

6. Ophthalmic Examination: No treatment-related changes were observed.

7. Organ Weights: Females at the 3 and 10 mg/kg dose levels exhibited higher adrenal gland weights (10 and 13% increases) compared to controls. However there were no differences in organ/body weight ratios in these groups and no associated gross or microscopic abnormalities observed with respect to the adrenal gland in these animals.

8. Gross/Microscopic Pathology: No gross or microscopic changes related to treatment with RS-25,259-197 were observed.

In conclusion, the 3 mg/kg dose was the no effect dose for this study, and provides an adequate margin of safety for both the 0.3 ug/kg initial clinical dose and for the highest planned clinical dose, 120 ug/kg.

2. Palonosetron Hydrochloride: 28-Day Subcutaneous Toxicity Study in Juvenile Rats

Report No: _____ Report Number 1063/18-D6154

Conducting laboratory and location: _____

Date Started: November 12, 1999

Date of Study Report: July 2000

GLP compliance: Statements of compliance with _____ Statutory Instrument 1999 No. 3106, The Good Laboratory Practice Regulations 1999 and OECD Principles on Good Laboratory Practice (revised 1997, Issued January 1998) were included; however, there were no signatures.

QA- Report: Yes (X) No ()

Methods: In a 28-day subcutaneous toxicity study, neonatal/juvenile rats received palonosetron at doses of 0, 5, 15, and 25 mg/kg/day. The vehicle or drug solution was administered by the subcutaneous route for 31 days, starting at day 4 postpartum. In the clinical setting, the intravenous route of administration will be used; however, given the small size of animals in the study, the subcutaneous route was chosen. Rats (i.e., offspring) were killed and necropsied on day after the last treatment. Parental female rats received no treatment and were sacrificed following weaning of offspring.

Dosing:

species/strain: 30 time-mated female rats [CrI:CD(SD)IGSBR strain] were obtained from _____ to provide offspring for this study. The parental female rats were 8-10 weeks old and weighed at least 160 g at mating. Pregnant female rats were delivered by day 15 of gestation. These animals were allowed to deliver naturally and the day pups were first observed was designated as day 0 postpartum. On day 4 postpartum, litters were culled to 5

pups/sex/litter through random selection. Culled pups were discarded. Pups were examined on day 4 postpartum prior to allocation to the study. Offspring within each litter were allocated to the same treatment group. Litters were randomly allocated to treatment groups. The litter performance of parental female rats and the viability of offspring up to day 4 postpartum were poorer than expected with the result that less than required number of offspring were available for allocation. Accordingly, no satellite animals were allocated to the control group. The sex of each offspring was recorded on days 1 and 4 postpartum prior to allocation to treatment groups. Sexes were confirmed at weaning (day 21 postpartum) and prior to necropsy (day 34 postpartum).

- **#/sex/group or time point:** In the main study groups, there were 10 juvenile rats/sex/group.
- **age:** Rats were 4 days of age at the start of treatment.
- **weight:** On day 4 postpartum, mean body weights ranged from 7.3 to 8.3 g for male rats and 6.3 to 7.9 g for female rats.
- **satellite groups used for toxicokinetics or recovery:** In toxicokinetic groups that received palonosetron at 5, 15, or 25 mg/kg/day, there were 15 or 16 juvenile rats/sex/group.
- **dosage groups in administered units:** 0, 5, 15, and 25 mg/kg/day
- **route, form, volume, and infusion rate:** the subcutaneous route using a dose volume of 5 ml/kg administered Vehicle or drug solution.

Drug, lot#, radiolabel, and % purity: Palonosetron, batch number 30893-P105 (Purity 99.4%)

Formulation/vehicle: The vehicle was sodium chloride and sodium phosphate adjusted to pH 7.4 with sodium hydroxide or hydrochloric acid in Water for Irrigation.

Observations and times:

- **Clinical signs:** Parental animals and offspring were monitored daily for clinical signs of toxicity. During the treatment period, pups were observed immediately and at 1 and 4 hr after dosing. Animals were observed for moribundity/mortality twice per day. Offspring that died or were sacrificed in a moribund condition during the treatment period were submitted to a macroscopic examination.
- **Body weights:** Pup body weights were measured on day 1 postpartum and daily during the treatment period (days 4 to 34 postpartum).
- **Food consumption:** Food consumption was not measured due to the age of the animals.
- **Ophthalmoscopy:** Not performed.
- **EKG:** Not performed.
- **Hematology:** Not performed.
- **Clinical chemistry:** Not performed.
- **Urinalysis:** Not performed.
- **Gross pathology:** On the day following the last dose, surviving offspring were sacrificed and submitted to necropsy examination.
- **Organs weighed:** Organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, pituitary gland, prostate gland, spleen, testes + epididymides, and thyroids + parathyroids.
- **Histopathology:** Gross lesions, principally at injection sites, from all animals and tissues from the control and high dose groups were embedded in paraffin wax BP, sectioned at 5 μ m, stained with hematoxylin and eosin, and examined by a pathologist. Deaths and moribund

sacrifices during the treatment period were considered unrelated to treatment, and histopathological examination was not extended to these animals. Tissues examined from the control and high dose groups were as follows: adrenal glands, brain, cecum, colon, duodenum, eyes, femur with bone marrow and articular surfaces, heart, ileum, jejunum, kidneys, liver, lungs with mainstem bronchi, mammary (females only), mandibular lymph nodes, mesenteric lymph nodes, esophagus, optic nerves, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerves, skin, spinal cord (cervical, lumbar, and thoracic), spleen, sternum with bone marrow, stomach, testes and epididymides, thymus, thyroids and parathyroid, trachea, urinary bladder, and uterus.

- **Toxicokinetics:** Blood for measurement of plasma levels of palonosetron and its metabolite, RS-17825-007, was collected on days 1 and 28 (i.e., days 4 and 32 postpartum, respectively). Blood was collected from 4 or 5 rats/sex/group at 0.5, 1, 2, and 4 hr after dosing. Blood was obtained by decapitation on day 1 and from the orbital sinus on day 28. Samples were analyzed for palonosetron and RS-17825-007 by _____.

Animals in toxicokinetic groups were discarded without examination following blood collection.

- **Other:** Physical and functional development of rat pups was assessed. Eye opening was evaluated starting from day 13 postpartum. Evaluation of eye opening in the majority of pups was missed in error on day 16 postpartum. Air righting was evaluated on day 17 postpartum. Pupillary reflex and auditory startle response were evaluated on day 21 postpartum.

Results:

- **Clinical signs:** For male and female rats at 15 and 25 mg/kg/day, dose-related incidences of hair coat thinning, hair loss, and sores were observed at injection sites. For rats at 15 and 25 mg/kg/day, the percentage of animals with eyes open on day 15 postpartum was slightly reduced to 27 and 25%, respectively, as compared to 41% for the controls. By day 17 postpartum, all rats in control and treatment groups had opened their eyes. The incidences of male and female rats at 15 and 25 mg/kg/day with an absent pupillary reflex on day 21 postpartum were increased as compared to controls. For male rats at 15 and 25 mg/kg/day, the pupillary reflex was absent for 30 and 20% of animals, respectively, as compared to a poor response for 10% of controls. For female rats at 15 and 25 mg/kg/day, the pupillary reflex was absent for 10 and 30% of animals, respectively, as compared to a normal reflex for 100% of female controls. The sponsor reported background control ranges for offspring having no pupillary reflex of 0-42% for males and 0-46% for females. There were no treatment-related effects on air righting ability on postpartum day 17 or auditory response on postpartum day 21.

Pupillary reflex on postpartum day 21 for rats that received palonosetron by the subcutaneous route at doses of 0, 5, 15, and 25 mg/kg/day (mean % of pups showing a response).

Dose, Mg/kg/day	Absent		Poor		Normal	
	Male	Female	Male	Female	Male	Female
0	0	0	10	0	90	100
5	0	0	0	0	100	100
15	30	10	0	0	70	90
25	20	30	0	0	80	70

N =	0	0	6	9	0	0	8	9
-mineralization	0	0	0	4	0	0	0	3
-hemorrhage	0	0	3	6	0	0	3	5
-acanthosis	0	0	2	0	0	0	1	0
-dermatitis	0	0	3	8	0	0	7	9
-cellulitis	0	0	5	4	0	0	8	8
Left hip								
N =	0	2	6	9	0	0	8	9
-mineralization	0	0	0	3	0	0	0	6
-hemorrhage	0	0	3	7	0	0	4	6
-acanthosis	0	0	1	0	0	0	3	0
-dermatitis	0	0	4	8	0	0	5	9
-cellulitis	0	2	4	8	0	0	8	9
Optic nerve								
N =	10	0	0	9	5	0	0	9
-neuropathy	1	0	0	4	0	0	0	2
Kidney								
N =	10	2	0	9	5	0	0	9
-pelvic dilatation	0	0	0	4	1	0	0	5
-focal nephropathy	3	0	0	1	1	0	0	5
-papillitis	0	0	0	0	0	0	0	1

- **Toxicokinetics:** For male and female rats, plasma AUC values for palonosetron on days 1 and 28 increased with elevating dose, although, observed increases were greater than proportional to dose. Plasma AUC values for palonosetron were similar on days 1 and 28. There appeared to be no gender-related differences in plasma AUC values for palonosetron. Due to limited sample volumes available on day 1 of treatment, AUC values for palonosetron in the low and high dose group males were not determined and toxicokinetic analysis for RS-17825-007 was not performed at any dose level. The sponsor reported that low concentrations of the metabolite, RS-17825-007, were detected in the low and intermediate dose groups on day 28 to the extent that toxicokinetic analysis could be conducted.

Plasma pharmacokinetic parameters for palonosetron in rats that received palonosetron by the subcutaneous route at doses of 5, 15, or 25 mg/kg/day.

