CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-030

PHARMACOLOGY REVIEW(S)

Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research

Date:	September 24, 2008
Reviewer:	Lynnda Reid, Ph.D. Supervisory Pharmacologist
NDA #/SS#/date:	22-030 (N000) May 1, 2008
Sponsor:	Pfizer
Drug Product:	Fesoterodine fumarate (Toviaz®)
Indication:	Overactive bladder
RE:	MHT Labeling Consult

Introduction: This NDA was originally filed on March 17, 2006. It received an approvable action pending satisfactory inspection of the manufacturing facility on January 25, 2007. Labeling negotiations were, for the most part, completed at that time. The submission received on May 1, 2008 contained the Sponsor's final proposed label. A consult was sent to the Maternal Health Team (MHT) on June 27, 2008. The DRUP Pharm/Tox team filed their completed reviews with recommended pregnancy labeling on September 16, 2008. On September 19, 2008, the Division received the MHT review of the Pregnancy section containing labeling recommendations in non-PLR and PLR formats.

The Toviaz labeling agreed to by the Sponsor and DRUP is consistent with other antimuscarinic drug labels. The reproductive and developmental findings for fesoterodine are similar to all other antimuscarinic products. The recommended changes proposed by the MHT would make the Toviaz label significantly different than the other drugs of this class and may unfairly penalize it. In addition, the recommended division of the nonclinical data into summary and detailed observations is considered cumbersome and confusing in the non-PLR labeling format.

Regulatory Action: We recommend that the labeling format proposed by the Sponsor be retained. At the time this label is converted from non-PLR to PLR formatting, we will incorporate the recommended changes as appropriate.

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/s/

Lynnda Reid
9/25/2008 02:58:16 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-030
SERIAL NUMBER:	010
DATE RECEIVED BY CENTER:	01 May 2008
PRODUCT:	Fesoterodine
INTENDED CLINICAL POPULATION:	Men and women with overactive bladder with symptoms of urinary urgency, frequency and/or urge incontinence
SPONSOR:	Schwartz Pharma
DOCUMENTS REVIEWED:	Electronic File
REVIEW DIVISION:	Division of Reproductive and Urologic Products
PHARM/TOX REVIEWER:	Laurie McLeod-Flynn, Ph.D., D.A.B.T.
PHARM/TOX SUPERVISOR:	Lynnda Reid, Ph.D.
DIVISION DIRECTOR:	Scott Monroe, M.D.
PROJECT MANAGER:	Celia Peacock, MPH, RD

Date of review submission to Division File System (DFS): 09/11/08

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: There is no impediment to approval of this NDA from a pharmacology/toxicology perspective

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

C. Recommendations for labeling

Carcinogenesis, Mutagenesis, Impairment of Fertility

No evidence of drug-related carcinogenicity was found in 24-month studies with oral administration to mice and rats. The highest tolerated doses in mice (females 45 to 60 mg/kg/day, males 30 to 45 mg/kg/day) correspond to 11- to 19-fold (females) and 4- to 9-fold (males) the estimated human AUC values reached with fesoterodine 8 mg, which is the Maximum Recommended Human Dose (MRHD). In rats, the highest tolerated dose (45 to 60 mg/kg/day) corresponds to 3- to 8-fold (females) and 3- to 14-fold (males), the estimated human AUC at the MRHD.

Fesoterodine was not mutagenic or genotoxic in vitro (Ames tests, chromosome aberration tests) or in vivo (mouse micronucleus test).

Fesoterodine had no effect on reproductive function, fertility, or early embryonic development of the fetus at non-maternally toxic doses in mice. The maternal No-Observed-Effect Level (NOEL) and the NOEL for effects on reproduction and early embryonic development were both 15 mg/kg/day. Based on AUC, the systemic exposure was 0.6- to 1.5-fold higher in mice than in humans at the MRHD, whereas based on peak plasma concentrations, the exposure in mice was 5- to 9-fold higher. The Lowest-Observed-Effect Level (LOEL) for maternal toxicity was 45 mg/kg/day.

Pregnancy

Pregnancy Category C

Reproduction studies have been performed in mice and rabbits. No dose-related teratogenicity was observed at oral doses up to 75 mg/kg/day in mice (6 to 27 times the expected exposure at the MRHD based on AUC and greater than 77 times the expected C_{max}) and up to 27 mg/kg/day in rabbits (3- to 11- fold by AUC and 19- to 62– fold by C_{max}) or at subcutaneous doses up to 4.5 mg/kg/day in rabbits (9- to 11- fold by AUC and 43 to 56-fold by C_{max}). In mice treated orally with 75 mg/kg/day (6- to 27-times the expected exposure at the MRHD based on AUC and greater than 77-times the expected C_{max}), increased resorptions and decreased live fetuses were observed. One fetus with cleft palate was observed at each dose (15, 45 and 75 mg/kg/day), at an incidence within the background historical range. In rabbits treated orally with 27 mg/kg/day (3 to 11- fold by AUC and 19 to 62- fold by C_{max}), [redacted] incompletely ossified sternebrae (retardation of bone development) were observed in fetuses. In rabbits treated by subcutaneous (sc) administration with 4.5 mg/kg/day (9 to 11- fold by AUC and 43 to

b(4)

53- fold by C_{max}), maternal toxicity and [redacted] incompletely ossified sternebrae were observed in fetuses (at an incidence within the background historical range). At 1.5 mg/kg/day s.c., (3-fold by AUC and 11 to 13- fold by C_{max}), decreased maternal food consumption in the absence of any fetal effects was observed. Oral administration of 30 mg/kg/day fesoterodine to [redacted] mice in a pre- and postnatal development study resulted in decreased body weight of the dams and delayed ear opening of the pups. No effects were noted on mating and reproduction of the F_1 dams [redacted] or on the F_2 offspring. b(4)

There are no adequate and well-controlled studies using Toviaz in pregnant women. Therefore, Toviaz should be used during pregnancy only if the potential benefit outweighs the potential risk to the fetus.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Exaggerated pharmacological effects (including mydriasis, increased heart rate, and neurological effects) were the primary limiting toxicity for both mice and dogs. No treatment related histopathological changes were observed after treatment for 6 months in mice or 9 months in dogs.

Although a clearly defined effect on QT prolongation was not observed in dogs administered oral fesoterodine, effects were observed in dogs exposed intravenously to greater than 10 times the expected clinical exposure.

Fesoterodine was negative for genotoxicity and/or mutagenicity in a battery of *in vitro* and *in vivo* assays.

Two-year carcinogenicity bioassays were conducted in rats and mice up to a maximally tolerated dose of fesoterodine. There was adequate exposure to each of the major human metabolites. No treatment related increases in the type or incidence of neoplastic and/or hyperplastic lesions were observed.

Reproductive toxicology:

In a mouse fertility study (oral), at 45 mg/kg/day, no effect on male fertility or the male reproductive system was observed. In females, numbers of corpora lutea, implantation sites, live fetuses, and uterine weight were decreased at this dose. At 15 mg/kg/day (about equal to the expected clinical exposure), no effects on female fertility, the female reproductive system, or early embryonic development were observed.

In a mouse embryo/fetal study (oral), at 75 mg/kg/day (about 6-30 times the expected clinical exposure of an 8 mg dose via AUC), one dam died, and body weight, gravid uterine weight, and the number of live fetuses were decreased. Resorptions were increased. At 45 mg/kg/day, one dam died, but no effect on body weight was observed.

The number of live fetuses appeared to be decreased, but did not reach statistical significance. Lack of significance for resorptions in the mid dose group may have been due to the decrease in implantation sites seen in this group. At the lowest dose of 15 mg/kg/day (about equal to the expected clinical exposure), the number of resorptions was increased and the number of live fetuses was decreased. In addition, 1 fetus with cleft palate was observed in each of the treated groups, but not in the control group.

In a rabbit embryo/fetal study (oral), at 27 mg/kg/day (about 4-12 times the expected clinical exposure of an 8 mg dose via AUC), one dam died following dosing. Resorptions were increased at this dose and the total number of live fetuses was decreased. No malformations were observed, but the number of fetuses with incompletely ossified sternebrae were increased. At 9 mg/kg/day (about 0.2 times the expected clinical exposure), one dam aborted and was sacrificed. Although, the number of fetuses with incompletely ossified sternebrae appeared to be increased, statistical significance was not reached. A no effect level for maternal and fetal toxicity was not clearly identified in the study.

Although an increase in litter incidence of incompletely ossified sternebrae was not observed due to a high incidence in the control, this effect appears to be dose related and significant when fetal incidence is also considered.

Oral administration	Dams dosage (mg/kg/day)			
	0	3	9	27
Sternebrae incompletely ossified or reduced				
__fetal incidence (percent)	68 (63.1%)	76 (69.1%)	83 (71.6%)	82** (84.5%)
__litter incidence (percent)	16 (80.0%)	18 (94.7%)	18 (94.7%)	19 (100%)

In a rabbit embryo/fetal study (subcutaneous), at 4.5 mg/kg/day by subcutaneous administration (about 10-12 times the expected clinical exposure of an 8 mg dose via AUC of the active entity SPM 7605), mortality was observed in dams in conjunction with clonic convulsions, dyspnea, miosis, and a decrease in body weight and food consumption. No effects on number of corpora lutea, implantation sites, resorptions, placental and fetal weights, or number of live fetuses were observed. No external or skeletal malformations were observed. No treatment related external or skeletal variations were observed. No treatment related skeletal retardations were observed except incomplete ossification of the sternebrae. At 1.5 mg/kg/day (about 3-4 times), no maternal or fetal effects were observed except for a decrease in maternal food consumption. At 0.5 mg/kg/day, no maternal or fetal effects were observed.

Although an increase in litter incidence of incompletely ossified sternebrae was not observed due to a high incidence in the control, this effect appears to be dose related and significant when fetal incidence is also considered.

Oral administration	Dams dosage (mg/kg/day)			
	0	0.5	1.5	4.5
Incomplete ossification of the sternebrae				
__fetal incidence	32	31	33	46*
__litter incidence	14	14	12	16

In a mouse developmental study, at 60 mg/kg/day, one dam was found dead during the lactation period and decreased maternal body weight and food consumption were observed. Decreased litter weight and developmental delay (time to ear opening) were observed in the F1 generation. At 30 mg/kg/day, decreased maternal body weight was observed. A decrease in litter weight did not reach statistical significance at this dose, but developmental delay (time to ear opening) and increased activity level (not significant, but present at 60 mg/kg/day) were observed in the F1 generation. At 10 mg/kg/day, no effects on dams or the F1 generation were observed. No effects on reproductive performance of the F1 generation were observed nor any effects on the F2 generation, at any dose.

There was adequate exposure to each of the major human metabolites in reproductive studies.

B. Pharmacologic activity

Fesoterodine and its hydrolysis product SPM 7605 are specific but non-selective muscarinic receptor antagonists. In vivo, fesoterodine is rapidly and extensively metabolized to SPM 7605, which is much more inhibitory at muscarinic receptors than fesoterodine. SPM 5509, another major human metabolite is far less potent than SPM 7605. The pharmacology of the metabolites SPM 7789 and SPM 7790 were not studied.

C. Nonclinical safety issues relevant to clinical use

Exaggerated pharmacological effects (including mydriasis, increased heart rate, and neurological effects) were the primary limiting toxicity for both mice and dogs. An increase in QT interval was also observed at high doses in intravenous studies in dogs. The risk of these effects has been evaluated in clinical studies.

Low multiples of the expected clinical exposures were observed for some reproductive effects of fesoterodine in animals (oral studies); however, there is a history of similar effects in animals for anti-muscarinic drugs for overactive bladder, including tolterodine which produces the same primary active metabolite as fesoterodine. Several of the effects reported in animals, such as cleft palate in mice, are reported to be associated with stress during the gestational period. Although fesoterodine is not used at doses which are expected to cause stress in humans, labeling should recommend that it should not be used during pregnancy unless the potential benefit justifies the potential risk to the fetus. b(4)

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

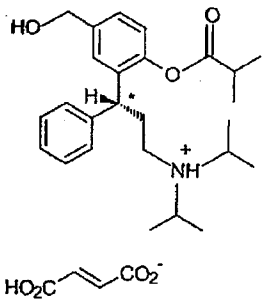
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22030
Review number: 2
Sequence number/date/type of submission: 010 / 01 May 2008
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Schwartz Pharma
Manufacturer for drug substance: Schwartz Pharma

Reviewer name: Laurie McLeod-Flynn
Division name: Division of Reproductive and Urologic Products
HFD #: 580
Review completion date: 9/9/08

Drug:

Trade name: Toviaz
Generic name: Fesoterodine
Code name: SPM 8272
Chemical name: 2-((R(+))-3-diisopropylamino-1-phenylpropyl)-4-(hydroxymethyl)-phenylester hydrogen fumarate
Molecular formula/molecular weight: $C_{30}H_{41}NO_7$ / 527.66
Structure:



Relevant INDs/NDAs/DMFs: IND # 51232

Drug class: anti-muscarinic

Indication: overactive bladder with symptoms of urinary urgency, frequency and/or urge incontinence

Clinical formulation: 4- and 8-mg sustained-release tablets

Route of administration: oral

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: There is no impediment to approval of this submission from a pharmacology/toxicology perspective.

Unresolved toxicology issues: No issues are considered to be unresolved.

Recommendations: There are no recommendations for further nonclinical studies.

Suggested labeling: see detailed suggestions on page 3 (Executive Summary).

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/s/

Laurie McLeod
9/16/2008 09:33:35 AM
PHARMACOLOGIST

Lynnda Reid
9/16/2008 11:44:50 AM
PHARMACOLOGIST

SUPERVISORY MEMO 2 FOOD AND DRUG ADMINISTRATION

Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research

Date:	September 15, 2008
Reviewer:	Lynnda Reid, Ph.D. Supervisory Pharmacologist
NDA #/SS#/date:	22-030 (N010) May 1, 2008
Sponsor:	Schwartz Pharma
Drug Product:	Fesoterodine fumarate (Toviaz®)
Indication:	Overactive bladder
Recommended Action:	Approval

Drug History: Fesoterodine fumarate, hereafter referred to as fesoterodine, is a new molecular entity being developed for the treatment of overactive bladder (OAB) with symptoms of urge urinary incontinence, urgency and urinary frequency. Fesoterodine is a muscarinic receptor antagonist and belongs to the antimuscarinic class of agents. Antimuscarinic drugs act by antagonizing the acetylcholine-induced stimulation of postganglionic muscarinic receptors. In the bladder, muscarinic receptors are thought to mediate the detrusor contractions responsible for normal voiding and the primary portion of the contraction in OAB associated with urgency and urge incontinence.

NDA 22-030 was initially filed on March 17, 2006. The original nonclinical data was reviewed by Dr. Laurie McLeod-Flynn. All required nonclinical studies were submitted including subchronic toxicology studies in mice, rats and dogs, 6 and 9 month chronic toxicology studies in mice and dogs, respectively, reproductive and developmental studies in mice and rabbits, full battery of genotoxicity studies, 2-year carcinogenicity studies in mice and rats, evaluation of skin and eye irritation potential, and in vitro assessment of phototoxicity.

The original NDA received an approvable action pending satisfactory inspection of the manufacturing facility. Supplement N010 contains no new nonclinical data and the original recommendation that the nonclinical data supported an approval still stands.

I concur with the labeling recommendations made by Dr. McLeod-Flynn in her review filed September 16, 2008.

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/s/

Lynnda Reid
9/16/2008 11:47:39 AM
PHARMACOLOGIST

Comments on NDA 22-030 Fesoterodine fumarate
From A. Jacobs 9/11/08

I concur that there are no outstanding pharm/tox issues.

I concur with the proposed pregnancy category: C

Perhaps concurrence with the carcinogenicity results (no drug-related effects) by the exec-cac should be referred to somewhere.

I have discussed this with the pharm/tox reviewer and supervisor.

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/s/

Abby Jacobs
9/11/2008 08:10:18 AM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY RESUBMISSION MEMO

NDA Number: 22030 **Applicant: Pfizer** **Stamp Date: 19 May 2008**

Drug Name: Toviaz **NDA Type: resubmission**

	Content Parameter	Yes	No	Comment
1	On its face, is the pharmacology/toxicology section of the NDA organized (in accord with 21 CFR 314 and current guidelines for format and content) in a manner to allow substantive review to begin?			NA
2	Is the pharmacology/toxicology section of the NDA indexed and paginated in a manner allowing substantive review to begin?			NA
3	On its face, is the pharmacology/ toxicology section of the NDA legible so that substantive review can begin?			NA
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted in this NDA (carcinogenicity, mutagenicity*, teratogenicity*, effects on fertility, juvenile studies, acute and repeat dose adult animal studies*, animal ADME studies, safety pharmacology, etc)?	X		The Pharmacology/Toxicology review is in DFS. There are no new issues from a P/T perspective.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	On its face, does the route of administration used in the animal	X		

	studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted a rationale to justify the alternative route?			
7	Has the sponsor submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions with the sponsor?			NA
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	If there are any impurity – etc. issues, have these been addressed? (New toxicity studies may not be needed.)			NA
11	Has the sponsor addressed any abuse potential issues in the submission?			NA
12	If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted?			NA

Any Additional Comments:

This will be a labeling review from a P/T perspective, unless a new safety issue is identified by the review team during the course of an NDA review.

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/s/

Laurie McLeod
6/6/2008 04:44:44 PM
PHARMACOLOGIST

Lynnda Reid
6/9/2008 11:17:28 AM
PHARMACOLOGIST

Comments on NDA 22-030 Fesoterodine fumarate
From A. Jacobs 1/22/07

I concur that there are no outstanding pharm/tox issues.

I concur with the proposed pregnancy category: C
In the labeling under pregnancy, consideration should be given to not including the increased incidences of fetuses with delayed ossification, since the incidence of litters (the preferred comparator) with delayed of ossification is not significantly increased.

I have discussed this with the pharm/tox supervisor.

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/s/

Abby Jacobs
1/22/2007 03:24:00 PM
PHARMACOLOGIST

SUPERVISORY MEMO FOOD AND DRUG ADMINISTRATION

Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research

Date: 12/20/06

Reviewer: Lynnda Reid, Ph.D.
Supervisory Pharmacologist

NDA #/SS#/date: 22-030 (N000) dated March 17, 2006

Sponsor: Schwartz Pharma

Drug Product: Fesoterodine fumarate

Indication: Overactive bladder

Drug History: Fesoterodine fumarate, hereafter referred to as fesoterodine, is a new molecular entity being developed for the treatment of overactive bladder (OAB) with symptoms of urge urinary incontinence, urgency and urinary frequency. Fesoterodine is a muscarinic receptor antagonist and belongs to the antimuscarinic class of agents. Antimuscarinic drugs act by antagonizing the acetylcholine-induced stimulation of postganglionic muscarinic receptors. In the bladder, muscarinic receptors are thought to mediate the detrusor contractions responsible for normal voiding and the primary portion of the contraction in OAB associated with urgency and urge incontinence.

Fesoterodine is hydrolyzed by nonspecific plasma esterases to the active phenol derivative SPM 7605, which is chemically identical to an active metabolite of tolterodine (the active component of Detrol, approved for OAB in 1998). Fesoterodine and SPM 7605 both show potent specific, but non-subtype selective, antimuscarinic properties.

Fesoterodine will be available as a sustained release (SR) tablet formulation based on a [redacted] containing 4 mg or 8 mg of the active fesoterodine fumarate, intended for once-daily oral administration. All inactive excipients are compendial. b(4)

Nonclinical data supporting approval of NDA 22-030: The nonclinical data submitted to support development under IND 51,232 and submission of NDA 22-030 were reviewed in detail by Dr. Laurie McLeod-Flynn. Studies included subchronic toxicology studies in mice, rats and dogs; 6 and 9 month chronic toxicology studies in mice and dogs, respectively; reproductive and developmental studies in mice and rabbits; full battery of genotoxicity studies; 2-year carcinogenicity studies in mice and rats; evaluation of skin and eye irritation potential; and in vitro assessment of phototoxicity. Dose

limiting findings in animals were related to exaggerated pharmacologic effects including mydriasis, increased heart rate, and neurological effects (e.g., ataxia, dyspnea).

Toxicology Studies: In single dose toxicology studies in mice and rats, the NOEL (no observed effect level) doses were 100 mg/kg following oral administration and 10 mg/kg following intravenous administration in both species. Toxicokinetics were not performed, but based on body surface area, the oral doses are approximately 60 and 120 fold higher, and the i.v. doses are approximately 6 and 12 fold higher in mice and rats, respectively, compared to the MRHD (maximum recommended human dose of 8 mg/day).

Mice dosed subchronically for 13 weeks at oral doses of 5 to 125 mg/kg/day, and chronically for 6 months at doses up to 100 mg/kg/day in males and 125 mg/kg/day in females evinced piloerection starting at 75 mg/kg. Dose-related changes in body weight gain were observed at all doses (≥5 mg/kg): primarily decreased weight gain in males and increased weight gain in females. At the highest doses, small but statistically significant changes were observed in triglyceride (↓) and urea (↑) levels in male mice, glucose levels (↑) in female mice, and platelet counts (↓) in both sexes. Exposures at the highest doses tested were approximately 85 fold higher in male mice and 185 fold higher in female mice than the expected exposures at the MRHD (based on mean SPM 7605 AUC values of ~ 45 ng·ml/hr in normal patients). When compared to exposure levels in poor metabolizers and patients with moderate hepatic impairment, exposures in mice were approximately 43 and 29 fold higher in male mice, and 94 and 63 fold higher in female mice, respectively (based on mean AUC values of ~ 89 ng·ml/hr in poor metabolizers and ~132 ng·ml/hr in patients with moderate hepatic impairment).

In rats dosed subchronically for 13 weeks at oral doses of 5 to 75 mg/kg/day, the following effects were observed: ALT, AST, triglycerides, total bilirubin, cholesterol and alkaline phosphatase were increased at ≥25 mg/kg/day and correlated with increased liver weights and morphological changes (pericholangitis with mild bile duct proliferation). Urinalysis showed significantly increased pH values in both sexes at 75 mg/kg and increased urine volume and decreased specific gravity in females. Systemic exposures in rats at the highest dose tested ranged from 9 to 14 fold higher than in normal patients, 4.5 to 7 fold higher than in poor metabolizers and 3 to 5 fold higher than in patients with moderate hepatic impairment. The NOAEL in rats was approximately equivalent to the MRHD in normal males and approximately 3 fold less than the MRHD in normal females.

Subacute dose range-finding studies (3 days) were performed in dogs at oral doses of 1, 3, 10 and 30 mg/kg. The NOAEL was determined to be 3 mg/kg/day while 30 mg/kg exceeded the MTD (maximum tolerated dose) as defined by ataxia, reduced motility, severe conjunctivitis and pale gingival, and decreased weight and food consumption. At ≥10 mg/kg, changes in hematology and chemistry parameters were observed consisting of increases in red and white cell parameters, platelets, bilirubin, triglycerides, inorganic phosphate, ALT and LDH. In the chronic 9-month study in dogs performed at doses of 0.5, 2.5 and 12.5 mg/kg/day, the NOAEL was 0.5 mg/kg/day, a dose approximately 4 to 6

fold lower than the MRHD. Adverse effects seen at ≥2.5 mg/kg/day consisted of increased heart rates 4 hours post dosing, sometimes accompanied by an increase in systolic blood pressure; sporadic changes in urea (↑ in males), creatinine (↓), and α2-glubulin (↑); and decreases in gall bladder contractility. Conjunctivitis, occasionally accompanied by adhesions of the eyelid, was observed in all high-dose animals due to decreased lacrimal secretion. Treatment with an artificial lacrimal fluid was effective in treating this response and examination of retinal tissue revealed no histopathological changes. Exposures in dogs (AUC of fesoterodine + SPM 7605) at the highest dose tested are approximately 80, 40 and 25 fold higher than exposures at the MRHD in normal metabolizers, poor metabolizers and in patients with moderate hepatic impairment, respectively.

In acute local irritation studies, fesoterodine was classified as an ocular irritant but not a skin irritant. Fesoterodine produced no signs of sensitization in guinea pigs or effects on the immune system as tested in chronic toxicology studies and in the plaque forming colony assay in mice. SPM-7605 exhibited no signs of phototoxicity in vitro.

Genotoxicity and Carcinogenicity: Fesoterodine was negative in the standard battery of genotoxicity studies. In 2-year carcinogenicity studies in mice and rats conducted with doses of 5, 15 or 45 mg/kg/day, there were no treatment-related increases in the type or incidence of neoplastic and/or hyperplastic lesions.

Reproductive and Developmental Toxicity: Fesoterodine was tested for effects on all stages of reproduction in mice, and for embryonic and teratogenic effects in rabbits. Administration of fesoterodine to male and female mice prior to mating resulted in no effects on fertility in males at 45 mg/kg/day (the highest dose tested) and in females at 15 mg/kg/day (a dose approximately equivalent to the MRHD for normal metabolizers). There were also no effects on reproductive performance or fertility in offspring exposed in utero to doses up 60 mg/kg/day. Female mice exposed to 45 mg/kg/day prior to mating and through implantation had decreased numbers of corpora lutea, implantation sties and live fetuses.

Female mice exposed during the period of organogenesis to doses of 15, 45 or 75 mg/kg/day demonstrated increased incidences of pre- and post-implantation loss including dose-related increase in resorptions, and dose-related increase in the number of live fetuses per litter. Fetal body weights were reduced starting at 45 mg/kg. There was also one fetus in each of the dosing groups with cleft palate. A pattern of increased resorptions and cleft palate is sometimes associated with maternal stress and has been seen with other muscarinic antagonists. In the multigenerational study in female mice exposed from implantation through weaning to doses of 10, 30 and 60 mg/kg/day, no incidences of cleft palate were observed. Offspring exposed in utero to 60 mg/kg/day had slight delays developmental parameters, i.e., ear opening, auditory startle reflex, passive avoidance response, mid-air righting reflex (also observed in the 30 mg/kg group), and open field test (also observed in the 30 mg/kg group). There was a slight decrease in neonatal survival starting at 30 mg/kg. The NOAEL for developmental toxicity was 10 mg/kg/day.

