CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-372

Pharmacology Review(s)

MEMORANDUM

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

DATE: July 23, 2003

FROM: Supervisory Pharmacologist
Division of Gastrointestinal and Coagulation Drug Products, HFD-180

SUBJECT: NDA 21,372 (Palonosetron)—Oral Carcinogenicity and Reproductive Toxicity Studies—Acceptability of Studies in Support of NDA for I.V. Injection

TO: NDA 21,372

The intended route of administration for palonosetron in humans is by intravenous injection. Generally, in toxicology studies, the drug is expected to be administered by the same route subject to practical considerations. In preclinical program, palonosetron was administered by oral gavage in mouse and rat carcinogenicity studies and reproductive toxicity studies in rats (Segment I. Fertility and reproductive performance, Segment II. Teratology and Segment III. Prenatal and postnatal) and rabbits (Segment II. Teratology). These studies are acceptable for the following reasons. Palonosetron has been developed under two INDS, one for i.v. (IND [redacted] (IND [redacted]) Dose selections for the carcinogenicity studies in mice and rats were based on maximum tolerated doses determined in 3-month oral toxicology studies. The Division and the CDER Executive CAC accepted them in 1994 and 1995. In the completed mouse carcinogenicity study, the systemic exposure to paolonosetron (plasma AUC) at the highest dose was about 150 to 289 times the human exposure (AUC =29.8 ng.hr/ml) at the recommended i.v. dose of 0.25 mg. In the rat carcinogenicity study, the systemic exposure at the high doses was 137 to 308 times the human exposure. Thus in both studies, the animals were exposed to sufficiently high doses. It is also impractical to administer the drug by i.v. injection daily for two years. The agency always accepted alternate routes of administration in the carcinogenicity studies as long as the dose selections are reasonably high.

In the reproductive toxicity studies, doses above 60 mg/kg/day were too toxic and lethal. Sufficiently high doses were employed in these studies assuring high systemic exposures as judged by the available toxicokinetic information from the other studies.

In conclusion, the oral carcingenicity and reproductive toxicity studies of palonosetron are acceptable in support of the NDA for palonosetron injection.

/S/

Jasti B. Choudary, B.V. Sc., Ph.D. Date
Supervisory Pharmacologist, HFD-180

Cc:
NDA
HFD-180
HFD-181/CSO
HFD-150/Dr. Leighton
HFD-180/Dr. Choudary
HFD-180/Dr. Justice
HFD-180/Dr. Korvick

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/s/

Jasti Choudary
7/23/03 12:28:25 PM
PHARMACOLOGIST

MEMORANDUM

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

DATE: July 10, 2003

From: Yash M. Chopra
Pharmacologist, HFD-180

Subject: Safety limits for the impurities of ——— in Palonosetron

To: NDA 21-372

1. Two potential impurities in the final drug substance of palonosetron were identified by sponsor as ——— Sponsor has asked to set the upper limit of these impurities as —% in the final drug substance.

2. The intended clinical dose of palonosetron injection is 5 ug/kg for nausea and vomiting induced by cancer chemotherapy. As per the sponsor request, the amount of each of the impurities administered in the suggested single clinical IV dose will be ———

3. The Division had suggested to sponsor to limit the amount of each of the impurities to —% and not —%. Sponsor did not conduct any preclinical toxicity studies with the purified impurities under NDA 21-372 and has now asked to set a limit of these impurities.

4. The safety limits for these impurities is estimated by computing the amounts of these impurities present in the 'no effect doses' of the available intravenous toxicity studies in a rodent and non-rodent. The 26-week IV toxicity study in rats (PALO-99-08) and 40-week IV toxicity study in dogs (PALO-99-10) were considered.

5. The 'no effect doses' in 26-week IV toxicity study in rats and 40-week IV toxicity study in dogs were 7 and 3 mg/kg/day, respectively. The batch # P30893-P105 of palonosetron was used in 26-week IV rat and 40-week IV dog toxicity studies and, a sample from this batch of the compound was reported to contain —% and —% of ——— respectively (according to the certificate of analysis attached with the study). The study #, doses used and % impurities, no effect dose and amounts of impurities computed from the data of the certificate of analysis are shown below in the table:

Study Name	Study #	Batch #	Doses Used (mg/kg/day)	% Impurities Present (Certificate Analysis)	No Effect Dose (mg/kg/day)	Computed Amount Impurities Administered in 'No effect Dose'
26-Week IV Tox in Rats	PALO-99-12	P30893-P105	0, 2, 7 & 10		7	ug/kg/day | ug/kg/day
40-Week IV Tox in Dogs	PALO-99-10	P30893-P105	0, 1, 3/10/6		3	ug/kg/day | ug/kg/day
		30893-P104	"		3	ug/kg/day | ug/kg/day

6. The amounts of [redacted] in the 'no effect dose' of 7 mg/kg/day in 26-week rat toxicity study were computed to be [redacted] ug/kg/day, respectively. As a rule of thumb, one-tenth of these doses were considered safe for humans. Thus the amounts of [redacted] ug/kg/day [redacted] in a clinical dose may be safe. In 40-week dog toxicity study, 'no effect dose' was 3 mg/kg/day and, the amounts of [redacted] administered were computed to be [redacted] ug/kg/day, respectively. . As a rule of thumb, one-fifth of these doses were considered safe for humans. Thus the amounts of [redacted] ug/kg/day of [redacted] in a clinical dose may be safe.

7. The amounts of the impurities present in the clinical iv dose of 0.25 mg (5 ug/kg/day) palnosetron could be [redacted] ng/kg/day of [redacted] These are small fractions of safe doses of these compounds identified in these studies and support the proposed limit of the impurities.

RECOMMENDATIONS:

None

/S/

Yash M. Chopra, M.D.,Ph.D., Date
Pharmacologist

COMMENTS:

/S/

Jasti B. Choudary, B.V.Sc, Ph.D. Date
Supervisory Pharmacologist,HFD-180

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/s/

Yash Chopra
7/10/03 05:28:23 PM
PHARMACOLOGIST

Jasti Choudary
7/11/03 07:51:45 AM
PHARMACOLOGIST

MEMORANDUM

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

DATE: July 10, 2003

FROM: Supervisory Pharmacologist
Division of Gastrointestinal and Coagulation Drug Products, HFD-180

SUBJECT: NDA 21,372 (Palonosetron)—Sponsor's Proposed Labeling—Changes In Preclinical Portions of Labeling.

TO: NDA 21,372

The following portions of the sponsor's proposed labeling should be replaced or changed as indicated. They relate to (1) Carcinogenesis, Mutagenesis, Impairment of Fertility, (2) Pregnancy. Pregnancy Category, (3) Nursing Mothers and (4) OVERDOSAGE. They are identified by the line numbers in the sponsor's draft labeling.

(1) Carcinogenesis, Mutagenesis, Impairment of Fertility---Lines 170 to 203.

"In a 104-week carcinogenicity study in CD-1 mice, animals were treated with oral doses of palonosetron at 10, 30 and 60 mg/kg/day. Treatment with palonosetron was not tumorigenic. The highest tested dose produced a systemic exposure to palonosetron (Plasma AUC) of about 150 to 289 times the human exposure (AUC= 29.8 ng.h/ml) at the recommended intravenous dose of — mg. In a 104-week carcinogenicity study in Sprague-Dawley rats, male and female rats were treated with oral doses of 15, 30 and 60 mg/kg/day and 15, 45 and 90 mg/kg/day, respectively. The highest doses produced a systemic exposure to palonosetron(Plasma AUC) of 137 and 308 times the human exposure at the recommended dose. Treatment with palonosetron produced increased incidences of adrenal benign pheochromocytoma and combined benign and malignant pheochromocytoma, increased incidences of pancreatic Islet cell adenoma and combined adenoma and carcinoma and pituitary adenoma in male rats. In female rats, it produced hepatocellular adenoma and carcinoma and increased the incidences of thyroid C-cell adenoma and combined adenoma and carcinoma.

Palonosetron was not genotoxic in the Ames test, the Chinese hamster ovarian cell (CHO/HGPRT) forward mutation test, the ex vivo hepatocyte unscheduled DNA synthesis (UDS) test or the mouse micronucleus test. It was, however, positive for clastogenic effects in the Chinese hamster ovarian (CHO) cell chromosomal aberration test.

Palonosetron at oral doses up to 60 mg/kg/day (about 1894 times the recommended human intravenous dose based on body surface area) was found to have no effect on fertility and reproductive performance of male and female rats.".

(2) Pregnancy. Teratogenic Effects: Category B---Lines204 to 211.

"Teratology studies have been performed in rats at oral doses up to 60 mg/kg/day (1894 times the recommended human intravenous dose based on body surface area) and rabbits at oral doses up to 60 mg/kg/day (3789 times the recommended human intravenous dose based on body surface area) and have revealed no evidence of impaired fertility or harm top the fetus due to palonosetron. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, palonosetron should be used during pregnancy only if clearly needed."

(3) Nursing Mothers---Lines 215 to 218.

It is not known whether palonosetron is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants and because of the potential for tumorigenicity shown for palonosetron in the rat carcinogenicity study, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother."

(4) OVERDOSAGE—LINES 269 to 277. The following should be added after line 277.

[DRAFT]

/S/

Jasti B. Choudary, B.V. Sc., Ph.D. Date
Supervisory Pharmacologist, HFD-180

Cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary

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/s/

Jasti Choudary
7/10/03 08:49:23 AM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY REVIEW OF NDA 21-372

Sponsor and/or agent: Helsinn HealthCare SA, Lugano (Switzerland)

Authorized US Agent: Craig Lehmann, Pharm. D.,
Austin, TX.

Review number/Date of submission: 000/September 26, 2002

Information to sponsor: Yes ( ) No (X)

Reviewer name: Yash M. Chopra, M.D., Ph.D.

Division name: Division of Gastrointestinal & Coagulation Drug Products, HFD-180
Date of Submission: September 26, 2002

Date of HFD180 Receipt: September 30, 2002

Review completion date: July 11, 2003

Drug:

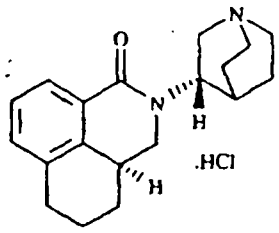
Trade name: Not Established
Generic name (list alphabetically): Palonosetron Hydrochloride
Code name:08-PALO, RS-25259-197
Chemical name: (3aS)-2-(S)-1-Azabicyclo[2,2,2]oct-3-yl]-2,3,3a,4,5,6-hexahydroxo-1-oxo-1*H*-benz[de]isoquinoline hydrochloride.
CAS registry number: 135729-62-3
Mole files number: NA

Manufacturer of the Drug: ———

Contact Name: ———

Molecular formula/molecular weight: $C_{19}H_{24}N_2O.HCl$

Structure:



Helsinn code: 08-PALO

Palonosetron HCl

(Syntex lab code: RS-25259-197)

INDs/NDAs/DMFs: Type II DMF #[redacted] IND[redacted] (Injection) & IND[redacted]

Drug class: Serotinin 5-HT_3 Antagonist

Indication: For the prevention of acute and delayed nausea and vomiting associated with initial and repeated courses of emetogenic chemotherapy.

Clinical formulation: Each 5 ml vial of Palonosetron Injection contains 0.25 mg palonosetron base as hydrochloride, 207.5 mg mannitol, disodium edetate and citrate buffer in sterilized water for injection. The pH of the solution is 4.5 to 5.5.

Route of administration: Intravenous

Proposed Clinical Use: An intravenous dose of 0.25 mg is recommended as a single dose approximately 30 minutes before the chemotherapy for the prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of emetogenic cancer chemotherapy including the highly emetogenic chemotherapy.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

None

Executive Summary

I. Recommendations

A. Recommendation on Approvability: From preclinical standpoint, approval of the application is recommended.

B. Recommendation for Nonclinical Studies: None

C. Recommendations on Labeling:
The suggested changes indicated in the revised text of the label should be adopted.

II. Summary of Nonclinical Findings

A. Pharmacological Activity:

Palonosetron a 5-HT_3 receptor antagonist (pki = 10.4 rat cortex) binds selectively with high affinity with cloned 5-HT_3 receptor sites of 293E1 cells and NG108 cells. It was seen to show a small effect on 5-HT_4 receptors in rat esophageal muscularis mucosa and very little affinity for dopamine, histamine and acetylcholine and other 5-HT receptor sites. It blocked cisplatin-induced emesis in ferrets and dogs at intravenous doses of 0.01 and 0.1 mg/kg, respectively without affecting the intestinal motility. At equivalent doses, palonosetron and ondansetron antagonized cisplatin induced emesis in ferrets. Palonosetron was efective in inhibiting cisplatin induced emesis in dogs, when administered up to 7 hr before cisplatin. The duration of effects for palonosetron effect was 6 hr prior to challenge. The inhibition of Bezold-Jarisch reflex by oral and intravenous routes of administration was more pronounced (ID_{50}= 0.04 ug/kg, iv; 3.2 ug/kg i.d.) than ondansetron and granisetron. It was found in brain after its systemic administration and exerted a mild CNS activity from intraperitoneal dose of 3 ug/kg in mice. It exerted no effects on respiratory functions and only a transient decrease in diastolic blood pressure with minimal effects on myocardium conduction and dog EKG parameters. The metabolite M9 (N-oxide of the compound) identified in animals and human, from 0.001 to 0.1 mg/kg, IV did not affect the blood pressure, heart rate, and ECG in conscious dogs but exerted a weak anti-emetic effect at 0.1 mg/kg, i.v. on chemotherapy induced emesis. Palonosetron affected the fast sodium ion channels and potassium channels at a high concentration of 10 ng/ml in in vitro rabbit purkinje fibre preparation. The minimum concentration (10 ng/ml) producing this effect is much higher than the 0.9 ng/ml Cmax attained at the recommended i.v. dose of 0.25 mg/day. Based on its anti-emetic effects for prolonged duration, the sponsor is seeking permisssion for its use in the control of immediate and delayed phases of chemotherapy induced vomiting. Sponsor did not conduct any preclinical study to demonstrate the efficacy of palonosetron on the delayed phase of emesis.

Intravenously administered palonosetron in rats, attained plasma peak concentration after 5 min of administration with rapid systemic clearance and volume of distribution of 17.2 l/kg. The systmic clearance in dog was larger and its the terminal half life was greater

than rat. The compound was disrtributed in bladder, ileum, lungs, adrenals, large and small intestines. Eight metabolites in the plasma of rat and 9 in the dog were detected and none of these conjugated before teir excretion. Metabolite 2, 3, 4, 1 and 5 (in order of rank) were isolated in rats and, metbolite 6 was the major metabolite in rats. The excretion was in feces up to 82% in first 24 hr (the excretion in urine complete in 8 hr and in feces by 24 hr). The compound was transformed in M6 (33.6%), M3 (4.7%) and parent compound (4.43%) in the dog. The chemical identification of the metabolites has been undertaken and completed by sponsor. The structures of metabolites was provided in the submssion. In monkeys, most of the administered compound by intravenous route was metabolized before excretion in urine. Metabolite M9 at intravenous doses of 0.001, 0.01, and 0.1 mg/ kg had no effects on blood pressure, heart rate, and ECG in conscious dogs. In man, a dose of 0.25 mg (5 ug/kg for 50 kg man), the proposed dose attains a plasma concentration of Cmax of 0.92 ng/ml and $AUC_{(0\text{-inf.})}$ of 29.8 ng.hr/ml, half life of 47.2 hr and clearance of 1.81 ml/min/kg.

B. Brief Overview of Toxicology

The acute tolerance of the intravenously administered compound was determined in rats, mice and dogs. A single iv dose of 30 mg/kg RS-25259-197 was lethal in mice and rats. Symptoms of toxicity were convulsions, inactivity, labored respiration, salivation, tremors and collapse. In dogs, an iv dose of 20 mg/kg was not lethal. The signs of toxicity were similar.

The toxicity of the prolonged duration with the compound was studied in rats, mice and dogs by intravenous, subcutaneous and oral routes of administration. Palonosetron was well tolerated in rats up to an intravenous dose of 3 mg/kg/day in 4 week iv toxicity study. In 28-day subcutaneous toxicity study in neonatal/ juvenile rats, subcutaneously administered palonosetron from 5 to 25 mg/kg/day produced treatment related but non-dose proportional plasma concentrations in both sexes and these were similar on day 1 and 28. The target organs (or tissues) of toxicity were injection sites, optic nerve neuropathy, spleen and kidneys. In the repeated study, the optic nerve neuropathy was not observed. A 26-week iv toxicity study in rats was done at the doses from 2, 7 and 14 (10) mg/kg/day and convulsions were seen in animals treated with 14 mg/kg/day dose but not after the dose adjustment was made to 10 mg/kg/day. A non-proportional plasma concentrations, bleeding at the site of injection, convulsions, reduced activity and deaths were observed suggesting central nervous system and site of injection as target organs of toxicity. In 1-month iv toxicity study in beagle dogs, sporadic bleeding in thymus, lungs and hemorrhage urinary baldder were seen in animals of 10 mg/kg/day group. In 28-day iv toxicity study in juvenile dogs, palonosetron at 0, 1, 3 and 6 mg/kg/day produced dose proportional plasma concentrations and no target organs of toxicity were identified. In a 40-week i.v. toxicity study in dogs, 1, 3 and 5/10/6 mg/kg were administered . At 10 mg/kg/day, animals had convulsions., ataxia, vomiting and diarrhea. Ataxia was seen even after the reduction of the dose. Central nervous system was the target organ of toxicity.

The 104-week carcinogenicity studies were conducted in mouse and rats. In the mouse study, oral gavage doses of 0, 0, 10, 30 and 60 mg/kg/day palonosetron were administered in 5 groups of animals (56/sex/group). A dose proportional plasma concentration of the compound. No increase in tumors incidences were reported in palonosetron treated animals. The exposure levels in the males and females included in the high dose treatment group on study week 26 were 289.3 and 149.7 times the human exposure at the recommended clinical dose.

In 104-week rat carcinogenicity study, oral gavage doses of 0, 0, 15, 30 and 60 mg/kg/day palonosetron in males and, 0, 0, 15, 45 and 90 mg/kg/day in females were administered in 5 groups of animals (65/sex/group). A non-dose proportional increase in the plasma concentrations were seen in rats. The plasma concentrations in females were more than males of the treatment groups during the study. On study week 26, the exposure of the compound in male and female animals was 136.9 and 308.2 times the plasma concentration (AUC values) achieved after the suggested clinical dose of 5 ug/kg in man. Palonosetron produced a treatment related but non-dose proportional plasma concentrations in animals. Increased incidences of benign pheochromocytoma in male and female animals and, increased incidences of combined benign and malignant pheochromocytoma, pancreatic islet cell adenoma and benign adenoma of pars distalis in high dose males were seen during the study. The higher incidences hepatocellular adenoma and thyroid C-cell adenoma were seen in high dose treatment group females.

The mutagenicity of the compound was determined in Ames assay, chromosomal aberration test in CHO cells, in vivo mouse micronucleus test, mammalian gene mutation assay and ex-vivo unscheduled DNA synthesis in Fisher 344 rats. It was clastogenic in a chromosomal aberration assay in CHO cells with and without S-9 mix.