Dose Mg/kg	Day 1						Day 28					
	AUC _{0.5-4hr} , ng hr/ml		C _{max} , ng/ml		T _{max} , hr		AUC _{0.5-4hr} , ng hr/ml		C _{max} , ng/ml		T _{max} , hr	
	M	F	M	F	M	F	M	F	M	F	M	F
5	NC	256.40	266.36	138.63	0.5	0.5	152.28	140.15	247.07	196.63	0.5	0.5
15	1357.68	558.71	1393.40	776.50	0.5	0.5	655.68	700.90	646.76	752.10	0.5	0.5
25	NC	1584.53	1297.56	1141.50	0.5	0.5	1701.29	1913.12	1432.59	1179.44	0.5	1.0

NC = Not Calculated

Key Study Findings: In a 28-day subcutaneous toxicity study, neonatal/juvenile rats received palonosetron at doses of 0, 5, 15, and 25 mg/kg/day. The vehicle or drug solution was administered by the subcutaneous route for 31 days, starting at day 4 postpartum. In the clinical setting, the intravenous route of administration will be used; however, given the small size of animals in the present study, the subcutaneous route was chosen. Rats (i.e., offspring) were killed and necropsied on day after the last treatment. Parental female rats received no treatment and were sacrificed following weaning of offspring. The no effect dose was 5 mg/kg/day. There was

no treatment-related mortality. The incidences of male and female rats at 15 and 25 mg/kg/day with an absent pupillary reflex on day 21 postpartum were increased as compared to controls. Eye opening was slightly delayed for rats at 15 and 25 mg/kg/day. Target organs (or tissues) of toxicity were injection sites, optic nerves, and kidneys. For male and female rats at 15 and 25 mg/kg/day, histopathological changes at injection sites included mineralization, hemorrhage, acanthosis, dermatitis, and cellulitis. For male and female rats at 25 mg/kg/day, the incidence of neuropathy in the optic nerves was increased. For male and female rats at 25 mg/kg/day, the incidence of pelvic dilatation in the kidneys was increased. The incidences of focal nephropathy and papillitis were increased for female rats at 25 mg/kg/day. Hematology and clinical chemistry parameters were not provided, although, it appears that blood was collected on day 30 of treatment.

3. Subcutaneous 28-Day Toxicity Study in Juvenile Rats with Palonosetron Hydrochloride:
(Study #PALO-02-05; PV10001)

Testing Laboratory:

Dates of Start and Completion of the study: April 10, 2002 & December 5, 2002

GLP # QAU Requirements: Statement of compliance to GLP regulations, 1999, 1999 # 3106 was attached.

Species and Strain: CrI:CD^RBR VAF/Plus SD IGS BR VAF/PLUS strain neonate rats

Batch #: TA/2002/163 (Batch #21000565)

Methods: Sixty mated females were allowed to litter and 136 pups/sex were divided in to 4 main groups (12/sex/group) and 4 satellite groups (22/sex/group). These were administered subcutaneous injection (between the shoulder blades) 0, 5, 15 or 25 mg/kg/day palonosetron (5 ml/kg) for 28 days (post-partum day 4 to day 34/35). The following table of sponsor (vol 58.1, pp 18 of 422) shows group size, doses and identification # of animals.

Group	Colour code	Number of animals		Identification numbers		Dose level (mg/kg/day) (base)
		Males	Females	Males	Females	
1	Main	12	12	61-72	109-120	0
	Satellite	22	22	157-178	245-266	
2	Main	12	12	73-84	121-132	5
	Satellite	22	22	179-200	267-288	
3	Main	12	12	85-96	133-144	15
	Satellite	22	22	201-222	289-310	
4	Main	12	12	97-108	145-156	25
	Satellite	22	22	223-244	311-332	

Sponsor conducted this repeat study on the request of the Agency (letter January 31, 011) as the previous study (report #1063/18-D6154) was not complete and it lack the measurements of hematology and clinical chemistry parameters. The doses and the methods used in the present study were the same as used in the previous 28-day study in juvenile rats (report #1063/18-D6154). In brief, the study included the assessment of the body weights changes, the developmental parameters of ear/eye opening (from day 3 to 6 for ear and from day 11 to 16 for eye opening), righting reflex (day 5), air righting reflex (three trials on day 22), startle response (on day 15), pupillary light reflex and ophthalmoscopy (on day 33 day of age) were also observed. The Approximately 0.5 ml of blood was collected from 4 satellite group from 3 pups/sex/time point on day 1, 4 and 35/36 of dosing (i.e., postpartum day 4) at 0.5, 1, 2 and 4 hr after dosing for the estimation of palonosetron and its metabolite RS 17825-007. At necropsy (day 35/36 of animal age), 0.5 ml the blood from the non-fasted main study groups for hematology and blood chemistry parameters. The tissues and organs from main study groups were the same as described the previous study. The microscopic examination was done on all tissues from control and high dose treatment groups of main study animals and, eyes and optic nerves from all the animals of all groups of the main study and, all tissues of animals from main study group dying or killed during the study and, all gross lesions from the main study groups animals.

Results:

a. **Observed Effects:** Two males (#67 and 72) and 1 female (#265) Thickening and scabies of the skin at the injection sites were seen in animals of 25 mg/kg/day treatment group. No other changes in the physical condition of the animals were observed.

The percent animals with ears open (day 5), startle response (day 15), eye opening (day 16) were 100% among all the groups. The average day for opening the eyes were 14.9, 15.2, 15.0 and 15.2, respectively in control and 3 treatment groups. The righting reflex on day 5 of observation, was 8.3% less in animals of 25 mg/kg/day treatment group than control group animals. The percent animals with eye open on day 14 were 22.2, 8.3, 16.7 and 16.7 in control and 3 treatment groups animals. The development parameters were similar in all study groups.

b. **Mortality:** Three animals were found dead during the study. These were 2 males in control group and 1 female of satellite group of 5 mg/kg/day treatment group. Two males of control group and 1 female of 25 mg/kg/day treatment group (satellite group) were sacrificed because of humane reasons.

c. **Body Weight/Food Consumption Changes:** The mean body weight gain of the males and females included in treatment groups and control group were not statistically different during the preweaning period. The mean initial (day 1) and final body weights (on postpartum day 20) of neonates of the control main study group during the pre-weaning period were males 7.7 g and 46.4 g and, females 7.1 and 44.2 g, respectively. During post weaning period (postpartum day 21 to 35), a retardation of 10.7 and 5.8% in the body weight gain of male and female animals included in 25 mg/kg/day treatment group was seen. On post weaning day 35, the bodyweights of animals of main study control and 3 treatment groups were 145.5, 143.8, 148.1 and 132.7 g for males and, 133.4, 138.5, 133.8 and 126.0 g for females, respectively.

TABLE
Table for Mean Weight (g) of rats during the study

	Control	Low Dose	Mid Dose	High Dose
Pre-Weaning				
Postnatal Day 3				
M	7.7	7.9	8.0	7.9
F	7.1	7.7	7.6	7.6
Postnatal Day 20				
M	46.4	45.9	47.5	43.6
F	44.2	47.3	45.9	43.0
Post-Weaning				
Day 21				
M	49.9	50.0	52.2	47.3
F	48.7	51.4	50.4	46.2
Day 35				
M	145.5	143.8	148.1	132.7
F	133.4	138.5	133.8	126.0

d. **Hematology/Coagulation/Bone Marrow Changes:** A slight decrease was seen in RBC count, hemoglobin and percent packed cell volume in treated animals. A dose related increase in total WBC counts in treated males (10.65, 11.02, 12.8 and 13.8X10³/ul) and females (9.87, 12.45, 11.68 and 12.98 X10³/ul) was also seen.

e. **Blood Chemistry/Urinalysis Changes:** Alkaline phosphatase (ALP) levels were decreased (p<0.001) by 6.6, 18.0 and 24.5% in males and, 6.0, 24.7 and 21.7 in females of 5, 15 and 25 mg/kg/day treatment groups. The albumin to globulins ratio was decreased in a dose related manner in males and females indicating the drug effect on liver. The serum low albumin and ALP values may be due to the inflammatory reactions caused by the compound.

f. **Physical Examination and Ophthalmic Test Changes:** The abnormality of eye lens opacity and posterior synechia was seen in 1 out of 12 male pups and, the delay in development of unilateral lens suture line was observed in 1 out of 12 female pups of 25 mg/kg/day treatment group. No optic nerve neuropathy was seen in the pups during the study.

g. **Organ Weight Changes:** The heart ($p < 0.01$), liver and kidney (0.05) absolute weights were decreased in males of 25 mg/kg/day treatment group. The relative weight of these organs to body weight was not affected. The thymus and lung organ absolute weights were decreased ($p < 0.05$ to 0.001) among females of 25 mg/kg/day treatment group.

Toxicokinetics: The subcutaneously administered compound was seen to attain the peak plasma levels within 0.5 to 1 hr of its administration on day 4 and 30. The plasma peak levels on a steady state ($AUC_{0-\infty}$) and the time for peak plasma concentration of the compound were similar in males and females on day 4 and 35 as shown in sponsor table 3 (scanned below).

Table 3: Results from the non-compartmental analysis of plasma concentration-time data (palonosetron).

Sex	Day	Group	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/ml)	AUC_{0-4} (ng/ml.h)	$AUC_{0-\infty}$ (ng/ml.h)	λ_z (/h)	$t_{1/2}$ (h)	n
Male	4	2	5	0.50	355.04	384.37	450.27	0.548	1.266	4
		3	15	0.50	1418.35	1564.25	1593.01	1.078	0.643	4
		4	25	0.50	1471.28	2333.88	2393.65	0.995	0.697	4
	35	2	5	0.50	262.64	245.20	259.99	1.690	0.410	3
		3	15	1.00	859.89	1104.28	1122.35	1.153	0.801	3
		4	25	1.00	1538.32	2220.54	2314.70	0.896	0.774	3
Female	4	2	5	0.50	414.58	557.41	608.29	0.675	1.026	4
		3	15	0.50	931.11	1420.54	1487.40	0.836	0.829	4
		4	25	0.50	1680.31	2839.61	3125.39	0.640	1.083	4
	35	2	5	0.50	249.01	276.36	286.77	2.293	0.302	2
		3	15	0.50	945.93	1126.97	1139.45	1.241	0.559	3
		4	25	1.00	1544.02	1797.22	1828.09	1.184	0.585	3

* Corresponds to the number of points used in the half-life determination.

