kg/day. An increased incidence of Harderian gland hypersecretion was found in all treatment groups. Plasma C_{max} and AUC values for darifenacin and UK-148,993 were approximately proportional to dose for both males and females. Plasma C_{max} and AUC values for darifenacin and UK-148,993 were greater in females than males. Based upon the correlation of impaired weight gain with decreased food consumption, the maximum tolerated dose was 100 mg/kg/day for both male and female rats. Plasma drug levels for human volunteers following single oral doses of 0.2 or 0.4 mg/kg were 34.8 and 75.7 ng.hr/mL. Human plasma AUC values ranged from 66 to 39 times lower than the average level observed for rats at 25 mg/kg, respectively; however, the respective dose differential was 125 and 62.5-fold.

In a 6 month chronic toxicity study, rats were treated with darifenacin by oral gavage at doses of 0, 3, 10, or 30 mg/kg/day. The no effect dose was 10 mg/kg/day. Mortality occurred at 30 mg/kg/day for 1 male and 4 females; however, the cause of death was unknown. The target organs of toxicity were the stomach and the Harderian glands. Bacterial overgrowth in the non-glandular stomach was observed in 2 males and 11 females from the 30 mg/kg/day group. An increased incidence of Harderian gland hypersecretion was found in the 10 and 30 mg/kg/day groups. Plasma AUC values for darifenacin and UK-148,993 in male treatment groups were approximately proportional to dose. For female treatment groups, the AUC values for darifenacin and UK-148,993 at 30 mg/kg/day were 20-24 and 6.7-8.2 times values observed at 3 and 10 mg/kg/day, respectively. AUC values for male treatment groups relative to corresponding female groups varied with dose.

In a 3 month dose range finding study for the mouse carcinogenicity study, mice received darifenacin in the diet at dose levels of 25, 50, and 100 mg/kg/day. The no effect level was 25 mg/kg/day on the basis of decreased body weight gain at doses of 50 and 100 mg/kg/day. The maximum tolerated dose was 50 mg/kg/day for male mice and 25 mg/kg/day for female mice. There was no treatment-related mortality. Body weight gain was impaired >10% for the male 100 mg/kg/day group and the female 50 and 100 mg/kg/day groups. The target organs of toxicity was the Harderian glands. There was an increased incidence of Harderian gland hypersecretion for darifenacin treatment groups; although, the dose response relationship was flat. Hepatic cytochrome P450 levels were increased in the 50 and 100 mg/kg/day groups; although, histopathology and electron microscopy revealed no significant changes. One female of the 100 mg/kg/day group was observed with a follicular adenoma of the thyroid gland, which is unusual for an animal of this age. Plasma AUC values for darifenacin and UK-148,993 levels were approximately proportional to dose for male mice. Plasma drug levels for human volunteers following single

oral doses of 0.2 or 0.4 mg/kg were 34.8 and 75.7 ng.hr/mL. Human plasma AUC values ranged from 19 to 8.9 times lower than the average level observed for rats at 25 mg/kg, respectively; however, the respective dose differential was 125 and 62.5-fold.

In a 14 day study, dogs received darifenacin by the intravenous route at doses of 0, 0.5, and 2.5 mg/kg/day. The no effect dose was 0.5 mg/kg/day. There was no mortality. The target organ of toxicity was the stomach. Two males of the 2.5 mg/kg/day group were observed with a chronic, multifocal gastric inflammation located in the pyloric zone. This histopathological change was not observed in oral toxicity studies with darifenacin in dogs.

In a 1 month study, dogs received darifenacin by oral gavage at doses of 0, 1, 4, and 16 mg/kg/day. Dose selection was based upon a 14 day dose range finding study. The no effect dose was 4 mg/kg/day. There was no treatment-related mortality. A target organ of toxicity was not identified. Relative liver weights were increased for the male and female 16 mg/kg/day groups; although, there were no corresponding histological changes. No feces were observed for any animals of the 16 mg/kg/day group during the first 4-6 days of treatment. Difficulty swallowing (i.e., dysphagia) was observed for 2 females of the 16 mg/kg/day group. Final ophthalmic examinations for two animals of the 16 mg/kg/day group found mild corneal edema and a moderate accumulation of mucoid material. On days 1, 15, and 30, plasma AUC values for darifenacin at 4 mg/kg/day were similar. On days 1 and 15, plasma AUC values for dogs treated with 4 and 16 mg/kg/day were approximately proportional to dose. On day 30, plasma AUC values for male and female dogs treated with 16 mg/kg/day were 7.45 and 6.73 times corresponding values at 4 mg/kg/day, respectively.

In a 6 month chronic toxicity study, beagle dogs received darifenacin by the oral route of administration in gelatin capsules at doses of 0, 1, 3, and 10 mg/kg/day. The no effect dose was 3 mg/kg/day. One male of the 10 mg/kg/day group was found dead on day 83. Death appeared to be related to an inhalation pneumonia, a severe enteritis, and a prerenal uremic syndrome. The sponsor considered the death to be unrelated to treatment; however, the death may have been caused by treatment-related difficulties in swallowing food (i.e., dysphagia) and subsequent aspiration of food into the lungs. The target organ of toxicity was the eyes. The conjunctivae was observed with hyperplasia of the epithelium and infiltration of lymphocytes and plasma cells into the subepithelium. Corneal changes consisted of hyperplastic epithelium with infiltration of lymphocytes and the presence of small neovessels in the stroma. These effects appeared to be due to an inhibition of lacrimal gland secretion by this anticholinergic agent. On days 15 and 177, plasma AUC and C_{max} values for darifenacin were proportional to dose. Plasma AUC and C_{max} values for darifenacin on day 177 were greater than those

observed on day 15. On days 15 and 177, plasma AUC and C_{max} values for UK-148,993 were proportional to dose. Plasma AUC and C_{max} values for UK-148,993 on day 177 were slightly greater than those observed on day 15. There were no significant differences in plasma AUC and C_{max} values between male and female dogs for either darifenacin or UK-148,993 on days 15 or 177.