C. Non-Clinical Safety Issues Relevant to Clinical Use: None

III. Administrative

A. Reviewer signature: /S/

B. Supervisor signature: Concurrence - /S/

Non-Concurrence - /S/
(See memo attached)

C. C.:
Original NDA
HFD-180
HFD-181/CSO
HFD-180/Dr.Chopra
HFD-180/Dr.Choudary
HFD-345/Dr. Viswanathan

PRECLINICAL PHARMACOLOGY AND TOXICOLOGY STUDIES:

Type of Study	Study/Report #	Name of Laboratory	Drug Batch #	Page #
PHARMACOLOGY	–	–	–	7
ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION				
In Vitro				
Binding of RS-29259-197 to Plasma Proteins	CL 6204		^{14}C-16398-GTH-27; Unlab PA15303-615 A9204010	40
In Vivo				
Absorption/Pharmacokinetics				
Pharmacokinetics of RS252559-007 After a single Oral Dose of RS-25259-197 in Male Rats	AT 6303	Syntex Res Lab., Palo Alto	^{14}C-151886-GTH-106 Unlab-13977-66 A9012010	41
Pharmacokinetics of RS252559-007 and Its N-Oxide metabolite following once Daily Oral Dose of RS-25259-197 in Male Rats for 5 Days	AT6302	,,	PA15303-51 A9201007	41
Pharmacokinetics of RS252559-007 After a single Oral Dose of RS-25259-197 administered to Dogs	AT 6304	,,	15188GTH-53 Unlab.5891-74; A9012002	42
Plasma Pharmacokinetics of RS252559-007 and Its N-Oxide metabolite following once Daily Oral Dose of RS-25259-197 in Female Dogs for 5 days	AT6301/ AM1023	,,	15303-51, A9201007	43
Distribution/Metabolism:				
Metabolic Disposition and Tissue Distribution of ^{14}C-RS252559-197 after single IV dose in Rats	AT 5975	,,	^{14}C-15188-GTH-53; Unlab 13977-86, A910210	44
Metabolic Disposition and Tissue Distribution to Brain, Eye and Intestine after a Single Intravenous Dose following single Intravenous and oral doses of ^{14}C-RS252559-197 in Pigmented Rats	AT 6285	,,	^{14}C-163898GTH-21 (IV), Unlab: 15303-615 A9204010	48
Metabolic Disposition following single Intravenous and oral doses of ^{14}C-RS52559-197 and Tissue Distribution after a Single Intravenous Dose in Rats	AT 6264	,,	^{14}C-15188-GTH-53 (IV), 151188-GTH-106 (PO); Unlab: 13977-86, A9102010	50
Metabolic Disposition of ^{14}C-RS252559-197 after single PO/IV Dose in Cynolmolgus Monkeys	DM 1078	,,	^{14}C-RS25259 IV: #17488GTH-75; 17488GTH-72; Unlab:15303-18; A9204029	53

TOXICOLOGY:				
Acute Toxicity Studies:				
I.V. (strain)				
Mice (Crl:CD-1)ICR BR VAF/Plus:/8 weeks)	AT 5921	Syntex Res, Palo Alto	PA13977-140 PA13977-86	59
Rat (Crl:SD)	711-R-91	"	PA13977-86	59
Rat (Crl:SD)	5939	"	PA13977-140 PA13977-86	59
Dog (Beagle)	710-D-91	"	PA13977-86	59
Dog (Beagle)	AT 5922	"	PA13977-86 PA13977-140	59
Oral:				59
Mice (Crl:CD-1)ICR BR VAF/Plus)	711-M-92	"	PA15303-61S	59
Rat (Crl:SD)	743-R-90	"	5935-107/ PA13977-86	59
Rat (Crl:SD)	AT6284	"	PA15303-61S	61
Dog (Beagle)	744-D-90	"	5935-107/ PA13977-86	59
Dog (Beagle)	AT 6268	"	PA15303-61S	61
SUBACUTE/SUBCHRONIC/CHRONIC TOXICITY STUDIES:				
Rats:				
1-Month IV Study in Rats	AT 5962	Syntex Res., PaloAlto	PA13977-86 PA13977-140	62
4-Week SC Study in Juvenile Rats	PALO-99-12	[redacted]	30893-105P	63
4-Week SC Study in Juvenile Rats	PALO-02-05	[redacted]	21000565	69
26-Week IV Toxicity Study in Rats	PALO-99-08	[redacted]	30893-P105	74
1-Month Oral Gavage Study in Rats	AT 6329	"	PA15303-61S	78
Dogs:				
1-Month IV Study in Beagle Dogs	AT 5963	"	13977-86, PA13977-140	81
1-Month Oral Gavage Toxicity Study in Beagle Dogs	AT 6328	"	PA15303-61S	88
4-Week IV toxicity in Juvenile Dogs	PALO-99-22	[redacted]	30893-P105	83
3-Month Oral (capsules) Study in Beagle Dogs	AT 6787	Syntex Res., PaloAlto	PA1530353ML PA16505-69S	89
40-Week IV Toxicity Study in Dogs	PALO-99-10	[redacted]	30893-P105	92
Carcinogenicity Studies:				
3-Month Oral Dose Ranging Study in Mice	# 719-M-94-252 59-97; AT6751	Syntex Discovery Research, Palo Alto	13977-86, PA13977-140	98
2-Year Mouse Carcinogenicity	PALO-99-18	[redacted]	30893-P104 30893-P106	104
Three Months Oral Dose Ranging Study in Rat with RS-25259-007	# AT 6665	Syntex, Palo Alto, CA		115

2-Year Rat Carcinogenicity	PALO-98-03	„	30893-P104 30893-P106	122
REPRODUCTIVE TOXICOLOGY:				
Segment I Male Fertility Toxicity Study in Rats	AT 6267	Syntex Res., Palo Alto	PA 15303-51	140
Segment I. Male Fertility Toxicity Study in Rats	AT 6700	„	PA 16505-69S	142
Segment I. Female Fertility & Embryonic Development Toxicity Study	AT6750	„	PA17555-40ML	144
Segment II. Teratology Study in Rats.	AT6756		PA 17555-40ML	146
Segment II. Teratology Study in Rabbits.	AT6755	„	PA 17555-40ML	150
Segment III. Pre- and Post- Natal Development in Rats	PALO-99-13		30893-P105	154
MUTAGENICITY:				
Ames Test	AM 0400		PA 15303-18	157
Mammalian Gene Mutation (HGPRT) Assay in CHO-K_1 BH4 cells	A0402	„	PA 15303-18	160
Chromosomal Aberration Assay in Chinese Hamster Ovarian CHO-K1 cells (ATTC CCL 61)	A 0401	„	PA 15303-18	157
In Vivo Mouse Micronucleus Assay	AM 0399		13977-86 & PA 13977-140	159
Unscheduled DNA Synthesis ex Vivo in Fisher 344 Rats	PALO-99-38		30893-P105	161
SPECIAL TESTS:				
Acute Vein Irritation Study in White Rabbits:	AT 5923	Syntex, Palo Alto	13977-86/PA13977-140; 25259-197-11529	163
Ear Vein Irritation Study in Rabbits	PALO-99-30/15-D6144		30893-P106; 1737331/21	163
In Vitro Compatibility Test in Human Whole Blood & plasma	CL 5911	Syntex Res., Palo Alto	13977-86/13977-140	164

Most of these studies were reviewed in IND [redacted] (RS 25259-197, injectable). The reviews of these studies were acceptable and are included in this review. The new pharmacology and toxicology studies submitted with the present application are also reviewed here. The new toxicity studies submitted were 28-day juvenile rat toxicity study, 6-month i.v. toxicity study in rats, 104-week oral gavage carcinogenicity studies in the mouse and the rat, prenatal and postnatal toxicity study in rat, 40-week toxicity study in dogs, ex-vivo unscheduled DNA synthesis in rat liver cells.

The following studies submitted with the application but were not reviewed here were:

GOOD LABORATORY PRACTICE & QAU REGULATIONS: Sponsor submitted statements of compliance with GLP and QAU regulations with each of the submitted toxicity studies.

PHARMACOLOGY:

Palonosetron a 5-HT_3 antagonist was seen to exert a minimal effect on 5-HT_4 receptors in rat esophageal muscularis mucosa and in guinea pig ileum preparations. It did not show any effect (agonist or antagonist) on hipocampal adenylate cyclase activity in guinea pigs up to a concentration of 100 µM. It (1 µM) showed a little agonistic effect and shifted substance P induced dose response (contraction) curve in guinea pig ileum to the right. It exerted antiemetic and anti-nauseating effects in animals treated with cancer chemotherapy and was claimed to be useful in chemotherapy induced emesis. The following preclinical studies submitted with the present application are reviewed below.

Mechanism of Action:

The chemotherapy induced nausea and vomiting had been shown to be due to the release of serotonin from the enterochromaffin cells of small intestines and stimulate 5- HT_3 receptors located on vagal afferents. This causes simulation of 5-HT_3 receptors in nuclear tractus solitaris, amygdaloid complex and area postrema and chemoreceptor trigger zone. Palonosetron, a selective 5-HT_3 receptors blocking agent was seen to block the 5-HT induced contractions in isolated of guinea pigs intestines, block rat cerebral cortex membrane 5-HT_3 receptors (pKi = 10.4). Its binding with 293El cells and NG108 cells expressing 5-HT_3 receptors, was 9.5 and 23.2 times more than granisetron. It blocked the 2-methyl-5-hydroxy-tryptamine induced bradycardia (von Bezold-Jarisch reflex) in anaesthetized rats, dogs and cats following intravenous, intraduodenal or dermal administration (ID_{50} = 0.04 ug/kg, IV; 3.2 ug/kg, i.m.). Palonosetron from 0.01 mg/kg in ferrets and, 0.1 mg/kg p.o. in dogs completely blocked cisplatin induced vomiting without an effect on the intestinal motility. It also was shown to bind with central 5-HT_3 receptors sites in the brain stem nuclei, dorsal motor nucleus of the vagus, limbic areas including the hippocampal subfields, nuclear tractus solitaris, amygdaloid complex and area postrema.

PHARMACODYNAMIC EFFECTS:

A. In Vitro Receptor Binding and Activation studies:

1. Using radioligand binding techniques, the affinity of RS-25,259-97 at 29 different receptor types/subtypes were examined. RS-25,259-197 exhibited the highest affinity (pKi) for 5-HT_3 receptors in rat cerebral cortex of 10.4±0.2 (mean ± S.E.M., n=4). Binding affinities less than 6 were observed for the following receptors serotonin 5-HT_{1A} 5-HT_{1c}, 5-HT_{1D}, 5-HT_2 and 5-HT_4 and their uptake sites; α-adrenoreceptors α_{1A}, α_{1B}, α_{2A}, α_{2B}; β-adrenoreceptors β_1, β_2; Dopamine D_1, D_2; angiotensin II subtypes 1 and 2; dihydropyridine-Ca^{2+} channel; saxitoxin binding site of Na+ channel; N-methyl-D-aspartate (NMDA) receptor channel; (γ-aminobutyric

acid (GABA), picrotoxin, and benzodiazepine binding sites on GABA receptor/channel complex; neurokinin NK_1; opioid delta, kappa, and mu; and muscarinic M_1, M_2 and M_3 receptors. Binding affinities, with respective test systems for these latter receptors are provided in Table 1, below. These data suggest that RS-25,259-197 acts as a potent and selective compound for the 5-HT_3 receptor.

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TABLE 1. Binding Affinities for RS-25,259-197 at Various Receptors and Test Systems

Receptor	pKi(SEM)	Test System
5-HT_3	10.4 (0.2)	Rat cerebral cortex
5-HT_{1A}	4.4 (0.1)	Rat cerebral cortex
5-HT_{1C}	4.6 (0.3)	Rat cerebral cortex
5-HT_{1D}	4.2 (0.3)	Bovine striatum
5-HT_2	4.8 (0.2)	Rat cerebral cortex
5-HT uptake	5.3 (0.0)	Rat cerebral cortex
α_{1A}	5.6 (0.3)	Rat submaxillary gland
α_{1B}	5.4 (0.1)	Rat liver
α_{2A}	5.4 (0.2)	Rabbit spleen
α_{2B}	5.4 (0.1)	Rat kidney
β_1	<4	Rabbit lung
β_2	<4	Rat lung
M_1	5.9 (0.1)	Rat cerebral cortex
M_2	4.9 (0.1)	Rat heart
M_3	5.3 (0.1)	Rat submaxillary gland
D_1	<4	Rat striatum
D_2	<4	Rat striatum
AII-1	<4	Rat liver
AII-2	<4	Bovine Cerebellum

Receptor	pKi (SEM)	Test System
Ca^{++} channel	<4*	Rat cerebral cortex
Na^{++} channel	<4*	Rat cerebral cortex
NMDA	<4*	Rat whole brain
$GABA_A$	<4*	Rat whole brain
$GABA_A$/BZD	<4*	Rat whole brain
$GABA_A$/Picrotoxin	<4*	Rat whole brain
NK_1	<4	Rat submaxillary gland
Delta opioid	4.0 (0.4)	Guinea pig brain (minus cerebellum)
Kappa opioid	4.2 (0.1)	Same as above
Mu opioid	3.7 (0.2)	same as above

* Values are pIC_{50}
(SEM) = Standard Error

2. The effects of RS-25,259-197 (100 um) on 5-HT_4 receptor mediated increases in adenylate cyclase activity was tested in guinea pig hippocampus. These studies showed that when tested either alone or in the presence of 0.4 uM 5-carboxyamido-tryptamine, a 5-HT_{1A} antagonist (added to eliminate any involvement of 5-HT_{1A}-like receptor), RS25259-197 had no effect on adenylate cyclase activity. In addition, RS-25,259-197 had no effect on serotonin (10 uM) induced stimulation of adenylate cyclase activity in this preparation in the presence or absence of the 5-HT_{1A} antagonist. Collectively, these studies suggest that RS25,259-197 has no activity (stimulatory or inhibitory) at either the 5-HT_{1A} or the 5-HT_4 type receptors.

3. Additional in vitro studies to examine the specificity of RS-25,259-197 for 5-HT_3 type receptors were conducted. These studies showed that RS-25,259-197 (1 uM) did not antagonize the 5-HT-induced contraction of dog saphenous vein, rabbit aorta or proximal guinea pig ileum, events which have been shown to be mediated by 5-HT_1, 5-HT_2, and 5-HT_3, receptor subtypes, respectively. In contrast, RS25,259-197 (1 uM) produced potent and insurmountable antagonism of 5-HT_3 mediated contraction in proximal segments of guinea pig ileum. These data indicate that RS-25,259-197 acts as a selective and potent 5-HT_3 antagonist.

4. The potential for 5-HT_3 type receptors antagonism of RS-29-259-197, RS-17825-007, Metabolite M4 (5-S-hydroxypalonosetron) and ondansetron was assessed in increasing concentrations on isolated guinea pig ileum preparation containing 5-$HT_{1/2}$ receptor antagonist (methysergide) and 5-HT_4 receptor desensitizing agent (5-methoxytryptamine). All of the compounds showed antagonistic activity on 5-HT_3 receptors but RS-29-259-197 had the lowest IC_{50} value followed by ondansetron. The metabolite RS-17825-007 and Metabolite M4 showed low activities as shown in the following table of the sponsor (vol 12.381, p 007). Granisetron used as a standard preparation and its IC50 was 10^{-6} M. In conclusion, in vitro binding studies as well as isolated tissue studies showed that RS-25,259-197 acts as a potent and selective antagonist of the 5-HT_3 type receptor.

Test substances	NOEL	Emax, concentration (probability)	IC_{50}	5% CI
PALONOSETRON.HCl	$3x10^{-9}$M	100% at $3x10^{-7}$M (**)	$2.8x10^{-8}$M	$2.4x10^{-8}$M to $3.4x10^{-8}$M
RS-17825-007	$3x10^{-6}$M	98.3% at 10^{-4}M (**)	$1.2x10^{-5}$M	$8.1x10^{-6}$M to $1.8x10^{-5}$M
M4	$3x10^{-7}$M	100% at $3x10^{-5}$M (**)	$2.7x10^{-6}$M	$2.5x10^{-6}$M to $3.0x10^{-6}$M
ONDANSETRON	10^{-7}M	98.2% at $3x10^{-6}$M (**)	$7.1x10^{-7}$M	$5.1x10^{-7}$M to $9.7x10^{-7}$M

The effects are expressed as percentages of inhibition of contractions calculated in relation to reference contractions induced by serotonin (10^{-5}M)
NOEL: no observed effect level
Emax: maximal effect.
Probability: ** $P \leq 0.01$ when compared with the control group dosed with the vehicle, analysis of variance with NEWMANN-KEULS test if $P \leq 0.05$.
IC_{50}: concentration which induces a 50% inhibition of the maximal effect
5% CI: 5% confidence interval ($\alpha = 5\%$)

5. **Distribution of [^{3}H]-RS-25259-197 Binding Sites in Rat and Mouse Brain By Quantitative Autoradiaphy:**

Methods: Mounted sections of brains from 6 male Sprague Dawley Rats and 6 male mice were incubated with buffer containing 1.0 nM [^{3}H]-RS-25259-197 for 60 min. The distribution of [^{3}H] RS-25259-197 binding sites were then determined using autoradiographic analysis following exposure to x-ray film for a period of 24 weeks. Brain slices were then stained with 0.25% cresyl violet for histological verification of the regional binding data.

Results: The highest density of [^{3}H] RS-25259-197 binding sites in sections of mouse and rat brain was located in the brain vagal complex (nucleus tractus solitaires, area postrema, and dorsal motor nucleus of the vagus). Moderate levels of activity were also seen in the spinal trigeminal tract, amygdala, hippocampus and cerebral cortex. The distribution of [^{3}H] RS-25259-197 in both rat and mouse brain is consistent with the known distribution of 5-HT_3 receptors.

6. Activity of RS-25259-197 at the 5-HT_3 receptors in isolated guinea pig ileum: **(Report No. AT 6373)** A study on the activity of RS-25259-197 (1-100 nM) at 5-HT_3 receptors in isolated guinea pig ileum showed that RS-25259-197 was an insurmountable antagonist with apparent affinities (mean $\pm$ SEM of 8.7$\pm$ 0.2, 8.8 $\pm$ 0.2, and 8.5 $\pm$ 0.1 at antagonist concentrations of 1, 10, and 100 nM, respectively. These findings were similar to the initial affinity estimates reported in study AT 6063.

7. Evaluation of Possible 5-HT_4 Agonist or Antagonist Activity on Isolated Proximal Colon of Guinea-Pig: In isolated proximal portion of guinea pig colon, palonosetron at the concentration from 10^{-6} to 10^{-4} M did not increase the 5-methyltryptamine (MeOT) induced contractions indicating that it was devoid of agonistic activity. But it inhibited 5-MeOT induced contraction at a dose dependent manner (23.4% at $3X10^{-6}$ M and 93.9% at $3X10^{-4}$ M). Thus the study indicated that palonosetron exerted 5-HT_4 receptor antagonistic activity ($IC_{50} = 1.2X10^{-5}$ M).

8. Activity of RS-25259-197 at the Substance P receptor in isolated guinea pig ileum: In separate studies using the isolated guinea pig ileum, RS-25259-197 (1 µM) caused a significant rightward shift (less than 2 fold) in the dose response curve to Substance P, suggesting that RS-25259 has slight antagonistic effects at substance P receptors.

9. Activity at the 5-HT_4 Receptor in Isolated Rat Esophageal Muscularis Mucosa: Additional pharmacology tests on the activity of RS-25259-197 at 5-HT_4 receptors in isolated rat esophageal striated muscle showed that RS-25259-197 (0.1 nM to 1 µM) had neither relaxant (agonist) nor antagonistic effects at the 5HT_4 receptors.

10. Interaction of RS-25259-197 and its Three Enantiomers (RS-25259-198, RS-25233 197 and RS-25233-198) at 5-HT_3 receptors in guinea-pig ileum, in membrane of NG- 108-15 cells and in radiographic studies: (Res Publication Study Wong EHF et al. Br. J. Pharmacology 114: 851-59, 1995)
The concentration response curves of 5-10 uM 5-HT were prepared in the isolated guinea-ileum preparation (suspended in Tyrode containing 1 uM methysergide to antagonize 5-HT 1 and 2 receptors or 5-methoxytryptamine to desensitize 5-HT_4 receptors). Out of the 4 enantiomers, RS25259-197 showed unsurmountable antagonism and its pKB value was greater than the other 3 enantiomers (S,S>(R,S)>(S,R) as shown in Table 3 of the publication (vol 13:381, pp 0091).