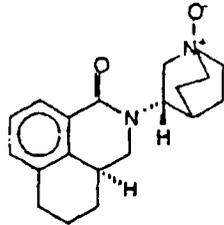
Table 4: Results from the non-compartmental analysis of plasma concentration-time data (RS-17825-007).

Sex	Day	Group	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/ml)	AUC_{0-4} (ng/ml.h)	$AUC_{0-\infty}$ (ng/ml.h)	λ_z (/h)	$t_{1/2}$ (h)	n
Male	4	2	5	1.00	13.79	27.11	30.64	0.586	1.182	3
		3	15	1.00	53.61	121.09	146.04	0.499	1.388	3
		4	25	2.00	85.20	203.54	224.33	0.704	0.985	3
	35	2	5	0.50	6.04	4.05	10.04	0.708	0.979	2
		3	15	1.00	22.68	24.91	27.74	1.605	0.432	2
		4	25	1.00	38.58	59.44	62.37	0.864	0.802	3
Female	4	2	5	1.00	17.93	33.20	36.73	0.868	1.038	3
		3	15	1.00	66.47	118.93	136.78	0.587	1.181	3
		4	25	1.00	78.79	198.44	251.69	0.441	1.571	3
	35	2	5	0.50	6.14	7.65	8.92	1.137	0.609	3
		3	15	0.50	28.77	33.41	40.31	1.024	0.677	3
		4	25	1.00	37.53	54.75	56.15	1.081	0.641	3

* Corresponds to the number of points used in the half-life determination.

The peak plasma concentration of the metabolite (RS-17825-187, with the following structure) was seen within 1 to 2 hr of the administration of the compound.

RS-17825-007



The toxicokinetic parameters of the metabolite were shown in sponsor table 4 (as scanned above).

h. **Gross Pathology Findings:** An abnormal skin color was seen in 2 and 4 males and, 1 and 4 females of 15 and 25 mg/kg/day treatment groups. The incidences of scales formation were 1 and 7 among males and, 2 and 6 females of 15 and 25 mg/kg/day treatment groups. Abnormal size ovaries and uterus were seen in 2 females of 25 mg/kg/day treatment group.

i. **Histopathological Changes:** Renal pelvic distension was present in 0 of 12, 1 of 1, 1 of 2, and 1 of 12 males and, 0 of 12, 1 of 1, 1 of 2, and 3 of 12 females belonging to 0, 5, 15 and 25 mg/kg/day treatment groups. The incidences of renal pelvis distension in animals died during the study were 1 of 1 and 1 out of 2 males of 5 and 15 mg/kg/day treatment groups. One of 2 females of 15 mg/kg/day treatment group died during the study. Extramedullary haemopoiesis in spleen of marked intensity was observed in 1 male and 5 females out of 12/sex of 25 mg/kg/day treatment group. Slight to moderate intensity dermal acanthosis at the site of injection was observed in 4 of 4 and 12 of 12 males and, 2 of 2 and 6 of 8 females belonging to 15 and 25 mg/kg/day treatment groups. Dermal ulceration was seen in 50, 100 and 75% males and, 0, 50 and 62.5% females of 5, 15 and 25 mg/kg/day treatment groups. Myofibre degeneration/pannicular necrosis of subcutaneous tissue from moderate to marked intensity was observed in 50% and 83.3% males and, 50 and 100% females belonging to 15 and 25 mg/kg/day treatment groups. Optic nerve neuropathy was not seen in any of the study animals.

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Histopathological findings that were considered to be related to treatment were seen at the subcutaneous injection sites as tabulated below :

	Males				Females			
	0	2	4	12	0	0	2	8
No Examined	0	5	15	25	0	5	15	25
Dose in mg/kg/day	0	2	4	12	0	0	2	6
Acanthosis	0	2	4	12	0	0	2	7
Scabbing	0	1	4	9	0	0	1	5
Ulceration	0	1	4	11	0	0	2	7
Subcutaneous inflammation	0	0	3	4	0	0	1	7
Haemorrhage/ congestion	0	0	2	10	0	0	1	8
Myofibre degeneration/ pannicular necrosis	0	0	0	7	0	0	1	5
Dermal/ Subcutaneous necrosis	0	0	1	11	0	0	2	6
Fibroplasia	0	0	0	6	0	0	0	5
Dystrophic mineralisation								

In summary, subcutaneously administered palonosetron caused renal pelvic distension, extramedullary haemopoiesis of marked intensity in spleen and dermal reactions including myofibril degeneration/necrosis of moderate to marked intensity in animals of 15 and 25 mg/kg/day treatment groups. Eye lens opacity and structural abnormality of posterior synechia was seen in 1 of 12 male pups and, delay in development of lens suture line was seen in 1 of 12 female pups of 25 mg/kg/day treatment group. No incidences of optic nerve degeneration were seen. A dose of 5 mg/kg/day was a 'highest tolerable dose' and kidney, spleen and site of injection were the target organs of toxicity.

4. 26-Week Chronic Intravenous Toxicity Study in Rats with 4 Week of Recovery Period: (Study # 1063/1-D6154; PALO 99-08)

Testing Laboratory: _____

GLP and QAU Requirements: The study was conducted in compliance with GLP and QAU audits requirements (the certificate was not signed)

Dates of Start and Completion of the Study: May 17, 1999 and January 12, 2000 (The date of completion of pathology not given)

Animals: CrI:CD(SD)IGSBR strain rats with mean body weight of 277.3 to 289.7 g (males) and 197.8 to 205.8 g (females) were used in the study.

Methods: Two hundred and sixty animals (130/sex) were randomly divided in to 4 groups i.e., 30, 20, 20 and 30 animals/sex in 0, 2, 7 and 14/10 mg/kg/day palonosetron (B. #1308911/30893-P105; vol. 1 ml/kg) treatment groups, respectively. Ten animals of control and high dose treatment groups were reserved for the recovery period of 4 weeks to determine the reversibility of treatment related effects. In addition, 10 rats/sex/group consisted of satellite treatment groups. The dose selection was based on a preliminary dose escalating toxicity study performed in 2 groups of rats (5/sex/group) and these animals were given a dose of 7 or 10 mg/kg/day for 7 days. These doses were escalated to 14 and 20 mg/kg/day, respectively in these two groups on day 8. Uncoordinated movements were seen after 14 mg/kg/day dose from day 11. Based on this observation a dose of 10 mg/kg/day was selected as the dose for the present study.

Group Number	Description/Dose (mg/kg/day)*	Animals/group				
		Main study		Satellite Study		
		Male	Female	Male	Female	
1	Control	0	20+10	20+ 10#	-	-
2	Low	2	20	20	10	10
3	Intermediate	7	20	20	10	10
4	High	10	20+10#	20+10#	10	10

*the dose levels are expressed in term of free base. A conversion factor (1.123) was applied to convert from the base to salt; # 10 animals/sex from the control and high dose groups maintained treatment free for 4 weeks following 26 weeks of treatment.

All animals were observed for the changes in the clinical signs, mortality, food consumption, and body weight changes. The ophthalmoscopic examination was done in animals of control and high dose treatment groups on week 25. Blood samples were collected on day 1, and on 1st day of week 5, 13 and 26. The blood samples from 3 animals/sex/satellite group of 10 mg/kg/day treatment group at 10, 30 min and 1, 2, 6 and 24 hr after dosing for toxicokinetic experiment. Blood samples for hematology and blood chemistry tests were drawn from 10 animals/main study treatment groups. Urine samples were collected from other 10 animals /sex/group (not used for blood parameters estimations) at week 12 and 25 and week 30 (after recovery period). All animals of the study (including satellite group) were necropsied and examined visually. The organs and tissues of each of the animals were separated, cleaned, weighed and/or preserved for microscopic examination. The organs weighed were adrenals, brain, kidneys, liver, pituitary, testes/ovaries, prostate, spleen, thyroid/parathyroid and uterus. The organs fixed in formalin, methanol and Davidson's stain before microscopic examination were adrenal, bone marrow

(femur), eye/optic nerve, zymbal glands, muscle Quad, skin and mammary glands, sciatic nerve, seminal vesicle, femur marrow and artificial surface, ileum, cecum, colon, rectum, uterus, prostate, urinary bladder, sternum+Marrow, testes+epididymedis, liverX2, salivary glands, mesenteric/mandibular lymph nodes, spleen, thyroid/parathyroid, pancreas, duodenum, jejunum, spinal cord (cervical, thoracic, lumbar), lungs, bronchi, kidneys, nasal turbinate, nasopharynx, esophagus, stomach, aorta, larynx, pituitary, ovaries, brain, heartX3, thymus, trachea and gross lesions.