In a Segment I study, the effects of darifenacin, administered to F_0 male and female rats at doses of 0, 3, 10, and 50 mg/kg/day, were examined on mating and fertility as well as the viability, development, and behavior of their offspring. The fertility of the F_1 offspring was also assessed. Mortality occurred for 4 animals in the 50 mg/kg/day group. Body weight gains for male and female rats receiving darifenacin at 50 mg/kg/day were impaired. Maternal toxicity was evident at a dose of 50 mg/kg/day. The mating indexes for the F_0 generation was not unaffected by darifenacin treatment; however, the fertility indexes for the 10 and 50 mg/kg/day groups were reduced to 78.6 and 83.3% as compared to 95.8% for the control. The number of viable pups/dam was reduced to 12.0 for the 50 mg/kg/day group as compared to 15.9 for controls. For visceral anomalies, urinary bladder dilatation was found for 58.2% of fetuses in the 50 mg/kg/day group. For dams allowed to deliver, offspring body weight and survival for the 50 mg/kg/day group on days 1, 4, and 21 were reduced as compared with control.

In a Segment II study, pregnant, female rats received darifenacin by oral gavage at doses of 0, 3, 10, and 50 mg/kg/day from days 6 to 17 of gestation. Darifenacin was not teratogenic or embryotoxic. There were no treatment-related external, skeletal, or visceral abnormalities. Darifenacin concentrations in maternal plasma and fetal tissue at 6 hr after dosing were similar; however, levels in amniotic fluid were significantly lower.

In a Segment II study, pregnant, female rabbits received darifenacin by oral intubation at 0, 3, 10, and 30 mg/kg/day from days 6 to 18 of gestation. Darifenacin was embryotoxic at 30 mg/kg/day. The number of fetuses/dam for the 30 mg/kg/day group was reduced and embryomortality was increased. However, darifenacin did not appear to be teratogenic. The number of fetuses/dam for the 30 mg/kg/day group was reduced and embryomortality was increased. Evidence of maternal toxicity at 30 mg/kg/day included spontaneous abortions for two females and weight loss during the treatment period.

In a Segment III study, pregnant, female rats received darifenacin by oral gavage at doses of 0, 3, 10, and 50 mg/kg/day from day 15 of gestation until day 20 post-partum. Maternal toxicity was evident at 50 mg/kg/day as evidence by impaired body weight gain. Slight developmental delays were observed for pups at all dose levels; however, dose response relationships were not evident and changes were small suggesting little biological

significance. The gestational length for the 50 mg/kg/day group was increased. The death in utero rates for the 10 and 50 mg/kg/day groups were increased to 3.7 and 12.9% as compared to a control value of 1.2%. Fetal body weights for the male 3, 10, and 50 mg/kg/day groups and the female 10 and 50 mg/kg/day groups at birth were lower than corresponding control levels. Pup viability for the 50 mg/kg/day group declined from 87.1% at day 1 pp to 31.4% at day 4 pp. Several females of the 50 mg/kg/day group (8 of 9) were observed with hypogalactia as evidenced by little or no milk in mammary glands at necropsy. Through day 28 pp, pup body weights for the 10 and 50 mg/kg/day groups were depressed below control values; however, body weights for the 3 mg/kg/day group were not different from controls. The grasping reflex was delayed for the 10 and 50 mg/kg/day groups. The appearance of incisors and opening of palpebrae fissures was delayed for all treatment groups; although, a dose response relationship was not evident. An increased incidence of hydroureter(s), sometimes accompanied by dilatation of renal pelvis(es) or dilatation of urinary bladder, was found for pups of the 50 mg/kg/day group that died between days 1 and 4 pp. Atropine has been shown to abolish the release of prostacyclin, which stimulates myometrial contractility in the rat.

The genotoxic potential of darifenacin was evaluated in the Ames Test, Chinese hamster ovary/hypoxanthine-guanine phosphoribosyl transferase assay, human lymphocyte chromosomal aberration assay, and mouse bone marrow metaphase analysis. Darifenacin was not mutagenic with tester strains TA 1535, TA 1537, TA 100, and TA 98; however, the assay was incomplete. It is necessary to include either the strain TA 102 or Escherichia coli WP2 uvrA, which detect mutations at A-T sites. Darifenacin was not mutagenic in the CHO/HGPRT assay in the presence or absence of metabolic activation. The exact criteria for determination of a positive response were not included in the protocol. Darifenacin did not produce chromosomal aberrations in human lymphocytes in vitro with or without metabolic activation. Darifenacin at 125 mg/kg did not produce chromosomal aberrations in the mouse bone marrow metaphase analysis at 6, 24, and 48 hr after exposure.

Dermal irritation of darifenacin or its hydrobromide salt, darifenacin-04 were assessed in the rabbit following 7 consecutive days of topical application. Darifenacin or darifenacin-04 were administered by occluded dermal application at doses of 0.4, 4, or 40 mg base/kg/day. No erythema or edema was observed with rabbits that received darifenacin at 0.4 mg/kg/day.