Table 3 Saturation studies of [^{3}H]-RS 25259-197 and [^{3}H]-RS 42358-197 in membranes from NG-108-15 cells, human kidney 293E1 cells transfected with cloned 5-HT$_3$ receptor homomeric subunit and human hippocampus

Preparation	[^{3}H]-RS 25259-197 K_d (nM)	[^{3}H]-RS 25259-197 B_{max} (fmol mg^{-1})	[^{3}H]-RS 42358-197 K_d (nM)	[^{3}H]-RS 42358-197 B_{max} (fmol mg^{-1})
NG 108-15 cells	0.05 ± 0.02	610 ± 60	0.20 ± 0.01	660 ± 70
293E1 cells	0.07 ± 0.01	1068 ± 33	0.20 ± 0.03	888 ± 79
Human hippocampus	0.15 ± 0.07	6.9 ± 2.4	-	-

Values are means ± s.e.mean, n = 3 animals

The radioligand binding assays of 5-HT$_3$ receptors in membranes from NG 108-1 5 cells was done according to the methods of Wong et al., (1993), Palonosetron and RS-42348-197 were used from the concentrations of 4 pM to 4 nM. The competition studies with the compounds were conducted at 0.1 to 0.4 uM of radioligand. The non-specific binding was blocked by zacopride. The compound RS25259-197 showed three to fourfold higher affinity than RS-42358-197. The density of label sites in the preparations showed that the amount of these receptors was different in the brain areas but these were similar pharmacologically as shown in the above table. The labeled receptors in membranes from NG 108-15 cells, rat cortex, rabbit ileum or guinea pig ileum, RS-25259-107 showed affinity value of 10.1, 10.2, 10.1 and 8.3, respectively and the *Hill coefficient* was not different from unity. The labeled ^{3}H-RS25259-97 was taken up in different densities indicating different amounts of 5-HT$_3$ receptors in rat brain areas. The highest density of these receptors was present in nuclear tractus solitaris and area postrema and the medium amounts of the receptors were present in spinal trigeminal tract ventral dentate gyrus and basal medial amygdala. Only a low density of receptors was present in hippocampus, parietal cortex, medium raphe and cerebellum.

B. <u>In Vivo studies (Antagonism of 5-HT Mediate Reflex Bradycardia)</u>:

1. <u>Rats; Topical Application (study 1)</u>: The effects of topical application of the HCl salt, RS-25,259-197 (0.1-1000 ug/chamber) and its free base, RS-25259-007 (0.1100 ug/chamber) on the 2-methyl-5-HT-induced Bezold-Jarisch reflex (B-Z; reflex bradycardia) were tested. Both RS25,259-197 and its free base, RS-25259-007 inhibited the 2 methyl-5-HT-induced reflex, in a dose-dependent manner. ID$_{50}$s for the HCl salt and the free base were 0.41 (0.01-0.88) ug/chamber and 0.43 (0.01-1.06) ug/chamber, respectively. Time to reaching peak effect was inversely related to the concentration of the compound in the chamber and peak effects following each concentration lasted throughout the 300 min observation period.

<u>Rats: Topical Application (Study 2)</u>: In a second study in the anesthetized rat RS-25,259-197 (HCl salt) produced similar dose-dependent inhibition of 2-methyl-5-HT-induced B-Z reflex bradycardia (ID$_5$. = 32.8 ug/chamber; approximately 76 fold greater than that previously observed). In the present study the test article was dissolved in deionized water in a volume of 0.4 ml, whereas in the previous study the test agents were dissolved in a vehicle containing 79% propylene glycol, 20% water and 1% azone in a 0.4 ml volume.

<u>Cats: Topical Application</u>: A third study concerning the effects of topically applied —— —— RS-25,259 (dissolved in 79% propylene glycol, 20% water and 1% azone in a 0.4 ml

volume) on the 2-methyl-5-HT-induced B-Z reflex was performed in anesthetized cats. In this, like the studies in rats, RS-25,259-197 produced a dose-dependent inhibition of the reflex (ID_{50} = 0.33; [0.12-0.55] mg/chamber).

Rats: Intravenous Administration: The effects of either single or multiple i.v. doses (0.001-100 ug/kg) of RS25,259-195, on the 2-methyl-5-HT induced Bezold-Jarisch reflex in anesthetized rats ($n \geq 8$/group) were evaluated. Comparative effects of both Ondansetron and Granisetron on the reflex were also tested. RS-25,259-197 produced a concentration-dependent inhibition of the 2-methyl-5-HT induced bradycardia. ID_{50} estimates (mean [95% confidence interval] in ug/kg) for RS-25,259-197 from two separate multiple dose studies and one single dose study were 0.02 (0.017-0.023), 0.02 (0.002-0.253), and 0.04 (0.024-0.063) respectively. RS-25,259-197 was more potent than either ondansetron (ID_{50}s = 3.2 [0.2-47.1], multiple doses; 2.2 [0.5-3.9]) or Granisetron (ID_{50}s = 0.36 [0.12-1.12], multiple doses; 0.10 [0.01-0.19], single doses. At equi-effective dose levels, RS-25,259-197 also exhibited a longer duration of action than either Ondansetron or Granisetron. These findings are summarized in TABLE 2, Below (Reproduced from Sponsors Table 2, Report No. AT 5618& Vol. 1.3, pp.71)

TABLE 2

COMPARISON OF RS-25259-197, ONDANSETRON AND GRANISETRON ON THE BRADYCARDIC RESPONSE TO 2-METHYL-5-HYDROXYTRYPTAMINE IN URETHANE-ANESTHETIZED RATS

Protocols Dose, µg/kg, iv	RS-25259-197	Ondansetron	Granisetron
	ID_{50}[a], µg/kg		
iv-single dose	0.04 (0.024-0.063)	2.2 (0.5-3.9)	0.10 (0.01-0.19)
iv-multiple dose	0.02 (0.017-0.023)	2.6 (1.5-4.7)	0.36 (0.01-1.12)
	0.02 (0.002-0.253)		
	Duration[b], min		
0.1	60		30
1	180	0	120
3		15	
10	>480	60	240
30		180	
100		180	360
300		360	420

[a]An estimated dose that induced a 50% inhibition, with its 95% confidence interval shown in the parentheses.
[b]Duration of inhibition, from time of compound administration to the time when the inhibitory effect returned to a level not significantly different from that of vehicle control.

C. **In Vivo Studies; Specificity with Regard to Involvement of 5-HT_3 Receptors in Chemical induced Emesis**:

1. **Effects of RS 25,259-197 on Cisplatin-induced Emesis in Ferrets**: The actions of intravenously administered RS25,259-197 (0.001, 0.003, 0.01, 0.03 and 0.1 mg/kg) on Cisplatin-induced emesis in ferrets were tested. These studies showed that even at the lowest dose tested (0.001 Mg/kg), RS-25,259-197 produced approximately 40 % inhibition of the emesis induced by 10 mg/kg Cisplatin, while at doses from 0.003-0.1 mg/kg, RS-25,259-197 was nearly 100% effective.
The effects of orally administered RS-25,259-197 (0.0003, 0.001, 0.003, 0.01, 0.03 and 0.1 mg/kg) on Cisplatin-induced emesis in ferrets were also tested. These studies showed that oral

doses (0.003 mg/kg to 0.1 mg/kg) of RS-25,259-197 produced from 50% up to around 95% inhibition of the emesis induced by cisplatin (10 mg/kg i.v.) in ferrets.
The duration of the antagonistic effects of RS-25,259-197 (0.1 mg/kg, p.o.) on cisplatin-induced emesis in ferrets was studied. These studies showed that administration of RS-25,259-197 at 1, 3, and 6 hours prior to challenge with cisplatin (10 mg/kg, i.v.) produced > 98% inhibition of the cisplatin-induced emesis.

2. **Anti-emetic Effects in Ferrets**: Male ferrets were used to study the effects of RS-25259-197 (0.001 to 0.1 mg/kg, i.v.) on emesis induced by Cisplatin (10 mg/kg i.v., administered 30 min following dosing with RS-25259-197). Results from these studies showed that, at all doses tested, RS-25259-197 reduced the mean number of emetic episodes (measured over a 4 hour period) compared to the incidence in vehicle control animals. In general, inhibition was complete at doses of RS-25259-197 $\geq$ 0.003 mg/kg (Report No. AT 6664).

3. **Effects of RS-25,259-197 on Cisplatin-induced Emesis in Dogs**: The antiemetic effects of RS-25,259-197 (0.0003-0.3 mg/kg) administered orally 1 hr prior to cisplatin (3 mg/kg, i.v.) challenge in dogs were studied. These studies showed that RS-25,259-197 inhibited the cisplatin-induced emesis in dogs, with significant inhibition observed, beginning at 0.01 ng/kg (72% inhibition) and with 100% inhibition seen at the 0.1 mg/kg dose. In comparison, orally administered Ondansetron (0.001-1.0 mg/kg) produced significant inhibition only at the two highest doses tested (0.3 and 1.0 mg/kg), producing 80 and 87% inhibition of the number of emetic episodes, respectively.

The duration of action of intravenously administered RS-25,259-197 on cisplatin-induced emesis in dogs was evaluated in two separate studies. The first study evaluated the antiemetic effects of RS-25,259-197 at 0.03 mg/kg, i.v. when administered 24, 12, 8, 7, 6, 5, 4, 3, 2, or 1 hours prior to challenge with cisplatin (3.0 mg/kg, i.v.). For comparison, ondansetron (0.3 ng/kg, i.v.) was administered at 8, 7, 6, 5, 4, 3, 2, or 1 hrs prior to cisplatin challenge. Results from the first study showed that RS-25,259-197 significantly inhibited the cisplatin-induced emesis in dogs, when administered at up to 7 hrs prior to cisplatin challenge, with greater inhibition observed at times closer to the Cisplatin challenge (i.e. RS-25,259-197 produced 38.1% when given at 7 hr and 88% when administered at 1 hour prior to cisplatin challenge). In comparison, inhibition of Cisplatin-induced emesis by ondansetron (0.3 mg/kg) was more variable, with significant inhibition seen only following ondansetron administration at 7,1 4 and 1 hr prior to cisplatin challenge. The percent inhibitions produced by ondansetron at these three times were 43, 40 and 69.3%, respectively.

The second study evaluated the antiemetic effects of RS-25,259-197 at 0.1 mg/kg, i.v. when administered 12, 10, 8, 6, 4, 2, or 1 hours prior to challenge with cisplatin (3.0 mg/kg, i.v.). This study showed that administration of RS-25,259-197 (0.1 mg/kg, i.v.) from 10-1 hours prior to cisplatin challenge produced from 50-97 % inhibition of Cisplatin-induced emesis in dogs. In comparison, Ondansetron (0.15 mg/kg, i.v.) only produced 43% inhibition, and only when administered at 1 hr prior to cisplatin challenge. Additionally, Granisetron (0.04 mg/kg, i.v.) produced no significant inhibition at any time tested (12-1 hrs) pre cisplatin challenge.

Two additional studies in dogs evaluated the therapeutic effects of RS-25,259-197 on cisplatin-induced emesis when administered following cisplatin administration at the onset of emesis.

In the first study, the ability of RS-25,259-197 at doses of 0.001-0.3 mg/kg to inhibit cisplatin-induced emesis when added one-hour post Cisplatin administration were tested. These studies showed that i.v. doses of RS-25,259-197 inhibited the number of Cisplatin-induced emetic episodes compared to vehicle controls. Although somewhat variable, the inhibitory effects of RS-25,259-197 were generally dose proportional and ranged from 47% inhibition at 0.001 mg/kg to a maximal of 90% inhibition at the 0.03 mg/kg dose. When similarly tested, Ondansetron produced comparable inhibition at doses of 0.03 to 1.0 mg/kg.

In another study, the ability of RS-25,259-197 at 0.1 mg/kg, i.v. to inhibit cisplatin-induced emesis when administered at the onset of emesis (i.e. post Cisplatin administration) was tested. These studies showed that in dogs, RS-25,259-197 (0.1 mg/kg, i.v.) reduced the number of emetic episodes in dogs by 82% when administered intravenously at the onset of emesis induced by cisplatin (3.0 mg/kg, i.v.). In comparison, i.v. administered Ondansetron (1.0 mg/kg) produced only 45% inhibition of the cisplatin-induced emetic episodes.

4. **Correlation of Anti-Emetic Activity and Plasma Levels of RS-25259-197 and its N-oxide (RS-72033-007) in Cancer Chemotherapy Treated Dogs: (Study # Syntex AT 6313)**

Methods: Twenty four beagle dogs of either sex were divided in 4 groups and administered oral doses of 0, 100, 316 or 1000 ug/kg RS-25259-197 in capsules 30 min prior to 3.0 mg/kg cisplatin. Blood samples were collected to estimate the plasma concentrations of RS-25259-197. Venous blood samples (sample volume not provided) were collected (via jugular vein) at 0 (predose), 15, 30 min, 1, 2, 4, 8, 24 and 48 hours after dosing. Following ——— plasma levels of RS-25259-007 (free base of RS-25259-197) and RS-17825-007 (N-oxide of RS-25259) of were quantitated using ——— Pharmacokinetic data for RS-25259-007 was calculated based on generated plasma concentration-time curves for each.

Results: The plasma concentrations (AUC) of RS-25259-197 was seen to increase in a dose related manner in the animals and the number of emetic episodes were reduced in the similar manner in animals of all study groups. The number of emesis were reduced from 15.83 in Cisplatin treated animals to 1.83, 4.50 and 5.67 in 3 treated groups of animals. The anti-emetic effect was not related to the plasma concentration of RS-25-259-197.

5. **Effects of RS-25,259-197 on Chemically-induced Emesis in Dogs by Agents Other Than cisplatin, Which also Induce Emesis mediated by Activation of 5-HT, Subtype Receptors:**

a. Actinomycin D: RS-25,259-197 (0.001-0.03 mg/kg, i.v. and 0.003-0.1 mg/kg, p.o.) was also shown to inhibit emesis induced by Actinomycin D (0.15 mg/kg, i.v.) in dogs. RS-25,259-197 induced inhibition of emesis following iv administration ranged from around 40% at the 0.001 and 0.003 mg/kg doses, up to around 70 % at the 0.03 dose. Following p.o. dosing, RS-25,259-197 produced around 57% inhibition at the 0.003 and 0.091 mg/kg doses up to 93% at the o.1

mg/kg dose. In comparison, Ondansetron (0.01-0.03 mg/kg, i.v. and 0.03-1.0 mg/kg, p.o.) produced comparable inhibition of the actinomycin D (0.15 mg/kg, i.v.) -induced emesis in dogs.

b. Dacarbazine: Additional studies in dogs examined the antiemetic effects of RS-25,259-197 (0.001-0.03 mg/kg, i.v. and 0.003-0.1 mg/kg, p.o.) on Dacarbazine (30 mg/kg i.v.)-induced emesis. These studies showed that at i.v. doses 0.003 -0.03 (administered 2 hr prior to challenge with Dacarbazine), RS-25,259-19, produced from 35.4- 92% inhibition of the dacarbazine-induced emetic episodes. Likewise, oral doses of RS-25,259-197 (0.01-0.1 mg/kg) produced from 52-100% inhibition of the same, respectively. In comparison, ondansetron (0.1 and 0.3 mg/kg, i.v.) produced 53 and 87% inhibition of Dacarbazine-induced emetic episodes, respectively, and from 56-99% inhibition following oral doses in the range of 0.1-1.0 mg/kg.

c. Mechlorethamine: other studies examined the effects of RS-250259-197 administered i.v. (0.001-0.03 mg/kg) and orally (0.003-0.1 mg/kg) on emesis induced by mechlorethamine (0.4 mg/kg, i.v.) in dogs. Ondansetron (0.01-0.3 mg/kg i.v. and 0.03- 1.0 mg/kg, p.o.) was likewise studied for comparison. Intravenous doses of RS-25,259-197 (0.01 and 0.03 mg/kg) significantly inhibited the mechlorethamine-induced emesis in dogs (70 and 98% respectively). Inhibition of the mechlorethamine-induced emesis by RS-25,259-197 following oral dosing ranged from 53% at the 0.003 mg/kg dose to 100% at the 0.1 mg/kg dose. In comparison, ondansetron administered i.v. and p.o., also inhibited the mechlorethamine-induced emesis in dogs, up to 53% at the 0.3 mg/kg i.v. dose and by 70% and 100% at the 0.3 and 1.0 mg/kg oral doses, respectively.

6. Effects of RS-25,259-197 on Chemically-induced Emesis in Dogs by Agents Which Induce Emesis Mediated by Mechanisms other than Activation of 5-HT_3 Subtype Receptors:

a. **Apomorphine:** Studies were conducted which examined the ability of RS-25,259-197 (0.001-1.0 mg/kg, p.o.) to affect apomorphine (0.1 mg/kg, s.c.)-induced emesis in the dog model. Apomorphine is a dopamine agonist and induces emesis via activation of the D2 receptor subtype in the chemoreceptor trigger zone. These studies showed that neither RS-25,259-197, ondansetron (1.0 mg/kg. p.o.), nor granisetron (1.0 mg/kg, p.o.) significantly altered the apomorphine-induced emesis in dogs at the doses tested. In contrast the apomorphine-antagonist, Haloperidol (5.0 mg/kg, p.o.) decreased (98% inhibition) the apomorphine-induced emesis in the dog.

b. **Copper sulfate:** Comparative effects of RS-25,259-197 (0.001-1.0 mg/kg, p.o.), Ondansetron (1.0 mg/kg, p.o.), granisetron (1.0 mg/kg, p.o.) and Haloperidol (5.0 mg/kg, p.o.) on emesis induced by Copper sulfate (a local irritant) in dogs were tested. Results from these studies showed that while the lowest does of RS-25,259-197 (0.001 mg/kg) augmented the copper sulfate induced emesis (89% increase), the three higher doses tested (0.01, 0.1 and 1.0 mg/kg) had no effect. By comparison, none of the other three agents significantly affected the copper sulfate-induced emesis in the dog, suggesting that none of these agents protect against the local irritant action of Copper sulfate.

In conclusion, the combined results from studies on the effects of RS-25-259-197 on chemically-induced emesis suggest that the antiemetic effects of RS-25,259-197 are selective for agents in which the induced emesis involves 5-HT3 receptor activation.

SECONDARY PHARMACOLOGY

A. Cardiovascular Pharmacology:

Hemodynamic Effects: The hemodynamic effects, including lead II EKG, of RS-25,259-197 or Ondansetron (ascending doses of 1, 10, 100 and 1000 ug/kg) administered intravenously in closed chest anesthetized dogs were studied. RS-25,259-197 produced a significant decrease in heart rate (12 bpm) at 45 min following administration of the 1000 ug/kg dose. A significant decrease in right arterial pressure was also detected at 30 min post dose of RS-25,259-197 at 1 ug/kg, however this change was small (1 mm Hg) and isolated and was not considered to represent a physiologically relevant event. Ondansetron produced no significant hemodynamic effects at any dose tested except for a 1 ml/min increase in systolic volume at 60 min post administration of the high dose (1000 mg/kg). This latter finding with Ondansetron also was not considered to be physiologically relevant, since these changes were isolated and not associated with any changes in either HR or CO at this time point.

Effects of RS-25,259 on lead II EKG parameters did not appear in the results or discussion sections of this report. However, in response to a request by the Division for submission of EKG data from this study (in a letter dated July 15,1992), the Sponsor, in Amendment # 001, dated July 31, 1992, submitted tables containing QT and QTc, (corrected for heart rate) interval data derived from this study. Summary data showed that rising dose administration of RS-25,259 (1-1000 ug/kg) had no significant effects on QT or QTc intervals, compared to control values. However, an increase in the QT interval (0.05 sec average increase from pre-dose values) at the 1000 ug/kg dose was evident in 5/6 dogs tested. Three of six dogs tested at the 1000 mg/kg dose had QT intervals of 0.27-0.29 sec, the normal range for QT intervals in dogs is 0.15-0.25 sec. Prolonged QT intervals can occur under conditions of hypocalcemia, hypokalemia, quinidine toxicity ethylene glycol poisoning, strenuous exercise, hypothermia and central nervous system disorders. The prologation of QT-wave was seen in very high and toxic dose. The overall means in the study were not significantly different from control values, therefore not of any importance.

In conclusion, intravenously administered RS-25,259-197 (1, 10, 100, and 1000 ug/kg) in dogs produced a small decrease in heart rate at the 1000 ug/kg dose, but had no effect on hemodynamic function. An increase in QT interval at the 1000 ug/kg dose was also seen in 3/6 dogs tested. However, overall means were not significantly different from control values at this dose. Data on the effects of RS-25,259-197 on other EKG intervals (PR and QRS), amplitudes (P, Q, R, and T-waves) as well as representative EKG tracings at each dose and times were not provided. These data are currently needed in order to fully assess any effects, which RS-25,259 might have on cardiac conduction in this study.