Results:

a. Observed Effects: Four out of 10 animals included in 14 mg/kg/day treatment group had convulsions on first day of treatment. Uncoordinated movements and/or reduced activity were seen in these animals immediately after dosing. Therefore the dose 14 mg/kg/day was reduced to 10 mg/kg/day from study day 2.

b. Mortality: Eight animals of the study died and these were 1 female belonging to control and 7 animals of 14/10 mg/kg/day treatment group. The animal number, week of death and comments on each of the animals died, are shown in the following table (table taken from sponsor submission vol 1:1, pp 050):

Mortality Table			
Group Number	Animal no/sex	Week of Death	Comments
1	125F	30	Died during bleed procedure
4	76m	14	Found Dead post dose
	77M	14	Found dead post dose
	173F	14	Found dead post dosing.
	199F	26	Died during bleed procedure
	223M(S)	22	Found Dead post dosing
	229M(S)	12	Found dead prior to dosing
	259F(S)	23	Found Dead post dosing

(S) = satellite group animal

Three animals (# 173, 77 and 76 died approximately 4 hr post dosing with no clinical signs. Animal # 223 showed convulsions before death. The other animals had no signs before deaths.

c. Body Weight/Food Consumption/Water consumption: The animals included in 2, 7 and 14/10 mg/kg/day treatment groups gained similar body weights. On week 26, the mean final weights were 517.6, 517.4, 522.1 and 502.1 g for males and 298.3, 301.4, 301.4 and 301.1 g for females included in 0, 2, 7 and 14/10 mg/kg/day treatment groups, respectively. On week 26, the mean food consumption/animal was 23.3, 24.1, 23.9 and 22.6 g/day for the males and, 16.8, 17.4, 17.5 and 17.3 g/day in females included in 0, 7 and 14/10 mg/kg/day treatment groups.

d. Hematology/Coagulation/Bone Marrow Changes: No treatment or dose related statistically significant changes were seen in study animals.

e. Clinical Chemistry/Urinalysis Changes: No dose or treatment related changes of clinical importance were reported during the study.

f. Drug Plasma Concentrations: Intravenously administered palonosetron attained a peak plasma concentration in a dose-related manner in rats. On day 1, the plasma concentrations (C_{max}) of 310.1 ng/ml was lower than other the concentration observed at other intervals because compound did not achieve the peak at this time. From week 5 to 26, the plasma concentrations were dose proportional (see table). The concentrations of the compound from on study week 5 to 26 were not very different, thus the compound did not accumulate. The plasma concentrations of males were slightly higher than females. The pharmacokinetics parameters of the compound in rats on day 1 and week 13 of the study are shown below (table data extracted from sponsor's tables in vol 1.1, pp 47):

Dose	Sex	AUC _(0-24hr)		C _{max} (ng/ml)				T _{max} (hr) (mg/kg/day)			Day 1	Wk 5	
		Wk 26	Day 1	Wk 5	Wk 26	Day 1	Wk 5	Wk 26					
2	M		291.4	412.1	613.3	310.1	382.9	330.8	0.167	0.167	0.167		
7	M			939.8	1446.1	2024.5	1226.5	1922.2	1733.9	0.167	0.167	0.167	
10	M			2075.2/ 1253.8	2290.5	3787.4	1854.0	2736.1	2460.7	0.167	0.167	0.167	0.167
2	F			268.3	291.8	410.8	323.9	351.2	380.1	0.167	0.167	0.167	0.167
7	F			928.2	1027.0	1493.1	1206.7	1176.9	1337.5	0.167	0.167	0.167	0.167
10	F			1919.2/ 1182.8	1622.3	2151.7	2586.3/ 1417.6	1682.9	1779.4	0.167	0.167	0.167	0.167

The plasma concentrations (C_{max}) from study week 5 to week 26 were similar in males and females therefore the data of week 13 were not shown in the above table. C_{max} seemed to be not achieved on day 1 and the values were dose dependent though not proportional. AUC values were dose related but non-proportional during the study (see above table). Mean AUC_(0.167-24hr), C_{max} values in males and female rats were similar.

The estimated toxicokinetic parameters of metabolite RS-17825-007 indicated that the metabolite was formed in a dose related manner. The amount of metabolite was consistently more in males than females, therefore no conclusion was made. The AUC values were 1262.18, 715.5, 931.3 and 890.1 ng.hr/ml in males and 965.5, 868.9, 663.2 and 708.62 ng.hr/ml in females on day 8, week 14, 27 and 40 of the study of the animals belonging to 10 mg/kg/day treatment group. The peak plasma concentration of the metabolite was reached within 0.389 to 0.625 hr in males and 0.278 to 0.5 hr in females of the study.

g. Organ Weight Changes: No organ weight changes were seen among males and females included in treatment groups of the study during 26 weeks of treatment and recovery periods.

h. Pathological Changes: Ovarian cyst was seen in 1 female of 10 mg/kg/day treatment group and, red color ovaries were reported in 0, 1, 3 and 2 out of 20, 20, 20 and 19 females included in 0, 2, 3 and 14/10 mg/kg/day palonosetron treatment groups. The injection sites of 1 and 1 out of 20 and 19 males and, 0 and 1 female out of 20 and 18 females included in 3 and 14/10 mg/kg/day treatment groups.

i. Histopathological Changes: No treatment related effects were seen in animals excepting agonal congestion/ hemorrhage was seen in ovaries of 0, 20, 100 and 10.5% females included in 0, 2, 7 and 10 mg/kg/day treatment groups.

In summary intravenously administered 14 mg/kg/day palonosetron produced convulsions, reduced activity and deaths among animals. The dose was adjusted and the study doses of 2, 7 and 10 mg/kg/day produced non-proportional plasma concentrations and bleeding at the site of injection. The identified target organ of toxicity were site of injection and central nervous system and, 10 mg/kg/day and 7 mg/kg/day were identified as the 'highest tolerable and 'no effect' doses, respectively.

5. One Month Oral Toxicity Study with RS-25259-197 in Rats:
(Report No. 6329)

Testing Laboratories: Syntex,
Palo Alto, CA

Study Started: July 16, 1992

Study Completed: April 2, 1993

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

Animals: CrI:CD^RBR VAF/Plus Sprague Dawley Rats; 8 weeks of age; Males: 232-299 g, Females: 196-250 g

Lot No./Drug Batch Nos.: # PA15303-61S/12005, 12006, 12008, and 12009

Methods: Five groups of rats (10/sex/group) were orally administered RS-25259-197 (by gavage) at doses of 0 (vehicle), 6, 18, 60, and 180 mg/kg/day for 1 month in a total dose volume of 10 ml/kg. The vehicle used in the present study 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 purified water, USP). Doses were selected on the basis of a 2-week dose range finding study in which administration of RS-25259-197 at doses of 10, 30, and 100 mg/kg/day produced no mortality or pathologic changes. All rats were examined daily for mortality and clinical signs of toxicity. Body weights were determined at predose, weekly and terminally, whereas food consumption measurements were recorded weekly. Ophthalmoscopic examinations were conducted on all animals at predose and in the last week of treatment. Blood samples (volume not indicated) were collected from all animals immediately before euthanasia by cardiocentesis for determination of hematology and clinical chemistry. Additional blood samples (1.5 ml) were collected approximately 15 min post dosing on the first day (5 lowest numbered rats/group) and during the last dosing week (5 highest numbered rats/group) for determination of plasma drug levels. Attempts were made to collect urine samples for urinalysis near the end of the last week of dosing. Rats in all groups underwent complete gross examinations, with determination of organ weights for heart, liver, adrenal glands, pituitary glands, spleen, thymus, brain, ovaries, uterus/cervix, prostate gland/seminal vesicles, testes, and kidneys. Tissues from control and high dose animals and from rats, which

died or were killed in extremis, as well as any tissue, which showed gross alterations, were also subjected to histological examinations.

Results:

1. **Observed Effects:** Treatment related observed effects were only evident at the 180 mg/kg dose and were limited to a rough coat (1 of 10 males and 3 of 10 females) and urogenital staining (5 of 10 females). The Sponsor also indicated that treatment-related salivation occurred, but that the incidence of this latter observation was not recorded.

2. **Mortality:** One rat at the 18 mg/kg dose and 3 rats at the 60 mg/kg dose died on day 1. However, death in all four rats attributed to the anesthesia used to obtain blood samples for plasma drug levels. In addition, 1 rat at the 180 mg/kg dose died of an intubation error during the third week of dosing. Thus, no apparent treatment-related mortality was observed in the study.

3. **Body Weight/Food Consumption:** At the 180 mg/kg high dose, RS-25259-197 produced a suppression of body weight gain (28.4% suppression in females and 29.6% suppression in males). However, no effects on body weights were observed at the lower doses. No treatment-related changes in food consumption were observed.

4. **Hematology:** Male and female rats which received the 180 mg/kg dose, showed decreased hemoglobin (-4.7% and -9.68%), hematocrit (-4.57 and -9.54%) and lower platelet counts (-25.0% and -24.97%), respectively. Females at the 180 mg/kg dose also showed lower mean corpuscular volume (-7.26%) and mean corpuscular hemoglobin (-7.49%). Finally, males at the 180 mg/kg dose had higher leukocyte counts (56.42%) as a result of increased lymphocyte (55.05%) and monocyte counts (from 48.7/mm³ to 308/mm³). Overall, however, the aforementioned effects were considered mild as most fell within the range of historical control values.

5. **Blood Chemistry:** Treatment-related alterations in blood chemistry parameters were observed at the 60 and/or 180 mg/kg doses and included: increased triglyceride levels (54.3 and 200% in males at the 60 and 180 mg/kg doses and 114.1% in females at the 180 mg/kg doses); increased total bilirubin levels (114.2 and 128.6% in males at the 60 and 180 mg/kg doses, respectively); increased phosphorus (26.4% in females at the 180 mg/kg doses); decreased aspartate aminotransferase levels (-38.6% in males at the 180 mg/kg dose and -23.1% and -35.4% in females at the 60 and 180 mg/kg doses) and dose-dependent decreases in alkaline phosphatase levels (-22.5 to 51.6% males and females at the 60 and 180 mg/kg doses). In addition, males and females at the 180 mg/kg dose levels showed lower sodium (from 145.97 and 144.35 meq/L to 140.31 and 140.18 meq/L, respectively), chloride (from 99.4 and 100.7 meq/L to 93.7 and 95.4 meq/L, respectively), total protein (-14.8 and -17.9%, respectively), albumen (-9.41% and -11.1%, respectively), and globulin (-24.5% and -33.3%, respectively), with increased A/G ratios (21.31% and 44.6%, respectively). Finally, lower cholesterol levels (-39.8%) were seen in females at the 180 mg/kg dose. In general, most of the aforementioned changes were within the range of historical control values, with the exceptions of the changes in

chloride and total protein (males and females at the 180 mg/kg doses), triglycerides (males at the 180 mg/kg dose) and globulin (females at the 180 mg/kg doses).