Female rabbits were exposed during the period of organogenesis to oral doses of 3, 9 or 27 mg/kg/day and subcutaneous doses of 0.5, 1.5 and 4.5 mg/kg. Systemic exposures were approximately the same at the 27 mg/kg/day p.o. dose and the 4.5 mg/kg/day s.c. dose (approximately 10 times the MRHD). Following oral administration, dose-related increases in the incidence of post-implantation loss, early resorptions, and incomplete ossification of sternebrae were observed and a NOAEL was not defined. Although the subcutaneous route was significantly more toxic to the dams, with deaths, clonic convulsions, dyspnea, miosis and deceased body weight and food consumption observed at 4.5 mg/kg, the only fetal effect at this dose was incomplete ossification of the sternebrae. The developmental NOAEL was 1.5 mg/kg (approximately 3-4 times the MRHD) following s.c. administration.

Conclusion/Recommendation: Nonclinical studies were limited by the exaggerated antimuscarinic pharmacological effects of fesoterodine, i.e., reduced GI motility, reduced secretion of the lacrimal gland, mydriasis, conjunctivitis, negative papillary reflex and changes in heart rate. Similar effects are seen with other muscarinic antagonists approved for the treatment of overactive bladder, and all effects are reversible upon cessation of treatment. Mice and dogs were considered more relevant species since there were a number of metabolites in rats which were not observed in mice, dogs or humans. From a pharmacology/toxicology perspective, the drug appears well characterized and there are no nonclinical findings which would indicate that this drug should not be approved for the chronic treatment of overactive bladder in adults.

Labeling: I concur with the recommended labeling changes purposed by the primary reviewer, Dr. McLeod-Flynn. I concur with labeling under pregnancy category C due to drug-related adverse effects observed on fetal viability in both mice and rabbits, but no definitive frank teratogenicity, and delayed development in offspring exposed in utero.

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/s/

Lynnda Reid
12/20/2006 04:16:18 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-030
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	3/17/06
PRODUCT:	Fesoterodine
INTENDED CLINICAL POPULATION:	Men and women with overactive bladder with symptoms of urinary urgency, frequency and/or urge incontinence
SPONSOR:	Schwartz Pharma
DOCUMENTS REVIEWED:	Electronic File
REVIEW DIVISION:	Division of Reproductive and Urologic Products
PHARM/TOX REVIEWER:	Laurie McLeod-Flynn
PHARM/TOX SUPERVISOR:	Lynnda Reid
DIVISION DIRECTOR:	Daniel Shames
PROJECT MANAGER:	Jean Makie

Date of review submission to Division File System (DFS): 11 December 2006

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: There is no impediment to approval of this NDA from a pharmacology/toxicology perspective

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

C. Recommendations on labeling

Recommended changes:

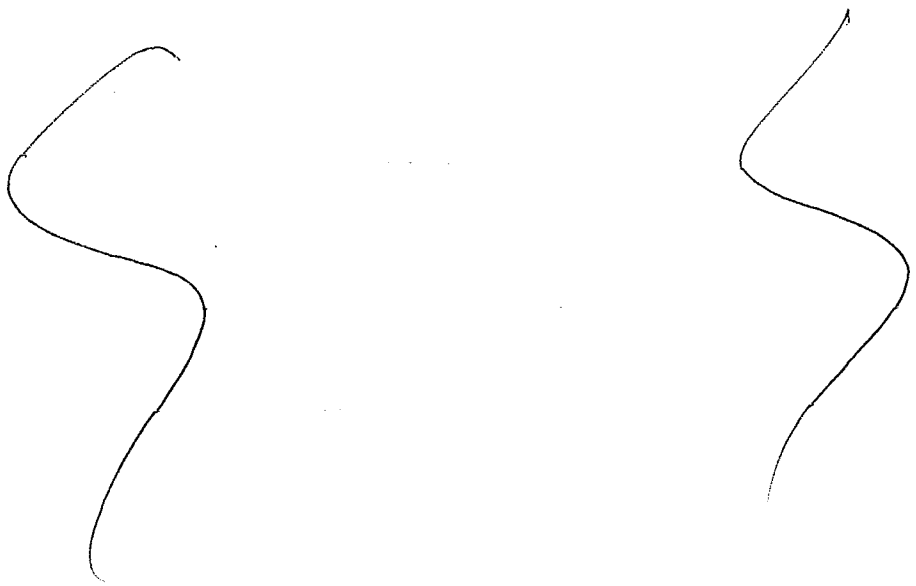
Carcinogenesis, Mutagenesis, Impairment of Fertility

b(4)

Pregnancy

Pregnancy Category C

b(4)



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Nursing Mothers

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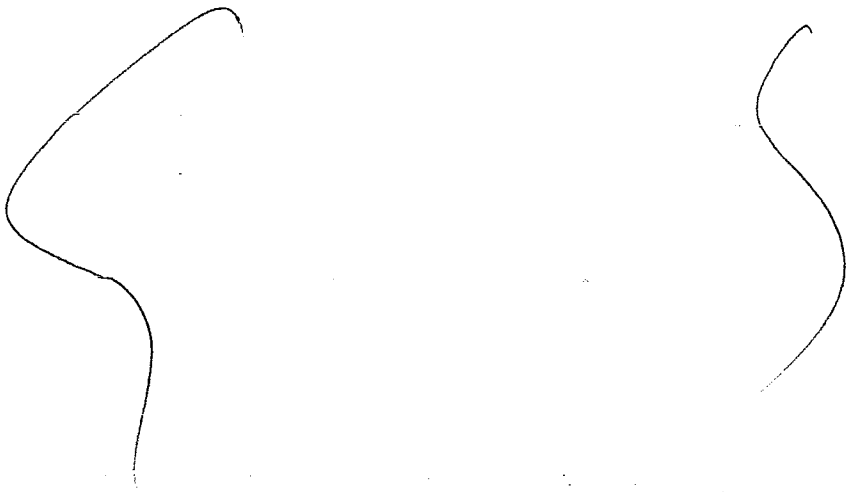
Carcinogenesis, Mutagenesis, Impairment of Fertility



b(4)

Pregnancy

Pregnancy Category C

b(4)

Nursing Mothers

b(4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Exaggerated pharmacological effects (including mydriasis, increased heart rate, and neurological effects) were the primary limiting toxicity for both mice and dogs. No treatment related histopathological changes were observed after treatment for 6 months in mice or 9 months in dogs.

Although a clearly defined effect on QT prolongation was not observed in dogs administered oral fesoterodine, effects were observed in dogs exposed intravenously to greater than 10 times the expected clinical exposure.

Fesoterodine was negative for genotoxicity and/or mutagenicity in a battery of *in vitro* and *in vivo* assays.

Two-year carcinogenicity bioassays were conducted in rats and mice up to a maximally tolerated dose of fesoterodine. There was adequate exposure to each of the major human metabolites. No treatment related increases in the type or incidence of neoplastic and/or hyperplastic lesions were observed.

Reproductive toxicology:

In a mouse fertility study (oral), at 45 mg/kg/day, no effect on male fertility or the male reproductive system was observed. In females, numbers of corpora lutea, implantation sites, live fetuses, and uterine weight were decreased at this dose. At 15 mg/kg/day (about equal to the expected clinical exposure), no effects on female fertility, the female reproductive system, or early embryonic development were observed.

In a mouse embryo/fetal study (oral), at 75 mg/kg/day (about 6-30 times the expected clinical exposure of an 8 mg dose via AUC), one dam died, and body weight, gravid uterine weight, and the number of live fetuses were decreased. Resorptions were increased. At 45 mg/kg/day, one dam died, but no effect on body weight was observed. The number of live fetuses appeared to be decreased, but did not reach statistical significance. Lack of significance for resorptions in the mid dose group may have been due to the decrease in implantation sites seen in this group. At the lowest dose of 15 mg/kg/day (about equal to the expected clinical exposure), the number of resorptions was increased and the number of live fetuses was decreased. In addition, 1 fetus with cleft palate was observed in each of the treated groups, but not in the control group. As any of these effects may be considered a symptom of maternal stress, it should be considered that no NoAEL was observed for maternal or fetal effects in the study.

In a rabbit embryo/fetal study (oral), at 27 mg/kg/day (about 4-12 times the expected clinical exposure of an 8 mg dose via AUC), one dam died following dosing. Resorptions were increased at this dose and the total number of live fetuses was decreased. No malformations were observed, but the number of fetuses with incompletely ossified sternebrae were increased. At 9 mg/kg/day (about 0.2 times the expected clinical exposure), one dam aborted and was sacrificed. Although, the number of fetuses with incompletely ossified sternebrae appeared to be increased, statistical significance was not reached. A no effect level for maternal and fetal toxicity was not clearly identified in the study.

In a rabbit embryo/fetal study (subcutaneous), at 4.5 mg/kg/day by subcutaneous administration (about 10-12 times the expected clinical exposure of an 8 mg dose via AUC of the active entity SPM 7605), mortality was observed in dams in conjunction with clonic convulsions, dyspnea, miosis, and a decrease in body weight and food consumption. No effects on number of corpora lutea, implantation sites, resorptions, placental and fetal weights, or number of live fetuses were observed. No external or skeletal malformations were observed. No treatment related external or skeletal variations

were observed. No treatment related skeletal retardations were observed except incomplete ossification of the sternebra(e). At 1.5 mg/kg/day (about 3-4 times), no maternal or fetal effects were observed except for a decrease in maternal food consumption. At 0.5 mg/kg/day, no maternal or fetal effects were observed.

In a mouse developmental study, at 60 mg/kg/day, one dam was found dead during the lactation period and decreased maternal body weight and food consumption were observed. Decreased litter weight and developmental delay (time to ear opening) were observed in the F1 generation. At 30 mg/kg/day, decreased maternal body weight was observed. A decrease in litter weight did not reach statistical significance at this dose, but developmental delay (time to ear opening) and increased activity level (not significant, but present at 60 mg/kg/day) were observed in the F1 generation. At 10 mg/kg/day, no effects on dams or the F1 generation were observed. No effects on reproductive performance of the F1 generation were observed nor any effects on the F2 generation, at any dose.

There was adequate exposure to each of the major human metabolites in reproductive studies.

B. Pharmacologic activity

Fesoterodine and its hydrolysis product SPM 7605 are specific but non-selective muscarinic receptor antagonists. In vivo, fesoterodine is rapidly and extensively metabolized to SPM 7605, which is much more inhibitory at muscarinic receptors than fesoterodine. SPM 5509, another major human metabolite is far less potent than SPM 7605. The pharmacology of the metabolites SPM 7789 and SPM 7790 were not studied.

C. Nonclinical safety issues relevant to clinical use

Exaggerated pharmacological effects (including mydriasis, increased heart rate, and neurological effects) were the primary limiting toxicity for both mice and dogs. An increase in QT interval was also observed at high doses in intravenous studies in dogs. The risk of these effects has been evaluated in clinical studies.

Low multiples of the expected clinical exposures were observed for some reproductive effects of fesoterodine in animals (oral studies); however, there is a history of similar effects in animals for anti-muscarinic drugs for overactive bladder, including tolterodine which produces the same primary active metabolite as fesoterodine. Several of the effects reported in animals, such as cleft palate in mice, are reported to be associated with stress during the gestational period. Although fesoterodine is not used at doses which are expected to cause stress in humans, labeling should recommend that it should not be used during pregnancy unless the potential benefit justifies the potential risk to the fetus.

b(4)

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

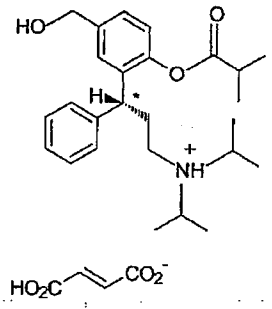
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22030
Review number: 1
Sequence number/date/type of submission: 000/ 17 March 2006 /original submission
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Schwartz Pharma
Manufacturer for drug substance: Schwartz Pharma

Reviewer name: Laurie McLeod-Flynn
Division name: Division of Reproductive and Urologic Products
HFD #: 580
Review completion date: 4 December 2006

Drug:

Trade name: _____
Generic name: Fesoterodine
Code name: SPM 8272
Chemical name: 2-((R(+))-3-diisopropylamino-1-phenylpropyl)-4-(hydroxymethyl)-phenylester hydrogen fumarate
Molecular formula/molecular weight: $C_{30}H_{41}NO_7$ / 527.66
Structure:



Relevant INDs/NDAs/DMFs: IND # 51232

Drug class: anti-muscarinic

Indication: overactive bladder with symptoms of urinary urgency, frequency and/or urge incontinence

Clinical formulation: 4- and 8-mg sustained-release tablets

Route of administration: oral

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

Studies reviewed within this submission:

Neuropharmacological screening of mice according to Irwin following oral administration of SPM 8272
Examination of the influence of SPM 8272 on the spontaneous motility of mice following oral administration
Examination of the influence of SPM 8272 on pentetrazol-induced convulsions in mice following oral administration
Examination of the influence of SPM 8272 on electroshock-induced convulsions in mice following oral administration
Evaluation of an analgesic effect in the phenylbenzoquinone writhing test in the mouse following a single oral administration
Examination of the influence of SPM 8272 on the hexobarbital sleeping time in mice following oral administration
SPM 8272: Evaluation of interactions with hypothermia and ptosis induced by reserpine in the mouse following a single oral administration
Evaluation of effect on the autonomic nervous system following a single oral administration in conscious rats
Evaluation of haemodynamic effects and electrocardiogram following intravenous dosing in the anaesthetized dog
SPM 8272, SPM 7605, and SPM 9078: Evaluation of effect on cardiac action potential in isolated canine Purkinje fibres
Effects of SPM 7605 on cloned hERG channels expressed in mammalian cells
Electrophysiological examination of activity of SPM 7605, SPM 8272, and SPM 9078 on the SCN5A-sodium channel expressed in CHO cells
Examination of SPM 8272, SPM 7065, SPM 9078 of L-type Ca^{2+} inward current in isolated ventricular myocytes of the guinea pig
Evaluation of effect on respiration in the unrestrained conscious rat following oral administration
Examination of the influence of SPM 8272 on the diuresis and saluresis in rats following oral administration
Examination of the influence of SPM 8272 on the intestinal motility following oral administration (charcoal propulsion test in the mouse
Examination of SPM 8272 and SPM 7605 for spasmolytic and spasmogenic properties in the isolated guinea pig ileum
Evaluation of SPM 8272 and SPM 9078 on gastrointestinal transit after oral administration in the mouse

In vitro pharmacology: study of six compounds
In vitro pharmacology: human muscarinic receptors: study of SPM 8272, SPM 8290 and SPM 16086

Determination of dissociation kinetics for SPM 7605 and tiotropium from human recombinant muscarinic receptor subtypes M1-M5 by radioligand displacement analysis
Receptor specificity of SPM 8272 *in vitro*
Receptor specificity of SPM 7605 *in vitro*
Receptor specificity of SPM 9078 *in vitro*
Interaction of SPM 5509, SPM 7605, SPM 8272, and SPM 9078 with cloned human muscarinic acetylcholine receptors: determination of agonistic and antagonistic activities
In vitro and *in vivo* characterization of the effects of fesoterodine (SPM 8272) and of its active metabolite SPM 7605 on rat bladder – comparison with tolterodine, oxybutynin and atropine

6-Month chronic toxicity study of SPM 8272 by oral administration to CD-1 mice
9-Month chronic toxicity study of SPM 8272 by oral administration to Beagle dogs

13-Week subchronic toxicity study of SPM 8272 by oral administration to CD-1 mice with an interim dissection after 2 test weeks
13-week MTD study of SPM 8272 by oral administration to Sprague-Dawley rats
13-week subchronic toxicity study of SPM 8272 by oral administration to Beagle dogs
13-week subchronic toxicity study of SPM 7605 by 24-hr continuous i.v. infusion to Beagle dogs

Mutagenicity study of SPM 8272 in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay (*in vitro*)
Mutagenicity study of SPM 8272 in the *Salmonella typhimurium* reverse mutation assay (*in vitro*)
In vitro assessment of the clastogenic activity of SPM 8272 in V79 cells
In vitro assessment of the clastogenic activity of SPM 8272 in cultured human peripheral lymphocytes
Micronucleus test of SPM 8272 in bone marrow cells of the NMRI mouse by oral administration

104-week carcinogenicity study of SPM 8272 by oral administration to CD rats
104-week carcinogenicity study of SPM 8272 by oral administration to CD-1 mice

Examination of the influence of SPM 8272 on the fertility and early embryonic development to implantation of CD-1 mice by oral administration to the animals of the F0 generation
Study of embryo-fetal development in CD-1 mice with SPM 8272 by oral administration
Study of embryo-fetal development in rabbits with SPM 8272 by oral administration
Study of embryo-fetal development in rabbits with SPM 8272 by subcutaneous administration
Examination of SPM 8272 for effects on the pre- and postnatal development (including maternal function) following oral administration to the dams of CD-1 mice of the F0 Generation

The disposition of total radioactivity in the mouse following single oral and single intravenous administration of [^{14}C] SPM 8272.
The distribution of total radioactivity in the rat following single oral and single intravenous administration of [^{14}C] SPM 8272.
Pharmacokinetic feasibility study with SPM 8272 in the female rabbit.
The distribution of total radioactivity in the dog following single oral and single intravenous administration of [^{14}C] SPM 8272.
Examination of the bioavailability of SPM 8272 in Beagle dogs.
Pharmacokinetic feasibility study with SPM 8272 and/or SPM 7605 in the Beagle dog.
Plasma protein binding of SPM 8272 and SPM 7605 in human, dog, monkey, mouse, rabbit and rat plasma samples.

Evaluation of the *in vivo* metabolism in mice following repeated oral administration of SPM 8272
Evaluation of the *in vivo* metabolism in rats following repeated oral administration of SPM 8272
Evaluation of the *in vivo* metabolism in dogs following oral administration of SPM 8272 and intravenous administration of SPM 8272 or SPM 7605
Evaluation of the in vivo metabolism in rabbits following single and repeated oral or subcutaneous administration of SPM 8272
The metabolic profiling and identification of samples from mouse, rat, and dog studies with (^{14}C) SPM 8272
Metabolism of the ester (SPM 8272)(pro-drug) in Caco-2 cell monolayers grown on flasks
In vitro metabolic profiling study with [^{14}C] SPM 8272 in rat, mouse, dog and human liver microsomes
In vitro metabolic profiling study with SPM 8272 in hamster, rabbit, primate and human liver microsomes
Investigation of the human cytochrome P450 isoforms involved in the metabolism of SPM 7605
Investigation of the cytochrome P450 3A4 induction potential of the compound SPM 8272 in cryopreserved human hepatocytes
Determination of the cytochrome P450 induction potential of fesoterodine in human hepatocytes
SPM 8272: Effect on cytochrome P450 and related parameters in male and female CD-1 mice following oral administration at dose levels of 0, 5, 25, and 75 mg/kg/day (increased to 100 mg/kg/day in males and 125 mg/kg/day in females from week 16) for 6 months
SPM 8272: Effect on cytochrome P450 and related parameters in male and female Beagle dogs following oral administration at dose levels of 0, 0.5, 2.5 and 12.5 mg/kg/day for 9 months
Interaction of the compounds SPM 8272, SPM 7605, SPM 5509, SPM 6923, and SPM 9078 with the cytochrome P450 isoenzymes 1A2, 2C9, 2C19, 2D6, and 3A4

In vitro assessment of the phototoxicity of SPM 7605 in cultured BALB/c 3T3 NRU cells
UV/VIS absorption of SPM 7605

28-day immunotoxicological study of SPM 8272 by repeated oral administration to CD-1 mice-plaque forming colony test
Examination of SPM 8272 and SPM 7605 (infusion solutions) for compatibility and hemolytic properties in citrate-anticoagulated human blood (*in vitro*)

Investigation of stability of SPM 8272 in mouse plasma.
Stability of SPM 8272 in mouse, rabbit and human plasma *in vitro*.
Stability of SPM 8272 in dog and rat plasma *in vitro*
Stability of fesoterodine (SPM 8272) in hamster and monkey plasma *in vitro*.
Determination of SPM 7805 in mouse plasma by LC-MS/MS.
Amendment to determination of SPM 7805 in mouse plasma by LC-MS/MS.
Validation of a method for the determination of SPM 5509, SPM 7789 and SPM 7790 in mouse plasma by HPLC-electrospray MS/MS.
Validation of a method for the determination of SPM 5509, SPM 7789, and SPM 7790 in mouse plasma by HPLC-electrospray MS/MS.
Validation of a HPLC-method for the determination of SPM 7500 in rat plasma with "in line clean up" in a concentration range of 25-2500 ng/ml.
Determination of SPM 7605 in rat plasma by HPLC-electrospray MS/MS in a concentration range of 0.05-20 ng/0.2 ml.
Validation of a method for the determination of SPM 7605 in rat plasma by HPLC-electrospray MS/MS (validation at an [redacted] mass spectrometer).
Determination of SPM 7605 in rat plasma by HPLC-electrospray MS/MS in a concentration range 0.05-20 ng/0.2 ml.
Validation of a method for the determination of SPM 5509, SPM 789 and SPM 7790 (three major metabolites of fesoterodine) in rat plasma by HPLC-electrospray MS/MS.
Amendment 1-Validation of a method for the determination of SPM 5509, SPM 789 and SPM 7790 (three major metabolites of fesoterodine) in rat plasma by HPLC-electrospray MS/MS.
Amendment 2-Validation of a method for the determination of SPM 5509, SPM 7789 and SPM 7790 (three major metabolites of fesoterodine) in rat plasma by HPLC-electrospray MS/MS.
Determination of SPM 7605 in rabbit plasma by LC-MS/MS.
Amendment 1-Determination of SPM 7605 in rabbit plasma by LC-MS/MS: validation study report.
Validation of a method for the determination of SPM 5509, SPM 7789 and APM 7790 (three major metabolites of fesoterodine) in rabbit plasma by HPLC-electrospray MS/MS.
Amendment 1-Validation of a method for the determination of SPM 5509, SPM 7789 and APM 7790 (three major metabolites of fesoterodine) in rabbit plasma by HPLC-electrospray MS/MS.
Validation of a HPLC method for the determination of SPM 7500 in dog plasma with "in line clean up" in a concentration range of 25-2500 ng/ml.
Determination of SPM 8272 and SPM 7605 in dog plasma by HPLC-electrospray MS/MS in the concentration range 0.05-50 ng/0.2 ml.

Validation of a method for the determination of SPM 8272 and SPM 7605 in dog plasma by HPLC-electrospray MS/MS in the concentration range 0.05-100 ng/0.2 ml.
Validation of a method for the determination of SPM 5509, SPM 7789 and SPM 7790 (three major metabolites of fesoterodine) in dog plasma by HPLC-electrospray MS/MS.
Amendment 1-Validation of a method for the determination of SPM 5509, SPM 7789 and SPM 7790 (three major metabolites of fesoterodine) in dog plasma by HPLC-electrospray MS/MS
Validation of a chromatographic method (HPLC) for the ester (SPM 8272), tolterodine (SPM 9078) and the metabolite.
Development and validation of an in vitro method to study inhibitory drug-drug interactions with the human cytochrome P450 isoenzymes 1A2, 2C9, 2C19, 2D6, and 3A4.
Amendment-Development and validation of an *in vitro* method to study inhibitory drug-drug interactions with the human cytochrome P450 isoenzymes 1A2, 2C0, 2C19, 2D6 and 3A4.
Validation of cryopreserved human hepatocyte culture as an in vitro test system to investigate the potential of test compounds for cytochrome P450 induction
Method re-validation for the determination of SPM 8272 in aqueous test substance carrier mixtures and in gelatin capsules

Acute skin irritation test (patch test) of SPM 8272 in rabbits
Acute eye irritation study of APM 8272 by instillation into the conjunctival sac of rabbits
Local tolerance test of SPM 8272 and SPM 7605 in rabbits after a single intravenous, intraarterial, paravenous, subcutaneous and intramuscular administration
Examination of SPM 8272 in the skin sensitization test in guinea pigs according to Magnusson and Kligman (maximization test)

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Fesoterodine (SPM 8272) and its hydrolysis product SPM 7605 are specific but non-selective muscarinic receptor antagonists. In vivo, fesoterodine is rapidly and extensively metabolized to SPM 7605, which is much more inhibitory at muscarinic receptors than fesoterodine. SPM 5509, a major human metabolite is far less potent than SPM 7605. The pharmacology of the metabolites SPM 7789 and SPM 7790 were not studied.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

In vitro pharmacology: study of six compounds (#817007)(December 2001).

Effects of the test compounds on the specific radioligand binding to the human muscarinic receptors and IC_{50} values for the reference compounds

	M_1 *(h)*		M_2 *(h)*		M_3 *(h)*		M_4 *(h)*		M_5 *(h)*	
Test compounds	1 µM		1 µM		1 µM		1 µM		1 µM	
SPM 8272	52		62		26		65		33	
SPM 5509	94		95		59		85		75	
SPM 6923	-		-		-		-		-	
SPM 7605	100		100		98		97		98	
SPM 7833	95		88		66		-(1)		-(1)	
SPM 9078	100		99		97		-(1)		-(1)	
Reference compounds	IC_{50} (nM)	*(nH)*	IC_{50} (nM)	*(nH)*	IC_{50} (nM)	*(nH)*	IC_{50} (nM)	*(nH)*	IC_{50} (nM)	*(nH)*
pirenzepine	17	*(1.1)*								
methoctramine			26	*(1.0)*						
4-DAMP					3.1	*(1.7)*	2.1	*(1.2)*	3.4	*(1.7)*

For the test compounds, the results are expressed as a percent inhibition of control specific binding (mean values ; n = 2).
The symbol - indicates an inhibition of less than 10%.