2. Effects on Renal Functions: The Effect of orally administered RS-25,259-197 (0.001-1 mg/kg) on renal function in conscious saline loaded rats (8/group). RS-25,259-197 (0.001 and 1 mg/kg, p.o.) significantly decreased urinary sodium excretion by ≥ 62% at one hour after saline loading compared to controls. The urinary sodium/potassium ratio was also significantly decreased (56%) at 1 hr in the 0.001 group as a result of the decrease in sodium excretion for this time period. Chloride excretion was also reduced (≥ 71%) in the 0.001, 0.01 and the 0.1 mg/kg groups at the 1 hr time point. However, urinary volume, osmolality, potassium, and creatinine excretion as remained unchanged at all doses tested. RS-25,259-197 also had no significant effect on GFR or free water clearance. By comparison, hydrochlorothiazide (10 mg/kg, p.o.), a standard diuretic produced expected increases in urinary volume, sodium, potassium and chloride excretion, as well as free water clearance. The changes induced by hydrochlorothiazide occurred without significant alterations in either urinary creatinine clearance or GFR.

In conclusion, RS-25,259-197 produced significant, but non dose-dependent, reductions in urinary sodium and chloride excretion in saline loaded rats. Since these changes were variable and occurred in the absence of changes in urinary volume, osmolality, creatinine clearance, GFR and free water clearance, they most likely are of little physiological concern.

B. Gastrointestinal Pharmacology: The effects of RS-25,259-197 on gastric emptying in rats were evaluated. RS-25,259-197 (0.001- 1.0 mg/kg i.p. administered 30 min prior to test meal) had no significant effect on gastric emptying of a test meal in rats compared to vehicle control. In contrast, the positive control, metoclopramide (10 mg/kg i.p.) significantly enhanced gastric emptying.

C. CNS Pharmacology:

1. Effects on Gross Behavior in Mice: Studies on the effects of RS-25,259-197 on the gross behavior in mice showed that i.p. administration of RS-25,259-197 at concentrations of 0.003-10 mg/kg induced a mild increase in locomotion (50%). In contrast a reference compound. chlorpromazine (30 mg/kg i.p.) induced significant decreases in body temperature, responsiveness and caused marked CNS depression.

2. Anxiolytic Effects in Mice: Black:white Box Model of Anxiety: The effects of RS-25,259-197 (1.0 ng/kg, 1.0 ug/kg, and 1.0 mg/kg, i.p.) on mouse behavior in the mouse two compartment exploratory black:white box model of anxiety were evaluated by [redacted] (Study I) and Syntex (Study 2). Study 1 showed that RS-25,259-197 did not influence behavioral responses in mice and therefore, at the doses used, did not possess anxiolytic activity in mice. Study 2 which tested RS-25,259-197 at doses of 0.0003, 0.003 and 3000 ug/kg, p.o. showed that RS-25,259-197 produced significantly decreased time in the dark but was without effect on other parameters such as shuttle activity, latency, and the percentage of activity in the dark. These latter findings are suggestive of weak anxiolytic properties for RS-25,259-197. The differences between the two studies could be represent differences in the strains of mice used or differences in the route of administration.

3. Effects on Feeding Behavior in Rats: Studies which evaluated the role of 5-HT_3 receptors in feeding behavior in the rat showed that oral doses (1-300 ug/kg) RS-25,259-197, granisetron and ondansetron were without affect on feeding behavior in rats. And although serotonin has been implicated in the regulation of food intake, these studies suggest the lack of involvement of 5-HT3 receptors in this behavior. The positive controls, D-amphetamine (3 and 6 mg/kg) and Fenfluramine (1 and 10 mg/kg) decreased feeding in rats. (Report # AT 5452) (Vol. 1.3, pp 19-23)

4. Proconvulsant Activity in Mice:

Palonosetron at single doses of 1, 30 and 60 mg/kg did not induce convulsions in animals. These animals were administered Metrazol or electroshock after 45 min of the administration of the compounds. The palonosetron pre-treatment did not induce convulsions in animals at any of these doses. The animals treated with amphetamine (30 mg/kg, po) and bemegride (40 mg/kg, po) showed convulsions against metrazol or electroshock. Thus these drugs were not proconvulsants.

In conclusion, the CNS effects of RS-25,259-197 appear to be minimal, with concentrations up to 10 mg/kg only producing mild increases in locomotion (50%). Conclusions with regard to possible anxiolytic activity of RS-25,259-197 cannot currently be drawn, due to conflicting results from two separate studies in mice, one, which showed no effect, and the other which showed only weak anxiolytic effects. Finally, studies, which showed no effect of RS-25,259-197 on feeding behavior, suggested the lack of involvement of 5-HT_3 receptor subtypes in this behavior in rats.

D. Interaction of RS-25259-197 with Tumor Models

1. Interaction of RS-25259-197 with Cisplatin Chemotherapy in Murine P338 Leukemia Model (Study No. AT 6777)
Methods: The effects of RS-25259-197 on the anti-tumorigenic action of Cisplatin were evaluated using a murine P388 leukemia model. Briefly, eleven groups of female $CD2F_1$ mice (10 mice/ groups) were implanted intraperitoneally with 10^6 murine P388 leukemia cells and administered RS-25259-197 (p.o.; 1, 10, and 30 mg/kg RS-25259) and/or Cisplatin (i.p. 8 and 5.3 mg/kg) alone or in combination on days 2, 5, and 9 following tumor cell implantation. In the combination groups, RS-25259-197 given 30 min prior to Cisplatin. A control group of 20 mice received both vehicles only. The effects of RS-25259-197 on the anti-tumorigenic activity of Cisplatin were assessed in terms of its effect on the percent increase of median life span of the tumor implanted mice (%ILS) and net $\log_{10}$ cell kill. The net $\log_{10}$ cell kill was calculated using tumor doubling time (T_d) determined from an internal tumor titration consisting of implants from serial 10-fold dilutions according to the following formula:

$$\text{Net } \log_{10} \text{ cell kill} = \frac{(T-C)-(\text{duration of treatment in days})}{3.32 \times T_d}$$

Where (T-C) is the difference in the median day of death between the treated (T) and the control (C) groups and T_d is the mean tumor doubling time (days) calculated from a log linear least-squares fit of the implant sizes of the titration group and the corresponding median days of death.

Results: Cisplatin alone was effective against the leukemia producing a dose-dependent increased %ILS (200 and 240 %) and reduced tumor burden of 6.7-$\log_{10}$ at both the 8 and the 5.3 mg/kg doses, respectively. RS-25259-197 alone was ineffective although the lowest dose 1 mg/kg was associated with a 50% increased %ILS (this may have been due to an error in Cisplatin dosing in 5 of the 10 animals in this group). Finally, pretreatment with oral RS-25259-197 had no effect on the antitumor activity of Cisplatin against the leukemia.

In conclusion, RS-25259 given orally at doses of 1, 10, or 30 mg/kg/dose (measured as the free base) had no effect on the antitumor activity of Cisplatin in the murine P388 leukemia model.

2. **In Vivo anti-tumorigenic activity of doxorubicin administered with palonosetron to mice bearing an i.p. lymphocytic DBA/2 lymphocytic leukemia L1210 cells**: A dose of 10 mg/kg, i.p. palonosetron in mice bearing lymphocytic leukemia L1210 did not interfere with tumor pathology in positive control animals. It did not affect the anti-tumor activity of 10 mg/kg doxorubicin or increased the lethality. The animals treated with palonosetron showed increased survival time (day 15 against day 11 of the negative control). The treatment did not affect the anti-tumor activity of doxorubicin.

3. In mice bearing i.m. Lewis Lung carcinoma, palonosetron neither interfered with the tumor pathology nor produced alteration in the antitumor activity of Cisplatin. The survival rate in animals treated with 10 mg/kg, i.p. palonosetron was similar to the negative control; i.e. 135 for the group administered the combination of Cisplatin and palonosetron in comparison to 137% for the group receiving Cisplatin.

4. **Interaction of Cyclophosphamide with Palonosetron to Mice Bearing a SC Melanotic Melanoma B16**: From C57BL/6 female mice [redacted] treated with MMB16 tumor cells, tumor cell homogenate was collected on day 16. These cells homogenate (0.5 ml) was implanted subcutaneously in BDF1 test mice. Palonosetron at 10 mg/kg/day, ip did not influence the pathology of the tumor and the anti-tumor activity of cyclophosphamide (as the T/CX100 survival index was never <85%) in these mice during the treatment and subsequent observation period of 15 days. In NCI protocol, the acceptable median negative control survival time is between 14 to 22 days.

1. **Palonosetron Hydrochloride: EKG measurements from 28-Day intravenous toxicity study in juvenile dogs**: (Study #PALO-99-22; [redacted] 1063/17)

In this study the effects of palonosetron treatment on cardiac conductivity of male and female juvenile dogs included in 28-Day intravenous toxicity study were determined. During the study, palonosetron was administered in 4 groups of neonate dogs (4/sex/group) at the intravenous doses of 0, 1, 3 and 6 mg/kg/day. EKG tracings of all study animals were measured 2 hr

postdosing on week 4. Sponsor in the present submission had done hand measurements (on January 12, 2001) of mean PQ interval in msec from lead II EKG (the tracing used for heart rate measurement). The other EKG parameters of cardiac conduction could not be recorded because of high heart rate in juvenile dogs. The data were compared with the control group animals.

The mean PQ duration in study male neonate dogs were 59±8, 61±10, 59±7 and 63±8 ms in 0 (control), 1, 3 and 6 mg/kg/day Palonosetron treatment groups. In female neonate dogs, mean PQ intervals were 60±10, 61±7, 58±9 and 62±8 ms in 0 (control), 1, 3 and 6 mg/kg/day Palonosetron treatment groups. There was no statistically significant difference between the control and the treatment group animals. Sponsor reiterated that the other EKG parameters from the tracings could not be measured because of high heart rate in juvenile dogs.

Cardiovascular system:

The effects of palonosetron on hERG tail currents recorded from mammalian cells, HEK293 cells, transfected with hERG (cloned human *ether-a-go-go*) cDNA were assessed using whole-cell-patch-clamp technique. The currents expressed by hERG are responsible for delayed rectifier potassium current (I_{kr}). The results indicated that palonosetron (0.03, 0.3, 3, and 30 µM or 10, 100, 1000, 10000 ng/ml) significantly inhibited hERG tail currents in a concentration dependent manner with IC_{50} of 2.04 µM. At the lowest concentration of 0.03 µM (10 ng/ml), the hERG tail currents were inhibited by ~17%. This concentration (10 ng/ml) is close to the maximum plasma level (7.17 ng/ml) of palonosetron given intravenously at 0.75 mg to subjects in clinical trials. The results were summarized in table scanned below (Table 1 on page 150 of Amendment #120).

Table 1 Concentration-Response Relationship for Palonosetron on HERG Tail Currents

Concentration (μM)	Tail Current (% Control)
0.03	82.7 ± 2.1
0.3	60.0 ± 4.1 **
3	31.4 ± 2.8 **
30	6.1 ± 1.3 **

** $P < 0.01$; ANOVA followed by Dunnett's test.
Data are given as mean ± s.e. mean for n=4 cells per group before correction for mean vehicle rundown.

In another similar study, the effects of palonosetron on hERG tail currents were compared with ondansetron. The results indicated that palonosetron dose-dependently inhibited hERG tail currents with IC_{50} of 1.9 μM. The results were summarized in a table on page 7 of Amendment #120. This table is attached below.

Concentration (μM)	Mean fraction of hERG current ($I_{Test}/I_{Control}$)
0.00	0.94
0.20	0.84
0.70	0.75
2.00	0.44
3.00	0.44
10.00	0.13

Ondansetron also significantly inhibited the hERG currents in a dose dependent manner with an IC_{50} (1.8 μM), similar to that of palonosetron.

The effects of palonosetron on hHNa currents (fast sodium current) recorded from HEK293 cells transfected with hHNa cDNA were assessed using whole-cell-patch-clamp technique. The results indicated that palonosetron (1, 3, 10, and 100 μM) significantly inhibited hHNa current in a dose dependent manner with IC_{50} of 6.5 μM.

The *in vitro* electrophysiological effects of palonosetron were assessed in canine Purkinje fibers. Free running Purkinje fibers were isolated from left ventricle of Beagle dogs. The following action potential parameters were recorded at two stimulation rates (60 or 12 pulses per minute): resting potential (RP), amplitude (APA), maximal rate of depolarization (Vmax), and duration of action potential (APD_{50}, APD_{70}, and APD_{90}). The results indicated that at 60 pulses per minute palonosetron prolonged the action potential duration at 0.3 and 3 μM (100 and 1000 ng/ml) in a

dose dependent manner and this effect was more obvious at low rate (12 pulses per minute). However, at higher concentration of 30 μM (10000 ng/ml), palonosetron significantly decreased the action potential duration. The prolongation of action potential duration is consistent with the results that palonosetron inhibited the hERG tail currents in the whole-cell-patch-clamp studies in HEK293 cells transfected with hERG cDNA. The results were summarized in Tables 1 and 2 on pages 269 and 270 of Amendment #120. These tables are attached below.

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EFFECT OF PALONOSETRON ON CARDIAC ACTION POTENTIAL IN ISOLATED CANINE PURKINJE FIBRES UNDER NORMAL STIMULATION RATE (60 ppm)

TABLE I

Treatment		APA (mV)	RP (mV)	Vmax (V/s)	APD50 (ms)	APD70 (ms)	APD90 (ms)
Predose values (Tyrode)	Mean	118	-92	496	247	290	327
	SEM	2	1	57	10	7	8
	N	6	6	6	6	6	6
Tyrode	Mean	7	-1	3	-6	-2	0
	SEM	1	1	23	5	2	2
	N	6	6	6	6	6	6
Palonosetron 10 ng/mL	Mean	10	-1	10	-8	-2	2
	SEM	2	1	31	6	3	3
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
Palonosetron 100 ng/mL	Mean	0	-1	-2	-9	8	13
	SEM	2	1	26	9	4	5
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
Palonosetron 1000 ng/mL	Mean	8	-1	-39	-38	13	36
	SEM	1	1	41	14	6	5
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	*
Palonosetron 10000 ng/mL	Mean	-5	4	-231	-183	-135	-72
	SEM	2	1	53	12	10	16
	N	6	6	6	6	6	6
	P	**	NS	**	**	**	**

Predose values: control period with Tyrode.
ppm: pulses per minute.
APA: amplitude of the action potential.
RP: resting potential.
Vmax: maximal rate of depolarisation.
APD50: duration of the action potential to 50% of repolarisation.
APD70: duration of the action potential to 70% of repolarisation.
APD90: duration of the action potential to 90% of repolarisation.
Mean: mean value.
SEM: Standard Error of the Mean.
N: number of preparations.
Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion).
NS: $P > 0.05$, *: $P \leq 0.05$, **$P \leq 0.01$ when compared to the vehicle control period (Tyrode): analysis of variance with NEWMAN KEULS's test if $P \leq 0.05$.
Note: values of APA, RP, Vmax, APD50, APD70 and APD90 were analysed 25 minutes after starting each infusion period.

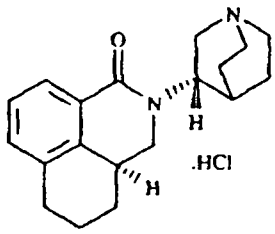
EFFECT OF PALONOSETRON ON CARDIAC ACTION POTENTIAL IN ISOLATED CANINE PURKINJE FIBRES UNDER LOW STIMULATION RATE (12 ppm)

TABLE 2

Treatment		APA (mV)	RP (mV)	Vmax (V/s)	APD50 (ms)	APD70 (ms)	APD90 (ms)
Predose values (Tyrode)	Mean	115	-88	442	287	352	395
	SEM	2	2	46	18	15	15
	N	6	6	6	6	6	6
Tyrode	Mean	2	1	58	-15	-13	-7
	SEM	2	1	51	3	3	3
	N	6	6	6	6	6	6
Palonosetron 10 ng/mL	Mean	5	0	36	-20	-9	-1
	SEM	2	1	32	14	5	5
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
Palonosetron 100 ng/mL	Mean	3	4	-13	-5	16	24
	SEM	5	1	29	10	3	5
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
Palonosetron 1000 ng/mL	Mean	3	1	8	1	84	125
	SEM	4	2	24	24	18	12
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	**	**
Palonosetron 10000 ng/mL	Mean	-17	9	-168	-262	-175	-75
	SEM	4	2	25	23	16	24
	N	6	6	6	6	6	6
	P	**	**	**	**	**	**

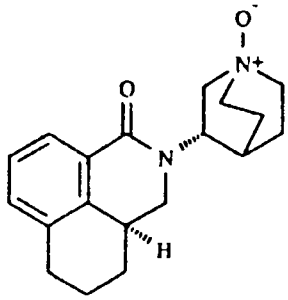
Predose values: control period with Tyrode.
ppm: pulses per minute.
APA: amplitude of the action potential
RP: resting potential.
Vmax: maximal rate of depolarisation.
APD50: duration of the action potential to 50% of repolarisation.
APD70: duration of the action potential to 70% of repolarisation.
APD90: duration of the action potential to 90% of repolarisation.
Mean: mean value.
SEM: Standard Error of the Mean.
N: number of preparations.
Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion).
NS: $P > 0.05$, **$P \leq 0.01$ when compared to the vehicle control period (Tyrode): analysis of variance with NEWMAN KEULS's test if $P \leq 0.05$.
Note: values of APA, RP, Vmax, APD50, APD70 and APD90 were analysed 30 minutes after starting each infusion period.

It is also noted that palonosetron inhibited maximal rate of depolarization (Vmax) at 3 and 30 µM. This is consistent with the results that palonosetron inhibited hHNa current in the whole-cell-patch-clamp study in HEK293 cells transfected with hHNa cDNA.
The effects of RS-17825-007, a main metabolite (M9) of palonosetron, and ——— ——— on the action potentials were also studied in this model. M9 is N-oxide metabolite of palonosetron. The structures of palonosetron and M9 are attached below.



Helsinn code: 08-PALO

Figure 2: structure of M9 (RS-17825-007, 08-PALO-D1)



The structure of is attached below.

Helsinn code:

(Syntex lab code:

The results indicated that the metabolite RS-17825-007 had no obvious effects on the action potential at concentrations tested (10, 100, 1000, and 10000 ng/ml). However, the , prolonged action potential duration and decreased the maximal rate of

depolarization. The results were summarized in Tables 5 and 6 on pages 275 and 276 of Amendment #120. These tables are attached below.

EFFECT OF . ON CARDIAC ACTION POTENTIAL IN ISOLATED CANINE PURKINJE FIBRES UNDER NORMAL STIMULATION RATE (60 ppm)

TABLE 5

Treatment		APA (mV)	RP (mV)	Vmax (V/s)	APD50 (ms)	APD70 (ms)	APD90 (ms)
Predose	Mean	118	-90	441	223	266	307
values	SEM	3	1	54	15	14	15
(Tyrode)	N	6	6	6	6	6	6
Tyrode	Mean	3	-2	72	-5	2	3
	SEM	3	1	55	2	2	2
	N	6	6	6	6	6	6
	Mean	-1	-2	10	-6	-2	-2
10 ng/mL	SEM	4	1	49	4	3	3
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
	Mean	-4	0	-56	-4	6	6
100 ng/mL	SEM	4	1	49	7	1	2
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
	Mean	4	-3	13	-15	16	31
1000 ng/mL	SEM	4	1	34	8	4	4
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	*
	Mean	-15	5	-159	-147	-84	-3
10000 ng/mL	SEM	10	4	25	34	23	15
	N	6	6	6	6	6	6
	P	NS	NS	**	**	**	NS

Predose values: control period with Tyrode.
ppm: pulses per minute.
APA: amplitude of the action potential.
RP: resting potential.
Vmax: maximal rate of depolarisation.
APD50: duration of the action potential to 50% of repolarisation.
APD70: duration of the action potential to 70% of repolarisation.
APD90: duration of the action potential to 90% of repolarisation.
Mean: mean value.
SEM: Standard Error of the Mean.
N: number of preparations.
Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion).
NS: $P > 0.05$, *: $P \leq 0.05$, **$P \leq 0.01$ when compared to the vehicle control period (Tyrode): analysis of variance with NEWMAN KEULS's test if $P \leq 0.05$.
Note: values of APA, RP, Vmax, APD50, APD70 and APD90 were analysed 25 minutes after starting each infusion period.