6. **Urinalysis:** No treatment-related effects on urinalysis parameters were observed.

7. **Ophthalmic Examinations:** No treatment-related ocular effects were observed.

8. **Organ Weights:** Treatment-related organ weight changes included; at the 180 mg/kg dose: reduced absolute and relative weights for testes (-19.51% and -8.98%, respectively) and accessory sex organs (-26.9% and -17.83%, respectively) in males; reduced absolute and relative weights for thymus (-22.7% and -20.93%, respectively) in females, and higher absolute and relative weights for livers in both males (10.44% and 25.7%, respectively) and females (24.9% and 32.21%, respectively) at the 180 mg/kg dose. Finally increased adrenal gland weights were observed for males at the 18, 60 and 180 mg/kg doses (increases in absolute and relative weights of 21.3, 13.1, and 42.6% and 21.5, 13.2, and 60.4%, respectively) and at the 180 mg/kg dose in females (15.8% and 23.04%, respectively).

9. **Gross Pathology:** Gross pathological findings included: enlarged adrenal glands in 2 of 10 females each at the 60 and 180 mg/kg doses; a small thymus in 1 of 10 females at the 180 mg/kg dose group and small testes, epididymis, prostate gland, and seminal vesicles in 1 of 10 males in the 180 mg/kg dose group.

10. **Histopathology:** All 10 males at the 180 mg/kg dose had centrolobular hepatocellular swelling, due to foamy cytoplasm. Liver sections from 3 of the aforementioned animals also showed increased positivity of periodic acid-Schiff reagent (indicative of increased glycogen content), with necrosis of the liver seen in 1 of 10 males at the 180 mg/kg dose. Periportal hepatocellular swelling was also observed in 3 of 10 rats at the 60 mg/kg dose and 1 of 10 males at the 18 mg/kg dose. Eight of 10 males at the 180 mg/kg dose also showed degeneration/necrosis of the seminiferous epithelium in the testes and immature spermatogenic cells in the epididymis and prostate atrophy in 1 of 10 males at the 180 mg/kg doses. Finally, 2 of 10 males at the 180 mg/kg dose had islet hemorrhage/fibrosis syndrome of the pancreas. In contrast, histological findings in females were limited animals at the 180 mg/kg dose and included thymic lymphoid depletion (5 of 10 females) and possibly necrosis of the glandular tissue of the salivary glands (2 of 10 females). The four rats that died on the first day of dosing all had pulmonary hemorrhage, edema and periportal hepatocellular swelling. These latter findings were attributed to anesthesia and blood collection. The high dose female which died showed esophageal changes consistent with an intubation error.

11. **Plasma Levels of the Drug:** Plasma concentrations measured at 15 min after dosing on day 1 and during week-4 are presented in Table 5 below.

Table 5. Mean Plasma Concentrations of RS-25259-007 at 15 min Following Oral Administration of RS-25259-197 in Rats.

Dose (mg/kg)	SEX	Plasma Concentration (ng/ml)	
		Day 1	Week 4

6	M	4.21 ± 2.45	5.81 ± 2.20
6	F	6.02 ± 2.46	165 ± 190
18	M	33.2 ± 10.3	56.8 ± 43.4
18	F	306 ± 61.1	392 ± 174
60	M	1210 ± 502	457 ± 124
60	F	2010 ± 1050	1450 ± 790
180	M	4150 ± 1360	1990 ± 1020
180	F	5050 ± 1700	2460 ± 1550

Briefly, the data in Table 5 show that increasing oral doses of RS-25259-197 in the rat produced increases in mean plasma concentration which were linear, but disproportional to dose (higher than expected). Females showed greater plasma concentrations compared to males. Plasma concentrations on day 1 and at the 4 week interval were comparable at the 6 and 18 mg/kg doses,

In conclusion, oral administration of RS-25259-197 to rats at doses of 6, 18, 60, and 180 mg/kg produced salivation and a rough coat and suppression of body weight gain at the 180 mg/kg dose, with no treatment-related mortality observed. Alterations in hematology [reduced Hb, Hct, platelets (both sexes), MCV and MCHC (females) and increased leukocytes (males)], seen at the 180 mg/kg dose were mild and generally within the range of historical controls. Rats dosed at the 60 and 180 mg/kg doses RS-25259-197 showed alterations in blood chemistry including: increased levels of triglycerides, total bilirubin, and phosphorus and decreased liver enzymes (alkaline phosphatase and aspartate aminotransferase). In addition, decreased sodium, chloride, total protein, albumin, globulin and cholesterol levels were observed at the 180 mg/kg doses. Target organs of toxicity currently identified included; in males at the 180 mg/kg dose: the liver (increased weights, hepatocellular swelling and glycogen deposition) and testes (reduced weights, degeneration/ necrosis of the seminiferous epithelium and immature spermatogenic cells in the epididymis) and in females at the 180 mg/kg dose: the thymus (reduced weights/small thymus and thymic lymphoid atrophy). The 18 mg/kg dose was the no effect oral dose in rats.

DOGS

1. Intravenous 1-Month Toxicity Study with RS-25259-197 in Beagle Dogs: (Report No 5963)

Testing Laboratories: Syntex Research, Palo Alto, CA

Study Started: July 15, 1991

Study Completed: October 25, 1991 (Report Date February 28, 1992)

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

Animals: Beagle Dogs; 10-16 months of age

Lot No./Drug Batch Nos.: 13977-86 and 13977-140

Methods: Male and female dogs (3/sex/group) were i.v. administered either vehicle or RS-25259 at doses of 0 (vehicle), 1, 3 or 10 mg/kg/day) in a total dose volume of 1 ml/kg for 1 month. Animals were observed daily for clinical signs of toxicity with body weights were measured at predose, weekly and terminally. Food and water consumption was not recorded, but the sponsor did not observed anorexia. Ophthalmoscopic examinations were conducted at predose and during the last week of treatment. Blood and urine samples were collected from all animals at predose and at the end of the last week of treatment for determination of hematology and clinical chemistry and urine analysis. At the end of the study, a complete necropsy was done on each dog. Protocol specified tissue were weighed and prepared for histopathological examination. Organ weights and organ to body weight ratios were calculated.

Results:

1. **Achieved Doses:** Same as intended
2. **Observed Effects:** Dogs given 1 and 2 mg/kg exhibited no overt signs of toxicity. At 10 mg/kg RS-25,259 produced transient salivation and/or red ear discoloration, which abated within 15 min post dosing. Although emesis was present in all groups, there appeared to be a greater incidence in RS-25259-197 treated groups, however, this was not dose related.
3. **Mortality:** None.
4. **Body Weights:** Slight body weight loss (<10%) was seen in all groups and was not treatment-related. The sponsor did not food and water intake, but reported no anorexia.
5. **Hematology:** There were no treatment related hematological changes observed
6. **Blood Chemistry/ Urinalysis:** A decrease in serum calcium and cholesterol were observed at 10 mg/kg dose in males, these values were within the normal range.
7. **Electrocardiography and Ophthalmic Examinations:** No treatment-related effects on ophthalmoscopic changes were observed. The sponsor in response to the division inquiry stated that the effects on conduction parameters were not monitored in the current study. Such information was vital for the safety of the compound and should be included in all future toxicity studies in dogs.
8. **Organ Weights:** An increase in the absolute weight (142%) and relative weights (73%) of prostate gland in 1 male of 3 mg/kg dose was observed, however, this finding was isolated and appeared to be unrelated to drug treatment.

9. Gross/Microscopic Pathology: Sporadic necropsy findings of multifocal red/purple discoloration of lungs, thymic atrophy and hemorrhagic foci in the mucosa of urinary bladder (result of catheterization for the collection of urine) were also observed. No treatment-related changes were observed.

10. Plasma Levels of the Drug: Not measured

In conclusion, the 3.0 mg/kg dose was the no effect dose in the present study.

2. Palonosetron Hydrochloride: 28-Day Intravenous Toxicity Study in Juvenile Dogs

Report No: _____ Report Number 1063/17-D6154

Conducting laboratory and location: _____

Date Started: January 14, 2000

Date Completed: July 2000

GLP compliance: Statements of compliance with _____ Statutory Instrument 1999 No. 3106, The Good Laboratory Practice Regulations 1999 and OECD Principles on Good Laboratory Practice (revised 1997, Issued January 1998) were included; however, there were no signatures.

QA- Report: Yes (X) no ()

Methods: In a 28-day intravenous toxicology study, juvenile dogs received palonosetron at doses of 0, 1, 3, or 6 mg/kg/day. Dose levels were based upon a dose range finding study (CLE study 1063/16) in which juvenile beagle dogs were found to tolerate intravenous doses up to 6 mg/kg/day for a 2-week treatment period.

Dosing:

- **Species/strain:** Eight "purpose-bred" female dogs with sufficient offspring at approximately 1 week of age were removed from the _____ and transferred to the testing laboratory. The day of parturition was termed day 0 of age. The mother and offspring were acclimatized for 1 week prior to the start of the study. Offspring were randomly assigned to treatment groups. Offspring, which were not assigned to treatment groups, were maintained in the litters throughout the study. Two litters were used for each group with offspring from each litter receiving treatment at the same dose level. The adult female dogs, at approximately 2 weeks after the completion of the dosing period, were screened for clinical signs of toxicity and exposure to the test article, and subsequently returned to the _____

- **#/sex/group or time point:** 4 juvenile dogs/sex/group

- **age:** At the start of treatment, the age of animals ranged from 9 to 18 days with a mean age of 13 days.