(1) : results not confirmed in additional experiments for IC_{50} determinations.

IC_{50} and K_i values determined for the test compounds and the reference compounds at the human muscarinic receptors

	M_1 *(h)*			M_2 *(h)*			M_3 *(h)*		
Test compounds	IC_{50} (nM)	K_i (nM)	*(nH)*	IC_{50} (nM)	K_i (nM)	*(nH)*	IC_{50} (nM)	K_i (nM)	*(nH)*
SPM 8272	737	624	*(1.2)*	824	562	*(1.0)*	not tested		
SPM 5509	124	105	*(1.1)*	143	97	*(1.3)*	684	489	*(1.0)*
SPM 7605	2.1	1.8	*(1.3)*	2.4	1.7	*(0.9)*	8.8	6.3	*(1.0)*
SPM 7833	47	40	*(1.1)*	182	124	*(1.1)*	473	338	*(0.8)*
SPM 9078	3.6	3.0	*(1.2)*	9.4	6.4	*(1.3)*	17	12	*(1.0)*
Reference compounds									
pirenzepine	16 / 16	13 / 14	*(1.1) / (1.1)*						
methoctramine				22	15	*(0.9)*			
4-DAMP							2.4	1.7	*(1.4)*

	M_4 *(h)*			M_5 *(h)*		
Test compounds	IC_{50} (nM)	K_i (nM)	*(nH)*	IC_{50} (nM)	K_i (nM)	*(nH)*
SPM 8272	399	177	*(1.1)*	not tested		
SPM 5509	211	94	*(1.2)*	510	306	*(1.1)*
SPM 7605	2.2	1.0	*(1.2)*	8.7	5.2	*(1.2)*
SPM 7833	79	35	*(0.9)*	160	96	*(0.9)*
SPM 9078	4.2	1.9	*(1.3)*	7.7	4.6	*(1.2)*
Reference compound						
4-DAMP	1.7 / 2.5	0.75 / 1.1	*(1.3) / (1.5)*	4.7 / 3.1	2.8 / 1.9	*(1.6) / (1.5)*

In vitro pharmacology: human muscarinic receptors: study of SPM 8272, SPM 8290 and SPM 16086 (#8155)(March 2004).

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Assay Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
M_1 *(h)*			
8155-1	SPM 8272	1.0E-06	48
8155-2	SPM 8290	1.0E-06	12
8155-3	SPM 16086	1.0E-06	34
M_2 *(h)*			
8155-1	SPM 8272	1.0E-06	66
8155-2	SPM 8290	1.0E-06	9
8155-3	SPM 16086	1.0E-06	51
M_3 *(h)*			
8155-1	SPM 8272	1.0E-06	56
8155-2	SPM 8290	1.0E-06	9
8155-3	SPM 16086	1.0E-06	40
M_4 *(h)*			
8155-1	SPM 8272	1.0E-06	65
8155-2	SPM 8290	1.0E-06	5
8155-3	SPM 16086	1.0E-06	41
M_5 *(h)*			
8155-1	SPM 8272	1.0E-06	34
8155-2	SPM 8290	1.0E-06	8
8155-3	SPM 16086	1.0E-06	16

IC_{50} Determination: Summary Results

Assay Compound I.D.	Client Compound I.D.	IC_{50} (M)	K_i (M)	n_H
M_1 *(h)*				
8155-1	SPM 8272	1.1E-06	9.5E-07	*0.8*
M_2 *(h)*				
8155-1	SPM 8272	4.2E-07	2.9E-07	*0.9*
8155-3	SPM 16086	2.2E-06	1.5E-06	*0.6*
M_3 *(h)*				
8155-1	SPM 8272	8.0E-07	5.7E-07	*1.2*
M_4 *(h)*				
8155-1	SPM 8272	1.7E-06	7.5E-07	*1.3*

b(4)

Determination of dissociation kinetics for SPM 7605 and tiotropium from human recombinant muscarinic receptor subtypes M1-M5 by radioligand displacement analysis (#04/SP/01)(January 2004). Dissociation of SPM 7605 and tiotropium were measured by displacing with atropine. Half lives were determined for the five muscarinic receptors M1-M5.

receptor	t ½ [h] of SPM 7605						t ½ [h] of tiotropium	ratio
	part 1	part 2	part 3			mean	part 2	tiotropium / SPM 7605
	exp 1	exp 1	exp 1	exp 2	exp 3		exp 1	
M1	0.322	0.439	-	-	-	0.381	21.9	57
M2	(0.094)	(0.139)	0.136	0.163	0.143	0.147	3.6	24
M3	(0.200)	(0.132)	0.188	0.181	0.191	0.187	20.8	111
M4	0.162	0.192	-	-	-	0.177	21.1	119
M5	0.457	0.426	-	-	-	0.442	43.2	98
() = data not included in mean; - = not determined								

Receptor specificity of SPM 8272 *in vitro* (#1006653)(March 2000).

Significant responses (≥50% inhibition or stimulation for Biochemical assays) were noted in the primary assays listed below:

PRIMARY TESTS

CAT. #	PRIMARY BIOCHEMICAL ASSAY	SPECIES	CONC.	% INH.	IC_{50}*	K_i	n_H
252600	Muscarinic M_1	hum	1 µM	76	0.216 µM	0.052 µM	0.67
252800	Muscarinic M_2	hum	1 µM	51	0.987 µM	0.209 µM	1
252900	Muscarinic M_4	hum	1 µM	84	0.163 µM	0.023 µM	0.84
253000	Muscarinic M_5	hum	1 µM	71	0.396 µM	0.285 µM	0.506

ABOVE PRIMARY TESTS IN RANK ORDER OF POTENCY

CAT. #	PRIMARY RADIOLIGAND ASSAY	SPECIES	CONC.	% INH.	IC_{50}*	K_i	n_H
2900	Muscarinic M_4	hum	1 µM	84	0.163 µM	0.023 µM	0.84
252600	Muscarinic M_1	hum	1 µM	76	0.216 µM	0.052 µM	0.67
252800	Muscarinic M_2	hum	1 µM	51	0.987 µM	0.209 µM	1
253000	Muscarinic M_5	hum	1 µM	71	0.396 µM	0.285 µM	0.506

CAT. #	TARGET	BATCH*	SPP.	n=	CONC.	% INHIBITION -100 -50 0 50 100 %	IC_{50}	K_i	n_H	R
200510	Adenosine A_1	14875	hum	2	1 µM	-9				
200610	Adenosine A_{2A}	14874	hum	2	1 µM	-2				
203500	Adrenergic α_1, Non-Selective	14872	rat	2	1 µM	7				
203900	Adrenergic α_2, Non-Selective	14868	rat	2	1 µM	-2				
204010	Adrenergic β_1	14888	hum	2	1 µM	0				
204110	Adrenergic β_2	15091	hum	2	1 µM	10				
210010	Angiotensin AT_1	14756	hum	2	1 µM	15				
212610	Bradykinin B_2	14867	hum	2	1 µM	-1				
214600	Calcium Channel Type L, Dihydropyridine	14769	rat	2	1 µM	-11				
219500	Dopamine D_1	14795	hum	2	1 µM	18				
219600	Dopamine D_2	14794	hum	2	1 µM	29				
226010	Estrogen ERα	15189	hum	2	1 µM	11				
226500	$GABA_A$, Agonist Site	14768	rat	2	1 µM	22				
226800	$GABA_A$, Chloride Channel,TBOB	15012	rat	2	1 µM	6				
232010	Glucocorticoid	15062	hum	2	1 µM	15				
233000	Glutamate, NMDA, Phencyclidine	14950	rat	2	1 µM	15				
235050	Glutamate, Non-Selective	15211	rat	2	1 µM	5				
239000	Glycine, Strychnine-Sensitive	15010	rat	2	1 µM	-9				
239500	Histamine H_1, Central	14790	gp	2	1 µM	15				
239800	Histamine H_2	14965	rat	2	1 µM	4				
243000	Insulin	15008	rat	2	1 µM	-8				
♦ 252600	Muscarinic M_1	14854	hum	2	1 µM	76	0.216 µM	0.052 µM	0.67	
				2	0.1 µM	34				
				2	10 nM	-14				
				2	1 nM	4				
				2	0.1 nM	5				
252700	Muscarinic M_2	14853	hum	2	1 µM	47	>1 µM			
				2	0.1 µM	13				
				2	10 nM	-4				
				2	1 nM	2				
				2	0.1 nM	1				
♦ 252800	Muscarinic M_3	14852	hum	2	1 µM	51	0.987 µM	0.209 µM	1	

Appears This Way
On Original

CAT. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION	IC50	Ki	nH	R
						% (-100 -50 0 50 100)				
252800	Muscarinic M1	14852	hum	2	0.1 µM	8	0.587 µM	0.209 µM	1	
				2	10 nM	5				
				2	1 nM	12				
				2	0.1 nM	-6				
♦ 252900	Muscarinic M4	14851	hum	2	1 µM	84	0.163 µM	0.023 µM	0.84	
				2	0.1 µM	38				
				2	10 nM	11				
				2	1 nM	3				
				2	0.1 nM	8				
♦ 253000	Muscarinic M5	14850	hum	2	1 µM	71	0.396 µM	0.285 µM	0.906	
				2	0.1 µM	20				
				2	10 nM	10				
				2	1 nM	-5				
				2	0.1 nM	14				
257110	Neuropeptide Y2	15006	hum	2	1 µM	19				
258600	Nicotinic Acetylcholine, Central	15313	rat	2	1 µM	7				
260110	Opiate δ	14765	hum	2	1 µM	4				
260210	Opiate κ	14764	hum	2	1 µM	4				
260410	Opiate µ	14763	hum	2	1 µM	2				
264500	Phorbol Ester	14952	mouse	2	1 µM	8				
268000	Progesterone	14915	cal	2	1 µM	-5				
268700	Purinergic P2x	14984	rabbit	2	1 µM	2				
268800	Purinergic P2y	14792	rat	2	1 µM	13				
271000	Serotonin 5-HT1	15004	rat	2	1 µM	3				
271600	Serotonin 5-HT2	15350	rat	2	1 µM	23				
278300	Sigma, Non-Selective	14762	gp	2	1 µM	30				
279500	Sodium Channel, Site 2	14760	rat	2	1 µM	31				
255510	Tachykinin NK1	14939	hum	2	1 µM	-16				
265000	Testosterone	14754	rat	2	1 µM	-6				

*Batch: Represents compounds tested concurrently in the same assay(s).

♦ Denotes item meeting criteria for significance

†Results with ≥ 50% stimulation or inhibition are boldfaced. (Negative values correspond to stimulation of binding or enzyme activity)

R=Additional Comments

cal=calf; gp=guinea pig; hum=human

Receptor specificity of SPM 7605 *in vitro* (#1006654)(March 2000).

Significant responses (≥50% inhibition or stimulation for Biochemical assays) were noted in the primary assays listed below:

PRIMARY TESTS

CAT. #	PRIMARY BIOCHEMICAL ASSAY	SPECIES	CONC.	% INH.	IC50*	Ki	nH
252600	Muscarinic M1	hum	10 nM	72	3.88 nM	0.933 nM	0.966
252700	Muscarinic M2	hum	10 nM	55	8.14 nM	2.9 nM	0.931
252800	Muscarinic M3	hum	10 nM	51	8.11 nM	1.72 nM	1.09
252900	Muscarinic M4	hum	10 nM	59	7.5 nM	1.05 nM	1.24
253000	Muscarinic M5	hum	10 nM	69	3.76 nM	2.7 nM	0.785

ABOVE PRIMARY TESTS IN RANK ORDER OF POTENCY

CAT. #	PRIMARY RADIOLIGAND ASSAY	SPECIES	CONC.	% INH.	IC50*	Ki	nH
252600	Muscarinic M1	hum	10 nM	72	3.88 nM	0.933 nM	0.966
252900	Muscarinic M4	hum	10 nM	59	7.5 nM	1.05 nM	1.24
252800	Muscarinic M3	hum	10 nM	51	8.11 nM	1.72 nM	1.09
253000	Muscarinic M5	hum	10 nM	69	3.76 nM	2.7 nM	0.785
252700	Muscarinic M2	hum	10 nM	55	8.14 nM	2.9 nM	0.931

CAT. #	TARGET	BATCH*	SPP.	n=	CONC.	% INHIBITION -100 -50 0 50 100 %	IC_{50}	K_i	n_H	R
200510	Adenosine A_1	14875	hum	2	1 µM	1				
200610	Adenosine A_{2A}	14874	hum	2	1 µM	9				
203500	Adrenergic α_1, Non-Selective	14872	rat	2	1 µM	-6				
203900	Adrenergic α_2, Non-Selective	14858	rat	2	1 µM	8				
204710	Adrenergic β_2	15091	hum	2	1 µM	-4				
210010	Angiotensin AT_1	14756	hum	2	1 µM	14				
212510	Bradykinin B_2	14867	hum	2	1 µM	5				
214600	Calcium Channel Type L, Dihydropyridine	14769	rat	2	1 µM	8				
219500	Dopamine D_1	14795	hum	2	1 µM	17				
219600	Dopamine D_2	14794	hum	2	1 µM	31				
226010	Estrogen ERα	15189	hum	2	1 µM	6				
226500	$GABA_A$, Agonist Site	14768	rat	2	1 µM	20				
226800	$GABA_A$, Chloride Channel,TBOB	15012	rat	2	1 µM	4				
232010	Glucocorticoid	15062	hum	2	1 µM	20				
233000	Glutamate, NMDA, Phencyclidine	15704	rat	2	1 µM	14				
235000	Glutamate, Non-Selective	15211	rat	2	1 µM	3				
239000	Glycine, Strychnine-Sensitive	15010	rat	2	1 µM	9				
239500	Histamine H_1, Central	14790	gp	2	1 µM	15				
239800	Histamine H_2	14985	rat	2	1 µM	17				
243000	Insulin	15008	rat	2	1 µM	-4				
♦ 252600	Muscarinic M_1	14854	hum	2	1 µM	99	3.88 nM	0.933 nM	0.966	
♦				2	0.1 µM	97				
♦				2	10 nM	72				
				2	1 nM	19				
				2	0.1 nM	11				
♦ 252700	Muscarinic M_2	14853	hum	2	1 µM	99	8.14 nM	2.9 nM	0.931	
♦				2	0.1 µM	90				
				2	10 nM	55				
				2	1 nM	13				
				2	0.1 nM	-9				
♦ 252800	Muscarinic M_3	14852	hum	2	1 µM	101	8.11 nM	1.72 nM	1.09	
♦				2	0.1 µM	104				

CAT. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION -100 -50 0 50 100						IC50	Ki	nH	R
						%	↓	↓	↓	↓	↓				
♦ 252800	Muscarinic M1	14852	hum	2	10 nM	51						8.11 nM	1.72 nM	1.09	
				2	1 nM	15									
				2	0.1 nM	8									
♦ 252900	Muscarinic M4	14851	hum	2	1 µM	95						7.5 nM	1.05 nM	1.24	
♦				2	0.1 µM	97									
♦				2	10 nM	59									
				2	1 nM	8									
				2	0.1 nM	0									
♦ 253000	Muscarinic M5	14850	hum	2	1 µM	100						3.76 nM	2.7 nM	0.785	
♦				2	0.1 µM	97									
♦				2	10 nM	69									
				2	1 nM	21									
				2	0.1 nM	17									
257110	Neuropeptide Y1	15006	hum	2	1 µM	24									
258600	Nicotinic Acetylcholine, Central	15501	rat	2	1 µM	20									
260110	Opiate δ	14765	hum	2	1 µM	16									
260210	Opiate κ	14764	hum	2	1 µM	-1									
260410	Opiate µ	14763	hum	2	1 µM	-9									
264500	Phorbol Ester	14952	mouse	2	1 µM	28									
268000	Progesterone	14915	cal	2	1 µM	11									
268700	Purinergic P2x	14984	rabbit	2	1 µM	14									
268800	Purinergic P2y	14792	rat	2	1 µM	12									
271000	Serotonin 5-HT1	15004	rat	2	1 µM	0									
271600	Serotonin 5-HT2	15350	rat	2	1 µM	16									
278300	Sigma, Non-Selective	14762	gp	2	1 µM	-7									
279500	Sodium Channel, Site 2	14760	rat	2	1 µM	-25									
255510	Tachykinin NK1	14909	hum	2	1 µM	18									
285000	Testosterone	14754	rat	2	1 µM	12									

*Batch: Represents compounds tested concurrently in the same assay(s).

♦ Denotes item meeting criteria for significance

†Results with ≥ 50% stimulation or inhibition are boldfaced. (Negative values correspond to stimulation of binding or enzyme activity)

R=Additional Comments

cat=calf; gp=guinea pig; hum=human

Receptor specificity of SPM 9078 *in vitro* (#149991)(March 2000).

Significant responses (≥50% inhibition or stimulation for Biochemical assays) were noted in the primary assays listed below:

PRIMARY TESTS

CAT. #	PRIMARY BIOCHEMICAL ASSAY	SPECIES	CONC.	% INH.	IC50*	Ki	nH
252600	Muscarinic M1	hum	0.1 µM	93	0.021 µM	5.04 nM	1.62
252700	Muscarinic M2	hum	0.1 µM	83	0.039 µM	0.014 µM	1.67
252800	Muscarinic M3	hum	0.1 µM	80	0.05 µM	0.011 µM	1.98
252900	Muscarinic M4	hum	0.1 µM	80	0.033 µM	4.61 nM	1.26
253000	Muscarinic M5	hum	0.1 µM	93	9.97 nM	7.16 nM	0.682
278300	Sigma, Non-Selective	gp	1 µM	81			

ABOVE PRIMARY TESTS IN RANK ORDER OF POTENCY

CAT. #	PRIMARY RADIOLIGAND ASSAY	SPECIES	CONC.	% INH.	IC50*	Ki	nH
252900	Muscarinic M4	hum	0.1 µM	80	0.033 µM	4.61 nM	1.26
252600	Muscarinic M1	hum	0.1 µM	93	0.021 µM	5.04 nM	1.62
253000	Muscarinic M5	hum	0.1 µM	93	9.97 nM	7.16 nM	0.682
252800	Muscarinic M3	hum	0.1 µM	80	0.05 µM	0.011 µM	1.98
252700	Muscarinic M2	hum	0.1 µM	83	0.039 µM	0.014 µM	1.67

CAT. #	TARGET	BATCH	SPP.	n=	CONC.	% INHIBITION	IC_{50}	K_i	n_H	R
200510	Adenosine A_1	14875	hum	2	1 µM	12				
200610	Adenosine A_{2A}	14874	hum	2	1 µM	9				
203500	Adrenergic α_1, Non-Selective	14872	rat	2	1 µM	12				
203900	Adrenergic α_2, Non-Selective	14868	rat	2	1 µM	6				
204010	Adrenergic β_1	14888	hum	2	1 µM	-1				
204110	Adrenergic β_2	14889	hum	2	1 µM	1				
210010	Angiotensin AT_1	14756	hum	2	1 µM	19				
212610	Bradykinin B_2	14867	hum	2	1 µM	16				
214600	Calcium Channel Type L, Dihydropyridine	14769	rat	2	1 µM	-3				
219500	Dopamine D_1	14795	hum	2	1 µM	-2				
219600	Dopamine D_2	14794	hum	2	1 µM	29				
226010	Estrogen ERα	15189	hum	2	1 µM	-1				
226500	$GABA_A$, Agonist Site	14768	rat	2	1 µM	3				
226800	$GABA_A$, Chloride Channel,TBOB	15012	rat	2	1 µM	3				
232010	Glucocorticoid	15062	hum	2	1 µM	15				
233000	Glutamate, NMDA, Phencyclidine	14990	rat	2	1 µM	1				
235000	Glutamate, Non-Selective	15211	rat	2	1 µM	1				
239000	Glycine, Strychnine-Sensitive	15010	rat	2	1 µM	8				
239500	Histamine H_1, Central	14790	gp	2	1 µM	23				
239800	Histamine H_1	14965	rat	2	1 µM	12				
243000	Insulin	15008	rat	2	1 µM	9				
♦ 252600	Muscarinic M_1	14854	hum	2	1 µM	98	0.021 µM	5.04 nM	1.62	
♦				2	0.1 µM	93				
				2	10 nM	23				
				2	1 nM	2				
				2	0.1 nM	-5				
♦ 252700	Muscarinic M_2	14853	hum	2	1 µM	97	0.039 µM	0.014 µM	1.67	
				2	0.1 µM	83				
				2	10 nM	9				
				2	1 nM	3				
				2	0.1 nM	6				
♦ 252800	Muscarinic M_3	14852	hum	2	1 µM	102	0.05 µM	0.011 µM	1.98	

	CAT. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION -100 -50 0 50 100 %	IC_{50}	K_i	n_H	R
♦	252800	Muscarinic M_3	14852	hum	2	0.1 µM	80	0.05 µM	0.011 µM	1.98	
					2	10 nM	4				
					2	1 nM	8				
					2	0.1 nM	7				
♦	252900	Muscarinic M_4	14851	hum	2	1 µM	98	0.033 µM	4.61 nM	1.26	
♦					2	0.1 µM	80				
					2	10 nM	18				
					2	1 nM	1				
					2	0.1 nM	0				
♦	253000	Muscarinic M_5	14850	hum	2	1 µM	98	9.97 nM	7.16 nM	0.682	
♦					2	0.1 µM	93				
					2	10 nM	38				
					2	1 nM	26				
					2	0.1 nM	10				
	257110	Neuropeptide Y_2	15006	hum	2	1 µM	5				
	258600	Nicotinic Acetylcholine, Central	14949	rat	2	1 µM	15				
	260110	Opiate δ	14765	hum	2	1 µM	22				
	260210	Opiate κ	14764	hum	2	1 µM	6				
	260410	Opiate µ	14763	hum	2	1 µM	5				
	264500	Phorbol Ester	14932	mouse	2	1 µM	21				
	268030	Progesterone	14915	cal	2	1 µM	-1				
	268700	Purinergic P_{2X}	14984	rabbit	2	1 µM	19				
	268800	Purinergic P_{2Y}	14792	rat	2	1 µM	-3				
	271000	Serotonin 5-HT_1	15004	rat	2	1 µM	24				
	271600	Serotonin 5-HT_3	15350	rat	2	1 µM	11				
♦	278300	Sigma, Non-Selective	14762	gp	2	1 µM	81				
	279500	Sodium Channel, Site 2	14760	rat	2	1 µM	44				
	255510	Tachykinin NK_1	14909	hum	2	1 µM	25				
	285000	Testosterone	14754	rat	2	1 µM	27				

*Batch: Represents compounds tested concurrently in the same assay(s).

♦ Denotes item meeting criteria for significance

†Results with a 50% stimulation or inhibition are boldfaced. (Negative values correspond to stimulation of binding or enzyme activity)

R=Additional Comments

cal=calf; gp=guinea pig; hum=human

Study title: Interaction of SPM 5509, SPM 7605, SPM 8272, and SPM 9078 with cloned human muscarinic acetylcholine receptors: determination of agonistic and antagonistic activities. (Study no. 02/SP/04, December 2002) SPM 5509, SPM 7605, SPM 9078 and SPM 8272 are full antagonists at all tested human muscarinic acetylcholine receptor displaying different potencies with the following rank order: SPM 7605 > SPM 9078 >SPM 8272 > SPM 5509. The rank order for potency at human M1 to M5 receptors with the respective pKi values in parenthesis of SPM 7605 is: SPM 7605: M1 (9.1) = M2 (9.1) > M3 (8.9) > M5 (8.7) = M4 (8.6) The rank order for potency at human M1 to M5 receptors with the respective pKi values in parenthesis of SPM 9078 is: SPM 9078: M1 (8.9) = M2 (8.8) = M3 (8.8) > M5 (8.6) = M4 (8.5) The rank order for potency at human M1 to M5 receptors with the respective pKi values in parenthesis of SPM 8272 is: SPM 8272: M2 (8.0) > M3 (7.9) > M1 (7.8) > M5 (7.7) = M4 (7.7)

Summary of EC_{50}s:

Receptor	ACh (n=4) mean ± SD	SPM 5509 (n=1)	SPM 7605 (n=1)	SPM 8272 (n=1)	SPM 9078 (n=1)
M1	1400 ± 41	n.a.	n.a.	n.a.	n.a.
M2	129 ± 31	n.a.	n.a.	n.a	> 10,000 *
M3	43 ± 18	n.a.	n.a.	n.a	> 10,000 *
M4	957 ± 60	n.a.	n.a.	n.a	> 30,000 *
M5	3225 ± 330	n.a.	n.a.	n.a	> 30,000 *

* maximum stimulation did not reach saturation.