EFFECT OF ON CARDIAC ACTION POTENTIAL IN ISOLATED CANINE PURKINJE FIBRES UNDER LOW STIMULATION RATE (12 ppm)

TABLE 6

Treatment		APA (mV)	RP (mV)	Vmax (V/s)	APD50 (ms)	APD70 (ms)	APD90 (ms)
Predose	Mean	109	-83	420	247	316	361
values	SEM	2	2	52	27	25	27
(Tyrode)	N	6	6	6	6	6	6
Tyrode	Mean	2	0	48	-10	-1	5
	SEM	3	1	53	7	5	3
	N	6	6	6	6	6	6
10 ng/mL	Mean	0	-1	-29	5	4	10
	SEM	6	2	46	8	7	7
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
100 ng/mL	Mean	0	1	-33	-14	8	15
	SEM	4	2	55	17	7	7
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
1000 ng/mL	Mean	5	-1	-7	17	89	126
	SEM	3	2	63	21	18	27
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	**	**
10000 ng/mL	Mean	-14	2	-101	-151	-83	5
	SEM	6	2	60	36	30	19
	N	6	6	6	6	6	6
	P	NS	NS	NS	**	**	NS

Predose values: control period with Tyrode.
ppm: pulses per minute.
APA: amplitude of the action potential.
RP: resting potential.
Vmax: maximal rate of depolarisation.
APD50: duration of the action potential to 50% of repolarisation.
APD70: duration of the action potential to 70% of repolarisation.
APD90: duration of the action potential to 90% of repolarisation.
Mean: mean value.
SEM: Standard Error of the Mean.
N: number of preparations.
Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion).
NS: $P > 0.05$, **$P \leq 0.01$ when compared to the vehicle control period (Tyrode): analysis of variance with NEWMAN KEULS's test if $P \leq 0.05$.
Note: values of APA, RP, Vmax, APD50, APD70 and APD90 were analysed 30 minutes after starting each infusion period.

In this study, the effects of cisapride on action potential were also studied as a positive control. The results indicated that cisapride significantly prolonged the action potential duration by ~10-16% at 3 x 10^{-7} M. The results were summarized in Tables 7 and 8 on pages 278 and 279 of Amendment #120. These tables are attached below.

EFFECT OF CISAPRIDE ON CARDIAC ACTION POTENTIAL IN ISOLATED CANINE PURKINJE FIBRES UNDER NORMAL STIMULATION RATE (60 ppm)

TABLE 7

Treatment		APA (mV)	RP (mV)	Vmax (V/s)	APD50 (ms)	APD70 (ms)	APD90 (ms)
Predose values (Tyrode)	Mean	123	-91	489	223	267	308
	SEM	3	1	64	12	10	9
	N	6	6	6	6	6	6
Tyrode	Mean	-4	-1	-50	-3	3	5
	SEM	3	1	47	5	4	3
	N	6	6	6	6	6	6
Cisapride 3.10^{-7}M	Mean	-5	0	-36	6	27	39
	SEM	2	1	23	9	5	6
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	**	**

Predose values: control period with Tyrode.
ppm: pulses per minute.
APA: amplitude of the action potential.
RP: resting potential.
Vmax: maximal rate of depolarisation.
APD50: duration of the action potential to 50% of repolarisation.
APD70: duration of the action potential to 70% of repolarisation.
APD90: duration of the action potential to 90% of repolarisation.
Mean: mean value.
SEM: Standard Error of the Mean
N: number of preparations.
Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion).
NS: $P > 0.05$, **$P \leq 0.01$ when compared to the vehicle control period (Tyrode): analysis of variance with NEWMAN KEULS's test if $P \leq 0.05$.
Note: values of APA, RP, Vmax, APD50, APD70 and APD90 were analysed 25 minutes after starting each infusion period.

EFFECT OF CISAPRIDE ON CARDIAC ACTION POTENTIAL IN ISOLATED CANINE PURKINJE FIBRES UNDER LOW STIMULATION RATE (12 ppm)

TABLE 8

Treatment		APA (mV)	RP (mV)	Vmax (V/s)	APD50 (ms)	APD70 (ms)	APD90 (ms)
Predose values (Tyrode)	Mean	119	-88	431	245	305	359
	SEM	2	2	52	17	12	14
	N	6	6	6	6	6	6
Tyrode	Mean	-14	3	-81	-25	-9	8
	SEM	2	2	47	20	14	6
	N	6	6	6	6	6	6
Cisapride 3.10^{-7}M	Mean	-8	3	-27	-9	37	59
	SEM	4	2	33	14	14	14
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	*	**

Predose values: *control period with* Tyrode.
ppm: pulses per minute.
APA: amplitude of the action potential.
RP. resting potential.
Vmax: *maximal* rate of depolarisation.
APD50: duration of the action potential to 50% of repolarisation.
APD70: duration of the action potential to 70% of repolarisation.
APD90: duration of the action potential to 90% of repolarisation.
Mean: mean value.
SEM. Standard Error of the Mean.
N: number of preparations.
Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion).
NS. $P > 0.05$, *: $P \leq 0.05$, **$P \leq 0.01$ when compared to the vehicle control period (Tyrode): analysis of variance with NEWMAN KEULS's test if $P \leq 0.05$.
Note: values of APA. RP. Vmax. APD50. APD70 and APD90 were analysed 30 minutes after starting each infusion period.

To further investigate the cardiovascular effects of palonosetron, an *in vivo* study on blood pressure, heart rate, and EKG was conducted in conscious dogs. Three male and female dogs were implanted with telemetric transmitters, electrodes, and sensor catheter. These dogs were given palonosetron intravenously at 0.01, 0.1, and 1 mg/kg. Mean systolic and diastolic blood pressure, heart rate, and EKG were measured for 15 seconds at intervals of 15 minutes beginning 24 hours before and until 72 hours after dosing. No observed effects on these parameters were noted at doses up to the high dose of 1 mg/kg (20 mg/m^2). This dose is approximately 36 folds of clinical dose of 0.75 mg or 0.015 mg/kg if 50 kg body weight is assumed (0.56 mg/m^2).

The effects of the metabolite M9 and [redacted] on blood pressure, heart rate, and EKG were also studied in conscious dogs. Intravenous administration of M9 at doses of 0.001, 0.01, and 0.1 mg/kg had no effects on blood pressure, heart rate, and EKG. In a clinical pharmacokinetic study, about 12.9% of the parent dose were excreted as M9 in urine. The M9 dose of 0.1 mg/kg (2 mg/m^2) is approximately 27 folds of the M9 level following the clinical dose of palonosetron of 0.75 mg (0.56 mg/m^2 x 13% = 0.073 mg/m^2). The [redacted]

—— had no effects on blood pressure, heart rate, and EKG at doses of 0.0001, 0.001, and 0.01 mg/kg. The high dose of 0.01 mg/kg (0.2 mg/m^2) is approximately 119 folds of this intermediate present in palonosetron following clinical dose of 0.75 mg (—— —— : is present in palonosetron).

The effects of palonosetron on intra-atrial, intra-ventricular, and atrioventricular conduction were studied in anesthetized dogs. A pair of stimulating and two pairs of recording electrodes were placed in right atrium and right ventricle of the Beagle dogs anesthetized with sodium pentobarbitone. The times of the spontaneous or electrically-induced impulses between the points of electrodes in right atrium and ventricle were recorded following intravenous administration of palonosetron at cumulative doses of 1, 3, 10, 30, 100, 300, and 1000 µg/kg. Systolic and diastolic blood pressure, heart rate, and EKG were also recorded. The results indicated that at the doses tested palonosetron did not produce any obvious effects on the conduction and EKG but significantly decreased the blood pressures at doses of 300 and 1000 µg/kg. The decrease in blood pressures was observed immediately after dosing and returned to the pre-dose levels 10 minutes after dosing. At dose of 1000 µg/kg, diastolic blood pressure was decreased from pre-dose level of 69.5 mmHg to 35 mmHg.

2. **Evaluation of Effects on Cardiac Action Potential in Isolated Rabbit Purkinje Fibers** (Study #2002056PELM/PALO-01-33).

Methods: The effects of 0.03 to 30 uM concentrations palonosetron on action potential were assessed on the isolated rabbit Purkinje fiber preparation. The effects were compared with a positive control, cisapride. The lowest concentration of the preparation used was based on the plasma concentration achieved after a clinical dose of 10 ug/kg, iv and the highest dose was the dose, which mimics the overdose. The resting potential, amplitude of action potential, maximal rate of depolarization, duration of action potential (APD) and to produce early after depolarization (EAD)-induced triggered activity were estimated on the Purkinje fiber preparations. The duration of action potential was measured at 50, 70 and 90% repolarization (APD_{50}, APD_{70}, and APD_{90}) after 25 min after the start of the infusion. Cisapride was used at a concentration of 3×10^{-7} M as a reference control.

Results: Palonosetron at concentrations of 0.3uM to 3 uM produced dose-dependent increases in action potential duration under low and normal stimulation rates and these effects were only statistically significant at 3 uM or higher concentrations. These effects were more pronounced with a stimulation rate of 12 pulses/min (low) than 60 pulses/min (normal). Both palonosetron and cisapride at concentrations of 0.3 uM produced decreases of action potential duration (APD_{50}, APD_{70}, and APD_{90}). The early after depolarization was reported in 2 preparations at 30 uM under low stimulation rate suggesting its effect on the sodium channels. Increase of action potential duration observed at 3 uM was not associated with significant effect of action potential amplitude and maximal rate of depolarization (V_{max}) and resting potential. The reference control, cisapride at 3×10^{-7} M produced the previously observed increase in action potential duration at 50, 70 and 90% reploarization, suggesting that the validity of the model. Thus palonosetron increased the action potential duration at higher concentrations.

Table 2.1: Effect of PALONOSETRON.HCl on cardiac action potential in isolated rabbit Purkinje fibres under normal stimulation (60 ppm) (mean values)

TREATMENT		APA (mV)	RP (mV)	Vmax (V/s)	APD50 (ms)	APD70 (ms)	APD90 (ms)
Predose	Mean	121	-91	257	217	258	288
values	SEM	4	1	21	15	17	18
(Tyrode)	N	6	6	6	6	6	6
0.1% sterile	Mean	1	0	1	7	5	3
water	SEM	2	1	6	5	3	4
in Tyrode	N	6	6	6	6	6	6
PALONOSETRON.HCl	Mean	4	-1	0	15	11	9
0.03 μM	SEM	3	1	7	5	4	4
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
PALONOSETRON.HCl	Mean	4	0	4	22	20	22
0.3 μM	SEM	3	1	6	4	3	3
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	*
PALONOSETRON.HCl	Mean	0	0	10	47	66	80
3 μM	SEM	4	1	17	11	9	8
	N	6	6	6	6	6	6
	P	NS	NS	NS	**	**	**
PALONOSETRON.HCl	Mean	-11	4	-98	27	131	190
30 μM	SEM	7	1	21	47	43	37
	N	6	6	6	6	6	6
	P	NS	NS	**	NS	*	**
Tyrode	Mean	1	1	-13	74	193	233
	SEM	3	1	14	31	33	36
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	**	**
	Threshold	17	4	54	102	92	88

Electronic signature : table created by sra on 28-FEV-2002 at 09.24.04.210
Predose values: control period with Tyrode
ppm: pulses per minute.
APA: action potential amplitude.
RP: resting potential
Vmax: maximal rate of depolarisation.
APD50: action potential duration at 50% of repolarisation
APD70: action potential duration at 70% of repolarisation.
APD90: action potential duration at 90% of repolarisation.
Mean: mean value.
SEM: Standard Error of the Mean.
N: number of preparations.
Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion)
NS:$P>0.05$,*$P<0.05$,**$P\leq 0.01$, when compared to the vehicle period (Tyrode): analysis of variance with NEWMAN KEULS test if $P\leq 0.05$.
Note: values of APA, RP, Vmax, APD50, APD70 and APD90 were analysed 25 minutes after starting each infusion period.
Threshold : smallest difference being statistically significant ($P \geq 0.05$) calculated from Newman-Keuls's test.

Proarrhythmic Activity of Palonosetron and Ondansetron After Intravenous Administration in Anasthetized Rabbits: (PALO-01-35)

In 2 groups of 6 male anesthetized New Zealand white rabbits (2.4 to 3.35 kg), 10 mg/kg alonosetron or 10 mg/kg ondansetron was administered as an infusion (1mg/kg/min for 10 min). A decrease in blood pressure (systolic max 56% at 10 min and diastolic of max 64% at min) and

Table 2.2: Effect of PALONOSETRON.HCl on cardiac action potential in isolated rabbit Purkinje fibres under low stimulation rate (12 ppm) (mean values)

TREATMENT		APA (mV)	RP (mV)	Vmax (V/s)	APD50 (ms)	APD70 (ms)	APD90 (ms)
Predose	Mean	118	-88	270	248	309	346
values	SEM	3	1	25	20	32	33
(Tyrode)	N	6	6	6	6	6	6
0.1% sterile	Mean	-2	2	-19	8	5	-1
water	SEM	3	2	12	8	6	7
in Tyrode	N	6	6	6	6	6	6
PALONOSETRON.HCl	Mean	2	1	-10	16	12	12
0.03 µM	SEM	3	1	10	7	8	8
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
PALONOSETRON.HCl	Mean	3	1	-3	49	54	60
0.3 µM	SEM	1	1	15	10	14	15
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
PALONOSETRON.HCl	Mean	3	1	-9	313	364	396
3 µM	SEM	1	1	12	69	83	85
	N	6	6	6	6	6	6
	P	NS	NS	NS	**	**	**
PALONOSETRON.HCl	Mean	-4	2	-58	729	1472	1579
30 µM	SEM	5	1	18	343	295	289
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	**	**
Tyrode	Mean	4	0	-9	1220	1890	1949
	SEM	3	1	22	453	308	310
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	**	**
	Threshold	12	6	66	1005	729	725

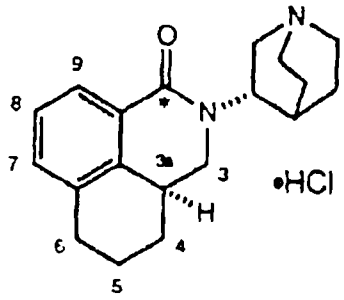
Electronic signature : table created by sra on 28-FEV-2002 at 09:38:45.620
Predose values: control period with Tyrode.
ppm: pulses per minute.
APA: action potential amplitude
RP: resting potential.
Vmax: maximal rate of depolarisation.
APD50: action potential duration at 50% of repolarisation.
APD70: action potential duration at 70% of repolarisation
APD90: action potential duration at 90% of repolarisation.
Mean: mean value.
SEM: Standard Error of the Mean.
N: number of preparations.
Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion).
NS:P>0.05,**P≤0.01. when compared to the vehicle period (Tyrode): analysis of variance with NEWMAN KEULS test if P<0.05.
Note: values of APA, RP, Vmax, APD50, APD70 and APD90 were analysed 25 minutes after starting each infusion period.
Threshold : smallest difference being statistically significant ($P \leq 0.05$) calculated from Newman-Keuls's test.

decrease in RR (25% decrease of the predose) interval (a measure of heart rate), QT interval (19% of the mean control) and QTc (32% of the predose mean values) was reported in 5 of the 6 rabbits within 1 to 20 min of administration. Three out of 6 animals showed cardiac abnormalities of Q, R, S and T waves associated with U-waves. Ondanasetron at 10 mg/kg dose (1 mg/kg/min for 10 min), iv produced a reduced RR interval. An increase in QT, QTc was also seen in the animals in 3 of 6 rabbits. Occasional ventricular ectopic beat between 9 and 16 min of the administration was also reported. Palonosetron produced cardiac abnormalities of QTc and ventricular tachycardia in these dogs. The polymorphic tachycardia representing the Torse de pointes was not seen in any of the study animals.

The following pharmacokinetics and toxicity studies on RS-25259-197 were submitted with the present application and are reviewed below:

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME):

^{14}C-RS-25259-197 was used in ADME studies of the rat and the dog. The site of carbon labeling is shown in the following structure:



RS-25259-197

(a) site of ^{14}C labeling •-Site of ^{3}H labeling

In Vitro:

Binding of RS-25259-197 to plasma protein in vitro: (Report No. CL 6204)

Methods: The in vitro binding of [^{14}C]RS-25259-197 to plasma proteins from Sprague Dawley rats, beagle dogs, and humans was studied using equilibrium dialysis (24 hr at 37°C).

Results: Results showed that RS-25259-197 only moderately bound to rat plasma proteins, with binding decreasing from 49 to 46% over a concentration range of 255 to 2011 ng/ml (mean binding = 48%). Greater binding was observed in dog plasma proteins, with binding decreasing from 68.5 to 63.4 over a concentration range of 75 to 600 ng/ml (mean binding = 66.2). Finally binding in human plasma proteins was fairly constant (mean = 61.8%) over a concentration range of 5 to 412 ng/ml. Thus, protein binding in plasma from rat, dog and human was nonspecific and non-saturable over the concentration range studied.

In Vivo:

Pharmacokinetics of RS-25259-197 in Male Rats Following Single Dose Oral Administration: (Report No. AT 6303)

Methods: Male Sprague Dawley rats (3/group/time point) were administered [^{14}C]RS-25259-197 by gavage at single doses of 0.5, 60, and 180 mg/kg (calculated dose equivalents of 0.432, 53.6 and 160 mg/kg free base). The 0.5 mg/kg dose, was a mixture of [^{14}C]RS-25259-197 (hydrochloride salt, lot no. 15188-GTH-106) and unlabelled RS-25259-197 (hydrochloride salt, lot No. 13977-86, A9012010) dissolved in pH 5, 10 mM sodium acetate buffer, made isotonic with normal sodium chloride. The 60 and 180 mg/kg RS-25259-197 doses (hydrochloride salt, lot no. PA 15303-51 A9201007) were prepared in pH 7.4, 0.1 M sodium phosphate buffer. Doses were delivered in volumes of 2.5, 10, and 10 ml/kg for the 0.5, 60, and 180 mg/kg doses respectively. Blood samples were collected, via cardiac puncture from 3 rats/group/time point) at 0, 15, 30 min, 1, 2, 3, 4, 8, 24, and 96 hours after dosing. Samples were analyzed for plasma levels of RS-25259-007 (free base of RS-25259-197) and RS-17825-007 (the N-oxide of RS-25259-007) using ——— following ——— Mean Cmax and AUC (0-24 hr) for the free base and the N-oxide metabolite were calculated based on generated plasma concentration time curves for each.

Results: RS-25259-197 was rapidly absorbed following oral dosing and attained maximal plasma concentrations at 15 min post dosing at all doses tested. Increasing oral doses of RS-25259-197 (0.5, 60, and 180 mg/kg) resulted in linear increases in Cmax (0.346, 2170, and 5600 ug/ml) and AUC (0-24 hr) (0.454, 2520, and 11300 ug.hr/ml) values for the free base (RS-25259-007), respectively. However, plasma concentrations and AUC values were disproportionally increased at the 60 and 180 mg/kg doses compared respective values at the 0.5 mg/kg dose. The disproportional increase in Cmax and AUC values could indicate saturation of elimination pathways at the higher doses. Cmax levels for the N-oxide metabolite, RS-17825-007 were also attained at 15 min following dosing (Cmax = 137 and 421 ug/ml at the 60 and 120 mg/kg doses, respectively). Respective AUC values for RS-17825-007 averaged 124 and 431 ug.hr/ml at these doses. Half-life data for either the free base or the N-oxide metabolite could not be determined from the data generated.

Pharmacokinetics of RS-25259-197 in male rats following oral administration for 5 days: (Report No. AT 6302)

Methods: Male Sprague Dawley rats (4/group/time point) were orally (by gavage) administered RS-25259-197 (60 mg/kg) for a period of 5 days. RS-25259-197 (hydrochloride salt, lot no. PA 15303-512, A9201007) was dissolved in pH 7.4, 0.1 M sodium phosphate buffer and delivered in a volume of 10 ml/kg. Heparinized blood samples were collected, via cardiac puncture (predose) or from the orbital sinus at 0, 15, 30 min, 1, 2, 3, 4, 8 and 24 hours after dosing on day 1 24, and 96 hours after dosing on days 1 and 5. Samples were analyzed for plasma levels of RS-25259-007 (free base of RS-25259-197) and RS-17825-007 (the N-oxide of RS-25259-007) using

[redacted] following [redacted] Mean Tmax, Cmax and AUC (0-24 h) values for the free base and the N-oxide metabolite were calculated based on generated plasma concentration time curves for each.