- **weight:** Mean group body weights ranged from 691-1087 g for male dogs and 686-971 g for female dogs. Due to the small number and variable ages of offspring at the time of randomization into groups, it was not possible to ensure the offspring's body weights were within $\pm 20\%$ of the mean at the start of treatment.

- **satellite groups used for toxicokinetics or recovery:** None.
- **dosage groups in administered units:** 0, 1, 3, and 6 mg/kg/day.

- **route, form, volume, and infusion rate:** The vehicle or drug solution was administered by bolus intravenous injection at a rate of approximately 5 mL/min into the cephalic vein using a dose volume of 1 ml/kg.

Drug, lot#, radiolabel, and % purity: Palonosetron, batch number 30893-P105 (Purity 99.4%).

Formulation/vehicle: The vehicle was sodium chloride and sodium phosphate adjusted to pH 7.4 with sodium hydroxide or hydrochloric acid in Water for Irrigation.

Observations and times:

- **Clinical signs:** All animals were monitored daily for clinical signs of toxicity. In addition, each offspring was given a daily detailed physical examination. Mothers and offspring were observed twice daily for moribundity/mortality.

- **Body weights:** Body weights of offspring were measured weekly.

- **Food consumption:** Food consumption was not measured due to the age of the animals.

- **Ophthalmoscopy:** Ophthalmic examinations were performed on all offspring during week 4 of treatment.

- **EKG:** Electrocardiographic examinations of all offspring were performed during week 4 of treatment at 2 hr after dosing. Examinations were performed using fixed limb leads, I, II, and III, augmented leads, aVR, aVL, and aVF, and chest leads, V₁ to V₄. Heart rate was derived from lead II with the exception of animal #23 where V₂ was used.

- **Hematology:** Blood for determination of hematology parameters was collected at 1 week prior to the start of dosing and during week 4 of treatment.

- **Clinical chemistry:** Blood for determination of clinical chemistry parameters was collected at 1 week prior to the start of dosing and during week 4 of treatment.

- **Urinalysis:** Not performed.

- **Gross pathology:** On the day following the last treatment, each surviving offspring was sacrificed. All dosed offspring from control and treatment groups were submitted to necropsy examination. Bone marrow smears were prepared at necropsy; however, they were not examined.

- **Organs weighed:** Absolute organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate, spleen, testes + epididymides, thyroids + parathyroids, and uterus.

- **Histopathology:** Gross lesions from all offspring and tissues, listed below, were embedded in paraffin wax BP, sectioned at a nominal 5 µm, stained with hematoxylin and eosin, and examined by the study pathologist. Tissues submitted to histopathological examination were as follows: adrenal glands, bone marrow smear (sternum), brain, cecum, colon, duodenum, eyes with optic nerves, femur with bone marrow and articular surface, heart, ileum, jejunum, kidneys, liver, lungs with mainstem bronchi, mammary, mandibular lymph nodes, mesenteric lymph nodes, esophagus, ovaries, pancreas, pituitary gland, prostate, salivary glands, sciatic nerves, skin, spinal cord (cervical, lumbar, and thoracic), spleen, sternum with bone marrow, stomach, testes + epididymides, thymus, thyroids + parathyroid, trachea, urinary bladder, and uterus.

- **Toxicokinetics:** Blood for measurement of plasma levels of palonosetron and its metabolite, RS-17825-007, was collected on days 1 and 28 at 0.167, 1, 3, 8, and 24 hr after

dosing. Samples were analyzed for palonosetron and RS-17825-007 by

- **Other:** The study offspring received a course of routine treatment for endo-parasites and a course of vaccinations as follows: 3-4 weeks of age, vaccination for kennel cough with Intrac[®] (live modified Bordetella bronchiseptica vaccine); and 4-5 weeks of age, oral worming on three consecutive days Panacur[®] (Fenbendazole). Pups from one litter that received palonosetron at 1 mg/kg/day (study animals 7, 8, 23, and 24) were also treated for apparent eye infections. Animal #7 received Synulox[®] (clavulanate potentiated amoxicillin) for 8 days starting 4 days prior to the initiation of treatment, Orbenin[®] eye cream (benzathine cloxacillin) for 5 days starting 1 day prior to the initiation of treatment, and chloromycetin for 8 days starting on day 9 of treatment. The right eye of this animal did not respond to treatment, and the animal was sacrificed for humane reasons on day 18. Animal #8 received Synulox[®] for 5 days prior to the start of treatment. Animals #23 and #24 both received Synulox[®] for 4 days prior to the start of treatment.

Results:

- **Clinical signs:** There were no treatment-related clinical signs. The sporadic observations of loose feces were noted for dogs at 3 mg/kg/day. There were sporadic observations of loose feces and vomiting for dogs at 6 mg/kg/day.

- **Mortality:** There was no treatment-related mortality. One dog that received palonosetron at 1 mg/kg/day was sacrificed on day 18 of treatment for humane reasons due to an eye infection. Tissues from this animal were submitted to gross pathological and histopathological analyses.

- **Body weights:** Body weight gains were impaired for male treatment groups, although, there was no dose response relationship. Body weights for male controls on days 1 and 28 of treatment were 691 and 2104 g, respectively. Body weight gains for male dogs at 1, 3, and 6 mg/kg/day were 78.6, 72.8, and 84.5% of the control, respectively. Body weights for female controls on days 1 and 28 of treatment were 686 and 1718 g, respectively. Body weight gains for female dogs at 1, 3, and 6 mg/kg/day were 87.2, 83.3, and 112.0% of the control, respectively.

- **Ophthalmoscopy:** No treatment-related ophthalmic changes were observed; however, no data was provided for independent verification.

- **EKG:** Electrocardiographic examinations during week 4 at 2 hr after dosing found no treatment-related changes of heart rate. The sponsor provided no data for electrocardiographic intervals of the heart, although, the description of procedures suggested that it might have been possible to measure these intervals from the data collected.

- **Hematology:** Small changes of hematological parameters were observed that appeared to have no biological significance. Hemoglobin concentration, red blood cell counts, and hematocrit for female dogs at 6 mg/kg/day were increased to 114.6, 108.2, and 114.5% of control values (8.2 g/dl, $4.02 \times 10^6/\text{mm}^3$, and 27.6%), respectively. Platelet concentrations for male dogs

-agonal congestion/ hemorrhage	0	0	0	0	0	0	0	1
Heart -epicarditis	0	0	0	1	0	0	0	0
Kidney -inflammatory cell foci	0	0	0	0	0	0	0	1
Stomach -barbiturate lysis ^a	0	0	0	0	0	0	0	1
Liver -barbiturate lysis ^a	0	0	0	1	0	1	0	0
Gall bladder -lymphoid hyperplasia	0	0	0	0	0	0	0	1

No description for the term "barbiturate lysis" could be found.

- **Toxicokinetics:** Plasma AUC values for palonosetron on days 1 and 28 were proportional to dose. Plasma AUC values for RS-17825-007 on day 1 were greater than proportional to dose. Plasma AUC values for RS-17825-007 on day 28 were approximately proportional to dose. Plasma AUC values for palonosetron and RS-17825-007 on day 1 were greater than those observed on day 28, which may be the result of augmented clearance associated with repetitive dosing or increased maturity of animals. C_{max} values for palonosetron on day 28 were greater than those observed on day 1. The plasma T_{max} for palonosetron on day 28 was 0.167 hr for all male and female treatment groups. There were no gender-related differences in AUC values for palonosetron or RS-17825-007.

Plasma pharmacokinetic parameters for palonosetron on days 1 and 28 in dogs that received palonosetron at intravenous doses of 1, 3, and 6 mg/kg/day.

Dose Mg/kg	Day 1						Day 28			
	AUC _{0.167-8hr} , ng hr/mL		C _{max} , ng/mL		T _{max} , hr		AUC _{0.167-8hr} , ng hr/mL		C _{max} , ng/mL	
	M	F	M	F	M	F	M	F	M	F
1	325.07	390.46	222.04	221.48	0.167	0.445	262.21	253.40	317.64	330.90
3	1113.94	1337.16	704.82	845.36	0.167	0.167	859.45	853.33	1073.26	953.17
6	2014.63	2517.05	1276.94	1604.13	0.167	0.167	1422.27	1579.49	1682.28	1957.95

Plasma pharmacokinetic parameters for RS-17825-007 on days 1 and 28 in dogs that received palonosetron at intravenous doses of 1, 3, and 6 mg/kg/day.

Dose Mg/kg	Day 1						Day 28					
	AUC _{0.167-8hr} , ng hr/mL		C _{max} , ng/mL		T _{max} , hr		AUC _{0.167-8hr} , ng hr/mL		C _{max} , ng/mL		T _{max} , hr	
	M	F	M	F	M	F	M	F	M	F	M	F
1	250.96	437.61	68.83	117.93	1.000	1.667	66.76	98.69	66.32	70.52	0.167	0.167
3	978.35	1208.56	315.16	377.74	1.000	1.000	387.28	390.27	240.19	235.82	0.167	0.167
6	2615.76	2922.82	854.43	957.64	1.000	1.000	818.35	707.64	525.48	461.56	0.167	0.167

Key Study Findings: In a 28-day intravenous toxicology study, neonatal/juvenile dogs received palonosetron at doses of 0, 1, 3, or 6 mg/kg/day. At the start of treatment, animals ranged in age from 9 to 18 days with a mean age of 13 days. The dose of 6 mg/kg/day could be considered a tolerated dose. There was no target organ of toxicity. Electrocardiographic examinations during week 4 at 2 hr after dosing found no treatment-related changes of heart rate. The sponsor provided no data for electrocardiographic intervals of the heart, although, the description of procedures suggested that it might have been possible to measure these intervals from the data collected.

3. 1-month Oral Subacute Toxicity Study in Dogs (Study No. 17-D-92-25259-197; AT6328)

Testing Laboratory: Syntex Research, Palo Alto, CA.