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RECOMMENDATION:

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2.

A One Month Gavage Study in Beagle Dogs-Dose Levels: 0, 1, 4, or 16 mg active moiety/kg/day (Protocol No. 89-751-02)

1. Mean and standard deviation for the following at 0, 15 and 37 days are needed:

Heart rate
RR interval
body temperature
Systolic blood pressure
Globulin
Albumin/Globulin (A/G) ratio
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin content (MCHC)
Differential counts
Urinalysis

2. Line listing for urinalysis are scattered over several pages. If listings could be categorized by group and individual time points, it would greatly aid the review of this data.

IND
Page 124

6-Month Oral Toxicity in Sprague Dawley Rats (Study number 92028).

Mean and standard deviation for monocyte, eosinophil, and basophil counts are needed.

The Excretion and Metabolism of [¹⁴C]-Darifenacin in the Rat Following Single Intravenous (2.5 mg/kg) and Oral (10 mg/kg) Administration (UK-88525/DM/13/92; Volume 13, DM9, Page 3769).

1. Tables 3, 4, 5, and 6 are missing.

Timothy W. Robison, Ph.D. 1-16-98
Date

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HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Robison
HFD-180/Dr. Talarico
HFD-345/Dr. Viswanathan

R/D Init: J. Choudary 11/30/97

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B. CAC minutes and amendments to minutes

Executive CAC

Date of Meeting: 24 October 2000

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-900, Member
Abby Jacobs, Ph.D., HFD-540, Alternate Member
Alex Jordan, Ph.D., Team Leader
Laurie McLeod, Ph.D.

Author of Draft: Laurie McLeod

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

IND # 45457

Drug Name: Darifenacin

Sponsor: Pfizer

Background: Darifenacin is an M₃ muscarinic receptor antagonist for use in urge incontinence. It was negative for genotoxicity in a standard battery of assays.

Mouse Carcinogenicity Study and Mouse Dose Selection

In a 3-month dietary study in mice at doses of 0, 25, 50, and 100 mg/kg/day, a 6% decrease in body weight gain was observed at the high dose level. A dose-related increase in mydriasis and partially closed eyes was observed at all dose levels. An increased accumulation of Harderian gland secretions showed a similar incidence at all treatment levels. An adaptive increase in liver weight and an induction of cytochrome P450 were also observed at the mid and high doses; there were no histopathological changes to the liver.

The dose groups chosen for the carcinogenicity study were 50 mice / sex of 0, 0, 3, 20, and 100 mg/kg/day.

In the carcinogenicity study, the high dose was a maximum tolerated dose if based on the results that both males and females in the high dose group demonstrated 10% decreases in body weight gain over the two-year study period in the absence of significant changes in food consumption. An AUC ratio of 25 times the clinical dose was achieved.

Protein binding was approximately equivalent for mice and humans. The major metabolite of Darifenacin, UK-148,993, is present in the plasma of both mouse and man, in similar concentrations as those of Darifenacin

Rat Carcinogenicity Study and Rat Dose Selection

In a 3-month dietary toxicity study, rats exposed to 25, 50, or 100 mg/kg/day had reduced food intake and 10% decreases in body weight gain at all doses. Hepatic centrilobular hypertrophy was observed at 50 and 100 mg/kg/day. Mydriasis and partially closed eyes occurred, with increasing severity, at all doses. In a 1-month dietary study, rats exposed to 25 mg/kg/day showed a 10% decrease in body weight gain. Doses of 10 or 15 mg/kg/day induced 3 to 4 % decreases in body weight. Mydriasis was observed at 15 and 25 mg/kg/day. In a six-month oral gavage study in rats, treatment related decreases in body weight gain were also observed.

The dose groups chosen for the carcinogenicity study were 50 rats / sex of 0, 0, 1.5, 5, and 15 mg/kg/day.

In the carcinogenicity study, the high dose was a maximum tolerated dose if based on the results that males and females in the high dose group demonstrated 22% and 29% decreases in body weight gain, respectively, over the 2 year study period. Food consumption was decreased by approximately 9% in males and 5-7 % in females. Both males and females in the mid dose group demonstrated a decrease in body weight gain compared to controls. Food consumption in this group reached sporadic significance with a magnitude of about 3-4%.

The high dose produced 20 and 12 times the expected human exposure in males and females, respectively, based on AUC.

Executive CAC Recommendations and Conclusions:

Mouse:

The committee's conclusion was that the doses were adequate (MTD) based on data in the rat that weight loss was caused by gavage as well as dietary administration of the drug. It also concluded that the doses were adequate based on AUC in animals compared to the AUC in humans given the projected maximal clinical dose of 0.2 mg/kg t.i.d.

*The Committee agreed that there were no tumor findings, pending statistical analysis of the data.

Rat:

The committee's conclusion was that the doses were adequate (MTD) based on data in the rat that weight loss was caused by gavage as well as dietary administration of the drug.

* The Committee felt that there were few or no findings, pending statistical analysis of the data.

Other comments and responses:

General:

*The Committee requested verification of the study durations since both studies are listed as being 96 weeks in length, but the start and stop times are 2 years apart. *Response: The studies are both 722 days in duration (a little over 103 weeks).*

Mouse:

*The committee requested copies of the histopathology tables.