Results: RS-25259-197 was rapidly absorbed following oral dosing in rats with maximal plasma concentrations of 1970 ug/ml versus 2030 ug/ml, attained at 15 and 30 min post dosing on days 1 and 5, respectively. Similar AUC (0-24 hr) values of 3340 and 2490 ug.hr/ml were also observed for the free base on days 1 and 5, respectively, with no evidence of accumulation observed. Cmax and AUC values for RS 25259-007 generated currently were comparable to those seen previously following single dose oral administration in rats (Report no. AT 6303). Average Cmax and AUC values for the N-oxide metabolite, RS 17825 (day 5 only) were 145 ug/ml and 79.1 ug.hr/ml, respectively, with maximal plasma levels attained at 30 min post dosing. These values were also comparable to those seen previously following single dose administration (Half lives for either RS 25259-007 or RS 17825 were not determined in the present study.

Pharmacokinetics of RS-25259-197 in dogs following single dose oral administration: (Report No. AT 6304)

Methods: Female beagle dogs (4/group; fasted for 18 hours prior to and for 4 hours after dose administration) were administered [^{14}C]RS-25259-197 by intragastric intubation at single doses of 0.5, 6.0, and 20 mg/kg (calculated dose equivalents of 0.481, 5.56 and 17.8 mg/kg free base). The 0.5 mg/kg dose, was a mixture of [^{14}C]RS-25259-197 (hydrochloride salt, lot no. 15188-GTH-53) and unlabelled RS-25259-197 (hydrochloride salt, lot No. 5891-79, A9012002) dissolved in pH 5, 10 mM sodium acetate buffer, made isotonic with normal sodium chloride. The 6.0 and 20 mg/kg RS-25259-197 doses (hydrochloride salt, lot no. PA 15303-61S A92404100) were prepared in pH 7.4, 0.1 M sodium phosphate buffer. Doses were delivered in volumes of 0.5, 1.1, and 1.0 ml/kg for the 0.5, 6.0, and 20 mg/kg doses respectively. Heparinized blood samples (sample volume not indicated) were collected by venipuncture (jugular vein) at 0, 5 (0.5 mg/kg dose only), 15, 30 min, 1, 2, 3, 4, 8, 24, 48, 72 and 96 hours after dosing. Samples were analyzed for plasma levels of RS-25259-007 (free base of RS-25259-197) using [redacted] following [redacted] Mean pharmacokinetic parameters of RS-25259-197 were calculated based on generated plasma concentration time curves.

Results: Calculated mean pharmacokinetic values for RS-25259-007 following single oral doses in dogs are presented in Table 1, below (Taken from the Sponsors summary table, page 197, Vol. 2 of 4 from Amendment 013 dated June 16, 1993)

Table 1. Pharmacokinetics of RS-25259-007 Following Intragastric Administration of Single Doses in Fasted Female Dogs.

Parameter	Doses of RS-25259-197		
	0.5 mg/kg	6 mg/kg	20 mg/kg
AUC (0-96 hr) (μg-hr/mL)	29.9	295	2890
$T_{1/2}$ (hr)	2.12	4.54	9.97
C_{max} (μg/mL)	4.98	100	1440
T_{max} (hr)	1.38	0.500	0.375

N = 4 dogs per study.

Briefly, the data in Table 1 show that RS-25259-197 was rapidly absorbed following intragastric administration in dogs. Maximal plasma concentrations were attained at 1.38 hr after dosing at the 0.5 mg/kg low dose, but decreased to 0.375 hr following administration of the 20 mg/kg high dose. Increases in both Cmax and AUC values were linear but disproportional at the 20 mg/kg dose compared to respective values at the 0.5 mg/kg dose. Half-life in dogs also increased with increasing dose from 2.2 to 9.97 hr. at the 0.5 and 20 mg/kg doses, respectively. Thus, the data suggest possible saturation of the pathways for elimination of RS-25259-197 at higher doses.

Plasma Pharmacokinetics of RS-25259-007 and its' N-oxide metabolite in female dogs following oral administration for 5-days: (Report No. AT 6301/AM1023)

Methods: Four female beagle dogs were orally administered RS-25259-197 (hydrochloride salt) dose of 6 mg/kg/day (free base) for 5 days. RS-25259-197 was dissolved in 0.1 M sodium phosphate buffer solution and delivered in a total volume of 1.0 ml/kg. On days 1 and 5, venous blood samples (sample volume not provided) were collected (via jugular vein) at 0 (predose), 15, 30 min, 1, 2, 3, 4, 8, and 24 hours after dosing. Following ——— , plasma levels of RS-25259-007 (free base of RS-25259-197) and RS-17825-007 (N-oxide of RS-25259) of were separated and quantitated using ——— Pharmacokinetic data for RS-25259-007 and RS-17825-007 were calculated based on generated plasma concentration-time curves for each.

Results: Table 2 (below) shows the calculated pharmacokinetic data for RS-25259-007 and RS-17825-007 on days 1 and 5.

Table 2. Pharmacokinetic data for RS-25259-007 (free base) and RS-17825-007 (N-oxide of RS-25259) on Days 1 and 5 Following Repeated Single Daily Dosing for 5 Days in Dogs. (Sponsor Summary Table, Vol 2.4, pg. 92)

	Day 1	Day 5
RS-25259-007 Parameters:		
AUC (0-24 hr) (ng•hr/mL)	519 ± 437	659 ± 647
$T_{1/2}$ (hr)	1.74 ± 0.36	2.06 ± 0.56
C_{max} (ng/mL)	126 ± 43	175 ± 101
T_{max} (hr)	1.56 ± 1.20	0.813 ± 0.375
RS-17825-007 Parameters:		
AUC (0-8 hr) (ng•hr/mL)	550 ± 68	834 ± 197
$T_{1/2}$ (hr)	1.22 ± 0.79	1.05 ± 0.26
C_{max} (ng/mL)	387 ± 316	816 ± 345
T_{max} (hr)	0.438 ± 0.375	0.563 ± 0.315

Briefly the data in Table 2 show that RS-25259-197 was rapidly absorbed following oral dosing with maximum plasma levels of the free base occurring at 1.56 and 0.813 hr on days 1 and 5, respectively. Table 2 also shows comparable mean Cmax, AUC, and T1/2 values for the free base (RS-25259-007) on days 1 and 5, suggesting little effect of repeat dose administration on the pharmacokinetics of RS-25259-197. In contrast, AUC and Cmax values for the N-oxide metabolite of RS-25259 were approximately 1.5 and 2.1-fold higher on day 5 versus day 1, respectively. The significance of this latter observation is not currently apparent.

DISTRIBUTION/METABOLISM:

Rats:

Metabolic Disposition, and Tissue Distribution of ^{14}C- RS25,259-197 after a Single Intravenous Dose to Rats (Report No. AT 5975)

Methods: Fasted male rats were intravenously administered [14C]-RS-25,259-197 mixed with unlabeled RS-25,259-197 at a final dose of 0.469 mg/kg (specific activity = 135 uCi/mg). Total radioactivity in whole blood, plasma, urine, feces and selected tissues was determined using LSC at predose and up to 96 hours postdosing. Quantification of metabolises from plasma and brain was also performed using LSC following ——— Quantitation of metabolises in urine was performed by continuous flow radioactivity monitoring of the column effluent. Extracts of pooled urine were also tested for conjugation products following incubation with β-glucuronidase and sulfatase.

Results: Mean maximal concentration (Cmax) following single dose i.v. administration was 75.7 ng-Eq/ml in plasma and 114 ng/Eq/ml in blood 5 min after dose administration. Blood to plasma ratios averaged 1.49 ± 0.10 and remained constant between 0.083-8 hours, suggesting a partition of total radioactivity into red blood cells. AUC (0-24 hrs) for total radioactivity was 171 ng-Eq.hr/ml with an apparent terminal half-life (T1/2) of 1.79 hr. Pharmacokinetic parameters for RS-25,259-197 were as follows: Terminal half-life (t1/2) was 1.49 hrs. Systemic clearance (CL) was 7.98 L/kg/hr and volume of distribution (0) was 17.2 L/kg. Other pharmacokinetic parameters (AUC,[0-24 hrs], %AUC, Cmax and Tmax) for RS-25,259 and two of its major metabolises are presented in TABLE 1, below (Sponsors summary TABLE, Report # AT 5975, Vol 1.3, pp. 106).

Parameter	Total Radioactivity	RS-25259 -197	Metabolite 3	Metabolite 6
AUC 0-24 hr (ng-Eq•hr/mL)	171	58.9	19.7	46.3
% AUC	100	34.5	11.6	27.1
$T_{1/2}$ (hr)	1.79	1.49	2.13	1.50
C_{max} (ng-Eq/mL)	75.7	58.3	5.68	11.9
T_{max} (hr)	0.083	0.083	0.25	0.25

Chromatographic analysis of pooled plasma extracts revealed eight quantifiable metabolises of RS-25,259. Structural identification of these metabolises was not provided,

RS-25,259-197, metabolite # 6 and metabolite # 3 represented 34.5%, 27.1% and 11.6% of the total radioactivity based on a 0-24 hour AUC, respectively. Pharmacokinetic parameters for the two major metabolites, #6 and #3 are presented in table above. The other six minor metabolites represented a total of 12.9% of the total radioactivity, based on the AUC (0-24 hr) and < 4% individually, based on the same. Generally, all metabolites exhibited comparable T1/2 (when able to calculate) which averaged around 2 hours, with a range of 1.5 to 2.4 hours (observed for metabolites 6 and 8), respectively. Tmax for most metabolites occurred at 0.25 hours with the exceptions of metabolites 2 and 7 where Tmax occurred at 2 and 1 hours after dosing, respectively.

Greater than 82% of the administered radioactivity was recovered in excreta within the first 24 hours after dosing, with recovery in urine almost complete within 8 hours and that in feces occurring by 24 hours post dosing. Recovery of dose equivalents in urine and feces over the 96 hour period averaged 56.2% and 33.9%, of the total radioactivity administered. The metabolic profile of RS-25,259 in urine was qualitatively identical to that in plasma with the exception of metabolite #1, which was not quantifiable. However, quantitatively RS-25,259-197 was largely transformed to metabolite # 6. The quantitative metabolic, profile of RS-25,259-197 over 0-96 hr (in descending order of percentage of administered dose) was Metabolite 6 (33.4%), Metabolite 3 (4.68%), RS-25-259-197 (4.43%), Metabolite 5 (3.12%), Metabolite 8 (2.53%), Metabolite 4 (0.742%), Metabolite 7 (0.728%) and Metabolite 2 (0.258%). These metabolites accounted for 88.9% of the total radioactivity recovered in urine over the 96 hr period. Quantitation of metabolises in urine was performed by

Extracts of pooled urine were also tested for conjugation products following incubation with βƎ-glucuronidase and sulfatase.

Pharmacokinetic parameters for total radioactivity following intravenous dosing were as follows: Mean maximal concentration (Cmax) was 169 ng-Eq/ml with an individual range from 142 to 193 ng-Eq/ml. In blood, Cmax was 152 ngEq/ml. Tmax for plasma and blood was 0.875, and 0.646 hr, respectively. Blood/plasma ratios were 0.993 ± 0.077 and remained constant from 0.083-8 hours post dosing, suggesting some partitioning of drug into red blood cells. Mean AUC (0-24 hrs) was 1020 ng-Eq.hr/ml, ranging from 862-1210 ngEq.hr/ml. The apparent terminal half-life (T1/2) in dogs was 2.30 hr, ranging individually from 2.09-2.71 hr. This (T1/2) is slightly longer than that of 1.79 hr seen previously in rats.

Pharmacokinetic parameters for total radioactivity following oral (intubation) dosing were as follows: Mean maximal plasma concentration was 254 ng-Eq/ml with an individual range from 194 to 360 ng-Eq/ml. In blood, Cmax was 204 ngEq/ml. Tmax for plasma and blood was 1.5 hr for both. Blood/plasma ratios were 0.912 ± 0.097 and remained constant from 0.083-8 hours post dosing, suggesting some partitioning of drug into red blood cells. Mean AUC (0-48 hrs) was 1126 ng-Eq.hr/ml, ranging from 1100-1400 ng-Eq.hr/ml. The apparent terminal half-life (T1/2) in dogs was 2.07 hr and ranged from 1.45-2.64 hr. This (T1/2) was nearly identical to that observed following i.v. dosing in dogs but slightly higher than that of 1.79 hr seen previously in rats. Chromatographic analysis of pooled plasma extracts revealed nine quantifiable metabolises of RS-25,259, none of which have currently been identified.

RS-25,259-197 was rapidly eliminated from plasma following a single i.v. dose with a terminal $T_{1/2}$ = 1.87 hr. The Cmax of RS-25,259-197 was 122 ng/ml at 5 min post i.v. dosing and the mean AUC (0-24 hr) was 194 ng-Eq-hr/ml which represented 19% of the AUC (0-24 hr) for total radioactivity. For the RS-25,259-197 systemic clearance (CL) was 2.58 $L.kg^{-1}.hr^{-1}$ and volume of distribution (Ǝ) was 6.95 $L\text{-}kg^{-1}$. These values are less than that seen in the rat following i.v. dosing (Clearance in the rat was 7.98 $L.kg^{-1}.hr^{-1}$ and volume of distribution (Ǝ) was 17.2 $L.kg^{-1}$. Following a single oral dose in the dog, RS-25,295-197 was rapidly absorbed (Tmax = 1 hr) and eliminated from plasma with a terminal T1/2 of 2.17 hr. The Cmax for RS-25,259-197 following oral dosing was 16.4 ng/ml and the mean AUC(0-48 hr) was 24.2 ng-Eq-hr/ml (which represented only 1.92% of the AUC(0-24 hr) for total radioactivity). For the 0.5 mg/kg oral dose of RS-25,259-197, an estimate of bioavailability was 12.5%.

Following both single oral and i.v. doses of [14C] -RS-25,259-197, three major metabolites (Metabolite 1, Metabolite 2, and Metabolite 3) accounted for 53.5% (i.v.) and 67.4% (oral) of the AUC for total radioactivity in plasma. Comparisons of pharmacokinetic parameters for these three metabolises is shown in TABLE 2, below (Sponsors Summary Table, Report # AT 5976, Vol. 1.3, pp. 147)

TABLE 2. Comparison of the Pharmacokinetic Parameters of the Three Major Metabolites (Metabolites 1, 2, and 3) Following Intravenous and Oral Administration of [^{14}C]-RS 25,259-197

Parameter	Metabolite 1		Metabolite 2		Metabolite 3	
	IV	Oral	IV	Oral	IV	Oral
AUC (ng-Eq•hr/mL)	210	318	179	243	157	288
% AUC	20.6	25.2	17.5	19.3	15.4	22.9
$T_{1/2}$ (hr)	1.72	1.55	2.86	4.03	2.10	2.00
C_{max} (ng-Eq/mL)	40.2	44.5	21.6	30.8	26.0	55.1
T_{max} (hr)	2	2	3	2	2	2

As can be seen the Cmax and AUC values for each metabolite were higher following oral dosing compared to i.v. whereas terminal T1/2 values were similar by both routes. Ratios of metabolites 1, 2 and 3 to RS-25,259-197 following i.v. dosing were 1.08, 0.923 and 0.809. Respective ratios following oral dosing were 13.1, 10.0 and 11.9 (ratios based on AUC, 0-24 hr). Although the reasons for this unexpected discrepancy are not apparent, increased metabolism of RS-25,259-197 following oral dosing may be a contributing factor. This possibility is supported by the data, which showed that the % AUC for RS-25f259-197 was about 10 fold less following oral dosing compared to i.v.

The six remaining metabolises in dogs following i.v and oral dosing in dogs represented less than 8%, individually of the AUC for total radioactivity. Pharmacokinetic parameters for these remaining metabolites varied widely. Half lives for these metabolises were generally longer following I.V. vs oral dosing, whereas Cmax and AUC values for most metabolises were generally greater following oral vs i.v. dosing. Here again the mechanisms, which contribute to the observed differences in pharmacokinetic parameters, although not apparent could include a large first pass effect following oral dosing.

In dogs, following a single 0.5 mg/kg i.v. dose of [^{14}C] -RS25,2590-197, 77.3% and 13.5% of the total radioactivity (0168 hr) was recovered in urine and feces, respectively. Similar recoveries of 75.4% in urine and 18.5% in feces were observed following oral dosing in dogs. Recovery in urine was nearly complete by 24 hours post dosing and in feces by 48 hours in both cases.

The urinary metabolic profile (0~48 hr) following either i.v. or oral administration was qualitatively identical to the plasma metabolic profile. Metabolites #1, #2, #3, and #4 accounted for 53.8% and 57.5% of the total administered dose, following i.v. and oral administration, respectively. The remaining 4 urinary metabolises, individually ranged from 8.83-0.386% and from 7.54-0.0386% of the total administered dose following i.v. and oral dosing, respectively.

Here again, treatment of urine with either the glucuronidase or the sulfatase did not alter the profile, suggesting the absence of conjugated metabolises in urine. In both cases very little original RS-25,295-197 was recovered in the urine (for i.v. and oral dosing recovery was 0.90% and 0.035%, respectively).

Compared to the rat the urinary metabolites formed by dogs are very similar, qualitatively, but differ quantitatively. For instance dogs transform RS-25,259-197 to five major metabolises, with each individually amounting to 8-19% of the administered i.v. dose and 7-18% of the oral dose. In contrast Rats transform RS-25p259-197, principally into Metabolite # 6 which accounts for about 33% of an administered i.v. dose in the rat.

Metabolism and tissue distribution to brain, eye and intestines after a single i.v. dose of [^{14}C]RS-25259-197 in pigmented rats: (Report No. AT 6285)

Methods: Male Long Evans (pigmented) rats (3/time point for plasma collection and one group of 6/final time point for collection of excreta) were administered [^{14}C]RS-25259-197 by gavage and intravenously at single i.v. (via tail vein) doses of 0.5 mg/kg in a volume of 1.0 ml/kg. The dose formulation consisted of carbon-14 labeled RS-25259-197 (hydrochloride salt, lot no. 16398-GTH-21, 55.2 mCi/mmol) mixed with unlabelled RS-25259-197 hydrochloride (lot no. PA 15303-615 A9204010) in a pH 5 solution of 10 mM sodium acetate buffer, made isotonic with sodium chloride (final dose specific activity, dose concentration and radiochemical purity were 138 µCi/mg, 0.450 mg/ml and 99.9%, respectively). Blood samples were collected, via cardiac puncture at 0, 5, 15, 30 min, 1, 2, 3, 4, 8, 24, 48, 72, and 96 hours after dosing. Urine was collected from 6 rats/group at 0-4, 4-8, 8-24, 24-48, 48-72, and 72-96 hours after dosing. The following tissues were excised at 0 (predose), 5, 15, 30 min, 1, 2, 4, 8, 24, and 96 hr after dosing for determination of total tissue radioactivity: cerebrum/cerebellum, eyes, large intestine, medulla small intestine and ileum (last 5 cm of small intestine). Levels of total radioactivity in plasma and urine were determined using liquid scintillation counting (LSC). Levels of total radioactivity in intestinal tissues and eyes were also determined using LSC, following total dissolution in 3N and 6N potassium hydroxide, respectively. Separation and quantitation of RS-25259-197 and metabolites in plasma, urine, and brain homogenates were achieved using : — — and — — followed by LSC assay of the — Pooled urine extracts were also incubated with β-glucuronidase and sulfatase followed by — and LSC for determination of possible conjugated products. Pharmacokinetic parameters for RS-25259-197 and metabolites were calculated based on generated plasma concentration-time curves for each.

Results: [^{14}C]RS-25259-197 was extensively distributed following i.v. dosing in Long Evans Rats. Table 4 (succeeding page) presents the Cmax, Tmax and AUC values for distribution of total radioactivity in eye, ileum, small intestine, large intestine, cerebrum/ cerebellum, medulla and plasma.