Summary of IC_{50}s:

Receptor	Atropine (n=8)	SPM 5509 (n=2)	SPM 7605 (n=2)	SPM 8272 (n=2)	SPM 9078 (n=2)
M1	5.5 ± 0.5	302.9 ± 0.5	7.6 ± 0.4	144.3 ± 66.7	12.0 ± 2.9
M2	3.6 ± 0.3	115.3 ± 19.7	3.8 ± 0.0	59.3 ± 4.6	8.6 ± 0.6
M3	3.9 ± 0.6	1175 ± 95	15.1 ± 3.9	147.1 ± 31.2	20.2 ± 0.5
M4	11.6 ± 0.7	2010 ± 690	35.8 ± 3.6	269.8 ± 13.6	36.7 ± 5.4
M5	32.5 ± 3.6	2295 ± 675	34.9 ± 2.9	391.3 ± 31.1	48.0 ± 3.9

Study title: *In vitro* and *in vivo* characterization of the effects of fesoterodine (SPM 8272) and of its active metabolite SPM 7605 on rat bladder – comparison with tolterodine, oxybutynin and atropine. (Study no. F9376, March 2004) *In vitro* studies were conducted in isolated bladder strips contracted by carbachol or electrical field stimulation. *In vivo* the effects of intravenous fesoterodine and SPM 7605 (0.01, 0.1, and 1 mg/kg) on micturition parameters were investigated using continuous cystometry in conscious female Sprague-Dawley rats. Tolterodine and oxybutynin were used at doses of 0.1, 0.5 and 1 mg/kg, and atropine was tested at 0.5 and 1 mg/kg. Fesoterodine and SPM 7605 caused a rightward shift of the concentration-response curve for carbachol without any depression of maximum, and concentration-dependently reduced contractions induced by electrical field stimulation. The potency of fesoterodine, SPM 7605, atropine, tolterodine and oxybutynin were similar. In vivo, 0.01 mg/kg fesoterodine or SPM 7605 reduced micturition pressure and increased intercontraction intervals and bladder capacity.

Drug activity related to proposed indication:

2.6.2.3 Secondary pharmacodynamics

Evaluation of SPM 8272 and SPM 9078 on gastrointestinal transit after oral administration in the mouse. Study no. 04.221/2/A (May 2005). SPM 8272 and SPM 9078 were administered to mice (n=8) at oral concentrations of 0, 1, 3, 10, and 30 mg/kg. A suspension of charcoal was administered orally at 60 minutes, and the mice were sacrificed by cervical dislocation after an additional 20 minutes. The results were expressed as percent transit from the cardia to the caecum of the small intestine. Atropine sulfate (20 mg/kg) was used as a positive control. Neither SPM 8272 or SPM 9078 affected gastrointestinal transit under the conditions tested.

2.6.2.4 Safety pharmacology

Neurological effects: b(4)

Report No. 12718/99 (2 March 2000): Neuropharmacological screening of mice according to Irwin following oral administration of SPM 8272. SPM 8272 (AC8288, WE No. SPM 10964, 96.94% pure) was administered to NMRI/ \ NMRI BR mice (female, 18-22g, 8/dose) at doses of 0, 3, 10, and 30 mg/kg (by oral gavage, in aqua ad injectabilia). Tests for 40 neurological functions were performed at 15, 30, 60 and 120 minutes. No effect of SPM 8272 was observed at 3 mg/kg. At 10 and 30 mg/kg dose related effects were observed on awareness (alertness), mood (vocalization, restlessness, aggression), motor activity (reactivity, spontaneous activity, touch response, pain response), CNS excitation (startle response), and autonomic function (pupil size, respiration rate), which persisted beyond 120 minutes.

Report No. 12719/99 (3 March 2000): Examination of the influence of SPM 8272 on the spontaneous motility of mice following oral administration. SPM 8272 (AC8288, WE No. SPM 10964, 96.94% pure) was administered to NMRI/ \ :NMRI BR mice (female, 18-23g, 5/dose) at doses of 0, 3, 10, and 30 mg/kg (by oral gavage, in aqua ad injectabilia). No effect of SPM 8272 on the spontaneous motility of mice was observed at the highest dose of 30 mg/kg. b(4)

Report No. 12722/99 (28 February 2000): Examination of the influence of SPM 8272 on pentetrazol-induced convulsions in mice following oral administration. SPM 8272 (AC8288, WE No. SPM 10964, 96.94% pure) was administered to NMRI/ \ NMRI BR mice (female, 10-21 g, 5/dose) at doses of 0, 3, 10, and 30 mg/kg (by oral gavage, in aqua ad injectabilia). No anticonvulsive effect of SPM 8272 on pentetrazol-induced convulsions (or on mortality) in mice was observed at the highest dose of 30 mg/kg. b(4)

Report No. 12721/99 (24 February 2000): Examination of the influence of SPM 8272 on electroshock-induced convulsions in mice following oral administration. SPM 8272 (AC8288, WE No. SPM 10964, 96.94% pure) was administered to NMRI/(.NMRI BR mice (female, 20-23g, 5/dose) at doses of 0, 3, 10, and 30 mg/kg (by oral gavage, in aqua ad injectabilia). No effect of SPM 8272 on electroshock-induced subthreshold convulsions in mice was observed at the highest dose of 30 mg/kg. b(4)

Study No. 20000031P (24 February 2000): SPM 8272: Evaluation of an analgesic effect in the phenylbenzoquinone writhing test in the mouse following a single oral administration. SPM 8272 (AC8282) was administered to Swiss ICR mice (female, 18.2-22.8 g, 10/dose) at doses of 0, 3, 10, and 30 mg/kg (by oral gavage, in aqua ad injectabilia)., followed, after one hour, by 0.025% phenyl-p-benzoquinone (ethanolic solution, 37C, i.p.). After 5 minutes, observations were counted for 10 minutes. At the highest dose of 30 mg/kg, a slight decrease in the number of writhings (-27% vs. control) was observed.

Report No. 12720/99 (16 February 2000): Examination of the influence of SPM 8272 on the hexobarbital sleeping time in mice following oral administration. SPM 8272 (AC 8288, WE No. SPM 10964, 97.18 % pure) was administered to NMRI/ .NMRI BR mice (female, 21-27 g, 5/dose) at doses of 0, 3, 10, and 30 mg/kg (by oral gavage, in aqua ad injectabilia). No effect of SPM 8272 on hexobarbital sleeping time in mice was observed at the highest dose of 30 mg/kg. b(4)

Study No. 20000030 P (31 March 2000): SPM 8272: Evaluation of interactions with hypothermia and ptosis induced by reserpine in the mouse following a single oral administration. SPM 8272 (AC8282) was administered to Swiss ICR mice (female, 18.0-22.6 g, 8/dose) at doses of 0, 3, 10, and 30 mg/kg (by oral gavage, in aqua ad injectabilia) 4 hours following a dose of reserpine (2.0 mg/kg, i.p.). Rectal temperature was measured prior to reserpine administration, at time 0 prior to test article administration, and at 60 minutes following test article administration. The high dose of 30 mg/kg did not antagonize the hypothermia and ptosis induced by reserpine. Although the relevance is unknown, the lowest dose (but not higher doses) of SPM 8272 caused an increase in hypothermia.

Study No. 20000028 P (15 February 2000): SPM 8272: Evaluation of effect on the autonomic nervous system following a single oral administration in conscious rats. SPM 8272 (AC8282) was administered to conscious Sprague-Dawley /CD^{R}BR rats (female, 273.7-315.3 g, 6/dose) at doses of 0, 3, 10, and 30 mg/kg (by oral gavage, in aqua ad injectabilia). 3 mg/kg was a no effect level. A decrease in the slope of the baroreflex sensitivity curve in terms of heart rate at steady state mean arterial pressure was observed beginning at 10 mg/kg (see table below).

	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	Guanethidine (+ cont.) 100 mg/kg
Baroreflex sensitivity (b./min./mmHg)*	-5.654	-4.856	-4.072**	-2.766**	-2.508**
Hypertensive response (mmHg)**					
+ 1 µg/kg phenylephrine	13	19	12	20	20
+ 5 µg/kg phenylephrine	37	43	35	46	53**
+ 25 µg/kg phenylephrine	49	55	58	62*	67**
Sec. hypertensive response (mmHg)***					
+ 3 µg/kg serotonin	3	5	4	4	5
+ 10 µg/kg serotonin	10	8	8	9	10
+ 30 µg/kg serotonin	12	11	13	11	16
Bradycardic response (b./min.)****					
+ 3 µg/kg serotonin	35	38	10	33	31
+ 10 µg/kg serotonin	135	115	95	70	107
+ 30 µg/kg serotonin	225	198	185	98**	167
Mean arterial pressure (mmHg)(1 hr)	123	117	110	118	102**
Heart rate (b./min.)(1 hr)	375	403	410	457**	350

* the slope of the baroflex curve at steady state mean arterial pressure at the mid point of heart rate range
** variation in relation to basal values in responses induced by phenylephrine
*** variation in relation to basal values in responses induced by serotonin
**** variation in relation to basal values in responses induced by serotonin

Cardiovascular effects:

Study title: SPM 8272: Evaluation of Haemodynamic Effects and Electrocardiogram Following Intravenous Dosing in the Anaesthetized Dog
Study no: 20000026P
Submission 001, volume # 6, and page #1
Conducting laboratory and location: [redacted]

b(4)

Date of study initiation: 24 February 2000
GLP compliance: yes
QA reports: yes (x) no ( ):
Drug: lot # AC8282, 95.21% pure, administered over a 30 second interval, volume of 0.5 ml/kg
Formulation/vehicle: sterile water
Methods: The dogs were anesthetized with thiopental (20 mg/kg, iv) and halothane (4%), and maintained with 1.5% halothane. The animals were artificially ventilated (18-20 cycles/min.). Arterial pressure was measured via a catheter to the abdominal aorta via the femoral artery. A catheter to the left ventricle via the aorta was used to measure left ventricular pressure and its derived parameters. Pulmonary arterial pressure was measured via the right jugular vein and right atrium. Central venous pressure was also measured via the jugular vein to the right atrium. Doppler probes (20 MHz) were fitted around the left femoral artery and the left renal artery for the measurement of arterial flow rates. After opening the chest, Doppler probes were fitted around the ascending aorta (10MHz) and the circumflex coronary artery (20 MHz) for the measurement of cardiac output and its derived parameters and coronary flow rate, respectively. Two electrodes connected to a [redacted] transmitter were attached to visualize and record the electrocardiogram [redacted] using the [redacted] acquisition system. In

b(4)

addition, 4 electrodes connected to a [redacted] acquisition system were attached. All hemodynamic parameters were measured or calculated using the [redacted] acquisition system. b(4)

Dosing: 6 dogs were dosed first with vehicle and subsequently with 8, 80, and 800 µg/kg iv at 30 minute intervals.

Results: Inversion of the T-wave was observed in one dog at 800 µg/kg. No other changes in T-wave morphology was observed at any dose. No other disturbances in the electrocardiogram due to administration of SPM 8272 were observed [redacted] I, II, III, aVR, aVL, aVF).

N=6	0 µg/kg	8 µg/kg	80 µg/kg	800 µg/kg
Mean arterial pressure (maximum % change)	+4	-3	+2	-11** (t=2 min)
Systolic arterial pressure (maximum % change)	+4	-4	+2	-10** (t=2)
Diastolic arterial pressure (maximum % change)	+4	-3	+3	-12** (t=2)
Heart rate (maximum % change)	+2	+2	+3	-18** (t=2-20)
Central venous pressure (maximum % change)	+2	-10** (t=30)	-11** (t=10-30)	+12** (t=2-20)
Mean coronary flow (maximum % change)	+2	-1	+2	-15** (t=2-30)
Coronary resistance (maximum % change)	+3	-3	-4	+13** (t=5-30)
Mean renal flow (maximum % change)	-4	+6	-4	-10** (t=2)
Mean femoral flow (maximum % change)	-6	-5	-2	-20** (t=2-15)
Femoral resistance (maximum % change)	+9	+2	+3	+12* (t=2-5)
End diastolic LVP (maximum % change)	+5	-6** (t=10-30)	-11** (t=10-30)	+19* (t=5-20)
dLVP/dt(+) (maximum % change)	+2	-3	-1	-15** (t=2-20)
dLVP/dt(-) (maximum % change)	+6	-3	-2	-16** (t=2-15)
Left ventricular work (maximum % change)	-2	-4	-3	-18** (t=2-15)
Cardiac output (maximum % change)	-2	-3	-2	-10** (t=2-10)
Stroke volume (maximum % change)	-4	-4	-3	+17** (t=5-30)
PR interval (maximum % change)	-3	+2	-6 (t=20)	-7 (t=15-30)
QRS interval (maximum % change)	+2	+2	-1	+6* (t=10)
QT interval (maximum % change)	+1	-2	+2	+18** (t=5-30)
QTc interval (Fred.formula) (maximum % change)		-1	+2	+11** (t=5-30)

Toxicokinetics:

Nominal dose (µg/kg iv)	SPM 7605 Cmax	SPM 8272 Cmax
8	0.8	10.9
80	14.4	108.5
800	167.2	1550.8

Summary of individual study findings:

Dose in µg/kg (iv)	SPM7605, conc. in nM	SPM7605, conc. in ng/ml	Effect
8	~1.5	0.8	↓CVP, LVP in dog*
80	~30	14.4	↓CVP, LVP in dog*
200 (oral)	~30	15	Fesoterodine 12 mg#
800	~300	167.2	↑CVP, LVP, ↑QT in dog*

* SPM 8272 also present
\# maximum clinical dose
CVP = central venous pressure
LVP = left ventricular pressure

Study title: SPM 8272, SPM 7605, and SPM 9078: Evaluation of Effect on Cardiac Action Potential in Isolated Canine Purkinje Fibres

Study no: 20000029P
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: 09 February 2000
GLP compliance: yes
QA reports: yes (x) no ( ):
Drug: lot #FP 9092, 99.92 % pure
Formulation/vehicle: Tyrode's solution

Methods: Seven male dogs weighing between 8.9 and 11 kg were used to prepare 15 Purkinje fiber preparations. SPM 8272 and SPM 7605 were each studied in group of 6 preparations and SPM 9078 was studied in a group of 3 preparations.

Results: No early afterdepolarizations were observed at any concentration of SPM 8272, SPM 7605, or SPM 9078. The positive control Cisapride responded as expected.

SPM 7605	0 (Tyrode's)	$1.5x10^{-8}$M	$1.5x10^{-7}$M	$1.5x10^{-6}$M	$1.5x10^{-5}$M
Action potential amplitude (mV)					
__normal stimulation rate (60 PPM)	2	0	5	4	-12*
__low stimulation rate (12 PPM)	5	2	4	4	-2
Resting potential (mV)					
__normal stimulation rate (60 PPM)	0	0	1	1	4
__low stimulation rate (12 PPM)	-4	-1	1	1	1
Vmax (V/sec)					
__normal stimulation rate (60 PPM)	32	-17	30	53	-153*
__low stimulation rate (12 PPM)	12	-45	20	-30	-81
APD50 (ms)					
__normal stimulation rate (60 PPM)	-12	5	5	12	-97**
__low stimulation rate (12 PPM)	-13	-12	14	84**	-93**
APD70 (ms)					
__normal stimulation rate (60 PPM)	-4	3	21	48**	-53**
__low stimulation rate (12 PPM)	-5	4	44	175**	-5
APD90 (ms)					
__normal stimulation rate (60 PPM)	3	6	30	69**	-16
__low stimulation rate (12 PPM)	-1	6	59	221**	58

SPM 8272	0 (Tyrode's)	$1.5x10^{-8}$M	$1.5x10^{-7}$M	$1.5x10^{-6}$M	$1.5x10^{-5}$M
Action potential amplitude (mV)					
__normal stimulation rate (60 PPM)	-4	3	1	-1	-23**
__low stimulation rate (12 PPM)	-5	0	8	-4	-15
Resting potential (mV)					
__normal stimulation rate (60 PPM)	-2	-1	-1	-2	0
__low stimulation rate (12 PPM)	-1	-1	-3	0	3
Vmax (V/sec)					
__normal stimulation rate (60 PPM)	-43	-14	-29	-31	-228**
__low stimulation rate (12 PPM)	-39	11	37	-32	-151
APD50 (ms)					
__normal stimulation rate (60 PPM)	-3	1	13	4	-120**
__low stimulation rate (12 PPM)	-11	10	4	57	-121**
APD70 (ms)					
__normal stimulation rate (60 PPM)	-4	4	23	48	-90**
__low stimulation rate (12 PPM)	-7	9	55	184**	-55
APD90 (ms)					
__normal stimulation rate (60 PPM)	-5	8	27	71**	-41*
__low stimulation rate (12 PPM)	-6	9	94	264**	25

SPM 9078	0 (Tyrode's)	$1.5x10^{-9}$M	$1.5x10^{-8}$M	$1.5x10^{-7}$M	$1.5x10^{-6}$M
Action potential amplitude (mV)					
__normal stimulation rate (60 PPM)	1	5	7	7	3
__low stimulation rate (12 PPM)	-1	4	0	6	2
Resting potential (mV)					
__normal stimulation rate (60 PPM)	2	-3	-2	-3	1
__low stimulation rate (12 PPM)	0	0	2	-3	2
Vmax (V/sec)					
__normal stimulation rate (60 PPM)	-12	-43	43	66	-9
__low stimulation rate (12 PPM)	-1	20	9	74	138
APD50 (ms)					
__normal stimulation rate (60 PPM)	1	-8	1	22	26
__low stimulation rate (12 PPM)	-24	-5	-3	19	17
APD70 (ms)					
__normal stimulation rate (60 PPM)	-4	-4	3	31	54
__low stimulation rate (12 PPM)	-5	0	4	31	56
APD90 (ms)					
__normal stimulation rate (60 PPM)	1	-1	7	44	83
__low stimulation rate (12 PPM)	-3	1	7	50	91

Cisapride	$3x10^{-7}$ M
Action potential amplitude (mV)	
__normal stimulation rate (60 PPM)	-4
__low stimulation rate (12 PPM)	-1
Resting potential (mV)	
__normal stimulation rate (60 PPM)	4
__low stimulation rate (12 PPM)	1
Vmax (V/sec)	
__normal stimulation rate (60 PPM)	-6
__low stimulation rate (12 PPM)	-6
APD50 (ms)	
__normal stimulation rate (60 PPM)	34
__low stimulation rate (12 PPM)	33
APD70 (ms)	
__normal stimulation rate (60 PPM)	52
__low stimulation rate (12 PPM)	56
APD90 (ms)	
__normal stimulation rate (60 PPM)	63
__low stimulation rate (12 PPM)	92

Summary of individual study findings:

Dose in µg/kg (iv)	SPM7605, conc. in nM	SPM7605, conc. in ng/ml	Effect
8	~1.5	0.8	↓CVP, LVP in dog*
	15		APD NOEL in Canine Purkinje
80	~30	14.4	↓CVP, LVP in dog*
200 (oral)	~30	15	Fesoterodine 12 mg#
	150		n.s. ↑APD in Canine Purkinje
800	~300	167.2	↑CVP, LVP, ↑QT in dog*
	1500		↑APD in Canine Purkinje
	15000		↑APD in Canine Purkinje

* SPM 8272 also present CVP = central venous pressure
# maximum clinical dose LVP = left ventricular pressure

Study title: Effects of SPM 7605 on Cloned hERG Channels Expressed in Mammalian Cells. Study # 020314.TDA (GLP) performed by [redacted]. HEK293 cells stably expressing hERG were held at –80 mV. Onset and steady state block of hERG current due to test or control articles were measured using a pulse pattern with fixed amplitudes (conditioned prepulse depolarization: + 20 mV for 2 seconds; repolarization: -50 mV for 2 seconds) repeated at 10 second intervals. Peak tail current was measured during the 2 second step to –50 mV. Steady state was maintained for at least 30 seconds before applying test or control articles. Peak tail current in test and control articles was measured until a new steady state was achieved. b(4)

SPM 7605 inhibited hERG current by 17, 39, 66, and 87% respectively at 0.1, 0.3, 1, and 3 µM. The data were fit to a simple 1:1 binding curve with an estimated IC_{50} of 0.5 µM. The positive control Terfenadine (60 nM) produced 91 % block of hERG current and the solvent DMSO had no effect on hERG current at 0.1%, the concentration used in the assay.

b(4)

Study title: Effects of SPM 8272 on Cloned hERG Channels Expressed in Mammalian Cells (n=3). Study # 020313.TDA (GLP), [redacted] SPM 8272 (pro drug) inhibited hERG current by 3%, 24%, 71%, and 96% at 0.1, 1, 10, and 100 μM, respectively. The estimated IC_{50} was 3.6 μM.

Study title: Effects of SPM 9078 on Cloned hERG Channels Expressed in Mammalian Cells (n=3). Study # 020315.TDA (GLP), [redacted] SPM 9078 inhibited hERG current by 20%, 49%, 73%, and 97% at 0.003, 0.01, 0.03, and 1 μM, respectively. The estimated IC_{50} was 0.011 μM.

b(4)

Study title: Electrophysiological effects of test items on the current mediated by the hERG-potassium channel stably expressed in HEK 293 [redacted] cells (n=4). Study # E-01-001-022 (GLP), Ion Gate. The IC_{50} and Hill coefficients were 279±35 nM and 0.91±0.08 for SPM 7605 (active drug) and 3.3±0.6 μM and 0.97±0.13 for SPM 5509 (major human metabolite). Inhibition of hERG tail currents was 21.7±2.6 % at 300 μM SPM 6923, the highest concentration tested.

b(4)

Study title: Electrophysiological examination of activity of SPM 7605, SPM 8272, and SPM 9078 on the SCN5A-sodium channel expressed in CHO cells. Schwartz Biosciences/Iongate Project No. E-01-014-002. The human cardiac sodium channel SCN5A was transiently expressed in Chinese hamster ovary cells. Four or more replicates were tested for each of the following: SPM 7065 (1, 5, 10, 50, and 100 μM), SPM 8272 (1, 5, 10, and 100 μM), SPM 9078 (Tolterodine)(0.1, 1, 10, and 50 μM), Lidocaine (positive control)(10 μM), and DMSO (negative control solvent)(0.1%). All test compounds showed a concentration dependent inhibition of SCN5A under multiple conditions of analysis, as follows:

SPM 7065			
Method of analysis	IC_{50} (μM)	Hill coefficient	% inhibition at 10 μM
Imax, 1st pulse of series 11	20.9±3.1	1.4±0.2	22.8±3.5
Imax, averaged last 5 pulses of series 11	15.3±2.4	1.5±0.3	30.4±3.1
Qt, 1st pulse of series 11	19.7±2.3	1.5±0.2	25.3±1.5
Qt, averaged last 5 pulses of series 11	13.6±1.7	1.8±0.3	34.6±2.3

SPM 8272			
Method of analysis	IC_{50} (μM)	Hill coefficient	% inhibition at 10 μM
Imax, 1st pulse of series 11	10.6±1.4	2.9±1.3	43.0±4.9
Imax, averaged last 5 pulses of series 11	9.5±0.8	2.8±0.8	52.8±3.9
Qt, 1st pulse of series 11	10.2±0.9	3.0±0.8	44.7±3.4
Qt, averaged last 5 pulses of series 11	9.5±0.7	2.4±0.5	53.3±3.9

SPM 9078			
Method of analysis	IC_{50} (μM)	Hill coefficient	% inhibition at 10 μM
Imax, 1st pulse of series 11	7.0±1.0	1.0±0.1	55.9±4.7
Imax, averaged last 5 pulses of series 11	3.5±0.5	1.1±0.1	74.2±5.1
Qt, 1st pulse of series 11	6.0±1.1	0.9±0.1	55.1±6.5
Qt, averaged last 5 pulses of series 11	2.7±0.5	1.0±0.2	75.2±6.3

Study title: Examination of SPM 8272, SPM 7605, SPM 9078 of L-type Ca^{2+} inward current in isolated ventricular myocytes of the guinea pig — Report # 15566/02 (GLP). SPM 8272 and SPM 7605 were tested at 15 nM, 150 nM, 1.5 µM, and 15 µM in 0.9% NaCl. SPM 9078 was tested at 15 nM, 150 nM, 1.5 µM, and 15 µM in 0.01% aqueous DMSO. Nifedipine (1 µM) was used as a positive control. All test substances were compared with the appropriate vehicle controls. b(4)

At concentrations up to 1.5 µM SPM 7605, SPM 8272, and SPM 9078 did not influence the L-type Ca^{2+} inward current from –40 to 0 mV or the current-voltage relationships from –50 to 50 mV.

AT 15 µM, significant declines of the L-type inward current were observed:

SPM 7605	18%	p=0.0249
SPM 8272	44%	p<0.0001
SPM 9078	17%	p=0.0032

Considering the run-down of the control currents with vehicle, only test substances at the concentrations of 15 µM distinctly decreased the L-type inward current at the following potentials: from –10 to +40 mV (SPM 7605), from –20 to +30 mV (SPM 8272, $p \leq 0.05$), and from –10 to +30 mV (SPM 9078), indicating that the drug-induced suppression of current was not voltage-dependent.

Pulmonary effects:

Study No. 20000027 P (7 February 2000): SPM 8272: Evaluation of effect on respiration in the unrestrained conscious rat following oral administration. SPM 8272 (AC 8282) was administered to Sprague-Dawley/CD^{R}BR rats (female, 256.8-303.2 g, 8/dose) at doses of 0, 0.3, 1, 3, 10, and 30 mg/kg (by oral gavage, in aqua ad injectabilia). No effect of SPM 8272 on respiratory parameters in rats was observed at 1 mg/kg. A slight, non-dose-dependent respiratory stimulant effect (decrease in inspiration time) was observed at ≥ 3 mg/kg. An increase in peak inspiratory flow was also observed at 3 mg/kg only.