*The Committee asked for verification of the clinical dose. *Response: All calculations were based on the maximal projected clinical dose of 0.2 mg/kg t.i.d., although that dose was not listed in the pk table at the meeting.*

*The Committee felt that there were probably no findings, but recommended adding a table for tabulated benign hemangiomas, hemangiosarcomas, and hemangiomas plus hemangiosarcomas and requested that the statistician be asked to look for significance in all combinations.

MOUSE TUMOR FINDINGS:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	3	20	100	0	0	3	20	100
Bone marrow										
N-hemangiosarcoma	1	0	0	0	0	0	0	0	0	0
Sternum										
N-hemangiosarcoma	0	0	0	0	0	0	0	0	1	0
Liver										
M-hemangiosarcoma	1	1	0	0	0	0	0	0	0	0
Ovaries										
B-hemangioma						1	0	0	0	0
Skin and adnexa										
B-hemangioma	0	0	1	0	0	0	0	0	0	0
M-hemangiosarcoma	0	0	0	0	0	0	0	0	1	0
Spleen										
N-hemangiosarcoma	1	0	0	0	0	0	0	0	1	0
Uterus										
B-hemangioma						1	0	1	0	0
Total hemangiosarcoma	3	1	0	0	0	0	0	0	3	0
Total hemangioma	0	0	1	0	0	2	0	1	0	0
Total hemangioma plus hemangiosarcoma	3	1	1	0	0	2	0	1	3	0
Total animals with hemangioma or hemangiosarcoma	1	1	1	0	0	1	0	1	1	0

Rat:

*The committee requested copies of the histopathology tables.

*The Committee felt that there were probably no findings, but recommended adding a row in the table for tabulated benign hemangiomas plus hemangiosarcomas and that the statistician be asked to look for significance in all combinations.

RAT TUMOR FINDINGS:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	1.5	5	15	0	0	1.5	5	15
Adrenal										
B-cortical adenoma	1/50	3/50	5/49	7/50	3/50	5/50	1/50	3/50	1/50	8/50
Spleen										
B-hemangioma	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/50
M-hemangiosarcoma	0/50	0/50	0/50	0/50	2/50	0/50	0/50	0/50	0/50	0/50
Kidney										
M-hemangiosarcoma	1/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50	0/50
Skin and adnexa										
B-hemangiopericytoma	0/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50	0/50
N-hemangiosarcoma	0/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50	0/50
Total hemangiosarcoma	1/50	0/50	0/50	0/50	4/50	0/50	0/50	0/50	0/50	0/50
Total hemangioma	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/50
Total hemangioma plus hemangiosarcoma *	1/50	0/50	0/50	0/50	4/50	0/50	0/50	0/50	0/50	1/50
Total hemangioma, hemangiosarcoma, or hemangiopericytoma	1/50	0/50	0/50	0/50	5/50	0/50	0/50	0/50	0/50	1/50

* all tumors represent separate animals

*Regarding adrenal cortical adenomas, hemangiomas, and hemangiosarcomas, the committee requested that the sponsor's historical control data from the same time period be sent (see attachment).

/S/

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\

- /Division File, HFD 580
- /Alex Jordan, Team leader, HFD-580
- /Laurie McLeod, Reviewer, HFD-580
- /Adele Seifried, HFD-024
- /Evelyn Farinas, HFD-580

**Appendix:
Lesion-related Incidence Data**

Report parameters:

Data Base: RJTA Rats (Version from 05-Jun-2000)
 Species: Rat
 Strain: SPRD
 Breeder: ALL
 Sex: males + females
 Study start year: not specified
 Study duration: not specified
 Organ: Adrenal gland
 Lesion: Adenoma, cortical
 Modifier/Qualifier: not selected
 Primary sort key: Study Number (ascending)
 No. Studies/Groups: 30

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Adrenal gland						Adenoma, cortical					
Study No.	Group No.	Start [m/y]	Duration [month]	Strain	Breeder	Males			Females		
						total exam.	with lesion	%	total exam.	with lesion	%
1	1	03/1985	24	SPRD	H	68	7	10.3	67	8	11.9
10	1	08/1985	25	SPRD	D1	50	1	2.0	50	1	2.0
14	1	09/1983	28	SPRD	D1	50	2	4.0	50	2	4.0
15	1	10/1983	27	SPRD	D1	50	2	4.0	50	2	4.0
16	1	11/1985	29	SPRD	H	70	3	4.3	69	4	5.8
18	1	09/1985	30	SPRD	H	70	7	10.0	70	7	10.0
19	1	02/1986	29	SPRD	H	68	13	19.1	70	5	7.1
24	1	08/1985	25	SPRD	D1	50	0	0.0	50	4	8.0
29	1	04/1988	26	SPRD	D1	50	0	0.0	50	3	6.0
35	1	08/1987	24	SPRD	D1	70	2	2.9	70	5	7.1
40	1	09/1988	25	SPRD	D1	50	4	8.0	50	8	16.0
42	1	05/1988	25	SPRD	D1	50	0	0.0	50	0	0.0
45	1	09/1989	24	SPRD	D3	55	1	1.8	55	4	7.3
46	1	07/1986	24	SPRD	D2	50	2	4.0	50	5	10.0
47	1	02/1989	24	SPRD	H	71	18	25.4	69	6	8.7
48	5	11/1987	24	SPRD	K	50	7	14.0	50	9	18.0
50	1	09/1989	24	SPRD	D3	55	3	5.5	55	3	5.5
52	2	07/1986	24	SPRD	D2	50	2	4.0	50	4	8.0
57	1	02/1990	24	SPRD	D3	50	1	2.0	50	1	2.0
58	1	11/1987	25	SPRD	L	50	9	18.0			
62	1	08/1987	25	SPRD	D2	60	1	1.7	60	4	6.7
68	1	02/1990	24	SPRD	D3	50	1	2.0	50	6	12.0
70	1	07/1986	24	SPRD	D1	48	5	10.4			
96	1	11/1993	24	SPRD	D2	60	1	1.7	60	2	3.3
100	1	02/1995	24	SPRD	D1	50	3	6.0	49	4	8.2
101	1	10/1992	24	SPRD	D8	54	2	3.7	55	2	3.6
102	1	10/1992	24	SPRD	D8	55	2	3.6	55	3	5.5
106	1	11/1993	24	SPRD	D2	59	3	5.1	60	1	1.7
111	1	07/1995	24	SPRD	D4	60	1	1.7	53	3	5.7