Table 4. Pharmacokinetic Parameters for [^{14}C]RS-25259-197 in Tissue and Plasma from Long Evans Rats, Based on Total Radioactivity following Single IV Injection.

Eyes	Ileum	Small Intestine	Large Intestine	Cerebrum/ Cerebellum	Medulla	Plasma (ng-Eq/ml)

AUC (ng.hr/g) 0-96hr	119000	6980	6700	4640	221	171	166
Cmax (ng/g)	1760	1520	1580	612	137	120	65.5
Tmax (hr)	4	4	2	0.25	0.083	0.25	0.083

Briefly, the rank order of distribution of total radioactivity (based on 0-96 hr AUC) in Long Evans Rats was eye, ileum, small intestine, large intestine, medulla and cerebrum/cerebellum. Maximal levels in plasma, cerebrum/cerebellum, medulla and large intestine were attained at 5-15 min post dosing and at 2 to 4 hr post dosing in small intestine, eye and ileum. Elimination from plasma and brain was rapid (T1/2 = 1.62 hr), but was delayed in eye and intestine. The major difference in the distribution of radioactivity seen currently was the selective distribution of radioactivity in eye tissue where Cmax and AUC values of 1760 ug/g and 119000 ng.hr/g were observed. In comparison, Cmax and AUC values for eye tissue in previous distribution studies in Sprague Dawley rats were only 139 ug/g and 429 ng.hr/g, respectively (Report No. AT6264). The Sponsor attributed these differences to possible binding of the quinuclidene amine moiety of RS-25259-197 to melanin in the pigmented eye of the Long Evans rat.

Analysis of plasma extracts showed 8 metabolites, with Nos. 1 and 12 accounting for 20.8% and 12.6% of the total radioactivity (based on 0-24 hr AUC), respectively. Rank order of the remaining plasma metabolites (% of total radioactivity) was: No. 11 (8.66%), No. 14 (6.26%), No. 6 (3.77%), No. 4 (1.78%), No. 10 (1.78%) and No. 13 (1.26%). In plasma and urine, RS-25259-197 only represented 33.0% and 5.76% of the total radioactivity, respectively. Otherwise, the metabolic profile in urine was similar to that seen in plasma except for metabolite Nos. 10 and 14 which were not detected in urine. Urinary excretion was the major route of elimination in Long Evans rats with an average of 50.9% of the administered dose recovered (49.3% within the first 24 hours after dosing). Half-life values for total radioactivity and metabolite Nos. 1 and 12, were: 1.62 hr, 1.32 hr, and 1.69 hr, respectively. Finally, the clearance for RS-25259 in Long Evans rats was 8.67 $L.kg^{-1}.hr^{-1}$. The aforementioned values for half life and clearance, as well as the metabolic profile for RS-25259-197 seen in plasma and urine of Long Evans were qualitatively and quantitatively similar to those seen in Sprague Dawley rats (Report No. AT 6264). Such similarity suggests overall common metabolic pathways for RS-25259-197 are shared by both strains of rats.

Excretion of ^{14}C-RS25259-197 Derived Radioactivity and Its Metabolite Profile: The excretion patterns of the intravenously and orally administered radioactivity tagged compound were similar, i.e., 56.2 and 50.8 % were excreted in urine and, 33.9 and 40.7 % in feces respectively. The recovery of radioactivity was almost similar and complete within 24 hr of its administration as 82 and 89 % of intravenously and orally administered compound excreted during this period. The recovery was about 89.1 and 91.5 % in urine and feces at 96 hr. Seven metabolites were detected in the urine which were qualitatively similar to that of plasma excepting Metabolite 10 was not present in urine. Metabolite 1 constituted 33.4 and 30.1 % of the intravenously and orally administered compound.

In summary, orally administered RS-25259-197 was rapidly absorbed as RS-25259-007 and had a bioavailability of 6.41 %. It was distributed extensively in tissues like gastrointestinal tract, lungs, kidneys and adrenals and was detected in brain (medulla and cerebrum) in a sufficient amount after 30 min of its administration. In was metabolized in 8 metabolites out of which 2 (Metabolite 1-6-hydoxy-RS-25259 and Metabolite 12) were the major ones. About 51 to 56 and 34 % of the radioactivity was excreted in urine and feces respectively.

Metabolism of [^{14}C]RS-25259-197 in rats following single i.v. and oral doses and tissue distribution of [^{14}C]RS-25259-197 after a single i.v. dose in Rats: (Report No. AT 6264)

Methods: Male Sprague Dawley rats (3/group/time point for plasma collection and 6/group/final-time point for collection of excreta) were administered [^{14}C]RS-25259-197 by gavage and intravenously at single doses of 0.5 mg/kg. For the dose formulations, carbon-14 labeled RS-25259-197 (hydrochloride salt, lot no. 15188-GTH-53, 54.9 mCi/mmol; for the i.v. dose and lot no. 15188-GTH-106, 54.9 mCi/mmol; for the oral dose) were mixed with unlabelled RS-25259-197 hydrochloride (lot no. 13977-86, A9102010) in a pH 5 solution of 10 mM sodium acetate buffer and made isotonic with sodium chloride (final dose specific activity, dose concentration and radiochemical purity were 135 µCi/mg, 0.469 mg/ml and 99.9%, respectively for the i.v. dose and 133 µCi/mg, 0.194 mg/ml and 96.8%, respectively for the oral dose). Doses were delivered in volumes of 1.0 and 2.5 ml/kg for the i.v. and oral doses, respectively. Blood samples were collected, via cardiac puncture from 3 rats/group/time point at 0, 5 (i.v. dose only) 15, 30 min, 1, 2, 3, 4, 8, 24, 48, 72, and 96 hours after dosing. Urine was collected from 6 rats/group at 0-4, 4-8, 8-24, 24-48, 48-72, and 72-96 hours after dosing, with feces collected over 0-24, 24-48, 48-72 and 72-96 hours after dosing. The following tissues from rats which received the i.v. dose were excised at 0 (predose), 5, 15, 30 min, 1, 2, 4, 8, 24, and 96 hr after dosing for determination of total tissue radioactivity: abdominal fat, abdominal skin, adrenals, bladder, bone marrow both femurs, cecum, cerebrum/cerebellum, eyes, heart, kidneys, large intestines, liver, lungs, medulla, skeletal muscle, small intestine, spleen, stomach and testes. Levels of total radioactivity in urine and plasma were determined using liquid scintillation counting (LSC), whereas levels of total radioactivity in whole blood and feces were determined by [redacted] in scintillation fluid for LSC analysis. Samples were analyzed for plasma levels of RS-25259-007 (free base of RS-25259-197) and RS-17825-007 (the N-oxide of RS-25259-007) using [redacted] following [redacted] Total radioactivity in tissue samples was determined using LSC following complete dissolution using 3N potassium hydroxide. Separation and quantitation of RS-25259-197 and metabolites in plasma, urine, and brain homogenates were achieved using [redacted] and [redacted] which were then assayed for radioactivity using LSC. Pooled urine extracts were also incubated with β-glucuronidase and sulfatase followed by [redacted] and LSC for determination of possible conjugated products. Pharmacokinetic parameters for RS-25259-197 and metabolites were calculated based on generated plasma concentration time curves for each.

Results: Table 3 (succeeding page) provides the pharmacokinetic data for total radioactivity, RS-25259-197 and metabolites in pooled plasma from rats following intravenous and oral dosing. Briefly, analysis of total plasma radioactivity following i.v. and/or oral administration

showed that RS-25259-197 was rapidly absorbed following oral administration in rats, with mean maximal plasma concentrations (Cmax) of 119 ng-Eq/ml observed at 15 min following oral administration versus Cmax values of 75.7 ng-Eq/ml at 5 min following i.v. dosing. Identical AUC values (based on total radioactivity) of 171 ng-Eq.hr/ml following i.v. and oral dosing also suggested that the oral dose was completely absorbed. Blood to plasma concentration ratios (based on total radioactivity between 5 min and 8 hours) of 1.50 $\pm$ 0.09 and 1.1 $\pm$ 0.14 following i.v. and oral dosing, respectively, suggests that radioactivity partitions into red blood cells. Analysis of plasma for RS-25259-197 showed Cmax values of 58.3 ng/ml at 5 min after i.v. dosing, with an AUC (0-24 hr) value of 58.9 ng.hr/ml (34.5% of the 0-24 hr total radioactivity in AUC). In comparison, a Cmax value of 0.964 ng/ml was observed at 15 min after oral dosing and the 0-24 hr AUC was 3.94 ng.hr/ml (2.30% of the 0-24 hr total radioactivity in AUC). Thus, bioavailability of RS-25259-197 after oral dosing was 6.41% (based on ratios of AUC values for RS-25259-197). RS-25259-197 was rapidly eliminated from plasma following i.v. or oral dosing (T1/2 = 1.49 hr following i.v. dosing, but T1/2 could not be determined following oral dosing). Finally, systemic clearance and volume of distribution for RS-25259-197 were 7.96 $L.kg^{-1}.hr^{-1}$ and 17.1 $L.kg^{-1}$, respectively.

Table 3. Pharmacokinetic Data for Total Radioactivity, RS-25259-197 and Metabolites in Pooled Plasma from Rats Following Administration of Single Intravenous and Oral Doses of [^{14}C]RS-25259-197

Time (hr) Total	Metab	Metab 1	Metab 4	Metab 6	Metab 10	Metab 11	Metab 12	Metab 13	RS-14	Others 25259	
Intravenous											
AUC (0-24hr) (ng.hr/ml)	171	46.3	2.12	6.41	5.86	5.51	19.7	1.64	2.14	58.9	22.1
%AUC	100	27.1	1.24	3.76	3.43	3.23	11.6	0.96	31.3	34.5	12.9
$T_{1/2}$(hr)	1.79	1.50	ND	2.43	2.24	1.69	2.13	ND	ND	1.49	ND
Cmax (ng.Eq/mL)	75.7	11.9	0.803	1.6	1.15	1.36	5.68	1.12	1.16	58.3	6.72
Tmax (hr) 0 083	0.25	1	0.25	0.25	2	0.25	0.25	0.25	0.083	0.25	
Oral											
AUC (0-24hr) (ng.Eq.hr/mL)	71	71.5	3.94	6.92	5.52	3.08	30.6	2.94	9.29	3.94	33.4
% AUC	100	41.8	2.3	4.05	3.23	1.8	17.9	1.72	5.43	2.3	19.5
$T_{1/2}$(hr)	2.07	1.23	1.33	1.02	ND	ND	2.21	1.57	2.7	ND	ND
CmaX (ng-Eq/ml)	119	70.3	2.51	6.24	1.84	1.12	21.7	3.17	2.42	0.964	10.2
Tmax (hr)	0 083	0.25	0.25	0.25	1	0.5	0.25	0.25	0.25	0.25	0.25

Terminal half-life (T1/2) = -ln 2/β, where β is the terminal elimination rate constant determined by linear regression analysis of natural log concentration versus time. For RS-25259-197, systemic clearance (CL) = Dose/AUC = 7.96 $L.kg^{-1}.hr^{-1}$. Calculation based on a theoretical dose of 0.469 mg/kg delivered. For RS-25259-197, volume of distribution (β) = CL/β = 17.1 $L.kg^{-1}$, where β is the terminal elimination rate constant (0.465 hr^{-1}).

RS-25259-197 was extensively metabolized in the rat with eight quantifiable metabolites; all of them attained maximal plasma concentrations between 15 min and 2 hr following dosing. Half-life values for all metabolites ranged from 1.02 to 2.7 hr. Metabolite #1 (6-hydroxy-RS-25259; the only metabolite currently identified) was the major plasma metabolite and accounted for 27.1% and 41.8% of the total plasma radioactivity (based on 0-24 hr AUC) following i.v. and oral dosing, respectively. The second most abundant metabolite in plasma was metabolite #12, which accounted for 11.6% and 17.9% of the total plasma radioactivity (based on 0-24 hr AUC) following i.v. and oral dosing, respectively. The remaining six metabolites observed in plasma collectively accounted for 22.54% (individual range = 0.963 to 3.76%) and 18.58% (individual range = 1.72 to 5.43%) of the total radioactivity (based on 0-24 hr AUC) following i.v. and oral dosing, respectively.

Total recovery of radioactive dose equivalents in urine and feces averaged 56.2% and 33.9% following i.v. dosing, respectively and 50.8% and 40.7% following oral dosing, respectively. These latter findings indicate that urinary excretion was the major route of elimination following i.v. or oral dosing. RS-25259-197 represented 4.43% of the total radioactivity collected in urine following i.v. dosing, but was below the limit of quantitation in urine following oral dosing. The metabolic profile of RS-25259-197 observed in pooled urine samples was qualitatively identical with that seen in plasma, with metabolites #1 and #12 being most abundant. However, metabolite # 10 (detected in plasma) was not detected in urine. Treatment of urine extracts with β-glucuronidase and sulfatase, had no effect on the chromatographic profile, indicating the lack of β-glucuronide and sulfate conjugates in rats.

The distribution of [^{14}C]RS-25259-197 associated radioactivity was rapid and extensive, with tissue to plasma ratios greater than 1 observed for all tissues examined. Largest tissue to plasma concentration ratios was observed in gut and bladder between 4 and 8 hours after dosing. The rank order of tissue distribution (based on AUC, 0-96 hr) was: bladder, ileum, large intestine, caecum, small intestine (-ileum), kidneys, lungs, liver, adrenals, testes, stomach, spleen, skin, bone marrow, heart, skeletal muscles, eyes, total femur, plasma, cerebrum/cerebellum, abdominal fat, femur (without marrow) and medulla. Maximal tissue/plasma concentration ratios of total radioactivity in medulla and cerebrum-cerebellum were 1.65 and 1.17, respectively at 30 min after dosing, with only RS-25259 detected in radioactivity extracted from brain tissue. In general, depletion of radioactivity from tissues paralleled that from plasma with the exception of bladder, ileum, small intestine, testes, and abdominal skin, where maximal levels of radioactivity occurred at 1-4 hours after dosing. By 96 hours after dosing, only the eyes showed low, but quantifiable levels of radioactivity.

The pharmacokinetic data of RS-25259-007 in rats and dogs is summarized in Sponsor's Table I:

Table I
Pharmacokinetic Parameters of Single Dose of 14C- RS-25259-197 in Rats and Dogs

Animal/ Route	Cmax (ng.eq/ml)	Tmax (hr)	$T_{1/2}$ (hr)	Cl (l/kg/hr)	Vdss (l/kg)	AUC (ng.eq.hr/ml)
RS-25259-197						
1. Spague-Dawley Rats (Crl:CD BRV						
Single Dose						
i) i.v.						
(0.5 mg/kg)						
Compound	58.3	0.083	1.49	7.96	17.1	58.9 (0-24hr)
b.Radioact.	75.7	0.083	1.79	–	–	171 (0-24hr)
ii) p.o.						
(0.5 mg/kg)						
a. Compound	0.964	0.25	–	–	–	3.94 (0-24hr)
b. Radioact.	119	0.25	2.07	–	–	171
2. Beagle Dogs						
i) i.v.						
(0.5 mg/kg)						
a. Compound	122	0.083	1.87	2.58	6.95	194 (0-24hr)
b. Radioct.	169	0.875	2.3	–	–	1020 (0-24hr)
ii) p.o.						
(0.5 mg/kg)						
Compound	16.4	1.0	2.17	–	–	24.2 (0-24hr)
Radioact.	254	1.5	2.07	–	–	1260 (0-24hr)
RS25259-007						
1. Rats						
(0.5 mg/kg, p.o.)	0.346	0.25	–	–	–	0.454 (0-24hr)
2. Dogs						
(0.5 mg/kg)	4.98	1.38	2.12	–	–	29.9 (0-24hr)

In conclusion, orally and intravenously administered RS-25259-197 was absorbed as RS-25259-007 in rats and dogs. These compounds had similar plasma pharmacokinetics by these routes and have a low bioavailability of 6.4 % and 12.5 % in rats and dogs, respectively. It was metabolized in 8 and 9 compounds in rats and dogs respectively. Two metabolites, i.e., N-oxide and 6-hydroxy RS-25259 were identified. These metabolites were not excreted as conjugates.

Metabolic Disposition of [^{14}C]RS-25259-197 after single i.v. and oral doses administered to Cynomolgus Monkey: (Report No. DM 1078)

Methods: Four 18-hr fasted male cynomolgus monkeys were used and 3 were intravenously administered 0.5 mg/kg [^{14}C]RS-25259-197 and the remaining one animal was administered a

single dose of 0.5 mg/kg by oral intubation. For the dose formulations, [^{14}C]RS-25259-197 (hydrochloride salt, lot no. 17488-GTH-75, 19.2 mCi/mg for the i.v. dose and; lot no. 17488-GTH-72, 20.3 mCi/mg; for the oral dose) were mixed with unlabelled RS-25259-197 hydrochloride (lot no. 15303-18, A9204024) in a pH 5 solution of 10 mM sodium acetate buffer and made isotonic with sodium chloride (radiochemical purity was 99.9% and 96.8%, respectively for the i.v. and orally administered compound). The dose was administered in a volume of 1.0 ml/kg for the i.v. and oral doses. Blood samples for plasma were collected, by venipuncture via jugular vein at 0, 5 (i.v. dose only) 15, 30 min, 1, 2, 3, 4, 8, 24, 48 and 72 hours after dosing. Urine was collected from each of the animals at 0-4, 4-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hours after dosing, with feces collected over 0-24, 24-48, 48-72, 72-96, 120-144 and 144-168 hours intervals after dosing. One ml of plasma/urine were mixed with 0.01 ml sodium triphosphate and the pH of the solution was adjusted to 10 and the solutions applied in liquid scintillation counting spectrometer to assess the radioactivity of the compound and metabolite. Samples were analyzed for plasma levels of RS-25259-007 (free base of RS-25259-197) and RS-17825-007 (the N-oxide of RS-25259-007) using —— Separation and quantitation of RS-25259-197 and metabolites in plasma and urine, and brain homogenates were achieved using —— and ——, which were then assayed for radioactivity using LSC. Pooled urine extracts were also incubated with β-glucuronidase (pH 5.0) and sulfatase (pH 7.0) followed by —— and LSC for determination of metabolites. Pharmacokinetic parameters for RS-25259-197 and metabolites were calculated.

Results: The analysis of total plasma radioactivity at steady state following i.v. and/or oral administration in animals was 1620 ng Eq.hr/ml (69.8%) and 2300 ng Eq.hr/ml (or 78.4%) . The mean terminal half-life of intravenously and orally administered ^{14}C-RS-25259-197 was 6.55 hr and not determined during the study. The AUC values (based on total radioactivity) were similar following i.v. and oral dosing thus suggesting that the oral dose was completely absorbed. The peak concentration of the radioactivity was seen after 8 hr of the administration of the compound. RS-25259-197 was eliminated from plasma following i.v. or oral dosing with $T_{1/2}$ of 1.49 hr following i.v. dosing and $T_{1/2}$ could not be determined following oral dosing. Finally, systemic clearance and volume of distribution for RS-25259-197 were 1.03 $L.kg^{-1}.hr^{-1}$ and 6.6 $L.kg^{-1}$, respectively.