Renal effects:

— **Report No. 12731/99 (9 March 2000): Examination of the influence of SPM 8272 on the diuresis and saluresis in rats following oral administration.** SPM 8272 (AC 8288, WE No. SPM 10964, 96.94 % pure) was administered to Sprague-Dawley rats (female, 174-196 g, 10/dose) at doses of 0, 3, 10, and 30 mg/kg (by oral gavage, in aqua ad injectabilia). No treatment related effects on diuresis or saluresis were noted up to the highest dose of 30 mg/kg. b(4)

Gastrointestinal effects:

b(4)

Report No. 12731/99 (9 March 2000): Examination of the influence of SPM 8272 on the diuresis and saluresis in rats following oral administration. SPM 8272 (AC 8288, WE No. SPM 10964, 96.94 % pure) was administered to Sprague-Dawley rats (female, 174-196 g, 10/dose) at doses of 0, 3, 10, and 30 mg/kg (by oral gavage, in aqua ad injectabilia). No treatment related effects on diuresis or saluresis were noted up to the highest dose of 30 mg/kg.

b(4)

Report No. 12730/99 (29 February 2000): Examination of the influence of SPM 8272 on the intestinal motility following oral administration (charcoal propulsion test in the mouse. SPM 8272 (AC 8288, WE No. SPM 10964, 96.94 % pure) was administered to NMRI/ NMRI BR mice (female, 17-22 g, 5/dose) at doses of 0, 3, 10, and 30 mg/kg (by oral gavage, in aqua ad injectabilia). No treatment related effect on intestinal motility was observed.

b(4)

Report No. 12726/99 (29 February 2000): Examination of SPM 8272 and SPM 7605 for spasmolytic and spasmogenic properties in the isolated guinea pig ileum SPM 8272 (AC 8288, WE No. SPM 10964, 96.94 % pure) and SPM 7605 (RD 7664/2, WE No. SPM 10989, 99.28-99.33 % pure) were used to treat Dunkin-Hartley guinea pig intestinal sections (male, 220-230 g, 6 intestinal sections/ test substance). No agonistic properties were observed for SPM 8272 or for SPM 7065 up to the highest concentration tested of 5×10^{-4} or 1×10^{-4} g/ml, respectively, in the bath fluid.

Antagonistic properties:

Agonist	SPM 8272 antagonistic effect		SPM 7605 antagonistic effect	
	Low effect level (g/ml batch fluid)	100 % effect (g/ml batch fluid)	Low effect level (g/ml batch fluid)	100 % effect (g/ml batch fluid)
Acetylcholine (5×10^{-7} g/ml)	3×10^{-8}	1×10^{-6}	$<1 \times 10^{-15}$	1×10^{-15} (lowest concentration tested)
Histamine (5×10^{-8} g/ml)	1×10^{-7}	1×10^{-5}	1×10^{-8}	3×10^{-5}
Barium chloride (2×10^{-4} g/ml)	3×10^{-7}	3×10^{-5}	3×10^{-8}	1×10^{-4}
5-hydroxytryptamine (1.5×10^{-8} g/ml)	1×10^{-8}	1×10^{-5}	1×10^{-5}	1×10^{-4}

IC_{50}s:

Agonist		SPM 8272 IC_{50}	SPM 7605 IC_{50}
Acetylcholine	5×10^{-7} g/ml	1.1×10^{-7} g/ml	$<1 \times 10^{-15}$ g/ml
Histamine	5×10^{-8} g/ml	8.1×10^{-7} g/ml	8.1×10^{-7} g/ml
Barium chloride	2×10^{-4} g/ml	1.9×10^{-6} g/ml	9.8×10^{-6} g/ml
5-hydroxytryptamine	1.5×10^{-8} g/ml	2.2×10^{-7} g/ml	1.4×10^{-5} g/ml

Examination of the effect of SPM 8272 and SPM 7605 on the guinea-pig tracheal muscle. ✎ report no. 12727/98 (March 2000). In isolated guinea pig trachea, no agonistic effect on contraction was observed for either SPM 8272 or SPM 7605, up to the highest dose tested of 5×10^{-4} g/ml. Using acetylcholine (5×10^{-7} g/ml) as an agonist, SPM 7605 exhibited a complete antagonistic effect at 3×10^{-6} g/ml, and SPM 8272 (pro drug) exhibited a marginal effect at 3×10^{-5} g/ml and 31% inhibition of contraction at 5×10^{-4} g/ml.

b(4)

2.6.2.5 **Pharmacodynamic drug interactions.** No studies were performed in animals.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Primary pharmacodynamics *in vitro*:

Type of Study	Test System		Ligand		Findings
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
Fesoterodine	M_1	human recombinant CHO	[^{3}H]pirenzepine	2.00	624
	M_2	human recombinant CHO	[^{3}H]AF-DX 384	2.00	562
	M_3	human recombinant CHO	[^{3}H]-DAMP	0.20	>1000
	M_4	human recombinant CHO	[^{3}H]-DAMP	0.20	177
	M_5	human recombinant CHO	[^{3}H]-DAMP	0.20	>1000
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
SPM 5509	M_1	human recombinant CHO	[^{3}H]pirenzepine	2.00	105
	M_2	human recombinant CHO	[^{3}H]AF-DX 384	2.00	97
	M_3	human recombinant CHO	[^{3}H]-DAMP	0.20	489
	M_4	human recombinant CHO	[^{3}H]-DAMP	0.20	94
	M_5	human recombinant CHO	[^{3}H]-DAMP	0.20	306
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
SPM 6923	M_1	human recombinant CHO	[^{3}H]pirenzepine	2.00	>1000
	M_2	human recombinant CHO	[^{3}H]AF-DX 384	2.00	>1000
	M_3	human recombinant CHO	[^{3}H]-DAMP	0.20	>1000
	M_4	human recombinant CHO	[^{3}H]-DAMP	0.20	>1000
	M_5	human recombinant CHO	[^{3}H]-DAMP	0.20	>1000
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
SPM 7605	M_1	human recombinant CHO	[^{3}H]pirenzepine	2.00	2
	M_2	human recombinant CHO	[^{3}H]AF-DX 384	2.00	2
	M_3	human recombinant CHO	[^{3}H]-DAMP	0.20	6
	M_4	human recombinant CHO	[^{3}H]-DAMP	0.20	1
	M_5	human recombinant CHO	[^{3}H]-DAMP	0.20	5
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
SPM 7835	M_1	human recombinant CHO	[^{3}H]pirenzepine	2.00	40
	M_2	human recombinant CHO	[^{3}H]AF-DX 384	2.00	124
	M_3	human recombinant CHO	[^{3}H]-DAMP	0.20	338
	M_4	human recombinant CHO	[^{3}H]-DAMP	0.20	35
	M_5	human recombinant CHO	[^{3}H]-DAMP	0.20	96
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
Tolterodine	M_1	human recombinant CHO	[^{3}H]pirenzepine	2.00	3
	M_2	human recombinant CHO	[^{3}H]AF-DX 384	2.00	6
	M_3	human recombinant CHO	[^{3}H]-DAMP	0.20	12
	M_4	human recombinant CHO	[^{3}H]-DAMP	0.20	2
	M_5	human recombinant CHO	[^{3}H]-DAMP	0.20	5
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
SPM 5428	M_1	human recombinant CHO	[^{3}H]pirenzepine	2.00	330
	M_2	human recombinant CHO	[^{3}H]AF-DX 384	2.00	200
	M_3	human recombinant CHO	[^{3}H]-DAMP	0.20	810
	M_4	human recombinant CHO	[^{3}H]-DAMP	0.20	350
	M_5	human recombinant CHO	[^{3}H]-DAMP	0.20	600
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
SPM 7504	M_1	human recombinant CHO	[^{3}H]pirenzepine	2.00	170
	M_2	human recombinant CHO	[^{3}H]AF-DX 384	2.00	76
	M_3	human recombinant CHO	[^{3}H]-DAMP	0.20	200
	M_4	human recombinant CHO	[^{3}H]-DAMP	0.20	140
	M_5	human recombinant CHO	[^{3}H]-DAMP	0.20	330
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
SPM 8163	M_1	human recombinant CHO	[^{3}H]pirenzepine	2.00	8
	M_2	human recombinant CHO	[^{3}H]AF-DX 384	2.00	5
	M_3	human recombinant CHO	[^{3}H]-DAMP	0.20	15
	M_4	human recombinant CHO	[^{3}H]-DAMP	0.20	8
	M_5	human recombinant CHO	[^{3}H]-DAMP	0.20	13

Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
SPM 8290	M_1	human recombinant CHO	[^{3}H]pirenzepine	2.00	>1000
	M_2	human recombinant CHO	[^{3}H]AF-DX 384	2.00	>1000
	M_3	human recombinant CHO	[^{3}H]4-DAMP	0.20	>1000
	M_4	human recombinant CHO	[^{3}H]4-DAMP	0.20	>1000
	M_5	human recombinant CHO	[^{3}H]4-DAMP	0.20	>1000
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
Fesoterodine	M_1	human recombinant CHO	[^{3}H]pirenzepine	2.00	950
	M_2	human recombinant CHO	[^{3}H]AF-DX 384	2.00	290
	M_3	human recombinant CHO	[^{3}H]4-DAMP	0.20	570
	M_4	human recombinant CHO	[^{3}H]4-DAMP	0.20	758
	M_5	human recombinant CHO	[^{3}H]4-DAMP	0.20	>1000
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
SPM 8290	M_1	human recombinant CHO	[^{3}H]pirenzepine	2.00	>1000
	M_2	human recombinant CHO	[^{3}H]AF-DX 384	2.00	>1000
	M_3	human recombinant CHO	[^{3}H]4-DAMP	0.20	>1000
	M_4	human recombinant CHO	[^{3}H]4-DAMP	0.20	>1000
	M_5	human recombinant CHO	[^{3}H]4-DAMP	0.20	>1000
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
SPM 16066	M_1	human recombinant CHO	[^{3}H]pirenzepine	2.00	>1000
	M_2	human recombinant CHO	[^{3}H]AF-DX 384	2.00	1500
	M_3	human recombinant CHO	[^{3}H]4-DAMP	0.20	>1000
	M_4	human recombinant CHO	[^{3}H]4-DAMP	0.20	>1000
	M_5	human recombinant CHO	[^{3}H]4-DAMP	0.20	>1000
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	$t_{1/2}$ [h]
SPM 7605	M_1	human recombinant CHO	[^{3}H]SPM 7605	10	0.38
	M_2	human recombinant CHO	[^{3}H]SPM 7605	10	0.15
	M_3	human recombinant CHO	[^{3}H]SPM 7605	10	0.19
	M_4	human recombinant CHO	[^{3}H]SPM 7605	10	0.18
	M_5	human recombinant CHO	[^{3}H]SPM 7605	10	0.64
Receptor binding	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
Tolterodine	Adenosine A_1	human recombinant CHO	[^{3}H]DPCPX	1.00	>1000
	Adenosine A_{2A}	human recombinant HEK293	[^{3}H]CGS-21680	50.0	>1000
	Adrenergic α_1	rat brain	[^{3}H]prazosin	0.25	>1000
	Adrenergic α_2	rat cerebral cortex	[^{3}H]yohimbine	0.70	>1000
	Adrenergic β_1	human recombinant CHO	[^{125}I]iodocyanopindolol	0.03	>1000
	Adrenergic β_2	human recombinant CHO	[^{3}H]CGP-12177	0.10	>1000
	Angiotensin AT_1	human recombinant SF9	[^{125}I][Sar^1,Ile^8]Angiotensin II	0.10	>1000
	Bradykinin B_2	human recombinant CHO-K1	[^{3}H]icatibant	0.20	>1000
	Ca-channel (type L)	rat cerebral cortex	[^{3}H]nimodipine	0.10	>1000
	Dopamine D_2	human recombinant CHO	[^{3}H]SCH23390	1.40	>1000
	Dopamine D_{2L}	human recombinant CHO	[^{3}H]spiperone	2.00	>1000
	Estrogen $ER\alpha$	human recombinant SF9	[^{3}H]estradiol	0.50	>1000
	$GABA_A$ (agonist site)	rat brain	[^{3}H]muscimol	1.00	>1000
	$GABA_A$ (Cl^- channel)	rat cerebral cortex	[^{3}H]TBOB	3.00	>1000
	Glucocorticoid	human HeLa S3 cells	[^{3}H]dexamethasone	6.00	>1000
	Glutamate (NMDA)	rat cerebral cortex	[^{3}H]TCP	4.00	>1000
	Glutamate	rat brain	[^{3}H]L-glutamate	1.60	>1000
	Glycine (strychnine-sensitive)	rat spinal cord	[^{3}H]strychnine	2.00	>1000
	Histamine H_1 (central)	guinea-pig brain	[^{3}H]pyrilamine	3.00	>1000
	Histamine H_2	rat brain	[^{3}H]N-α-methylhistamine	1.00	>1000
	Insulin	rat liver	[^{125}I]insulin	0.03	>1000
	Muscarinic M_1	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	5
	Muscarinic M_2	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	14
	Muscarinic M_3	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	11
	Muscarinic M_4	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	5

	Muscarinic M_5	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	7
	Neuropeptide Y_2	Human KAN-TS cells	[^{125}I]PYY	0.01	>1000
	Nicotinic Acetylcholine	rat brain	[^{3}H]cytisine	2.00	>1000
	Opiate δ	human recombinant CHO	[^{3}H]naltrindole	0.90	>1000
	Opiate κ	human recombinant CHO-K1	[^{3}H]diprenorphine	0.60	>1000
	Opiate μ	human recombinant CHO-K1	[^{3}H]diprenorphine	0.60	>1000
	Phorbol Ester	mouse brain	[^{3}H]PDBu	3.00	>1000
	Progesterone	calf uterus	[^{3}H]R-5020	2.00	>1000
	Purinergic P2X	rabbit urinary bladder	[^{3}H]α, β-methylene-ATP	8.00	>1000
	Purinergic P2Y	rat brain	[^{35}S]ADP-βS	0.08	>1000
	Serotonin 5-HT$_1$	rat cerebral cortex	[^{3}H]serotonin	2.00	>1000
	Serotonin 5-HT$_2$	rat brain	[^{3}H]ketanserin	0.50	>1000
	Sigma σ	pig brain	[^{3}H]DTG	0.80	>1000
	Sodium channel (site 2)	rat brain	[^{3}H]batrachotoxinin	1.50	>1000
	Tachykinin NK$_3$	human recombinant CHO	[^{3}H]SR-140333	0.23	>1000
	Testosterone	rat ventral prostate	[^{3}H]mibolerone	2.00	>1000
Receptor binding SPM 7605	Receptor	Origin	Ligand Concentration [nM]		K_i [nM]
	Adenosine A_2	human recombinant CHO	[^{3}H]DPCPX	1.00	>1000
	Adenosine A_{2A}	human recombinant HEK293	[^{3}H]CGS-21680	50.0	>1000
	Adrenergic α_1	rat brain	[^{3}H]prazosin	0.25	>1000
	Adrenergic α_2	rat cerebral cortex	[^{3}H]rauwolscine	0.70	>1000
	Adrenergic β_2	human recombinant CHO	[^{3}H]CGP-12177	0.30	>1000
	Angiotensin AT$_1$	human recombinant SF9	[^{125}I][Sar^{1}Ile^8]Angiotensin II	0.10	>1000
	Bradykinin B$_2$	human recombinant CHO-K1	[^{3}H]bradykinin	0.20	>1000
	Ca-channel (type L)	rat cerebral cortex	[^{3}H]nitrendipine	0.10	>1000
	Dopamine D$_2$	human recombinant CHO	[^{3}H]SCH23390	1.40	>1000
	Dopamine D$_{2L}$	human recombinant CHO	[^{3}H]spiperone	2.00	>1000
	Estrogen ERα	human recombinant SF9	[^{3}H]estradiol	0.50	>1000
	GABA$_A$ (agonist site)	rat brain	[^{3}H]muscimol	1.00	>1000
	GABA$_A$ (Cl$^-$ channel)	rat cerebral cortex	[^{3}H]TBOB	3.00	>1000
	Glucocorticoid	human HeLa S3 cells	[^{3}H]dexamethasone	6.00	>1000
	Glutamate (NMDA)	rat cortical cortex	[^{3}H]TCP	4.00	>1000
	Glutamate	rat brain	[^{3}H]L-glutamate	1.60	>1000
	Glycine (strychnine-sensitive)	rat spinal cord	[^{3}H]strychnine	2.00	>1000
	Histamine H$_1$ (central)	guinea-pig brain	[^{3}H]pyrilamine	3.60	>1000
	Histamine H$_2$	rat brain	[^{3}H]N-α-methylhistamine	1.00	>1000
	Insulin	rat liver	[^{125}I]insulin	0.03	>1000
	Muscarinic M_1	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	1
	Muscarinic M_2	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	3
	Muscarinic M_3	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	2
	Muscarinic M_4	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	1
	Muscarinic M_5	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	3
	Neuropeptide Y_2	Human KAN-TS cells	[^{125}I]PYY	0.01	>1000
	Nicotinic Acetylcholine	rat brain	[^{3}H]cytisine	2.00	>1000
	Opiate δ	human recombinant CHO	[^{3}H]naltrindole	0.90	>1000
	Opiate κ	human recombinant CHO-K1	[^{3}H]diprenorphine	0.60	>1000
	Opiate μ	human recombinant CHO-K1	[^{3}H]diprenorphine	0.60	>1000
	Phorbol Ester	mouse brain	[^{3}H]PDBu	3.00	>1000
	Progesterone	calf uterus	[^{3}H]R-5020	2.00	>1000
	Purinergic P2X	rabbit urinary bladder	[^{3}H]α, β-methylene-ATP	8.00	>1000
	Purinergic P2Y	rat brain	[^{35}S]ADP-βS	0.08	>1000
	Serotonin 5-HT$_1$	rat cerebral cortex	[^{3}H]serotonin	2.00	>1000
	Serotonin 5-HT$_2$	rat brain	[^{3}H]ketanserin	0.50	>1000
	Sigma σ	pig brain	[^{3}H]DTG	0.80	>1000
	Sodium channel (site 2)	rat brain	[^{3}H]batrachotoxinin	1.50	>1000
	Tachykinin NK$_3$	human recombinant CHO	[^{3}H]SR-140333	0.23	>1000

	Testosterone	rat ventral prostate	[^{3}H]mibolerone	2.00	>1000
Receptor binding Fesoterodine	Receptor	Origin	Ligand	Concentration [nM]	K_i [nM]
	Adenosine A_1	human recombinant CHO	[^{3}H]DPCPX	1.00	>1000
	Adenosine A_{2A}	human recombinant HEK293	[^{3}H]CGS-21680	50.0	>1000
	Adrenergic α_1	rat brain	[^{3}H]prazosin	0.25	>1000
	Adrenergic α_2	rat cerebral cortex	[^{3}H]rauwolscine	0.70	>1000
	Adrenergic β_1	human recombinant CHO	[^{125}I]iodocyanopindolol	0.03	>1000
	Adrenergic β_2	human recombinant CHO	[^{3}H]CGP-12177	0.20	>1000
	Angiotensin AT_1	human recombinant SF9	[^{125}I][Sar^{1}Ile^8]Angiotensin II	0.10	>1000
	Bradykinin B_1	human recombinant CHO-K1	[^{3}H]bradykinin	0.20	>1000
	Ca-channel (type L)	rat cerebral cortex	[^{3}H]nitrendipine	0.10	>1000
	Dopamine D_1	human recombinant CHO	[^{3}H]SCH23390	1.40	>1000
	Dopamine D_2	human recombinant CHO	[^{3}H]spiperone	2.00	>1000
	Estrogen $ER\alpha$	human recombinant SF9	[^{3}H]estradiol	0.50	>1000
	$GABA_A$ (agonist site)	rat brain	[^{3}H]muscimol	1.00	>1000
	$GABA_A$ (Cl$^-$ channel)	rat cerebral cortex	[^{3}H]TBOB	3.00	>1000
	Glucocorticoid	human HeLa S3 cells	[^{3}H]dexamethasone	6.00	>1000
	Glutamate (NMDA)	rat cerebral cortex	[^{3}H]CPP	4.00	>1000
	Glutamate	rat brain	[^{3}H]L-glutamate	1.60	>1000
	Glycine (strychnine-sensitive)	rat spinal cord	[^{3}H]strychnine	2.00	>1000
	Histamine H_1 (central)	guinea-pig brain	[^{3}H]pyrilamine	3.00	>1000
	Histamine H_2	rat brain	[^{3}H]N-α-methylhistamine	1.00	>1000
	Insulin	rat liver	[^{125}I]insulin	0.03	>1000
	Muscarinic M_1	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	52
	Muscarinic M_2	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	>1000
	Muscarinic M_3	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	209
	Muscarinic M_4	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	25
	Muscarinic M_5	human recombinant SF9	[^{3}H]N-methylscopolamine	0.29	285
	Neuropeptide Y_2	Human IMR-TS cells	[^{125}I]PYY	0.01	>1000
	Nicotinic Acetylcholine	rat brain	[^{3}H]cytisine	2.00	>1000
	Opiate δ	human recombinant CHO	[^{3}H]naltrindole	0.90	>1000
	Opiate κ	human recombinant CHO-K1	[^{3}H]diprenorphine	0.60	>1000
	Opiate μ	human recombinant CHO-K1	[^{3}H]diprenorphine	0.60	>1000
	Platelet Ether	mouse brain	[^{3}H]PDBu	3.00	>1000
	Progesterone	calf uterus	[^{3}H]R-5020	2.00	>1000
	Purinergic P2X	rabbit urinary bladder	[^{3}H]α, β-methylene-ATP	8.00	>1000
	Purinergic P2Y	rat brain	[^{35}S]ADP-βS	0.03	>1000
	Serotonin 5-HT_1	rat cerebral cortex	[^{3}H]serotonin	1.00	>1000
	Serotonin 5-HT_2	rat brain	[^{3}H]ketanserin	0.30	>1000
	Sigma σ	pig brain	[^{3}H]DTG	0.30	<1000
	Sodium channel (site 2)	rat brain	[^{3}H]batrachotoxinin	1.50	>1000
	Tachykinin NK_1	human recombinant CHO	[^{3}H]SR-140333	0.23	>1000
	Testosterone	rat ventral prostate	[^{3}H]mibolerone	2.00	>1000
Receptor binding SPM 7605	Receptor	Origin	Ligand		K_i [nM]
	M (non-specific)	rat cerebral cortex	[^{3}H]QNB		0.24
	histamine H_1	guinea-pig cerebellum	[^{3}H]pyrilamine		976
	sigma σ_1	guinea-pig cerebral cortex	[^{3}H]pentazocine		1758
Receptor binding Tolterodine	Receptor	Origin	Ligand		K_i [nM]
	M (non-specific)	rat cerebral cortex	[^{3}H]QNB		0.34
	Histamine H_1	guinea-pig cerebellum	[^{3}H]pyrilamine		170
	sigma σ_1	guinea-pig cerebral cortex	[^{3}H]pentazocine		333
Receptor binding SPM 7605	Receptor	Origin	Ligand		K_i [nM]
	M_1	human recombinant CHO	[^{3}H]QNB		2.3
	M_2	human recombinant CHO	[^{3}H]QNB		2.0
	M_3	human recombinant CHO	[^{3}H]QNB		2.3
	M_4	human recombinant CHO	[^{3}H]QNB		2.8
	M_5	human recombinant CHO	[^{3}H]QNB		2.9

	M (non-specific)	guinea-pig urinary bladder	[^{3}H]QNB		2.9
	M (non-specific)	guinea-pig parotid gland	[^{3}H]QNB		5.2
	M (non-specific)	guinea-pig heart	[^{3}H]QNB		1.1
	M (non-specific)	guinea-pig cerebral cortex	[^{3}H]QNB		0.6
Receptor binding	Receptor	Origin	Ligand		K_i [nM]
Tolterodine	M_1	human recombinant CHO	[^{3}H]QNB		3.0
	M_2	human recombinant CHO	[^{3}H]QNB		5.8
	M_3	human recombinant CHO	[^{3}H]QNB		3.5
	M_4	human recombinant CHO	[^{3}H]QNB		5.0
	M_5	human recombinant CHO	[^{3}H]QNB		3.4
	M (non-specific)	guinea-pig urinary bladder	[^{3}H]QNB		2.7
	M (non-specific)	guinea-pig parotid gland	[^{3}H]QNB		4.8
	M (non-specific)	guinea-pig heart	[^{3}H]QNB		1.6
	M (non-specific)	guinea-pig cerebral cortex	[^{3}H]QNB		0.8
Receptor antagonism Fesoterodine reporter gene assay	Reporter gene assay	Origin	Ligand	Concentration [pM]	IC_{50} [nM]
	M_1	human recombinant CHO	acetylcholine	10	144
	M_2	human recombinant CHO	acetylcholine	0.5	59
	M_3	human recombinant CHO	acetylcholine	0.5	147
	M_4	human recombinant CHO	acetylcholine	10	270
	M_5	human recombinant CHO	acetylcholine	50	591
Receptor antagonism SPM 7605 reporter gene assay	Reporter gene assay	Origin	Ligand	Concentration [pM]	IC_{50} [nM]
	M_1	human recombinant CHO	acetylcholine	10	5
	M_2	human recombinant CHO	acetylcholine	0.5	4
	M_3	human recombinant CHO	acetylcholine	0.5	13
	M_4	human recombinant CHO	acetylcholine	10	36
	M_5	human recombinant CHO	acetylcholine	50	35
Receptor antagonism SPM 5508 reporter gene assay	Reporter gene assay	Origin	Ligand	Concentration [pM]	IC_{50} [nM]
	M_1	human recombinant CHO	acetylcholine	10	303
	M_2	human recombinant CHO	acetylcholine	0.5	115
	M_3	human recombinant CHO	acetylcholine	0.5	1175
	M_4	human recombinant CHO	acetylcholine	10	2010
	M_5	human recombinant CHO	acetylcholine	50	2295
Receptor antagonism Tolterodine reporter gene assay	Reporter gene assay	Origin	Ligand	Concentration [pM]	IC_{50} [nM]
	M_1	human recombinant CHO	acetylcholine	10	12
	M_2	human recombinant CHO	acetylcholine	0.5	9
	M_3	human recombinant CHO	acetylcholine	0.5	20
	M_4	human recombinant CHO	acetylcholine	10	37
	M_5	human recombinant CHO	acetylcholine	50	48
Functional tissue effects Fesoterodine	Carbachol and electrical field stimulation induced contraction of rat urinary bladder strips		pA_2 (carbachol) 8.7 60±15% inhibition of electrical field stimulation induced contraction of bladder strips at 0.1 µmol/L.		
Functional tissue effects SPM 7605	Carbachol and electrical field stimulation induced contraction of rat urinary bladder strips		pA_2 (carbachol) 8.8 47±13% inhibition of electrical field stimulation induced contraction of bladder strips at 0.1 µmol/L.		
Functional tissue effects Tolterodine	Carbachol and electrical field stimulation induced contraction of rat urinary bladder strips		pA_2 (carbachol) 8.5 42±6% inhibition of electrical field stimulation induced contraction of bladder strips at 0.3 µmol/L.		
Functional tissue effects Oxybutynin	Carbachol and electrical field stimulation induced contraction of rat urinary bladder strips		pA_2 (carbachol) 8.4 34±14% inhibition of electrical field stimulation induced contraction of bladder strips at 0.1 µmol/L.		
Functional tissue effects Atropine	Carbachol and electrical field stimulation induced contraction of rat urinary bladder strips		pA_2 (carbachol) 9.0 40±27% inhibition of electrical field stimulation induced contraction of bladder strips at 0.1 µmol/L.		
Functional tissue effects SPM 7605	Carbachol induced contraction of rat urinary bladder strips		pK_B: 9.04		