p 2

Adrenal gland		Adenocarcinoma, cortical											
Study No.	Group No.	Start [m/y]	Duration [month]	Strain	Breeder	Males			Females				
						total exam.	with lesion	%	total exam.	with lesion	%		
102	1	10/1992	24	SPRD	D8	55	0	0.0	-	55	0	0.0	-
106	1	11/1993	24	SPRD	D2	59	0	0.0	-	60	1	1.7	-
111	1	07/1995	24	SPRD	D4	60	0	0.0	-	53	0	0.0	-
125	1	02/1996	24	SPRD	H	50	0	0.0	-				
All 30 studies:						1673	3	0.2		1517	11	0.7	
Range MIN:								0.0				0.0	
Range MAX:								2.0				6.0	

End of Report

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Amendment to Darifenacin CAC minutes:

IND # 45457
 Drug Name: Darifenacin
 Sponsor: Pfizer

On October 24, 2000, CAC reviewed the drug Darifenacin, IND#45457. A statistical analysis from historical data was requested for hemangiomas and hemangiosarcomas in Sprague Dawley rats. The statistical review, done by Karl Lin, found positive trends in the total incidence of hemangiosarcoma and hemangiosarcoma-plus-hemangioma in male rats at the highest dose tested, 15 mg/kg/day or 20 times the clinical dose (AUC). In females, the only incidence of hemangioma was in the high dose group at 15 mg/kg/day or 12 times the clinical dose (not statistically significant). There was no effect in male rats at 5 mg/kg/day or 7 times the clinical exposure. Inclusion of historical data did not affect the results. There were no findings in mice at 100 mg/kg/day or greater than 40 times the human exposure (AUC).

As a result of these conclusions, the committee proposed that the following points should be included in the label for Darifenacin:

1

1

Amendment to Darifenacin CAC minutes:

CAC amendment #2

NDA # 21513
 Drug Name: Darifenacin
 7/29/03

After a secondary review, CAC concluded that the incidences of hemangiosarcomas or hemangiomas (combined) in male rats are not clearly related to the administration of darifenacin. The trend analysis is not significant for common neoplasms (the incidence in one of the controls was 2%) and the pairwise comparison is not significant for uncommon or common neoplasms. Thus, no darifenacin-related neoplasms were seen in rats or mice.

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Table 1: Tumor Incidence Rate and Location of Hemangiosarcoma* in Males in Darifenacin Carcinogenicity Rat Study (Amboise Study No. 93073)

Group	Incidence	Location
Controls	1 / 100	Kidney
1.5 mg/kg	0 / 50	-
5 mg/kg	0 / 50	-
15 mg/kg	4 / 50	Kidney (1), Spleen (2), Skin/adnexae (1)

Figures in () indicate the number of tumors.
 * No hemangiomas in this study in males. 1 hemangioepithelioma in skin/adnexae in males at 15 mg/kg

Table 2: Pfizer Amboise and RITA (Registry of Industrial Toxicology Animal Data) Historical Data Used for Statistical Analysis of Total Incidence of Hemangiosarcoma and Hemangiosarcoma & Hemangioma Combined in Males in Darifenacin Carcinogenicity Rat Study (Amboise Study No. 93073)

Historical Data	Hemangiosarcoma	Hemangiosarcoma & Hemangioma
Amboise 5 studies 1989-1996* (Data submitted to FDA Dec 2, 2001)	17 / 330 (5.15%) (range 0-0.83%)	10 / 330 (3%) (range 1.8-5%)
RITA (1983-1995)	16 / 1678 (0.95%) (range 0-0.8%)	48 / 1678 (2.86%) (range 0-12.9%)
RITA (1996-2001)	10 / 610 (1.64%) (range 0.8%)	22 / 610 (3.61%) (range 1.66-10%)

* All studies conducted in Amboise during this time period

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this page is the manifestation of the electronic signature.**

/s/

Laurie McLeod
9/17/03 11:19:06 AM
PHARMACOLOGIST

Suzanne Thornton
9/17/03 11:21:54 AM
PHARMACOLOGIST

NDA # 21513

45 Day Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

ITEM	YES	NO	COMMENT
1) Does this action of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	✓		
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	✓		
3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	✓		
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were submitted from the NDA.	✓		

<p>5) Were the studies adequately designed (ie., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?</p>	<p>✓</p>		
<p>6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (ie. Adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?</p>	<p>✓</p>		
<p>7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?</p>	<p>✓</p>		
<p>8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.57? Is information available to express human dose multiples in either mg/m² or comparative serum/plasma AUC levels?</p>	<p>✓</p>		

9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item #10 below why it is not.	✓		
10) Reasons for refusal to file:			

ISI
Reviewing Pharmacologist

1/14/03

ISI
Supervisory Pharmacologist

1/14

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this page is the manifestation of the electronic signature.**

/s/

Laurie McLeod
1/23/03 03:13:20 PM
PHARMACOLOGIST

Alexander W. Jordan
1/23/03 03:19:03 PM
PHARMACOLOGIST

NDA 21-513 Enablex Extended Release Tablets
Darifenacin hydrobromide, 7.5 and 15 mg

Nonclinical Inspection Review Summary

Not applicable to this application.