Pharmacokinetic Data for Total Radioactivity, RS-25259-197 and Metabolites in Pooled Plasma from Monkeys Following Administration of Single Intravenous and Oral Dose (1 monkey) of [^{14}C]RS-25259-197

	Total Others	Metab 1	Metab 3	Metab 4	Metab 5	Metab 7	Metab 8	Metab 9	Metab 10	Metab 11	Metab 14	RS-25259	
						Intravenous							
AUC (0-tot. hr) (ng.Eq.hr/ml)	1690	60.7	59.1	127	ND	21.6	330	156	88.8	135	84.4	487	ND
%AUC	100	3.8	3.76	7.98	0.23	0.90	20.8	9.8	5.6	8.54	4.72	30.6	3.12
$T_{1/2}$(hr)	6.5	5.4	5.2	4.2	ND	10.8	4.1	3.8	4.2	4.5	6.9	4.44	ND

Cmax (ng.Eq/mL)	150	5.6	4.7	9.4	2.1	1.2	31.5	18.3	6.9	10.0	7.2	112	7.9
Tmax (hr)	1.2	2	0.5	4	0.025	4	2	1	6	6	2	0.083	0.25

	Total	Metab 1	Metab 3	Metab 4	Metab 5	Metab 7	Metab 8	Metab 9	Metab 10	Metab 11	Metab 14	RS-25259	Others
						Oral							
AUC (0-48hr) (ng.Eq.hr/mL)	2300	254	136	699	39.7	42.3	76.5	54.6	143	403	196	44.4	210
% AUC	100	11.1	5.92	30.4	1.73	1.84	3.3	2.4	6.2	17.5	8.5	1.9	9.2
$T_{1/2}$(hr)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cmax (ng-Eq/mL)	178	22.1	10.4	51.4	3.9	4.4	7.3	5.7	11.6	31.0	16.1	5.6	9.4
Tmax (hr)	8	8	8	8	8	8	8	8	8	8	8	8	8

Terminal half-life (T1/2) = -ln 2/β, where β is the terminal elimination rate constant determined by linear regression analysis of natural log concentration versus time. For RS-25259-197, systemic clearance (CL) = Dose/AUC = 7.96 $L.kg^{-1}.hr^{-1}$. Calculation based on a theoretical dose of 0.469 mg/kg delivered. For RS-25259-197, volume of distribution (β) = CL/β = 17.1 $L.kg^{-1}$, where β is the terminal elimination rate constant (0.465 hr^{-1}). The bioavailability of the compound (F0) = 8.97%

Orally administered RS-25259-197 was metabolized in 10 metabolites and these attained maximal plasma concentrations at about 8 hr following dosing. Half-life values for the metabolites were not estimated by oral route and the bioavailability of the orally administered compound was about 8.97%. The half-life of intravenously administered compound ranged from 1.02 to 2.7 hr. The intravenously administered compound was changed to metabolites and the peak concentrations of metabolites were seen in 1 to 6 hr of the administration of the compound (as shown in the tables above). The retention times of the metabolites and their structures were identified by sponsor and

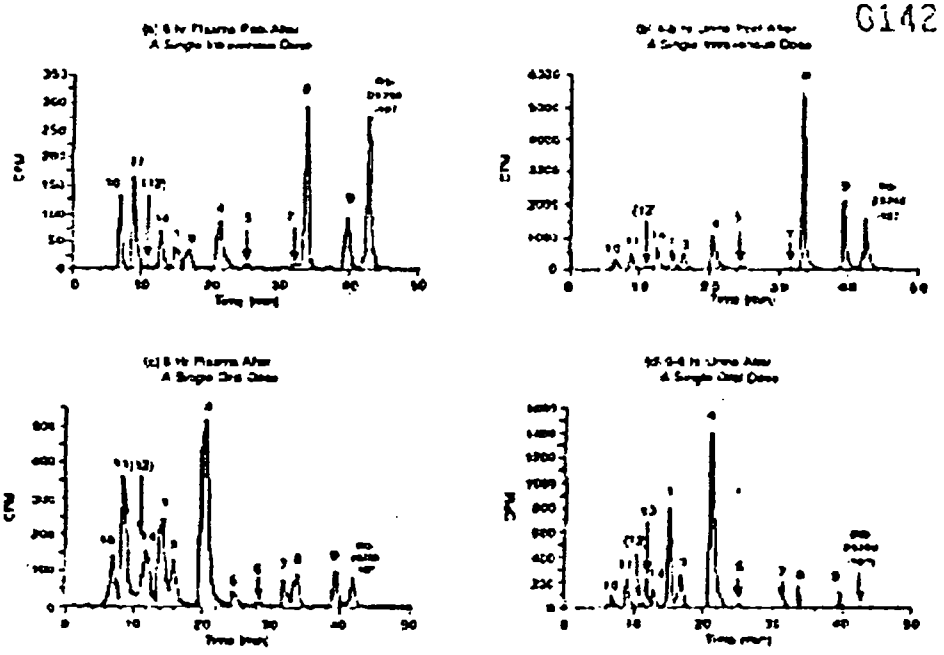


Figure 2. Representative chromatographic separation of RS-25259-197 and its metabolites in extracts of plasma and urine pooled from three cynomolgus monkeys given single 0.5 mg/kg IV dose (panels a and b) and from one monkey given a single 0.5 mg/kg PO dose (panels c and d) of [^{14}C]-RS-25259-197. Numbered peaks correspond to metabolites of RS-25259. RS-97253-007, 6-(S)-hydroxy-RS-25259, co-elutes with Metabolite 1. RS-17825-007, the N-oxide of RS-25259, co-elutes with Metabolite 9.

are shown below:

The radioactivity was tagged with the free base after iv administration and the second most abundant metabolite in plasma was metabolite #8, which accounted for 20.8% the total plasma radioactivity (based on 0-24 hr AUC). Following oral dosing, metabolite 4 was the major metabolite and its concentration was 30.4%. The remaining 9 metabolites observed in plasma collectively accounted for the remaining 69.6% (individual range = 1.84 to 11.1%) of the total radioactivity (based on 0-24 hr AUC) following oral dosing.

The identified urinary metabolites after iv administration of the compound were #8, 9, 4, 3 and 14 and were present in 13.8, 11.9, 11.7, 5.35 and 4.22% of the administered radioactivity. The recovery of radioactive compound after a single iv dose was 96.8%. After an oral dose, the metabolite #4, 1 and 11 were the main metabolites (as shown in the table above) in the urine. These latter findings indicate that urinary excretion was the major route of elimination following i.v. or oral dosing. RS-25259-197 represented 4.43% of the total radioactivity collected in urine following i.v. dosing, but was below the limit of quantitation in urine following oral dosing. The free compound was identified in urine of animals after an intravenous administration and not after its oral administration. Meatabolites 12 and 13 were identified in animals treated by an oral dose of the compound.

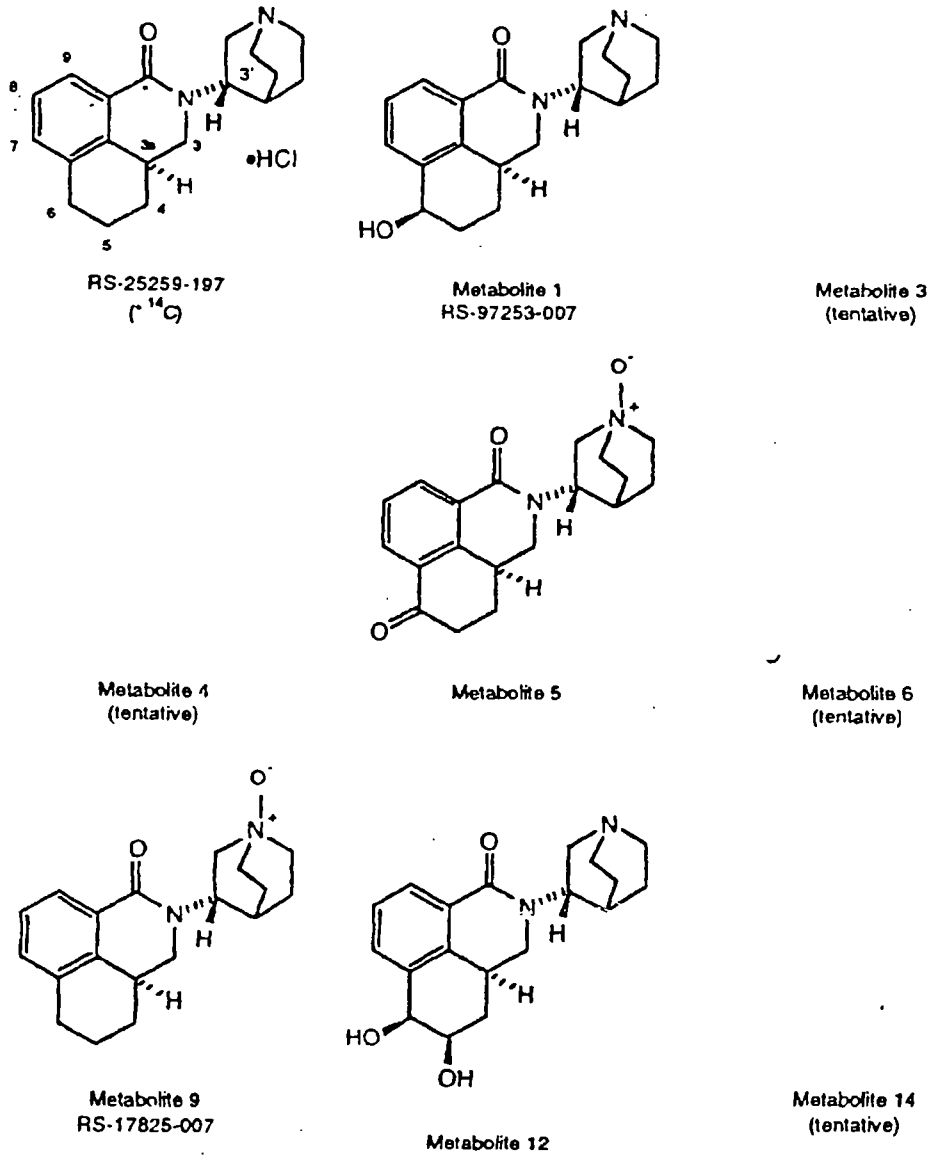


Figure 1. Structures of RS-25259-197 and metabolites. RS-25259-007 is the free base of RS-25259-007. Metabolites 7, 8, 10, 11 and 13 have not been structurally characterized. See Figure 5 for possible metabolites of RS-25259 which were not detected in animals.

Total recovery of radioactive dose equivalents in urine and feces averaged 56.2% and 33.9% following i.v. dosing, respectively and 50.8% and 40.7% following oral dosing, respectively. These latter findings indicate that urinary excretion was the major route of elimination following i.v. or oral dosing. RS-25259-197 represented 4.43% of the total radioactivity collected in urine following i.v. dosing, but was below the limit of quantitation in urine following oral dosing. The metabolic profile of RS-25259-197 observed in pooled urine samples was qualitatively identical with that seen in plasma, with metabolites #1 and #12 being most abundant. However, metabolite

10 (detected in plasma) was not detected in urine. Treatment of urine extracts with β-glucuronidase and sulfatase, had no effect on the chromatographic profile, indicating the lack of β-glucuronide and sulfate conjugates in rats.

The distribution of [^{14}C]RS-25259-197 associated radioactivity was rapid and extensive, with tissue to plasma ratios greater than 1 observed for all tissues examined. Largest tissue to plasma concentration ratios was observed in gut and bladder between 4 and 8 hours after dosing. The rank order of tissue distribution (based on AUC, 0-96 hr) was: bladder, ileum, large intestine, caecum, small intestine (-ileum), kidneys, lungs, liver, adrenals, testes, stomach, spleen, skin, bone marrow, heart, skeletal muscles, eyes, total femur, plasma, cerebrum/cerebellum, abdominal fat, femur (without marrow) and medulla. Maximal tissue/plasma concentration ratios of total radioactivity in medulla and cerebrum-cerebellum were 1.65 and 1.17, respectively at 30 min after dosing, with only RS-25259 detected in radioactivity extracted from brain tissue. In general, depletion of radioactivity from tissues paralleled that from plasma with the exception of bladder, ileum, small intestine, testes, and abdominal skin, where maximal levels of radioactivity occurred at 1-4 hours after dosing. By 96 hours after dosing, only the eyes showed low, but quantifiable levels of radioactivity.

The pharmacokinetic data of RS-25259-007 in rats and dogs is summarized in Sponsor's Table I:

Table I
Pharmacokinetic Parameters of Single Dose of 14C- RS-25259-197 in Rats and Dogs

Animal/ Route	Cmax (ng.eq/ml)	Tmax (hr)	$T_{1/2}$ (hr)	Cl (l/kg/hr)	Vdss (l/kg)	AUC (ng.eq.hr/ml)
RS-25259-197						
1. Sprague-Dawley Rats (Crl:CD BRV						
Single Dose						
i) i.v.						
(0.5 mg/kg)						
Compound	58.3	0.083	1.49	7.96	17.1	58.9 (0-24hr)
b.Radioact.	75.7	0.083	1.79	–	–	171 (0-24hr)
ii) p.o.						
(0.5 mg/kg)						
a. Compound	0.964	0.25	–	–	–	3.94 (0-24hr)
b. Radioact.	119	0.25	2.07	–	–	171
2. Beagle Dogs						
i) i.v.						
(0.5 mg/kg)						
a. Compound	122	0.083	1.87	2.58	6.95	194 (0-24hr)
b. Radioct.	169	0.875	2.3	–	–	1020 (0-24hr)
ii) p.o.						
(0.5 mg/kg)						

Compound	16.4	1.0	2.17	–	–	24.2 (0-24hr)
Radioact.	254	1.5	2.07	–	–	1260 (0-24hr)
RS25259-007						
1. Rats (0.5 mg/kg, p.o.)	0.346	0.25	–	–	–	0.454 (0-24hr)
2. Dogs (0.5 mg/kg)	4.98	1.38	2.12	–	–	29.9 (0-24hr)

In conclusion, orally and intravenously administered RS-25259-197 was absorbed as RS-25259-007 in rats and dogs. These compounds had similar plasma pharmacokinetics by these routes and have a low bioavailability of 6.4 % and 12.5 % in rats and dogs, respectively. It was metabolized in 8 and 9 compounds in rats and dogs respectively. Two metabolites, i.e., N-oxide and 6-hydroxy RS-25259 were identified. These metabolites were not excreted as conjugates.

TOXICOLOGY:

Acute Toxicity Studies:

Intravenous:

Strain of Animals Used: a) Rat - Crl:CD^{R}BR
b) Mice: Crl:CD-1
c) Dog – Beagle

a) Methods:

i) Rats: The animals used in the study were about 11 weeks of age. RS-25259-197 (injectable formulation) solution was made in phosphate buffer (pH 7.4) just before its administration to animals 10 ml/kg (vehicle). The animal in each group was observed daily for 14 days after the administration of the compound.

(ii) Mice: Thirty CD-1 mice (15/sex) were divided in 3 groups (5/sex/group), administered RS25259 solution in sodium phosphate buffer (pH 7.4$\pm$0.2) in a volume of 3 ml/kg. These animals were observed at 0 (pretest), 0.25, 0.5, 1, 3 and 6 hr postdosing and daily for 14 days. The autopsy of the animals that died during the study was done.

iii) Dogs: Beagle dogs used in the study were 17 to 31 months of age. The injectable formulation (RS-25259-197) of the compound after dilution in saline was used in the volume of 2 ml/kg. The observations on each of the animal/group were made up to 2 days of the study.

The results of the intravenous acute toxicity studies in the mouse, rat and dog have been tabulated below in Table.

TABLE Acute Toxicity Studies in the Rat, Mouse and Dogs

Species/strain	# animals/sex/ group	Dose Range Tested (mg/kg)	Highest Non-Lethal Dose (mg/kg)	Minimum Lethal Dose (mg/kg)	Observed Symptoms
Mice (CD-1)	5	0, 10, 30	10	30	Convulsions, gasping, palor, transient vocalization.
Rat (Crl:CD) i.v.	3	0, 10, 30, 100	10	30	All animals in 100 mg/kg group died, 3 males and 1 female treated at 30 mg/kg group died and showed labored respiration, convulsion, tremors etc.
Dog (Beagle): i.v.	1	10, 20	20	ND	Convulsions, labored respiration, tremors etc. were seen. Dogs collapsed but recovered.

In summary, these studies indicated that single doses of 10 and 20 mg/kg in rats and mice produced the common signs depression of CNS. The signs in the treated rats and mice were labored respiration, convulsions and inactivity. The CNS depression related salivation, labored respiration and tremors were seen in dogs. Both dogs collapsed and showed convulsions. Minimum i.v. lethal doses were 30 mg/kg in rats and mice. A dose of 20 mg/kg was not lethal in dogs.

Acute Oral Toxicology:

Rats and Dogs: (Study Nos. 6284 and 6268)

Methods: Acute oral toxicity studies were conducted in rats and dogs. In rats, RS-25259-197 was orally administered (by gavage) to groups of 5 rats/sex at single doses of 0 (vehicle), 250 and 500 mg/kg in total volumes of 5.0, 2.5 and 5.0 ml/kg, respectively. Similarly, in dogs RS-25259-197 was orally administered (by gavage) to groups of one dog/sex at single doses of 0 (vehicle), 50, and 100 mg/kg in total volumes of 1.0, 0.5, and 1.0 ml/kg, respectively. The vehicle used in both studies was a sodium phosphate buffer solution which consisted of 0.262 g% monobasic sodium phosphate monohydrate, USP and 1.150 g% dibasic sodium phosphate anhydrous, USP in 100 ml purified water, USP). Rats and dogs in both studies were observed for clinical signs of toxicity at 0-5 min, 0.5, 1, 3, and 6 hours after dosing on day one and daily thereafter for 14 days. Body weights were also determined for both species on the day of dosing and at 1, 7, and 14 days post-dosing. Blood samples were also collected from dogs, once prior to dosing and at 1, and 14 days after dosing for evaluation of hematology and clinical chemistry. Complete necropsies were performed on all animals in both studies, with histopathologic evaluations also conducted on all tissues found altered at necropsy.

Results: In rats, oral administration of RS-25259-197 at the 500 mg/kg dose was lethal producing death in 4 of 10 rats within 1 hour of dosing. Other clinical signs evident within 1 hour of dosing included collapse, convulsions, inactivity, labored breathing, salivation and tremors, with recovery generally occurring by 6 hours post dosing in surviving rats. One of 10 rats at the 250 mg/kg dose also showed ataxia, inactivity and tremors, but no death. No drug related effects on body weights, food consumption or gross pathology were observed in rats. Thus, the 250 mg/kg dose was the maximum non-lethal dose in rats. In dogs, at a dose of 100 mg/kg, RS-25259-197 produced emesis, convulsions (salivation, labored breathing, abnormal urination and defecation, cyanosis, and general rigidity) hyperemia of the sclera and inactivity followed by death within 1 hr after dosing in both dogs tested. At the 50 mg/kg dose both dogs tested showed emesis and inactivity, but recovered by 3 hours after dosing. No drug-related effects on body weights or clinical pathology parameters (hematology and clinical chemistry parameters) in surviving dogs at the 50 mg/kg dose were evident. Dogs which died at the 100 mg/kg dose exhibited gross findings of red lungs, dark red gastric mucosa, with excess mucus. Histological correlates in the 100 mg/kg dogs included pulmonary vascular congestion and minimal pulmonary edema in the mail and pulmonary and gastric vascular congestion, gastric epithelial cell erosion and mucus adhesion to the epithelial cell surface. Thus in dogs the maximum non-lethal dose was 50 mg/kg, producing only transient clinical signs, but no evidence of anatomic pathologic changes.

Subacute Toxicity

Rats

1. Intravenous 1-Month Toxicity Study in Rats with RS-25,259-197
(Study # AT 5962)
Testing Laboratories: Syntex Research, Palo Alto, California

Study Started: September 10, 1991

Study Completed: October 11, 1991

Report Date: February 28, 1992

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

Animals: Crl:CDBR VA/Plus Rats; 7-8 weeks of age; 175-225g

Drug Batch Nos.: 25,259-197-11530, -11531, and -11532

Methods: Male and female rats, 10 of each/group were intravenously administered either vehicle or RS-25,259-197 at 1, 3 or 10 mg/kg doses, daily over a one month treatment period. Animals were observed daily for clinical signs of toxicity, pre dose body weights along with weekly body weights and food consumption were also determined. Ophthalmologic examinations were performed at pre dose and during the last week of the study. Immediately prior to termination of the study hematologic, serum chemistry and urinalysis (last week of study) assays were performed. At the end of the study animals were sacrificed followed by a complete necropsy with preparation of tissues for microscopic examination. Both organ weights and organ to body weight ratios were calculated.

Results:

1. **Achieved Doses:** Same as intended

2. **Observed Effects:** Clinical signs of toxicity in rats following one month i.v. administration of RS-25,259-197 consisted of transient vocalization immediately after dosing during the fourth week of treatment occurred in 6/10 rats at the 10 mg/kg dose, but they became clinically normal within one minute.

3. **Mortality:** None

4. **Body Weight/Food Consumption/Water Consumption**: There were no treatment-related changes in body weights, food or water consumption during the course of the study.

5. **Hematology /Blood Chemistry:** There were no treatment-related differences in any of these parameters as all individual animal values were within reference ranges.