GLP & QAU Requirements: The statement of compliance was included.

Date of start and Completion of Study: July 10, 1992 and March 31, 1993.

Animals Used: Twenty four healthy beagle dogs (12/sex) approximately 10 months old and weighing between 8 to 14 kg were used in the study.

Methods: These dogs were divided into 4 groups (3/sex/group) and administered daily oral dose of either 0, 2, 6 or 20 mg/kg/day of RS25259-197 for 30 days. The compound was dissolved in vehicle (phosphate buffer in water) and administered in a volume of 1 ml/kg/day. The daily dose volumes (1 ml/kg/day) were adjusted according to mean body weights. Doses were based on the result of a 14-Day dose range finding study No. Syntex 708-D-92. In this study, only transient clinical changes were present in animals included in 20 and 40 mg/kg/day treatment groups. In the present study, general condition, food consumption and body weights were recorded before the first dose, once/week and at termination of the study. Slit lamp ophthalmic examination was performed before treatment and during last week of treatment. EKG tracing (limb lead II) were recorded twice before the initiation of treatment and once on day 1 and last day of study. Blood and urine samples for hematological and blood chemistry parameters were collected from fasted animals once before dosing and on the day of necropsy. The plasma levels of the compound were determined in the blood samples collected approximately 1 and 4 hr postdosing on the 1st day and during the last dosing week of the study. At termination of the study, each animal in all of the groups was necropsied and organs, endocrine glands and tissues were separated, examined for the pathological abnormalities, weighed and fixed in formalin. Additionally, the testes were examined from 2 and 6 mg/kg/day treatment groups. The tissues separated, examined and weighed were: liver, brain, heart, kidneys, ovaries, pituitary glands, prostate and uterus. A complete histopathological examination was performed on the tissue isolated from the animals of the control and 20 mg/kg/day treatment groups and those died during the study.

Results:

1. **Observed Effects:** Hypersalivation was seen in 1 and 3 animals out of 6 animals included in 6 and 20 mg/kg/day treatment groups, respectively.
2. **Mortality:** None.
3. **Body Weight/Food Consumption/ Water Consumption:** No significant retardation of body weight gain of clinical importance was seen in either male or female dogs treated up to 20 mg/kg/day dose for 4 weeks. No data on food intake of the animals was recorded
4. **Hematology/Coagulation/Bone Marrow Changes:** No treatment related changes in hematological parameters were seen in male and female animals belonging to any of the treatment groups.
5. **Blood Chemistry/Urinalysis Changes:** No changes in blood chemistry parameters were seen among animals included in treatment groups.
6. **Vital Signs/Physical Examination/Ophthalmic Examination:** No treatment related abnormal signs or an abnormality in slit lamp examination or EKG changes were observed.
7. **Organ Weight Changes:** Testes weights of dogs included in 20 mg/kg/day treatment groups was decreased. No other changes in the absolute and relative (to body weight ratio) weights were observed.
8. **Gross Pathology Changes:** None
9. **Histopathological Changes:** Autopsy reports showed thymic atrophy and hemorrhagic bleeding in lungs. These observations were sporadic thus considered not treatment related.

In summary, RS25259-197 at a daily dose of 20 mg/kg for 1 month produced a treatment related plasma concentration, hypersalivation and reduction in testis weight without any associated histopathological changes. Sponsor should have used higher than 20 mg/kg/day dose to identify the target organs of toxicity.

4. **3-Month Oral Toxicity Study in Dogs**
(Study # 43-D-94-25259-007; AT6787)

Testing Laboratory: Syntex Discovery Research, Palo Alto, CA.

GLP & QAU Requirements: The statement of compliance was included.

Date of start and Completion of Study: October 29, 1993 and November 17, 1994.

Animals Used: Beagle dogs, about 20 months old weighing between 10.7 and 11.8 kg (males) and 9.3 to 9.9 kg (females) were used in the study.

4. Hematology/Coagulation/Bone Marrow Changes: No treatment related hematological changes were seen in male and female animals belonging to any of the treatment groups.

5. Plasma Drug Levels/Blood Chemistry/Urinalysis Changes: The plasma concentrations of RS25259-197 and RS-17825-007 (N-oxide metabolite) increased in a dose related manner. AUC_{0-6hr} of the compound in animals included in 1, 5 and 20 mg/kg/day treatment groups are shown in the following table:

Table
Mean Plasma Concentration AUC_{0-6hr} of RS-25259-007 and RS-17825 in Dogs

Plasma Concentrations (ng.hr/ml)	Sex	Control	TREATMENT GROUPS (mg/kg/day)		
			1	5	20
RS-25259-007	F	--	18.0+15.3	239+200	1190+277
	M	--	40.3+19.7	187+95	1410+956
RS-17825-007	F	--	85.4+30.3	333+156	2480+411
	M	--	79.7+41.5	327+95	1640+591

Treatment related plasma concentrations of RS-25259-007 and RS-17825-007 were seen during the study. Sponsor did not determine dog plasma levels after the administration of the selected doses of the study, i.e., 2, 10 and 40 mg/kg/day, either on day 1 or at the termination of the study. The peak plasma concentrations was reported in 2 hr of the administration of the compound (study # DM 1015) and the half life of the compound was 2 hr.

6. Vital Signs/Physical Examination/Ophthalmic Examination: No treatment related abnormal sign/s were seen in slit lamp examination.

7. Organ Weight Changes: The organ weights of the animals were not affected during the study.

8. Gross Pathological Changes: None

9. Histopathological Changes: Glycogen depletion in liver was seen in 1/sex animal included in 40 mg/kg/day treatment group. There were no other treatment related histopathological changes reported in these animals.

In summary, RS-25259 attained a dose dependent plasma concentration up to a daily dose of 20 mg/kg. The highest dose of 20 mg/kg/day of RS25259-007 was tolerated well by both male and female dogs. The plasma concentration of the compound after the highest dose of 40 mg/kg/day of the study was not determined during the study. Since the half life of the compound was 2 hr, the spacing of two daily doses the compound should have been 2 hr instead of 6 hr. The highest dose could be greater than 40 mg/kg/day as the target organs of toxicity and MTD could not be identified in the study.

5. 40-Week Chronic Intravenous Toxicity Dogs with 4 Weeks of Recovery: (Study # 1063/5; PALO-99-10)

Testing Laboratory: _____

GLP and QAU Requirements: The study conducted in compliance with GLP and QAU audits requirements (the certificate was not signed)

Dates of Start and Completion of the Study: June 8, 1999 and August ,2000

Animals: Purebred beagles _____ months old with mean body weight of 7.06 to 8.44 kg (females) and 8.27 to 8.89 kg (males).

Methods: Twenty dogs/sex were divided in to 4 main study groups and two 4-week recovery groups. The main study groups (4/sex/group) were given intravenous bolus doses of 0, 1, 3 or 6 mg/kg/day palonosetron (Lot # 30893-P105, 99% pure) for 9 months (39 weeks). Two dogs in 0 and 6 mg/kg/day treatment groups were maintained for 4 weeks of recovery period to observe the reversibility of the adverse effect/s. The doses of the oral study in dogs were selected on the contention that sufficient plasma levels of Palonosetron will be achieved by oral doses and data could be used for the assessment of the safety of an intravenous clinical study. The peak plasma concentration (C_{max}) of 122 and 16.4 ng/ml and $AUC_{(0-24hr)}$ values of 194 and 24.22 ng.hr/ml of parent compound were achieved by an intravenous and oral dose of 0.5 mg/kg Palonosetron in dogs (data in following Table). Its estimated bioavailability as compared with intravenous dose was 12.5%. The ratios of total metabolites to unchanged drug in dog plasma by a single i.v. and oral dose of 0.5 mg Palonosetron was 1.9 and 42.4. The compound was metabolized in similar manner by these routes of administration and there were quantitative differences in the amounts of metabolites. The animals will be exposed to higher plasma concentration of parent compound by intravenous route than by oral route of administration. Therefore, data generated by an oral study will not give much useful information for a study with an intravenous dose of the compound.

Comparison of Pharmacokinetics of Single 0.5 mg/kg Dose of R-25259-007 in Dogs		
Parameter	I.V.	Oral
C_{max} (ng/ml)	122	16.4
T_{max} (hr)	0.083	1.0
Half-Life (hr)	1.87	2.17
$AUC_{(0-24 hr)}$ (ng \cdot hr/ml)	194	24.22+
Estimated Bioavail. (%)	—	12.5

NOTE: * = AUC value of 0-96 hr; ** = $AUC_{(0-24 hr)}$

The increased amounts of these plasma metabolites by oral route was due to large first pass effect in liver. Thus, orally and intravenously administered compound had different pharmacokinetics and consequences of higher plasma concentrations of metabolites achieved by oral route, is not yet known. Sponsor's contention on the change of route of administration in 9-

month dog study is not valid and not acceptable. Sponsor should conduct the needed 9-month chronic intravenous toxicity study in dogs. Sponsor should conduct this study according to previously submitted protocol (amendment # 56 dated November 10, 1998). The proposed 9-month chronic oral toxicity study in dogs can not substitute for the intravenous study.

The PK of metabolite RS17825-007 was also determined during the study. The dose schedule and the number of animals in each group are shown in the following table.

TABLE FOR STUDY DESIGN

Group	No. of Animals		Dose (mg/kg/day)	Main Study		Recovery*	
	Male	Female		Male	Female	Male	Female
1	6	6	0 (Vehicle)	4	4	2	2
2	4	4	1	4	4	--	--
3	4	4	3	4	4	--	--
4	6	6	6	4	4	2	2

* Recovery animals will be maintained for 4 weeks after dosing is terminated.