✓ 15/11

9/3/03

Statistical Review and Evaluation
(Carcinogenicity Studies)

IND No.: 45,457

Applicant: Pfizer

Name of Drug: Darifenacin

Documents Reviewed:

1. CDER CAC Executive Meeting Minutes on October 24, 2000
2. Historical Control Data submitted by the sponsor, not dated.

Reviewing Pharmacologist: Laurie McLeod, Ph.D., HFD-580

Statistical Reviewer: Karl K. Lin, Ph.D., HFD-715

Summary of Review

The positive trends in total incidence in hemangiosarcoma, and hemangiosarcoma +hemangioma in male rats are tested by the Cochran-Armitage test without and with the incorporation of the RITA historical control data submitted by the sponsor. The analysis results show that the positive trends in total incidence in hemangiosarcoma, and hemangiosarcoma +hemangioma are statistically significant in both analyses without and with incorporation of the historical control data. The analysis without the incorporation of the historical control data also shows that the positive trend in total incidence in hemangiosarcoma +hemangioma+hemangiopericytoma in male rats is statistically significant.

1. Introduction

There are two carcinogenicity studies, one in mice and one in rats, included in this IND submission. Two control groups and three treated groups were used in the two studies. 50 animals were used in each group/sex. The doses chosen for the mouse carcinogenicity study were 0, 0, 3, 20, and 100 mg/kg/day, and the dose groups chosen for the carcinogenicity study were 0, 0, 1.5, 5, and 15 mg/kg/day.

CDER Executive Carcinogenicity Assessment Committee (CAC) discussed the results of the two carcinogenicity studies on October 24, 2000 without a statistical review. It was concluded by CDER Executive CAC that the designs of the two studies were valid, and that there were no positive tumor findings in the mouse study. However, the Committee felt that there were few

positive findings in combined tumor incidence rate in hemangiosarcoma, hemangioma, and hemangiopericytoma in various tissues in male rats, and that historical control data should be requested from the sponsor for statistical review.

Dr. Laurie McLeod of HFD-580, reviewing pharmacologist of this IND, has asked Division of Biometrics II to perform a statistical analysis of the above tumor types with the incorporation of the large amount of historical control data submitted by the sponsor.

2. Reviewer's Analysis

Tumor data of hemangiosarcoma, hemangioma, and hemangiopericytoma in various tissues of the current rat study are included in Table 1 below. The data of female rats are not analyzed since there was only one female rat developed hemangioma in spleen.

Table 1
Tumor Incidence Rates of Hemangiosarcoma, Hemangioma,
and Hemangiopericytoma of the Rat study

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	1.5	5	15	0	0	1.5	5	15
Spleen										
B-hemangioma	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/50
M-hemangiosarcoma	0/50	0/50	0/50	0/50	2/50	0/50	0/50	0/50	0/50	0/50
Kidney										
M-hemangiosarcoma	1/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50	0/50
Skin and adnexa										
B-hemangiopericytoma	0/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50	0/50
N-hemangiosarcoma	0/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50	0/50
Total hemangiosarcoma	1/50	0/50	0/50	0/50	4/50	0/50	0/50	0/50	0/50	0/50
Total hemangioma	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/50
Total hemangioma plus hemangiosarcoma *	1/50	0/50	0/50	0/50	4/50	0/50	0/50	0/50	0/50	1/50
Total hemangioma, hemangiosarcoma, or hemangiopericytoma	1/50	0/50	0/50	0/50	5/50	0/50	0/50	0/50	0/50	1/50

* all tumors represent separate animals

2.1 Statistical Analysis Without Incorporation of Historical Control Data

The survival-unadjusted Cochran-Armitage trend test using the doses as weights (also called permutation trend test) is used in the analysis of the total tumor incidence data for two reasons. The first reason is that there is no electronic data set available to this reviewer to perform more

complicated survival-adjusted analysis. The second reason is that, according to the reviewing pharmacologist, the survivals are not significantly different among the treatment groups.

Results of the Cochran-Armitage trend tests on the total incidences of hemangiosarcoma, and of hemangiosarcoma and hemangioma combined in Table 2 (both have the same total incidences since there is no male rats developed hemangioma). The results show that there is a statistically significant trend in the total incidences of hemangiosarcoma, and of hemangiosarcoma and hemangioma combined in male rats (asymptotic $p = 0.0011$ and exact $p = 0.0062$).

The results of the Cochran-Armitage trend test on the total incidence rates of hemangiosarcoma, hemangioma, and hemangiopericytoma combined are included in Table 3. The results show that there is a statistically significant trend in the total incidences of hemangiosarcoma, hemangioma, and hemangiopericytoma combined in male rats (asymptotic $p = 0.0002$ and exact $p = 0.0014$).