Functional tissue effects Tolterodine	Carbachol induced contraction of rat urinary bladder strip	pK_B: 8.71
Functional tissue effects Oxybutynin	Carbachol induced contraction of rat urinary bladder strips	pK_B: 7.95
Functional tissue effects Atropine	Carbachol induced contraction of rat urinary bladder strips	pK_B: 9.1
Functional tissue effects SPM 7605	Carbachol induced contraction of guinea-pig urinary bladder strips	pA_2: 9.14
Functional tissue effects SPM 7605	Carbachol induced contraction of guinea-pig urinary bladder strips	IC_{50}: 5.7 nM
Functional tissue effects Tolterodine	Carbachol induced contraction of guinea-pig urinary bladder strips	IC_{50}: 14 nM
Functional tissue effects Oxybutynin	Carbachol induced contraction of guinea-pig urinary bladder strips	IC_{50}: 17 nM
Functional tissue effects Atropine	Carbachol induced contraction of guinea-pig urinary bladder strips	K_{DB}: 7.2 nM
Functional tissue effects SPM 7605	Carbachol-, KCl-, $CaCl_2$-, and electrical field stimulation induced contraction of human urinary bladder strips	pA_2 (carbachol) 9.04 No effect on KCl- and $CaCl_2$-induced contractions 57.5±7.1% and 70.8±1.0% inhibition of electrical field stimulation induced contractions at stimulation frequencies of 2 and 60 Hz, respectively.
Functional tissue effects Tolterodine	Carbachol-, KCl-, $CaCl_2$-, and electrical field stimulation induced contraction of human urinary bladder strips	pA_2 (carbachol) 9.04 No effect on KCl- and $CaCl_2$-induced contractions 52.5±10.2% and 66.2±4.1% inhibition of electrical field stimulation induced contractions at stimulation frequencies of 2 and 60 Hz, respectively.
Functional tissue effects Oxybutynin	Carbachol-, KCl-, $CaCl_2$-, and electrical field stimulation induced contraction of human urinary bladder strips	pA_2 (carbachol) 8.63 34% and 35% inhibition of KCl- and $CaCl_2$-induced contractions, respectively at 10 µmol/L 65.6±4.7% and 81.4±2.5% inhibition of electrical field stimulation induced contractions at stimulation frequencies of 2 and 60 Hz, respectively.
Functional tissue effects Atropine	Carbachol-, KCl-, $CaCl_2$-, and electrical field stimulation induced contraction of human urinary bladder strips	pA_2 (carbachol) 9.05 48.8±4.7% and 67.2±2.1% inhibition of electrical field stimulation induced contractions at stimulation frequencies of 2 and 60 Hz, respectively.

Primary pharmacodynamics *in vivo*:

Urodynamic measurement Fesoterodine	Rats, Sprague Dawley, female	Cystometry	0.01, 0.1, 1.0 mg/kg	Intravenous	Bladder capacity Intercontraction interval Micturition pressure	19% increased bladder capacity and intercontraction interval at 0.01 mg/kg 30% decreased micturition pressure at 0.01 mg/kg
Urodynamic measurement SPM 7605	Rats, Sprague Dawley, female	Cystometry	0.01, 0.1, 1.0 mg/kg	Intravenous	Bladder capacity Intercontraction interval Micturition pressure	10% and 33% increased bladder capacity and intercontraction interval, respectively at 0.01 mg/kg 63% decreased micturition pressure at 0.01 mg/kg
Urodynamic measurement Tolterodine	Rats, Sprague Dawley, female	Cystometry	0.1, 0.5, 1.0 mg/kg	Intravenous	Bladder capacity Intercontraction interval Micturition pressure	45% decreased micturition pressure at 0.1 mg/kg
Urodynamic measurement Oxybutynin	Rats, Sprague Dawley, female	Cystometry	0.1, 0.5 1.0 mg/kg	Intravenous	Bladder capacity Intercontraction interval Micturition pressure	46% decreased micturition pressure at 0.1 mg/kg

Urodynamic measurements Atropine	Rats, Sprague Dawley, female	Cystometry	0.5, 1.0 mg/kg	Intravenous	Bladder capacity Intercontraction interval Micturition pressure	67% decreased micturition pressure at 0.5 mg/kg
Urodynamic measurements SPM 7605	Rats, Sprague Dawley, female	Cystometry	0.003, 0.01, 0.03, 0.1 mg/kg	Intravenous	Micturition pressure	ID_{50}: 23 nmol/kg
Urodynamic measurements Tolterodine	Rats, Sprague Dawley, female	Cystometry	0.003, 0.01, 0.03, 0.1 mg/kg	Intravenous	Micturition pressure	ID_{50}: 54 nmol/kg
Urodynamic measurements Darifenacin	Rats, Sprague Dawley, female	Cystometry	0.003, 0.01, 0.03, 0.1 mg/kg	Intravenous	Micturition pressure	ID_{50}: 236 nmol/kg
Urodynamic measurements Oxybutynin	Rats, Sprague Dawley, female	Cystometry	0.003, 0.01, 0.03, 0.1 mg/kg	Intravenous	Micturition pressure	ID_{50}: 175 nmol/kg
Urodynamic measurements Atropine	Rats, Sprague Dawley, female	Cystometry	0.003, 0.01, 0.03, 0.1 mg/kg	Intravenous	Micturition pressure	ID_{50}: 14 nmol/kg
Acetylcholine-induced urinary bladder contraction SPM 7605	Cats, European short haired, female	Cystometry	2, 6, 20, 60, 200 nmol/kg	Intravenous	Urinary bladder contraction	ID_{50}: 13 nmol/kg

Functional tissue effects SPM 7605	Histamine induced contraction of guinea-pig ileum	IC_{50}: 6.1 µM
	Noradrenalin induced contraction of rat portal vein	IC_{50}: 100 µM
	Spontaneously beating guinea-pig right atrium	IC_{50}: 15.2 µM
	Electrically paced guinea-pig papillary muscle	IC_{50}: 5.4 µM
Functional tissue effects Tolterodine	Histamine induced contraction of guinea-pig ileum	IC_{50}: 340 nM
	Noradrenalin induced contraction of rat portal vein	IC_{50}: 2.8 µM
	Spontaneously beating guinea-pig right atrium	IC_{50}: 5.2 µM
	Electrically paced guinea-pig papillary muscle	IC_{50}: 6.5 µM
Functional tissue effects Oxybutynin	Histamine induced contraction of guinea-pig ileum	IC_{50}: 2.6 µM
	Noradrenalin induced contraction of rat portal vein	IC_{50}: 30 µM
	Spontaneously beating guinea-pig right atrium	IC_{50}: 9.7 µM
	Electrically paced guinea-pig papillary muscle	IC_{50}: 6.1 µM
Functional tissue effects Atropine	Histamine induced contraction of guinea-pig ileum	IC_{50}: 2.5 µM
	Noradrenalin induced contraction of rat portal vein	IC_{50}: 8.8 µM
	Spontaneously beating guinea-pig right atrium	IC_{50}: 54.0 µM
	Electrically paced guinea-pig papillary muscle	IC_{50}: 13.0 µM

Gastrointestinal transit Fesoterodine, Tolterodine	NMRI mice, male	Charcoal propulsion	1, 3, 10, 30 mg/kg	Oral	Distance of small intestine covered by charcoal	No significant effects of both substances
Electrical stimulation-induced salivary gland secretion SPM 7605	Cats, European short haired, female	Cystometry	2, 6, 20, 60, 200 nmol/kg	Intravenous	Salivary secretion	ID_{50}: 40 nmol/kg

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Absorption, distribution, metabolism and excretion of fesoterodine were studied in mice, rats, and dogs. Mice were most similar to human in terms of metabolic profile, and dogs were most similar in terms of routes of excretion (primarily in urine). Pigmented tissues were investigated in male mice and rats. Placental transfer was examined in pregnant mice and rats. Fesoterodine and its major human metabolites were also monitored in toxicity studies in mice, rats, rabbits and dogs. Fesoterodine and/or SPM 7605 (active entity / hydroxy metabolite), and carboxy (SPM 5509), carboxy-N-desisopropyl (SPM 7790) and N-desisopropyl metabolites (SPM 7789), as measured by LC-MS/MS, were adequately represented in the toxicity species. No inversion at the chiral centre of fesoterodine has been observed. The parameters for the method validations included accuracy, precision, selectivity, sensitivity, linearity, reproducibility, recovery, and stability. The analytes and matrix were the same as in clinical trials.

2.6.4.2 Methods of Analysis

[see also under individual study reviews]

Investigation of stability of SPM 8272 in mouse plasma (MA252)(July 2002). Liquid-liquid extraction in diethylether and back-extraction in HCl, followed by chromatographic separation on a ~ column under isocratic conditions, with UV detection, was used to isolate SPM 8272 SPM 8272 was extracted from water with a hydrolysis of about 2-3%; in mouse plasma, the hydrolysis was temperature dependent and could only be partially inhibited by the esterase inhibitor pyridostigmine bromide.

Stability of SPM 8272 in mouse, rabbit and human plasma *in vitro* (BA-385-02)(November 1999). SPM 8272 was rapidly hydrolyzed in mouse, rabbit, and human plasma at 37C in vitro; hydrolysis could not be prevented by addition of NaF. Strictly uniform handling of samples was recommended to prevent errors in measurement.

Stability of SPM 8272 in dog and rat plasma *in vitro* (BA-384-02)(October 1999). Stability in rat plasma was determined, preliminarily, to be lower than in dog plasma and to be unaffected by NaF.

Stability of fesoterodine (BA-495-02)(SPM 8272) in hamster and monkey plasma *in vitro* (March 2002). Hydrolytic activity in monkey and hamster plasma at 37C was shown to be as high as in mouse, rat, rabbit, and human plasma; no SPM 8272 was detectable after 30 minutes. NaF did not inhibit hydrolysis.

Determination of SPM 7605 in mouse plasma by LC-MS/MS (KA031)(March 2000). The method was found to be specific, sensitive, linear, precise, reproducible and with an adequate recovery. The analyte was stable during the procedure (methanolic samples

were stable at room temperature for 24 hours and plasma samples were stable for at least 24 hours, in the freezer at -20C for at least 14 days, and after up to 3 freeze-thaw cycles:

Calibrated Range	1.00 ng/mL – 1000 ng/mL
Defined LOQ	1.00 ng/mL
Linearity (mean r^2 of the standard curves)	0.99984
Intra-assay Accuracy [bias %]	between 4.4 and 8.2
Intra-assay Precision [cv %] (ICH: Repeatability)	between 0.5 and 6.4
Inter-assay Accuracy [bias %]	between 4.8 and 7.7
Inter-assay Precision [cv %] (ICH: Intermediate Precision)	between 2.2 and 6.7
Mean Recovery [%]	100.0

Amendment to determination of SPM 7805 in mouse plasma by LC-MS/MS (KA031)(March 2000). SPM 7605 was stable in mouse plasma at -20C for 260 days.

Validation of a method for the determination of SPM 5509, SPM 7789 and SPM 7790 (three major metabolites of fesoterodine: carboxy-, desisopropyl-, and desisopropyl-carboxy-, respectively) in mouse plasma by HPLC-electrospray MS/MS (1-500 ng/ml)(BA512-02)(April 2002). The quantitation limit was 1 ng/ml, Precision was 14.8% (SPM 5509), 7.1% (SPM 7789), 17.1% (SPM 7790) and accuracy was 99.5% (SPM 5509), 106.3% (SPM 7789), 103.0% (SPM 7790). The lowest concentration tested was 2 ng/ml; precision was 10.0% (SPM 5509), 5.8% (SPM 7789), 5.3% (SPM 7790) and accuracy was 97.5% (SPM 5509), 92.6% (SPM 7789), 99.8% (SPM 7790).

Amendment-Validation of a method for the determination of SPM 5509, SPM 7789, and SPM 7790 in mouse plasma by HPLC-electrospray MS/MS (BA512-02)(March 2003). The three major metabolites were determined to be stable in mouse plasma at <-50C for 9 months.

Validation of a HPLC-method for the determination of SPM 7500 in rat plasma with "in line clean up" in a concentration range of 25-2500 ng/ml (BA-326-03)(October, 1999). An HPLC method was described using an [redacted] column and UV detection. The linearity of calibration curves was high, and intraassay and interassay imprecisions and inaccuracies were reported to be ≤13.4%, and in most cases, below 6.3%. b(4)

Determination of SPM 7605 in rat plasma by HPLC-electrospray MS/MS in a concentration range of 0.05-20 ng/0.2 ml (BA-370-03)(May 2000). The quantitation limit was reported to be 0.05 ng/0.2 ml with an imprecision of 18.4% and an inaccuracy of 22.4%, after interassay validation.

Validation of a method for the determination of SPM 7605 in rat plasma by HPLC-electrospray MS/MS (validation at an [redacted] mass spectrometer)(BA-494-03)(February 2002). The calibration curves, in the range 0.25-100 ng/ml, were calculated by linear regression with 1/x weighting; the mean coefficient of correlation 'r' was 0.9999, and the mean precision of the slope 'b' 2.7%. the quantitation limit for SPM 7605 was 0.25 ng/ml with a precision of 3.0% and an accuracy of 101.3%, after interassay validation. b(4)

Determination of SPM 7605 in rat plasma by HPLC-electrospray MS/MS in a concentration range 0.05-20 ng/0.2 ml (BA-421-03)(December 2000). The method used in study BA-370-03 was revalidated using the [redacted] internal standard for SPM 7605 (SPM 8305). The maximum precision and accuracy of the concentration range of 0.05-20 ng/0.2 ml was 5.3% and 3.4%, respectively. b(4)

Validation of a method for the determination of SPM 5509, SPM 789 and SPM 7790 (three major metabolites of fesoterodine) in rat plasma by HPLC-electrospray MS/MS (BA-506-02)(February 2002). For each metabolite, in a concentration range of 0.4-400 ng/ml, calibration curves were calculated by linear regression with 1/x weighting The mean coefficients of correlation 'r' were 0.9988 (PM 5509), 0.9988 (SPM 7789) and 0.9989 (SPM 7790). The quantitation limit was 0.4 ng/ml (precision: 5.0% for SPM 5509, 14.3% for SPM 7789, 3.1% for SPM 7790 and accuracy: 102.1% for SPM 5509, 96.0% for SPM 7789, 101.5% for SPM 7790.) The lowest concentration examined during interassay validation was 0.8 ng/ml with a precision of 11.1% (SPM 5509), 6.3% (SPM 7789), 8.1% (SPM7790) and an accuracy of 107.4% (SPM 5509), 102.7% (SPM7789) and 107.0% (SPM7790).

Amendment 1-Validation of a method for the determination of SPM 5509, SPM 789 and SPM 7790 (three major metabolites of fesoterodine) in rat plasma by HPLC-electrospray MS/MS (BA-506-02)(June 2002). The metabolites were stable for 4 months at <-50C.

Amendment 2-Validation of a method for the determination of SPM 5509, SPM 7789 and SPM 7790 (three major metabolites of fesoterodine) in rat plasma by HPLC-electrospray MS/MS (BA-506-02)(February 2003). The metabolites were stable for 1 year at <-50C.

Determination of SPM 7605 in rabbit plasma by LC-MS/MS: validation study report (KA031)(March 2000). The analysis of samples was performed by LC-MS/MS-column switching technique, using the [redacted] system after on=line sample clean up with turbulent flow chromatography. After addition of [redacted] internal standard solution and centrifugation, the supernatant was directly injected into the chromatographic system. An electrospray ion source – atmospheric pressure ionization (API) – was used for ionization with positive ion mode. The chromatographic separation of the analyte was performed on a cyanopropyl system. b(4)

Characteristics of the method is given in the following table:

Calibrated Range	1.00 ng/mL – 1000 ng/mL
Defined LOQ	1.00 ng/mL
Linearity (mean r^2 of the standard curves)	0.99987
Intra-assay Accuracy [bias %]	between 3.2 and 6.5
Intra-assay Precision [cv %] (ICH: Repeatability)	between 1.2 and 2.0
Inter-assay Accuracy [bias %]	between 6.2 and 7.7
Inter-assay Precision [cv %] (ICH: Intermediate Precision)	between 1.4 and 4.3
Mean Recovery [%]	101.6

Amendment 1-Determination of SPM 7605 in rabbit plasma by LC-MS/MS: validation study report (KA031)(November, 2000). SPM was stable in rabbit plasma at -20C for 268 days.

Validation of a method for the determination of SPM 5509, SPM 7789 and APM 7790 (three major metabolites of fesoterodine) in rabbit plasma by HPLC-electrospray MS/MS (BA-516-02)(July 2002). For each metabolite, in a concentration range of 0.5-500 ng/ml, calibration curves were calculated by linear regression with 1/x weighting The mean coefficients of correlation 'r' were 0.9999 (SPM 5509 and SPM 7790) and 0.9993 (SPM 7789). The quantitation limit was 0.5 ng/ml (precision: 8.0% for SPM 5509, 5.0% for SPM 7789, 9.8% for SPM 7790 and accuracy: 93.8% for SPM 5509, 86.0% for SPM 7789, 103.5% for SPM 7790.) The concentrations examined during interassay validation were 1, 5, 25, 50, 100, and 500 ng/ml with a precision of 1.1-13.7% (SPM 5509), 1.7-12.2% (SPM 7789), 2.2-11.5% (SPM7790) and an accuracy of 95.5-100.9% (SPM 5509), 93.6-107.9% (SPM7789) and 95.6-101.8% (SPM7790).

Amendment 1-Validation of a method for the determination of SPM 5509, SPM 7789 and APM 7790 (three major metabolites of fesoterodine) in rabbit plasma by HPLC-electrospray MS/MS (BA-516-02)(March 2003). The metabolites were shown to be stable for 9 months at < -50C in rabbit plasma.

Validation of a HPLC method for the determination of SPM 7500 in dog plasma with "in line clean up" in a concentration range of 25-2500 ng/ml (BA-320-03)(November 1999). An HPLC method was described using an [redacted] column and UV detection. The linearity of calibration curves was high, and intraassay and interassay imprecisions and inaccuracies were reported to be ≤7.6%, and in most cases, below 4.6%.

Determination of SPM 8272 and SPM 7605 in dog plasma by HPLC-electrospray MS/MS in the concentration range 0.05-50 ng/0.2 ml (BA363-03)(May 2001). The quantitation limit following interassay validation was 0.25 ng/ml with a precision of

8.1% and an accuracy of 112.7% for SPM 8272 and a precision of 15.9% and accuracy of 102.0% for SPM 7605.

Validation of a method for the determination of SPM 8272 and SPM 7605 in dog plasma by HPLC-electrospray MS/MS in the concentration range 0.05-100 ng/0.2 ml (BA-464-03)(April 2002). Calibration curves in the range 0.25-500 ng/ml were calculated by linear regression with 1/x weighting. The mean coefficient of correlation 'r' was 0.9995 (SPM 8272) and 0.9994 (SPM 7605) and the mean precision of the slope 'b' 7.2% (SPM 8272) and 5.5% (SPM 7605). The quantitation limit for SPM 8272 and SPM 7605 was 0.25 ng/ml, the precision was 12.% and the accuracy was 102.0% for SPM 8272, and the precision was 9.3% and the accuracy was 94.4% for SPM 7605, following interassay validation.

Validation of a method for the determination of SPM 5509, SPM 7789 and SPM 7790 (three major metabolites of fesoterodine) in dog plasma by HPLC-electrospray MS/MS (BA-515-02)(June 2002). Calibration curves in the range 0.5-500 ng/ml were calculated by linear regression with 1/x weighting. The mean coefficient of correlation 'r' was 0.9997 (SPM 5509), 0.9994 (SPM 7789) and 0.9998 (SPM 7790). The quantitation limit was 0.5 ng/ml for calibration curves (precision: 6.2% (SPM 5509), 7.8% (SPM 7789), 5.2% (PM 7790) and accuracy: 92.2% (SPM 5509), 86.5% (SPM 7789), 99.6% (SPM 7790) ,following interassay validation. During the interassay validation performed at 1, 5, 25, 50, 100 and 500 ng/ml, the precision ranged from 3.9 to 6.7% (SPM 5509), from 2.6 to 5.5% (SPM 7789), from 4.2% to 7.3% (SPM 7790) and the accuracy form 96.1% to 100.5% (SPM 5509), from 91.8 to 102.3% (SPM 7789) and from 98.7% to 101.6% (SPM 7790).

Amendment 1-Validation of a method for the determination of SPM 5509, SPM 7789 and SPM 7790 (three major metabolites of fesoterodine) in dog plasma by HPLC-electrospray MS/MS (BA-515-02)(March 2003). The metabolites were stable for 9 months in dog plasma at < -50 C.

Validation of a chromatographic method (HPLC) for the ester (SPM 8272), tolterodine (SPM 9078) and the metabolite (SPM 7605)(March 2002). An HPLC method was validated with Krebs-ringer buffer for use in the Caco-2 cell monolayer assay.

Development and validation of an in vitro method to study inhibitory drug-drug interactions with the human cytochrome P450 isoenzymes 1A2, 2C9, 2C19, 2D6, and 3A4 (BA-443-02)(November 2002). An automated fluorometric assay was used employing recombinant human CYPs and converting substrates to fluorogenic compounds. CYP specific inhibitors and SPM 962 were used as validation compounds.

	validation compound (conc. range)	IC_{50} (mean ± imprecision)
CYP1A2:	furafylline (50000 – 23 nM)	1008 ± 11.7 %
	furafylline (15000 – 7 nM)	1228 ± 0.6 %
	SPM 962 (200000 – 91 nM)	31723 ± 9.8 %
CYP2C9:	sulfaphenazole (8000 – 3.7 nM)	239 ± 1.6 %
	sulfaphenazole (6000 – 2.7 nM)	275 ± 11.0 %
	SPM 962 (100000 – 46 nM)	15647 ± 14.5 %
CYP2C19:	tranylcypromine (20000 – 9 nM)	1871 ± 1.1 %
	tranylcypromine (18000 – 8 nM)	1755 ± 0.3 %
	SPM 962 (50000 – 23 nM)	1313 ± 3.7 %
CYP2D6:	quinidine (400 – 0.2 nM)	5.5 ± 4.2 %
	quinidine (300 – 0.1 nM)	10.3 ± 16.6 %
	SPM 962 (10000 – 4.6 nM)	372 ± 7.9 %
CYP3A4:	ketoconazole (1250 – 0.6 nM)	31.4 ± 5.8 %
	ketoconazole (900 – 0.4 nM)	59.6 ± 7.3 %
	SPM 962 (100000 – 46 nM)	14354 ± 15.3 %

Amendment-Development and validation of an *in vitro* method to study inhibitory drug-drug interactions with the human cytochrome P450 isoenzymes 1A2, 2C0, 2C19, 2D6 and 3A4 (BA-443-02)(July, 2003).

	K_m [µM]	
Cytochrome P450 isoenzyme /substrate	mean	SD
CYP1A2 / CEC	3.68	0.36
CYP2C9 / MFC	45.41	15.40
CYP2C19 / CEC	93.86	17.88
CYP2D6 / AMMC	0.25	0.02

Validation of cryopreserved human hepatocyte culture as an in vitro test system to investigate the potential of test compounds for cytochrome P450 induction (BA-518-02)(November 2002). It was determined that only a qualitative induction potential of CYP 1A and 3A could be determined in the cryopreserved cell culture.

Study title: Method re-validation for the determination of SPM 8272 in aqueous test substance carrier mixtures and in gelatin capsules (Study no. 13351/00)(October 2000). SPM 8272 in aqueous samples and in gelatin capsules were quantified using HPLC and UV detection and an internal reference standard. The following validation results were obtained.