Table 2

Results of Statistical Analysis of Total Incidences of Hemangiosarcoma
(or Hemangiosarcoma and Hemangioma Combined) in Male Rats

Min	Max	Mean	Std-dev	Observed
Standardized				
0.0000	75.00	21.50	12.54	60.00
3.070				
Asymptotic Inference:				
One-sided p-value:	Pr {	Test Statistic	.GE. Observed	= 0.0011
Two-sided p-value:	2 * One-sided			= 0.0021
Exact Inference:				
One-sided p-value:	Pr {	Test Statistic	.GE. Observed	= 0.0062
	Pr {	Test Statistic	.EQ. Observed	= 0.0029
Two-sided p-value:	Pr {	Test Statistic - Mean	.GE. Observed - Mean	= 0.0062
Two-sided p-value:	2*One-Sided			= 0.0123

Table 3

Results of Statistical Analysis of Total Incidences of Hemangiosarcoma, Hemangioma, and Hemangiopericytoma Combined in Male Rats

```
[ 1 2 by 5 tables and sum of scores from row <row1 > ]
Summary of Exact distribution of PERMUTATION statistic:
      Min      Max      Mean      Std-dev      Observed
Standardized
      0.0000      90.00      25.80      13.71      75.00
3.589
Asymptotic Inference:
One-sided p-value: Pr { Test Statistic .GE. Observed } = 0.0002
Two-sided p-value: 2 * One-sided = 0.0003
Exact Inference:
One-sided p-value: Pr { Test Statistic .GE. Observed } = 0.0014
                  Pr { Test Statistic .EQ. Observed } = 0.0007
Two-sided p-value: Pr { | Test Statistic - Mean |
                  .GE. | Observed - Mean | } = 0.0014
Two-sided p-value: 2*One-Sided = 0.0028
```

2.2 Statistical Analysis Incorporating Historical Control Data

The sponsor examined the RITA database (Registry of Industrial Toxicology Animal data) of the Fraunhofer Institute in Hanover, Germany, and submitted historical control data from 30 carcinogenicity studies using Sprague Dawley rats conducted during the period 1983-1998. The duration of these studies ranges from 24 to 30 months. The historical control data of hemangiosarcoma and hemangioma from Rita database are included in Table 4.

The statistical procedure described in Tarone (1982) is used in the analysis of the total incidence rates of hemangioma and hemangiosarcoma data of male rats with the use of the RITA historical control data submitted by the sponsor.

For a given experiment, the number of animals that develop a tumor in the control group follows the following binomial distribution with parameter p.

$$f(x) = \binom{n}{x} p^x (1-p)^{n-x} \quad \text{for } x = 0, 1, \dots, n.$$

p is the true spontaneous rate of the tumor, n is the total animals in the control group, and x is the number of animals in the control group developed the tumor.

It is proposed in the paper that the following beta distribution be used to model the distribution of the spontaneous tumor rate p of the control group that varies from experiment to experiment.

$$g(p) = \{\Gamma(\alpha+\beta)/\Gamma(\alpha)\Gamma(\beta)\} p^{\alpha-1} (1-p)^{\beta-1}, \quad \text{for } 0 < p < 1.$$

The mean and variance of the beta distribution are

$$E(p) = \alpha / (\alpha + \beta) \quad \text{and}$$

$$V(p) = (\alpha\beta) / \{(\alpha+\beta)^2(\alpha+\beta+1)\}.$$

The unknown parameters α and β are estimated by the method of moments, i.e., by equating the population mean and variance with the sample mean and variance and solving the two equations to find the estimates of α and β .

The summary statistics of total tumor incidence rates of hemangiosarcoma, and hemangiosarcoma and hemangioma combined of the RITA historical control data are included in Tables 5 and 6. The summary statistics are needed for estimating the unknown parameters α and β .

As mentioned above, the parameters α and β of the beta distribution are estimated by the method of moments. The beta distribution for modeling the spontaneous rate of hemangiosarcoma is determined by solving the following system of two equations simultaneously using the observed mean and variance from the historical data in Table 5.

$$\alpha/(\alpha+\beta) = 0.0284$$

$$(\alpha\beta)/\{(\alpha+\beta)^2(\alpha+\beta+1)\} = 0.00102.$$

The solution to the system of equations is $\alpha = 0.74$ and $\beta = 25.29$.

The beta distribution for modeling the spontaneous rate of hemangiosarcoma and hemangioma combined is determined by solving the following system of two equations simultaneously using the observed mean and variance from the historical data in Table 6.

$$\alpha/(\alpha+\beta) = 0.0097$$

$$(\alpha\beta)/\{(\alpha+\beta)^2(\alpha+\beta+1)\} = 0.0003.$$

The solution to the system of equations is $\alpha = 0.31$ and $\beta = 31.26$.

The positive trend in tumor incidence with the incorporation of historical control data is tested by the following statistic

$$\chi^2 = (\sum x_i d_i - p \cdot \sum n_i d_i)^2 / \{p \cdot q \cdot [\sum n_i d_i^2 - (\sum x_i d_i)^2 / n]\},$$

where $n = n_1 + \alpha + \beta$, $p' = (x_1 + \alpha) / n$, $q' = 1 - p'$, $n_i = \sum n_i$, and $x_i = \sum x_i$. The summation is from zero to r when there are $r + 1$ treatment groups in an experiment.

The test statistic χ^2 is distributed asymptotically as a chi square random variable with one degree of freedom.

Put the tumor data from the current study in Table 1 and the estimated values of α and β using the historical control data into the above equation, we get $\chi^2 = 10.004$ for hemangiosarcoma, and $\chi^2 = 8.085$. The p-values in both cases are less than 0.005.

The results of the asymptotic tests for positive trend incorporating the historical control data submitted by the sponsor show that positive trends in total incidence of hemangiosarcoma alone, and hemangiosarcoma and hemangioma combined are statistically significant in male rats.

No exact version of the above Tarone test for trend in tumor incidence is available. There are no exact p-values for comparison with the above asymptotic p-values. It is expected that if there are exact test methods available, the exact p-values may be a little bit larger.

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Table 4

RITA Historical Control Data of Hemangiosarcoma and Hemangioma of Male Rats

Male Rats										
		Kidneys	Liver		Lymph node mesenteric		Skin/Subcutaneous Tissue		Spleen	
Study #	Total Examined	Haemangioma	Haemangioma	Haemangiosarcoma	Haemangioma	Haemangiosarcoma	Haemangioma	Haemangiosarcoma	Haemangioma	Haemangiosarcoma
1	69	0	0	0	0	0	0	1	0	0
10	50	0	0	0	3	0	0	0	0	0
14	50	0	0	0	0	0	0	0	0	0
15	50	0	0	0	0	0	0	0	0	0
16	70	0	0	0	2	0	0	0	0	0
18	70	0	0	0	5	1	1	0	2	0
19	69	0	0	0	0	1	0	0	1	0
24	50	0	0	0	0	0	0	0	0	0
29	50	0	0	0	0	0	0	0	0	0
35	70	0	0	0	0	0	0	0	0	0
40	50	0	0	0	0	0	0	0	0	0
42	50	0	0	0	0	0	0	0	0	0
45	55	0	0	0	1	0	0	0	0	0
46	50	0	0	0	0	0	0	2	0	0
47	71	0	0	0	0	0	0	0	0	0
48	50	1	0	0	0	0	0	0	0	1
50	55	0	0	0	2	0	0	0	0	0
52	50	0	0	0	0	0	0	0	0	0
57	50	0	0	0	3	0	0	0	0	0
58	50	0	1	0	1	0	0	1	1	0
62	60	0	0	0	0	0	0	0	0	0
68	50	0	0	0	2	0	0	0	0	0
70	48	1	0	0	0	0	0	0	0	0
96	60	0	0	0	0	1	0	1	0	0
100	50	0	0	0	0	0	0	0	0	1
101	55	0	0	0	1	0	0	0	0	0
102	55	0	0	0	1	0	0	0	0	0
106	60	0	0	1	0	0	0	0	0	0
111	60	0	0	0	1	0	0	0	1	1
125	50	0	0	0	1	1	0	0	0	3
					23	4	1	5	5	6

Table 5

Summary Statistics of Total Incidence Rates of Hemangiosarcoma
Of the RITA Historical Control Data

<i>Hemansarcoma</i>	
Mean	0.009664596
Standard Error	0.003160223
Median	0
Mode	0
Standard Deviation	0.017309252
Sample Variance	0.00029961
Kurtosis	8.873692944
Skewness	2.68080227
Range	0.08
Minimum	0
Maximum	0.08
Sum	0.289937888
Count	30
Confidence Level(95.0%)	0.006463384

Table 6

Summary Statistics of Total Incidence Rates of Hemangiosarcoma
And Hemangioma Combined of the RITA Historical Control Data

<i>Hemangiosarcoma+Hemangioma</i>	
Mean	0.028412118
Standard Error	0.005834563
Median	0.019090909
Mode	0
Standard Deviation	0.031957219
Sample Variance	0.001021264
Kurtosis	2.473252257
Skewness	1.527590491
Range	0.128571429
Minimum	0
Maximum	0.128571429
Sum	0.852363542
Count	30
Confidence Level(95.0%)	0.011933028

3. References

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ISI

Karl K. Lin, Ph.D.
Expert Mathematical Statistician
(Applications in Pharmacology and Toxicology)

ISI

Concur: _____
S. Edward Nevius, Ph.D.
Director, Division of Biometrics II

cc: Original/IND 45,457 File
HFD-580/ L McLeod, A Jordan
HFD-715/E Nevius, K Lin

File: IND45457DarifenacinCarciReviewFinal.doc

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/s/

Karl Lin
4/30/01 03:30:56 PM
BIOMETRICS

S. Edward Nevius
5/2/01 03:04:49 PM
BIOMETRICS
Concur with review.

Amendment to Darifenacin CAC minutes:

IND # 45457

Drug Name: Darifenacin

Sponsor: Pfizer

On October 24, 2000, CAC reviewed the drug Darifenacin, IND#45457. A statistical analysis from historical data was requested for hemangiomas and hemangiosarcomas in Sprague Dawley rats. The statistical review, done by Karl Lin, found positive trends in the total incidence of hemangiosarcoma and hemangiosarcoma-plus-hemangioma in male rats at the highest dose tested, 15 mg/kg/day or 20 times the clinical dose (AUC). In females, the only incidence of hemangioma was in the high dose group at 15 mg/kg/day or 12 times the clinical dose (not statistically significant). There was no effect in male rats at 5 mg/kg/day or 7 times the clinical exposure. Inclusion of historical data did not affect the results. There were no findings in mice at 100 mg/kg/day or greater than 40 times the human exposure (AUC).

As a result of these conclusions, the committee proposed that the following points should be included in the label for Darifenacin:

L

J

|S|

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\

/Division File, HFD 580
/Alex Jordan, Team leader, HFD-580
/Laurie McLeod, Reviewer, HFD-580
/Adele Seifried, HFD-024
/Evelyn Farinas, HFD-580

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Joseph DeGeorge
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