Parameter	Method for the Determination of SPM 8272 in Aqueous Test Substance Carrier Mixtures and in Gelatine Capsule Samples
Linearity	r^2 = 1.000 (range 50 to 200 mg/l)
Accuracy	inaccuracy approx. ± 3% (mean)
intra-day and inter-day Precision	better than 3%
Stability	no apparent degradation within 72 hours of storage and within the entire autosampler run (approx. 17 hours at room temperature)
Sensitivity	Limit of Quantification (LOQ) = 9.1 mg/l Limit of Detection (LOD) = 3.0 mg/l
Specificity	as required

2.6.4.3 Absorption

The disposition of total radioactivity in the mouse following single oral and single intravenous administration of [^{14}C] SPM 8272 (February 2000).

Cumulative Excretion of Radioactivity

Total recoveries of radioactivity were generally good and were similar following both oral and intravenous administration. In male mice following oral administration at a target dose level of 5 mg/kg, fecal, urinary and cage wash recovery accounted for 51.5%, 36.2% and 5.34%, respectively. A mean total recovery of 93.1% of the administered dose was obtained in urine, feces, expired air and cage wash over the 0-168 hour collection period. In female mice fecal, urinary and cage wash recovery accounted for 54.6%, 26.0% and 11.4%, respectively. A mean total recovery of 92.1% of the administered dose was obtained in urine, feces, expired air and cage wash over the 0-168 hour collection period. Following intravenous administration at 2.5 mg/kg the excretion rate was rapid with the largest proportion of radioactivity recovered in feces (mean 44.9% and 58.9% in males and females, respectively). In male mice following intravenous administration at a target dose level of 2.5 mg/kg, urinary and cage wash recovery accounted for 43.2% and 3.17%, respectively. A mean total recovery of 91.3% of the administered dose was obtained in urine, feces, expired air and cage wash over the 0-168 hour collection period. In female mice following intravenous administration at a target dose level of 2.5 mg/kg, urinary and cage wash recovery accounted for 24.2% and 6.95% respectively. A mean total recovery of 90.1% of the administered dose was obtained in urine, feces, expired air and cage wash over the 0-168 hour collection period. No radioactivity was recovered in expired air following either oral or intravenous administration.

Plasma Kinetics

In male mice following oral administration Cmax (mean 1394 ng equiv/g) was reached on average at 0.42 hours post-dose. Plasma concentrations declined to mean values of 33.4 ng equiv/g at 8 hours post-dose and 7.83 ng equiv/g by 48 hours post-dose. In

female mice, Cmax (mean 1552 ng equiv/g) was reached on average at 0.333 hours post-dose. Thereafter the concentrations declined to mean values of 49.2 ng equiv/g at 8 hours and 3.75 ng equiv/g by 48 hours post-dose. Mean AUC values for male and female animals were 2505 and 2188 ng equiv.h/g, respectively. Following intravenous administration in male mice, a mean Cmax value of 1704 ng equiv/g was measured at 0.25 hours post-dose. Plasma radioactivity concentrations decreased to mean values of 410 ng equiv/g at 1 hour, 32.6 ng equiv/g at 8 hours and 7.62 ng equiv/g at 48 hours. Following intravenous administration in female mice, a mean Cmax value of 1391ng equiv/g was measured at on average 0.8 hours post-dose. Plasma radioactivity concentrations decreased to mean values of 264 ng equiv/g at 1 hour, 33.9 ng equiv/g at 8 hours and 17.1 ng equiv/g at 48 hours post-dose. Mean AUC values for male and female animals were 2225 and 2170 ng equiv.h/g, respectively. Oral bioavailability of [14C] SPM 8272 was approximately 56.3 and 50.4% for male and females, respectively.

Tissue Distribution by Whole Body Phosphor Imaging

Distribution of radioactivity in the male, female, pregnant female albino mice and the pigmented mice was similar. Radioactivity was widely distributed throughout the tissues investigated, with maximum tissue concentrations achieved at 0.5 hours post-dose in the majority of tissues. Highest concentrations of radioactivity were principally associated with the organs of biotransformation and excretion, the urinary bladder wall, kidney and liver. In the pregnant female mouse, radioactivity was detected in the fetus and tissues associated with fetal development until 8 hours post-dose. Melanin binding in the pigmented skin and eye of the pigmented mouse was evident until 72 and 168 hours, respectively. Elimination from tissues was rapid with radioactivity levels generally less than 15% of their maximum value by 8 hours post-dose. At 72 hours post-dose elimination was generally complete from all the tissues with only trace levels apparent in the kidney and liver of the pregnant female albino and male pigmented mice and additionally the pigmented skin and eye of the pigmented animal. Following oral or intravenous administration to male and female mice no sex differences were apparent in the routes and rates of elimination. The oral bioavailability of [14C] SPM 8272 was approximately 56.3 and 50.4% for male and females, respectively. Excretion occurred by biliary and urinary elimination. Examination of Cmax values and profiles following oral and intravenous administration indicates that the absorption of total radioactivity was approximately 40% and that circulating total radioactivity was cleared rapidly. Total radioactivity was distributed in the tissues but was rapidly cleared with no apparent retention. Fetal tissue was exposed to [14C] SPM 8272 and or its metabolites, no accumulation was evident and the total radioactivity was rapidly cleared. Melanin binding did occur but was in the process of being eliminated.

The distribution of total radioactivity in the rat following single oral and single intravenous administration of [^{14}C] SPM 8272 (March 2000).

Cumulative Excretion of Radioactivity

Following oral administration the excretion rate was moderate with the largest portion of radioactivity recovered in the feces (76.0 and 75.6% in males and females, respectively). A mean total recovery in urine and cage wash accounted for 9.6 and 1.6%, respectively, in males and 8.5 and 2.8%, respectively, in females. Mean total recoveries were 87.6% and 87.2% in urine, feces, expired air, cage wash, carcass and gastrointestinal tract over the 0-168 hour collection period in males and females, respectively. Following intravenous administration the excretion rate was moderate with the largest portion of radioactivity recovered in the feces (77.7 and 78.5% in males and females, respectively). Total recovery in urine and cage wash accounted for 16.3 and 1.2%, respectively, in males and 15.2% and 1.2%, respectively, in females. Mean total recoveries were 96.3% and 95.6% in urine, feces, expired air, cage wash, carcass and gastrointestinal tract over the 0-168 hour collection period in males and females, respectively.

Plasma Kinetics

The mean (± SD) pharmacokinetic parameters of total radioactivity following intravenous and oral administration are detailed below.

	Oral (5 mg/kg)		IV (2.5 mg/kg)	
Parameter	Male (n=3)	Female (n=3)	Male (n=2)	Female (n=1)
C_{max} (ng equiv/g)	246 ± 20.5	204 ± 36.3	13353 ± 16563	14462
t_{max} (h)	4.00	0.50	0.00	0.00
AUC_{last} (ng equiv.h/g)	3293 ± 221	4633 ± 882	6734 ± 4198	16092
$t_{1/2}$ (h)	23.3 ± 3.38	25.6 ± 2.62	43.9 ± 4.11	nc
F (%)	24.5	14.4	na	na

Median t_{max} are reported; na denotes not applicable; nc denotes not calculated

Tissue Distribution by Whole Body Phosphor Imaging

Distribution of radioactivity in the male, female, pregnant female albino rats and the pigmented rats was similar. Radioactivity was widely distributed throughout the tissues investigated, with maximum tissue concentrations achieved at 0.5 hours post-dose in the majority of tissues in the male albino rat and at approximately 4 hours in the other groups. Highest concentrations of radioactivity were principally associated with the organs of excretion and biotransformation. In the pregnant female rat, radioactivity was detected in the fetus and tissues associated with fetal development until 24 hours post-dose. Melanin binding in the pigmented eye of the pigmented rat was evident until 168 hours. At 24 hours post-dose elimination was generally complete from all the tissues with only low levels apparent in the tissues including kidney, liver, lung, spleen, pancreas of the albino rats fetus placenta and chorioallentoic placenta of the pregnant female rats, and additionally the pigmented eye of the pigmented animal. Following oral or intravenous administration to male and female rats no sex differences were apparent in the routes and rates of elimination. The absorption of radioactivity was good with a minimum of 15% absorbed. Excretion occurred by fecal elimination. The oral bioavailability of (14C) SPM 8272 was approximately 24.5 and 14.4% for male and females, respectively. Total radioactivity was distributed in the tissues but was rapidly cleared with no apparent

retention except for the pigmented eye. Fetal tissue was exposed to (14C) SPM 8272 and or its metabolites, no accumulation was evident and the total radioactivity was rapidly cleared. Melanin binding did occur.

Pharmacokinetic feasibility study with SPM 8272 in the female rabbit.

Rabbit plasma samples from the study —14902/01 were analyzed for their content of SPM 7605 by LC-MS/MS. b(4)

The analysis of samples were performed by LC-MS/MS-column switching technique, using the — API —system after on-line sample clean-up with turbulent flow chromatography (TFC). After addition of — internal standard solution and centrifugation, the supernatant was directly injected into the chromatographic system. An electrospray ion source - atmospheric pressure ionization (API) - was used for ionization with positive ion mode. The chromatographic separation of the analyte was performed on a cyanopropyl column.

Characteristics of the used method are given in the following table:

Calibrated Range	1.00 ng/mL – 1000 ng/mL
Defined LOQ	1.00 ng/mL
Linearity (r^2 of the standard curve)	0.99988
Accuracy [bias %]	between –3.5 and –0.6
Precision [cv %]	between 0.8 and 4.0

Study —14902/01 was conducted to investigate the pharmacokinetics of SPM 7605, the hydroxy metabolite of SPM 8272, in the female rabbit after oral and s.c. administration of SPM 8272. The following pharmacokinetic parameters were obtained for SPM 7605 after multiple oral SPM 8272 administrations at 3, 6 and 9 mg/kg/treatment three times daily or after SPM 8272 s.c. doses at 9 mg/kg/day for 4 consecutive days (medians, n=3/group): b(4)

SPM 8272 dose [mg/kg/day]	Route	Day	Admin.	C_{max} [ng/mL]	t_{max} [min]	AUC(0-24h) [b] [h*ng/mL]	F_{rel} [%]
9 (3 x 3)	oral	1	1(-3) [c]	4.21	20 [a]	28.0	1.24
		4	10(-12) [c]	5.86	20 [a]	29.6	1.16
18 (3 x 6)	oral	1	1(-3) [c]	7.83	40	68.1	1.51
		4	10(-12) [c]	9.27	20 [a]	98.9	1.94
27 (3 x 9)	oral	1	1(-3) [c]	9.30	20 [a]	99.0	1.46
		4	10(-12) [c]	28.7	20 [a]	238	3.12
9	s.c.	1	1	724	40	2256	100
		4	4	805	1	2547	100

a) first sampling time
b) estimated as 3-fold AUC(0-inf) for oral administration, AUC(0-inf) for s.c. administration
c) C_{max} was determined following the first administration of the given day, AUC refers to a 24-hour interval

The systemic exposure to SPM 7605 was markedly higher following s.c. administration of SPM 8272.

The distribution of total radioactivity in the dog following single oral and single intravenous administration of [^{14}C] SPM 8272 (April 2000).

Plasma Kinetics

The absorption rate following oral administration was fast with mean Cmax values of 368 ng equiv/g and 338 ng equiv/g, respectively, reached within 1 hour for male and female dogs. Thereafter, the concentrations declined to 8.88 (males) or 8.13 (females) ng equiv/g at 48 hours post-dose but were still detectable at 168 hours post-dose. Following intravenous administration, Cmax was observed in both male and female dogs at 5

minutes post-dose (mean values of 207 ng equiv/g and 202 ng equiv/g, respectively). Thereafter, plasma concentrations declined to 4.79 (males) or 4.37 (females) ng equiv/g at 48 hours post-dose. The mean oral bioavailability of [14C] SPM 8272 was approximately 98.5% for both sexes.

Whole blood Kinetics

Following oral administration mean Cmax values of 327 ng equiv/g and 307 ng equiv/g, respectively, were recorded at 1 hour post-dose for male and female dogs. Thereafter, the concentrations declined to mean values of 47.2 ng equiv/g and 39.1 ng equiv/g, respectively, for male and female dogs at 8 hours post-dose and were below detectable levels at 24 hours post-dose. Following intravenous administration the highest concentration was observed in male at 5 minutes post-dose and in female dogs at 30 minutes post-dose (mean values of 163 ng equiv/g and 172 ng equiv/g, respectively) and then declined steadily to mean values of 160 ng equiv/g (males) and 157 ng equiv/g (females) at 1 hour post-dose, and 9.93 ng equiv/g (males) and 7.63 ng equiv/g (females) at 48 hours post-dose. Radioactivity remained detectable in whole blood until the final time point of 168 hours where the values were 3.51 and 3.10 ng equiv/g in males and females, respectively.

Excretion of Radioactivity

Following oral administration the excretion rate was moderate with the largest proportion of radioactivity recovered in urine and cage wash (59.5% and 66.6% in males and females, respectively). Mean total excretion in feces was 26.3% and 24.6% in males and females, respectively. The majority of the dose was excreted in the first 48 hours post-dose with a mean total recovery of 85.8% and 91.3% in urine, feces, and cage wash over the 0-168 hour collection period in males and females, respectively. Following intravenous administration the excretion rate was moderate and the largest proportion of radioactivity recovered in urine and cage wash (mean recovery of 57.3% and 63.3% in males and females, respectively). Total excretion in feces accounted for mean values of 35.5% and 24.4% in males and females, respectively. The majority of the dose was excreted in the first 48 hours post-dose with a mean total recovery of 92.9% and 87.6% in urine, feces, and cage wash over the 0-168 hour collection period in males and females, respectively.

Tissue Distribution of Radioactivity

In general, radioactivity was poorly distributed with maximum concentrations of radioactivity detected in the majority of tissues at 1 hour post-dose. Highest concentrations of radioactivity were observed in the contents of the intestinal tract as would be expected following an oral dose. At 1 hour post-dose highest tissue concentrations were found in liver, small intestine wall and kidney. Elimination from organs and tissues was progressing at 4 hours post-dose with lower levels of radioactivity measured in the majority of tissues compared to their values at 1 hour post-dose. At 48 hours post-dose, the concentration of radioactivity in the majority of tissues was less than

20% of their maximum value except for thyroid (44%), brain (36%) and eye (25%). At 96 hours post-dose, although levels of radioactivity in the tissues were quantifiable, all values had reduced further. At all time points, with the exception of liver and kidney, the concentration in the tissues was less than approximately to that in the blood. Following oral or intravenous administration to male and female dogs no sex differences were apparent in the routes and rate of elimination. The mean oral bioavailability of [14C] SPM 8272 derived radioactivity was approximately 98.5% for both genders. Excretion occurred mainly by urinary elimination. Examination of Cmax values and profiles following oral and intravenous administration indicates that the absorption of total radioactivity was approximately 60% and that circulating total radioactivity was cleared rapidly. Total radioactivity was poorly distributed in the tissues and was rapidly cleared with no apparent retention.

Examination of the bioavailability of SPM 8272 in Beagle dogs (Study no. 12893/99)(February 2001). Following administration to Beagle dogs (N=5) of 200 ug i.v. or 200 ug p.o of SPM 8272, no changes in behavior or body weight were observed. Plasma concentrations of 108±26.8 ng/ml for SPM 8272 and 9.51±2.30 ng/ml for SPM 7605 were achieved (mean ±SD). SPM 8272 was rapidly cleared with a terminal half-life of about 25 minutes. The terminal half-life of SPM 7605 averaged 2 hours. Following oral SPM 8272 administration, maximum SPM 8272 and SPM 7605 plasma levels up to 1.73 and 3.97 ng/ml were determined, respectively. The systemic exposure to the metabolite SPM 7605 was between 2- and 13-fold higher than to SPM 8272. The oral bioavailability ranged from 0 to 3.6% for SPM 8272 and from 3.5 to 45% for SPM 7605.

Pharmacokinetic feasibility study with SPM 8272 and/or SPM 7605 in the Beagle dog (Study no. 14841/01)(October 2002). Beagle dogs (N=5) were administered (1) a single oral administration (4 mg/kg) followed by 30 hours of blood sampling, (2) a single 24 hour infusion (1 mg/kg) followed by 26 hours of blood sampling, or (3) 7 days of repeated oral doses (4 mg/kg) twice per day followed by 12 hours of blood sampling. No drug related effects on behavior or body weight were observed. The following pharmacokinetic data were collected:

SPM 8272

Dose regimen	Dose/treatment [mg/kg]	Day (treatment)	C_{max} [ng/mL]	t_{max} [h]	AUC [a] [h*ng/mL]
SPM 8272 SR tablet, oral (group 1)	3.8 - 4.2	1	2.84 (0.87-13.0)	6 (6-8)	31.3 (1.74-128)
SPM 8272 solution, oral (group 3)	2	1 (1)	3.99 (1.64-7.56)	1 (0.17-1)	5.75 (4.44-13.43)
	2	7 (13)	10.9 (4.64-43.0)	0.5 (0.17-2)	16.5 (12.9-42.8)

a) AUD(0-24h) for group 1, AUD(0-12h) for group 3

SPM 7605

Dose regimen	Dose/treatment [mg/kg]	Day (treatment)	C_{max} [ng/mL]	t_{max} [h]	AUC[a] [h*ng/mL]
SPM 8272 SR tablet, oral (group 1)	3.8 - 4.2	1	6.75 (4.10-9.16)	4 (2-8)	74.9 (30.0-110)
SPM 8272 solution, oral (group 3)	2	1 (1)	4.04 (3.68-8.55)	1 (0.5-4)	21.3 (15.7-22.0)
	2	7 (13)	16.5 (12.4-52.8)	0.5 (0.17-2)	70.0 (65.7-118)
SPM 7605, 24-hour infusion (group 2)	1	1	9.50 (6.44-13.3)	8 (8-24)	176 (133-232)

a) AUD(0-24h) for groups 1 and 2, AUD(0-12h) for group 3

2.6.4.4 Distribution

Plasma protein binding of SPM 8272 and SPM 7605 in human, dog, monkey, mouse, rabbit and rat plasma samples (BA 496-02)(March 2002). The binding of SPM 8272 ("C-SPM 8272) and SPM 7605 ("C-SPM 7605) to human, dog, monkey, mouse, rabbit and rat plasma proteins and to the individual proteins human serum albumin (HSA) and a1-acid glycoprotein (AGP) was analyzed by equilibrium dialysis.

Recovery after dialysis in human pooled plasma samples (n = 5):

^{14}C-SPM 8272: c = 100 ng/ml: 99 ± 10 %, c = 1000 ng/ml: 79 ± 3 %
^{14}C-SPM 7605: c = 118 ng/ml: 105 ± 4 %, c = 1182 ng/ml: 93 ± 5 %

Repeatability (n = 5): imprecision

^{14}C-SPM 8272: c = 100 ng/ml: 10 %, c = 1000 ng/ml: 3 %
^{14}C-SPM 7605: c = 118 ng/ml: 4 %, c = 1182 ng/ml: 5 %

Possible dependence of protein binding on SPM 8272 and SPM 7605 concentration was analyzed in the range c = 62.5 – 1000 ng/ml (SPM 8272) and c = 74 – 1182 ng/ml (SPM 7605) in human pooled plasma samples, HSA (c= 45 mg/ml), AGP (c = 1 mg/ml) and in a combination of HSA (c = 45 mg/ml) and AGP (c = 1 mg/ml). The relative amount of SPM 8272 and SPM 7605 bound to plasma, HSA and AGP was found to be independent of the concentration.

Determination of the relative amount of SPM 8272 and SPM 7605 bound to plasma proteins was performed in 5 individual human (healthy volunteers), dog, monkey, rabbit, rat and in pooled mouse plasma samples with c = 100 ng/ml (SPM 8272) and c = 118 ng/ml (SPM 7605). The % bound SPM 8272 (mean ± SD) was determined to be: human: 51±4, dog: 36±6, monkey: 37±4, mouse: 24±2, rabbit: 21±2, and rat: 20±2. The % bound SPM 7605 (mean ± SD) was determined to be: human: 53±2, dog: 30±4, monkey: 37±3, mouse: 25±2, rabbit: 21±2, and rat: 22±1.

Determination of the relative amount of SPM 8272 and SPM 7605 bound to individual proteins: HSA (c = 45 mg/ml) and AGP (c = 1 mg/ml) in the concentration range 62.5 – 1000 ng/ml (SPM 8272) and 74 – 1182 ng/ml (SPM 7605). The % bound SPM 8272 (mean + SD) was determined to be: HSA: 30±2, AGP: 72±7, and a combination of HSA

(c = 45 mg/ml) and AGP (c = 1 mg/ml): 91±6. The % bound SPM 7605 (mean + SD) was determined to be: HSA: 14±3, AGP: 16±5, and a combination of HSA (c = 45 mg/ml) and AGP (c = 1 mg/ml): 39±1.

SPM 8272 is completely hydrolyzed by esterases to SPM 7605 within 30 min in plasma of all species tested, except for the dog, and the dialysis time was 2 hours, so SPM 8272 was hydrolyzed to SPM 7605 in the plasma experiments. The two compounds bound therefore similarly to plasma proteins. However the amount of the compounds bound to the individual plasma proteins HSA and AGP was different, when analyzed with highly purified protein preparations to avoid esterase contamination. SPM 8272 was found to bind to > 91 % to a combination of HSA and AGP whereas SPM 7605 binds to this combination in the same range as to total plasma proteins, indicating that SPM 7605 binds mainly to HSA and AGP, with similar affinity.

2.6.4.5 Metabolism

Evaluation of the *in vivo* metabolism in mice following repeated oral administration of SPM 8272 (Study no. 634. 2004). Results by HPLC-electrospray MS/MS analysis showed the following metabolites in male and female mice following repeated administration.

Dose of SPM 8272 [mg/kg/day]	Sex	SPM 7605		SPM 5509		SPM 7789		SPM 7790	
		C_{max} [nM]	AUC_{last} [h nM]	C_{max} [nM]	AUC_{last} [h nM]	C_{max} [nM]	AUC_{last} [h nM]	C_{max} [nM]	AUC_{last} [h nM]
5	M	41.0	52.2	69.7	166	3.62	10.3	57.3	139
	M	38.4	43.6	86.3	162	3.03	4.53	44.6	72.2
	F	43.0	54.6	75.7	171	3.78	6.15	58.8	177
	F	29.3	62.0	89.8	125	1.72	3.89	43.6	77.4
15	M	135	283	163	450	23.7	69.8	127	402
	F	366	397	276	504	52.5	91.9	312	667
25	M	422	847	245	606	60.3	184	235	928
	F	755	1402	390	913	122	278	533	1528
45	M	1414	2661	580	1382	304	600	719	1812
	F	2764	3104	473	1412	466	750	934	3144
100	M	4890	11144	565	1683	817	2332	1158	6218
125	F	8697	24378	571	3099	1137	5348	1606	13622

M - males, F - females, the underlined AUC_{0-t} values are calculated from 0.5 to at most 12 hours post-dose

Dose of SPM 8272 [mg/kg/day]	Sex	Fractions related to SPM 7605					
		SPM 5509		SPM 7789		SPM 7790	
		C_{max}	AUC_{last}	C_{max}	AUC_{last}	C_{max}	AUC_{last}
5	Male	1.7	3.2	<0.1	0.20	1.4	2.7
		2.3	3.7	<0.1	0.10	1.2	1.7
	Female	1.8	3.1	<0.1	0.11	1.4	3.2
		3.1	2.0	<0.1	<0.1	1.5	1.3
15	Male	1.2	1.6	0.18	0.25	0.94	1.4
	Female	0.75	1.3	0.14	0.23	0.85	1.7
25	Male	0.58	0.71	0.14	0.22	0.56	1.1
	Female	0.52	0.65	0.16	0.20	0.71	1.1
45	Male	0.41	0.52	0.22	0.23	0.51	0.68
	Female	0.17	0.45	0.17	0.24	0.34	1.0
100	Male	0.12	0.15	0.17	0.21	0.24	0.56
125	Female	<0.1	0.13	0.13	0.22	0.18	0.56

Evaluation of the *in vivo* metabolism in rats following repeated oral administration of SPM 8272 (Study no. 667, 2004). Results by HPLC-electrospray MS/MS analysis showed the following metabolites in male and female Sprague-Dawley rats following repeated administration.

Duration of treatment	Dose of SPM 8272 [mg/kg/day]	Sex	SPM 7605			SPM 5509		
			C_{max} [nM]	t_{max} [h]	AUC_{last} [h nM]	C_{max} [nM]	t_{max} [h]	AUC_{last} [h nM]
13 weeks	15	M	40.7	1	365	25.3	1	226
		F	20.0, 108	0.5	89.6, 193	31.4, 64.7	0.5, 1	210, 315
	45	M	254	0.5	1025	96.0	0.5	570
		F	93.7, 147	0.5, 1	603, 726	70.2, 158	0.5, 1	677, 751
26 weeks	5	M	17.0-44.9	3-24	138-247	24.1-30.3	1-3	134-166
		F	11.6-73.7	0.5-3	43.3-123	31.9-63.0	0.5	103-128
	15	M	27.1-70.7	0.5-1	195-224	82.2-162	0.5-1	562-794
		F	25.5-32.8	0.5	111-164	77.7-127	0.5-1	356-559
	45	M	167-280	0.5	727-1128	439-558	0.5	1763-2210
		F	139-289	0.5-1	365-1109	224-419	0.5	1097-2415

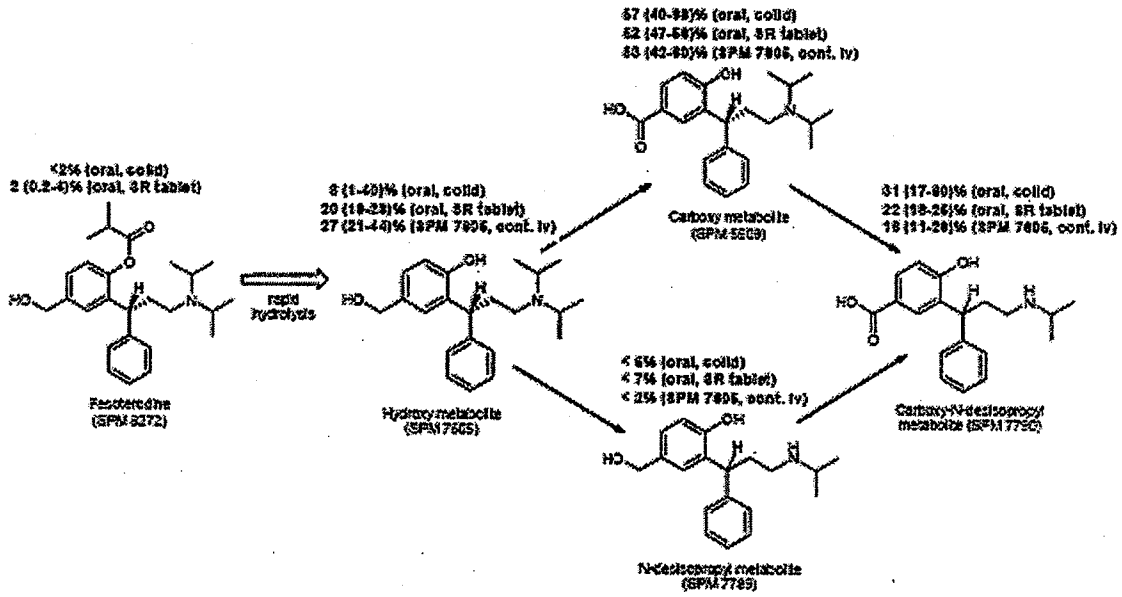
M - males, F – females, underlined denotes first sampling time

Duration of treatment	Dose of SPM 8272 [mg/kg/day]	Sex	SPM 7789			SPM 7790		
			C_{max} [nM]	t_{max} [h]	AUC_{last} [h nM]	C_{max} [nM]	t_{max} [h]	AUC_{last} [h nM]
13 weeks	15	M	11.1	1	26.8	82.0	1	568
		F	0.75, 1.70	0.5, 1	0.19, 0.42	6.72, 33.6	0.5, 1	46.1, 68.6
	45	M	475	0.5	1241	700	0.5	3565
		F	17.4, 25.3	1	94.7, 313	32.2, 60.7	1	426, 467
26 weeks	5	M	0-1.98	0.5-1	0-1.44	47.9-121	0.5-3	261-387
		F	1.22-11.3	0.5-1	0.31-3.53	11.9-22.8	0.5	29.1-59.5
	15	M	9.24-17.4	0.5-1	42.6-46.6	271-578	0.5-3	2133-2171
		F	4.12-4.62	0.5-1	6.45-8.29	30.9-40.0	0.5-1	129-160
	45	M	296-421	0.5-1	863-1450	3005-4845	0.5-1	9498-11217
		F	27.7-65.2	0.5-1	56-201	100-220	0.5-1	556-1561

M - males, F – females, underlined denotes first sampling time

Duration of treatment	Dose of SPM 8272 [mg/kg/day]	Sex	Fractions related to SPM 7605					
			SPM 5509		SPM 7789		SPM 7790	
			C_{max}	AUC_{last}	C_{max}	AUC_{last}	C_{max}	AUC_{last}
13 weeks	15	Male	0.6	0.6	0.3	<0.1	2.0	1.6
		Female	0.6, 1.6	1.6, 2.4	<0.1	<0.1	0.3, 0.3	0.4, 0.5
	45	Male	0.4	0.6	1.9	1.2	2.8	3.5
		Female	0.8, 1.1	0.9, 1.2	0.3, 0.1	0.4, 0.2	0.3, 0.4	0.6, 0.7
26 weeks	5	Male	0.6-1.4	0.7-1.0	<0.1	<0.1	1.7-3.6	1.1-2.8
		Female	0.6-5.4	0.9-3.0	0.1-0.2	<0.1	0.3-1.6	0.3-0.9
	15	Male	1.2-3.9	2.9-3.9	0.2-0.4	0.2	3.8-14	9.7-11
		Female	3.0-3.9	2.8-5.0	0.1-0.2	<0.1	1.0-1.6	1.0-1.3
	45	Male	1.9-2.7	2.0-3.0	1.3-2.0	1.0-1.3	14-29	9.9-13
		Female	1.5-2.7	1.7-3.0	0.2-0.2	0.1-0.2	0.7- 1.1	0.9-1.5

Evaluation of the *in vivo* metabolism in dogs following oral administration of SPM 8272 and intravenous administration of SPM 8272 or SPM 7605 (Study no. 669, 2004). Results by HPLC-electrospray MS/MS analysis showed the following metabolites in male and female Beagle dogs following repeated administration.



Formulation (Study no.)	Dose [mg/kg]	Admin.	Sex	Parameters		Median toxicokinetic parameters (n=2) SPM 8272	SPM 7605	SPM 5509	SPM 7789	SPM 7790
SPM 8272 solution twice daily (14841/01)	2	1	F	C_{max}	[nM]	13.1	17.9	420	4.03	357
				AUC_{last}	[h nM]	17.6	64.4	2104	15.6	2110
	2	13	F	C_{max}	[nM]	28.8	42.3	450	9.05	232
				AUC_{last}	[h nM]	50.8	199	2872	52.9	1733

Formulation (Study no.)	Dose [mg/kg/day]	Day	Sex			Median toxicokinetic parameters (n=2/sex/group) SPM 8272	SPM 7605	SPM 5509	SPM 7789	SPM 7790
SPM 8272 SR tablet (15409/02)	8	1	M	C_{max}	[nM]	8.17	53.8	1001	18.2	557
				AUC_{last}	[h nM]	61.7	841	14571	258	8338
			F	C_{max}	[nM]	86.6	125	907	17.2	477
				AUC_{last}	[h nM]	675	1942	14341	329	7089
		14	M	C_{max}	[nM]	323	1358	2017	173	671
				AUC_{last}	[h nM]	1119	7228	19200	1506	7886
			F	C_{max}	[nM]	169	677	1422	99.1	473
				AUC_{last}	[h nM]	840	6835	18493	1223	6730
SPM 8272 SR tablet (15409/02)	32	1	M	C_{max}	[nM]	42.5	460	2719	98.1	1164
				AUC_{last}	[h nM]	700	6523	39498	1433	18528
			F	C_{max}	[nM]	5.75	121	1585	55.7	723
				AUC_{last}	[h nM]	78.1	1691	25178	682	11565
		14	M	C_{max}	[nM]	171	2297	4278	414	2049
				AUC_{last}	[h nM]	2128	33211	71253	6422	35902
			F	C_{max}	[nM]	98.1	1574	3546	285	1339
				AUC_{last}	[h nM]	968	21613	63013	4251	24796

Formulation (Study no.)	Dose [mg/kg/day]	Week	Sex	Parameters		Median toxicokinetic parameters (n=2/sex/group) SPM 8272	SPM 7605	SPM 5509	SPM 7789	SPM 7790
SPM 8272 solid (13349/00)	0.5	39	M	C_{max}	[nM]	2.38	6.35	154	0	165
				AUC_{last}	[h nM]	2.96	14.6	1017	0	1275
			F	C_{max}	[nM]	0.89	6.59	304	2.21	180
				AUC_{last}	[h nM]	1.40	9.75	1357	2.48	1288
	2.5	39	M	C_{max}	[nM]	7.06	195	803	21.5	284
				AUC_{last}	[h nM]	11.3	467	5555	142	2469
			F	C_{max}	[nM]	41.0	80.8	706	19.9	305
				AUC_{last}	[h nM]	98.5	475	6555	181	3311
	12.5	39	M	C_{max}	[nM]	92.9	2177	3040	312	1072
				AUC_{last}	[h nM]	223	10728	34205	3181	13960
			F	C_{max}	[nM]	696	2907	3489	327	1202
				AUC_{last}	[h nM]	925	10873	31402	2214	13381

Formulation (Study no.)	Dose [mg/kg/day]	Day	Sex	Parameters		Toxicokinetic parameters (n=1/sex/group) SPM 8272	SPM 7605	SPM 5509	SPM 7789	SPM 7790
SPM 8272 4-hour iv (15540/02)	1	3	M	C_{max}	[nM]	189	63.3	319	1.95	200
				AUC_{last}	[h nM]	512	243	1225	5.98	781
			F	C_{max}	[nM]	193	55.1	347	2.50	282
				AUC_{last}	[h nM]	257	68.1	532	2.58	402
	5	3	M	C_{max}	[nM]	514	791	1684	32.5	442
				AUC_{last}	[h nM]	1452	2637	6053	118	1836
			F	C_{max}	[nM]	1075	822	1833	44.8	751
				AUC_{last}	[h nM]	3142	2760	7048	159	3230
	10	3	M	C_{max}	[nM]	1569	2490	3072	161	637
				AUC_{last}	[h nM]	4701	8991	11568	603	2570
			F	C_{max}	[nM]	1478	1952	2904	124	768
				AUC_{last}	[h nM]	2345	2463	4347	164	1041

Formulation (Study no.)	Dose [mg/kg/day]	Day	Sex	Parameters		Median toxicokinetic parameters (n=2/sex/group) SPM 8272	SPM 7605	SPM 5509	SPM 7789	SPM 7790
SPM 7605 cont. iv infusion (15409/02)	1.5	1	M	C_{max}	[nM]	NA	75.9	252	1.7	143
				AUC_{last}	[h nM]	NA	1227	4085	10	2354
			F	C_{max}	[nM]	NA	62.4	323	0	207
				AUC_{last}	[h nM]	NA	1145	5420	0	3871
		14	M	C_{max}	[nM]	NA	104	219	3.02	63.6
				AUC_{last}	[h nM]	NA	1975	4358	63.1	1257
			F	C_{max}	[nM]	NA	141	264	1.28	103
				AUC_{last}	[h nM]	NA	2491	5732	28.5	2351
SPM 7605 cont. iv infusion (15409/02)	6	1	M	C_{max}	[nM]	NA	290.3	858	11.5	510
				AUC_{last}	[h nM]	NA	4673	17200	108	8344
			F	C_{max}	[nM]	NA	367	1286	18.1	448
				AUC_{last}	[h nM]	NA	5587	21979	186	8832
		14	M	C_{max}	[nM]	NA	579	913	26.0	269
				AUC_{last}	[h nM]	NA	11851	19495	528	5897
			F	C_{max}	[nM]	NA	1020	1167	49.9	291
				AUC_{last}	[h nM]	NA	20368	24628	1042	6408

Evaluation of the in vivo metabolism in rabbits following single and repeated oral or subcutaneous administration of SPM 8272 (Study no. 628, 2003). Results by HPLC-electrospray MS/MS analysis showed the following metabolites in female rabbits following single and repeated administration.

Dose of SPM 8272 [mg/kg]	Day	SPM 7605		SPM 5509		SPM 7789		SPM 7790	
		C_{max} [nM]	AUC_{last} [h nM]	C_{max} [nM]	AUC_{last} [h nM]	C_{max} [nM]	AUC_{last} [h nM]	C_{max} [nM]	AUC_{last} [h nM]
3	1	12.3	21.0	164	230	4.17	1.73	274	590
	4[a]	17.2	23.7	157	207	16.4	9.49	758	1270
6	1	22.9	38.4	109	284	17.2	22.7	356	1069
	4[a]	27.1	73.8	318	480	13.6	28.1	1359	2164
9	1	27.2	82.6	470	683	10.6	24.5	1676	2664
	4[a]	84.0	201	746	1008	26.4	49.5	2908	4342

a - 10th administration

Dose of SPM 8272 [mg/kg]	Day	SPM 7605		SPM 5509		SPM 7789		SPM 7790	
		C_{max} [nM]	AUC [h nM]	C_{max} [nM]	AUC [h nM]	C_{max} [nM]	AUC [h nM]	C_{max} [nM]	AUC [h nM]
9	1	2120	6462	197	1044	0.00	0.00	543	2404
	4	2357	7204	266	1082	0.00	0.00	659	2667

Route	Dose of SPM 8272 [mg/kg]	Fractions related to SPM 7605					
		SPM 5509		SPM 7789		SPM 7790	
		C_{max}	AUC_{last}	C_{max}	AUC_{last}	C_{max}	AUC_{last}
Oral	3	6.1-13	8.2-11	0.0-0.4	0.0-0.1	14-42	13-40
		6.7-18	7.0-11	0.5-1.1	0.2-1.0	23-85	27-56
	6	3.1-13	3.7-11	0.0-0.8	0.0-0.7	10-33	20-29
		9.6-11	6.5-11	0.1-1.1	0.0-1.1	24-55	29-53
	9	2.7-17	1.8-8.8	0.0-0.8	0.0-0.8	7.4-78	4.8-44
		4.6-12	3.2-6.8	0.1-0.5	0.2-0.3	22-46	18-29
Subcutaneous	9	0.1-0.2	0.1-0.2	0	0	0.2-0.3	0.3-0.5
		0.1	0.1-0.2	0	0	0.3	0.3-0.5

The metabolic profiling and identification of samples from mouse, rat, and dog studies with (^{14}C) SPM 8272 (Study no. DHGY1008, 2000).

Total Radioactivity in Plasma Samples Following Oral and Intravenous Administration of SPM 8272

Species	Route	Sex	Time Point (h)	ng equiv/mL (Mean ± SD)	% administered dose
Mouse	Oral	Male	0.25	1634 ± 483	1.446 ± 0.471
			1	744 ± 190	0.650 ± 0.115
			4	141 ± 25.7	0.133 ± 0.025
		Female	0.25	1662 ± 494	1.470 ± 0.419
			1	461 ± 184	0.402 ± 0.165
			4	799 ± 1370	0.743 ± 1.287
	Intravenous	Male	0.083	987 ± 59.0	1.458 ± 0.071
			1	211 ± 96.0	0.310 ± 0.139
			4	36.2 ± 7.33	0.054 ± 0.011
		Female	0.083	949 ± 88.0	1.441 ± 0.271
			1	263 ± 48.7	0.406 ± 0.065
			4	36.5 ± 5.96	0.052 ± 0.010
Rat	Oral	Male	0.5	186 ± 32.2	0.189 ± 0.031
			1	261 ± 77.3	0.233 ± 0.069
			4	179 ± 27.2	0.164 ± 0.022
		Female	0.5	195 ± 30.5	0.178 ± 0.027
			1	182 ± 48.6	0.166 ± 0.045
			4	114 ± 20.4	0.104 ± 0.019
	Intravenous	Male	0.083	764 ± 70.9	1.361 ± 0.103
			0.5	508 ± 60.5	0.907 ± 0.096
			4	123 ± 25.1	0.227 ± 0.047
		Female	0.083	754 ± 49.6	1.387 ± 0.081
			0.5	392 ± 57.9	0.702 ± 0.091
			4	84.5 ± 17.9	0.150 ± 0.034
Dog	Oral	Male	0.25	168 ± 96.0	1.528 ± 0.869
			0.5	326 ± 76.5	2.976 ± 0.692
			1	350 ± 15.0	3.202 ± 0.176
		Female	0.25	102 ± 117	0.903 ± 1.021
			0.5	221 ± 147	1.968 ± 1.296
			1	330 ± 43.8	2.953 ± 0.407
	Intravenous	Male	0.083	206 ± 30.8	3.760 ± 0.565
			0.167	183 ± 50.6	3.531 ± 0.471
			1	170 ± 21.5	3.133 ± 0.390
		Female	0.083	159 ± 37.8	3.624 ± 0.686
			0.167	176 ± 14.2	3.204 ± 0.251
			1	160 ± 14.5	2.918 ± 0.245

The percent dose administered was calculated assuming that total plasma equates to 4.5% of the total bodyweight

Total Radioactivity in Urine Samples Following Oral and Intravenous Administration of SPM 8272

Results are as % of the administered dose

Species	Route	Sex	Time Point (h)	Mean	Standard Deviation
Mouse	Oral	Male	0-6	25.8	8.91
			6-24	9.42	1.17
		Female	0-6	17.3	3.95
			6-24	7.72	na
	Intravenous	Male	0-6	40.8	na
			6-24	15.3	8.67
		Female	0-6	4.04	na
			6-24	21.8	1.57
Rat	Oral	Male	0-6	3.3	0.600
			6-24	5.4	2.79
		Female	0-6	3.6	0.666
			6-24	3.6	1.89
	Intravenous	Male	0-6	10.3	4.10
			6-24	5.5	2.03
		Female	0-6	9.2	1.83
			6-24	5.2	0.819
Dog	Oral	Male	0-6	24.0	7.66
			6-24	26.8	11.9
		Female	0-6	36.5	5.91
			6-24	25.9	5.37
	Intravenous	Male	0-6	22.8	6.46
			6-24	27.7	8.65
		Female	0-6	32.9	13.9
			6-24	25.9	12.9

Total Radioactivity in Faeces Samples Following Oral and Intravenous Administration of SPM 8272

Results are as % of the administered dose

Species	Route	Sex	Time Point (h)	Mean	Standard Deviation
Mouse	Oral	Male	0-24	48.2	6.88
			24-48	2.74	2.41
		Female	0-24	50.2	1.59
			24-48	3.86	2.82
	Intravenous	Male	0-24	43.0	14.6
			24-48	1.28	0.48
		Female	0-24	56.2	5.72
			24-48	2.44	2.24
Rat	Oral	Male	0-24	47.7	22.9
			24-48	23.9	16.9
		Female	0-24	55.4	24.3
			24-48	5.7	2.70
	Intravenous	Male	0-24	74.2	1.65
			24-48	2.2	0.635
		Female	0-24	74.4	0.643
			24-48	2.2	0.231
Dog	Oral	Male	0-24	14.6	12.0
			24-48	9.7	7.49
		Female	0-24	18.2	3.55
			24-48	4.96	1.54
	Intravenous	Male	0-24	28.1	4.23
			24-48	3.32	1.44
		Female	0-24	19.6	3.28
			24-48	3.13	0.88

Metabolism of the ester (SPM 8272)(pro-drug) in Caco-2 cell monolayers grown on flasks. After a recovery of 85% of SPM-8272-related radioactivity from cell homogenates (Caco-2 *in vitro* model of intestinal mucosa), only SPM 7605, the ester hydrolysis product, was demonstrated to be present.

***In vitro* metabolic profiling study with [^{14}C] SPM 8272 in rat, mouse, dog and human liver microsomes (study no. DHGY1009, 2000).** HPLC conditions were developed to enable resolution of parent compound and metabolite standards and assessment of radiochemical purity. The metabolism of (^{14}C)-SPM 8272 (10 μM) was characterized in pooled rat, mouse, dog and human liver microsomes in the absence and presence of a NADPH regenerating system and in the absence and presence of microsomes. Protein content and incubation times were varied. Species differences in metabolic profile were demonstrated. SPM 8272 was shown to be extensively and rapidly metabolized and primarily cleared by non-P450 activity in rat, mouse, dog, and human. SPM 7605 was the primary metabolite, independent of co-factors, with a relative turnover rate of human>mouse>dog>>rat. SPM 5509 was detected in all species, independent of CYP450. SPM 7605 undergoes further CYP450 mediated metabolism, particularly in the rat.

***In vitro* metabolic profiling study with SPM 8272 in hamster, rabbit, primate and human liver microsomes (study no. DHGY1030, 2002).** SPM 8272 was extensively and rapidly metabolized and primarily cleared by non-cytochrome P450 activity with relative rate rabbit~human>>hamster>primate. SPM 7605 was the primary product, independent of a NADPH-regenerating system. In the presence of metabolic activation, SPM 7605 underwent further metabolism, particularly in primate, hamster, and rabbit. The human metabolites, SPM 7833, SPM 7605, SPM 7790, and SPM 5509 were also observed in primate, hamster, and rabbit. Some animal metabolites were not observed in human.

Investigation of the human cytochrome P450 isoforms involved in the metabolism of SPM 7605 (Study no. DHGY1029, 2002).

To investigate which CYP isoform(s) are involved in the metabolism of SPM 7605: (1)The rates of metabolite formation was measured in the presence of Supersomes™ over-expressing individual CYP isoforms. The following Supersomes™ with supplemental cDNA expressed reductase were selected: CYP1A1, CYP1A2, CYP1A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C19, CYP2D6*1, CYP2E1, CYP3A4, CYP3A5, CYP4A11 and control Supersomes ™ (containing no transfected CYP enzyme). (2) The effect of isoenzyme-selective chemical inhibitors on the metabolism of [^{14}C] SPM 8311 (fumarate salt of SPM 7605) was investigated. The following CYP chemical inhibitors were used: Furafylline (CYP1A2), Methoxalen (CYP2A6), Quercetin (CYP2C8), Sulphaphenazole (CYP2C9), Tranylcypromine (CYP2C19), Quinidine (CYP2D6), Diethyldithiocarbamate (DDC; CYP2E1) and Ketoconazole (CYP3A4). (3)[^{14}C] SPM 8311 metabolism was investigated in a panel of human liver microsomes. The formation of SPM 8311 metabolites was correlated with metabolite production for specific CYP probe substrates.

The path from 5509 to 7790 is 2D6 independent but the path from 7605 to 5509 can be inhibited by quinidine (2D6 dependent). 3A4 and 3A1 are involved in metabolism of 5509 to 7790.

Metabolism of SPM 7605 (0.7 µM) by Supersomes™ from baculovirus infected insect cells over-expressing specific CYP cDNAs

	Peak 1 Mean ± SD (pmol/min/pmol CYP)	Peak 2 Mean ± SD (pmol/min/pmol CYP)
Control	nd	0.00198 ± 0.000250
CYP 1A1	nd	0.00267 ± 0.002021
CYP 1A2*	0.000329*	0.00213 ± 0.000431
CYP 2A6*	0.000152*	0.00226 ± 0.000479
CYP 2B6	nd	0.00222 ± 0.000199
CYP 2C8[v]	0.000781 – 0.000116#	0.00203 ± 0.000364
CYP 2C9	0.000426 – 0.000607#	0.00202 ± 0.000384
CYP 2C19arg	nd	0.00190 ± 0.000340
CYP 2D6#	0.00115 – 0.00303#	0.162 ± 0.00588
CYP 2E1*	0.0000645*	0.00194 ± 0.000274
CYP 3A4*	0.00142*	0.00231 ± 0.000271
CYP 3A5#	0.000333 – 0.00138#	0.00268 ± 0.000496
CYP 4A11	nd	0.00185 ± 0.000150

Results are expressed as mean ± standard deviation of n = 3 results except for * and # where individual data for n = 1 and 2 are reported, respectively.

Inhibition of SPM 7605 (0.7 µM) metabolism in human liver microsomes with specific cytochrome P450 chemical inhibitors

A: Peak 1

Inhibitor (conc.)	Cytochrome P450 isoforms	Peak 1 % inhibition vehicle control activity
Furafylline (10 µM)	CYP1A2	19.5
Methoxsalen (2.5 µM)	CYP2A6	35.3
Quercetin (10 µM)	CYP2C8	23.8
Sulphaphenazole (10 µM)	CYP2C9	5.00
Tranylcypromine (50 µM)	CYP2C19	19.0
Quinidine (1 µM)	CYP2D6	-
DDC (50 µM)	CYP2E1	22.0
Ketaconazole (1 µM)	CYP3A4	46.9

B: Peak 2

Inhibitor (conc.)	Cytochrome P450 isoforms	Peak 2 % inhibition vehicle control activity
Furafylline (10 µM)	CYP1A2	-
Methoxsalen (2.5 µM)	CYP2A6	4.73
Quercetin (10 µM)	CYP2C8	-
Sulphaphenazole (10 µM)	CYP2C9	-
Tranylcypromine (50 µM)	CYP2C19	34.5
Quinidine (1 µM)	CYP2D6	82.3
DDC (50 µM)	CYP2E1	15.4
Ketaconazole (1 µM)	CYP3A4	10.5

Inhibition relative to appropriate vehicle control (Sulphaphenazole, Ketoconazole, Furafylline, DDC, Quercetin and Methoxsalen) or control where vehicle is water (Tranylcypromine and Quinidine)
- = no inhibition was measured

Results are expressed as mean ± standard deviation of n = 3 replicates.

Results of a multiple linear regression analysis to find CYP isoform activities that explain the variability in the rate of metabolism of SPM 7605

Metabolite	N	Selected Variables	Cumulative R^2	C_p	P-value in final model
Peak 1	18	CYP3A4	72.3%	9.5	<0.0001 ***
		CYP1A1	78.9%	3.8	0.0090 **
		CYP2E1	83.3%	4.1	0.0763
Peak 2	18	CYP2D6	38.7%	-1.9	0.0220 *

Notes:
1. N is the number of livers included in the analysis.
2. R^2 is the coefficient of determination as each term is added to the model.
3. C_p is Mallows' C_p statistic as each term is added to the model.
4. Statistical significance is flagged as *, ** and *** for p-value ≤ 0.0500, 0.0100 and 0.0010, respectively.

Results of a multiple linear regression analysis to find CYP isoform activities that explain the variability in the rate of metabolism of SPM 7605

Metabolite	N	Selected Variables	Cumulative R^2	C_p	P-value in final model
Peak 1	19	CYP1A2	20.2%	8.0	0.0947
		CYP1A1	34.1%	6.0	0.0409 *
		CYP2C9	46.4%	4.5	0.0848
Peak 2	19	CYP3D6	31.7%	2.3	0.0121 *

Notes:
1. N is the number of livers included in the analysis.
2. R^2 is the coefficient of determination as each term is added to the model.
3. C_p is Mallows' C_p statistic as each term is added to the model.
4. Statistical significance is flagged as *, ** and *** for p-value ≤ 0.0500, 0.0100 and 0.0010, respectively.