The dose selection of the 9-month chronic toxicity study was based on 1-month i.v. toxicity study in dogs conducted at the doses of 0, 1, 3 and 10 mg/kg/day. The animals of 10 mg/kg/day treatment group had transient ataxia. This was MTD and selected as the highest dose of the present study but animals treated with 10 mg/kg/day produced convulsions, ataxia and loose stools in the present study and the dose was reduced to 6 mg/kg/day. This was the high dose during the remaining period of the study. The animals in the high dose group were treated at the dose of 5 mg/kg/day for first week and no clinical observations were seen, the dose was increased to 10 mg/kg/day for 2 days during week 2 and the animals showed convulsions, the dose was reduced to 6 mg/kg/day from day 3 of 2nd week to week 40. The study animals were observed daily for toxicity, mortality and morbidity. The body weights were recorded prior to treatment and weekly during the treatment and recovery periods. The food consumption was recorded daily. Electrocardiogram recordings were taken by using fixed limb leads, I, II and III and the augmented leads aVR, aVL and aVF (heart rate determined from lead II). The hematological and blood chemistry parameters were determined on blood samples collected from jugular vein on day 0 and on week 13, 25, 39 and 44. Ophthalmoscopic examination was done prior to treatment and on week 39. The drug levels of palonosetron and RS-17825-007 in all animals were determined on day 1 and 8 and in week 14, 27 and 40. The body orifices were examined for discharge, blood etc. and all animals of the main study groups were killed and organs separated, cleaned weighed and preserved. The organs separated were blood, gall bladder, adrenal, ileum, aorta, cecum, bone marrow/smear (femur), eye+optic nerve, rectum, skin+mammary gland, uterus, urinary bladder, muscle quadriceps, prostate glands, sciatic nerve, seminal vesicle, testes+Epidy., articul. surface, ovaries, sternum+marrow, saliv.glands, liver, mandibular lymph nodes, mesenteric lymph node, thyr.+parathyrid, spleen, heart, pancreas, lungs+bronchi, duodenum, esophagus, trachea and gross lesions, were fixed in 10% neutral formalin, metanolor Davidson solution before examining for histopathological abnormalities. The data was statistically analyzed by using two-way analysis (ANOVA) and pairwise comparison with control group animals for each sex by using Dunnet's test.

Results:

- a. **Observed Effects:** Salivation, staggering gait and convulsions were seen in 2 animals # 35 and 37 included in 10 mg/kg/day treatment group. Animal # 35 was removed and replaced with a new animal. Slight ataxia was seen from the time of injection to 4 hr in 3 males and 2 females included in 6 mg/kg/day treatment group and , in 2 males of 3 mg/kg/day treatment group. Soft to loose feces was reported in 1 male of each of 3 and 6 mg/kg/day treatment groups during the treatment period.
- b. **Mortality:** One male of control group was replaced because of GI disturbances. All other animals survived up to the end of the study period. •
- c. **Body Weight/Food Consumption/Water Consumption Changes:** The body weight changes among males of treatment groups were comparable with control group males. There was a retardation of 9.9, 6.6 and 25.7% in the body weight gain in females included in 1, 3 and 6 mg/kg/day treatment groups. The food consumption of females included in 6 mg/kg/day treatment group was decreased. Among males, the food consumption was not significantly affected and it was 415.5, 400, 403.7 and 392.9 g among males included in 0, 1, 3 and 10.0 mg/kg/day treatment groups.
- d. **Hematology/Bone Marrow Changes:** The counts and the percent of reticulocytes were increased ($p < 0.05-0.01$) among males of 6 mg/kg/day treatment group and no change was seen among females of this group.
- e. **Clinical Chemistry/Urinalysis Changes:** No dose or treatment related changes of clinical importance were reported during the study.
- f. **Drug Plasma Concentrations:** A dose related plasma concentration was reported in dogs. On day 1, the plasma concentrations ($AUC_{0-\infty}$) were 288.1, 918.5 and 1520.7 ng.hr/ml in males and, 307.2, 903.1 and 1447.0 ng.hr/ml in females included in 1, 3 and 6 mg/kg/day treatment groups, respectively. On week 40, AUCs were: 430.8, 1474.2 and 2205.0 ng.hr/ml in males and, 447.5, 1411.4 and 2285.6 ng.hr/ml in females of 1, 3 and 6 mg/kg/day treatment groups. The toxicokinetic parameters of palonosetron in dogs on day 1 and week 13 are shown below (data from sponsor tables vol 1.3, pp 35 and 36):

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Table 5.2.2:6 Mean Toxicokinetic Parameters Derived from Individual Plasma Profiles in Dogs Treated with IV Palonosetron

Dose (mg/kg/d)	Mean AUC _(0-168 hr) in ng h/ml					Mean C _{max} in ng/ml				
	Day 1	Day 8	Week 14	Week 27	Week 40	Day 1	Day 8	Week 14	Week 27	Week 40
Male (n=4-6)										
1	288.1	344.2	433.6	373.7	430.8	387.3	344.9	374.2	302.3	330.9
3	918.5	1025.6	1480.3	1380.6	1474.2	1186.1	1183.3	1619.8	1224.2	1217.0
5 ^a	1520.7	-	-	-	-	1868.4	-	-	-	-
10 ^b	-	3108.4	-	-	-	-	3081.8	-	-	-
6 ^c	-	-	2343.0	2136.6	2208.0	-	-	2336.3	1886.9	1715.7
Female (n=4-6)										
1	307.2	369.8	451.8	505.4	447.5	382.9	448.6	482.8	459.8	369.8
3	903.1	965.3	1191.6	1377.6	1411.4	1560.8	1330.0	2152.6	1430.3	1544.2
5 ^a	1447.1	-	-	-	-	2160.6	-	-	-	-
10 ^b	-	2857.8	-	-	-	-	3082.7	-	-	-
6 ^c	-	-	2629.8	2283.2	2285.6	-	-	2354.3	2225.2	1627.1

^a High-dose during Week 1 (build-up phase).

^b High-dose for Day 1 and 2 of Week 2; discontinued due to excessive toxicity.

^c High-dose from Day 3 of Week 2 onwards.

Source: Tables TK 1 (p. 161), TK 2 (p. 162), TK 4 (p. 164) and TK 5 (p. 165) of study report PALO-99-10.

The plasma concentrations (C_{max}) on study week 14 and 40 and other time intervals during the study, i.e., day 8 and week 27 (data not shown in the table) were similar. C_{max} was achieved on day 8 and not on day 1 therefore its values were low at this time. AUC values were dose proportional during the study (see above in table). Mean AUC and C_{max} values in male and female animals were similar.

The AUC values of the metabolite RS-17825-007, were similar on study week 8, 14, 27 and 40 and these were: 1262.2, 715.5, 931.3 and 890.1 ng.hr/ml in males and 965.5, 868.9, 663.2 and 708.62 ng.hr/ml in females belonging to 6 mg/kg/day treatment group. The plasma concentration of the metabolite was reached within 0.39 to 0.625 hr in males and 0.28 to 0.5 hr in females.

Physical Examination/EKG/Ophthalmoscopic Examination Changes: Heart rate and blood pressure were not affected during the study. EKG tracings were used only to observe the possible effect of the compound on heart rate of the animals.

Organ Weight Changes: No treatment or dose related organ weight changes were seen among males and females included in treatment groups of the study during the treatment and recovery periods.

Pathological Changes: No treatment or dose related changes were reported in males and females included in study treatment groups during 26 weeks of treatment and recovery periods.

Histopathological Changes: The incidences of thymus cyst were slightly more (3 in treated and 1 in control group). Only 1 female included in 6 mg/kg/day treatment group had the cyst. There were no other adverse effects in treated males in 0, 1, 3 and 6 mg/kg/day treatment groups.

In summary, intravenously administered palonosetron attained dose proportional concentrations and these were generally similar in males and females during the study. The high dose produced adverse effect of ataxia, staggering gait and soft to loose stools and salivation in animals. A dose of 3 mg/kg/day was identified as the 'highest tolerable dose' and the central nervous system was the target organ of toxicity.

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CARCINOGENICITY STUDIES:

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3-Month Oral Dose Ranging Study in Mice
(Study # 719-M-94-25259-197; AT6751)

Testing Laboratory: Syntex Discovery Research, Palo Alto, CA.

GLP & QAU Requirements: The statement of compliance was included.

Date of start and Completion of Study: December 2, 1993 and September 9, 1994.

Animals Used: CrI:CD-1^R (ICR)BRVAF/Plus strain mice about 7 weeks old weighing between 29 to 36 g (males) and 21 to 31 (females) g were used in the study.

Methods: One hundred (50/sex) mice were divided into 5 groups (10/sex/group) and administered daily oral dose of either 0, 30, 60, 90 or 120 mg/kg/day of RS-25259-197 (as a free base) for 3 months. The compound was dissolved in vehicle containing sodium acetate and acetic acid (final pH 5.0±0.2) and administered in a volume of 5 ml/kg/day. The daily dose volumes of 5 ml/kg/day were adjusted according to mean body weights. Doses were based on the result of 2-week oral dose range finding study No. Syntex 708-M-94. In this study, no clinical toxicity was seen at 30 mg/kg/day but mortality was seen among animals included in 150 mg/kg/day treatment group and only a transient clinical signs of inactivity, labored respiration and rales were seen in 100 mg/kg/day treatment group. In the present study, general condition, food consumption and body weights were recorded before the treatment, once/week of the study and at termination of the study. Slit lamp ophthalmic examination was performed once before treatment and during week 12 of the study. Blood samples for the estimation of the plasma concentration of the compound were collected from 5 mice/sex/group approximately 30 min after 85th dose. Blood samples for hematological assays and plasma for clinical chemistry parameters were collected from fasted animals immediately before the necropsy of animals. Urine samples were collected at the end of the study. At termination of the study, each animal in all of the groups was necropsied and tissues were separated, examined for the pathological abnormalities. Adrenals, brain, eye, sciatic nerve, spinal cord, heart, liver, pancreas, pituitary glands, salivary glands, stomach, accessory sex organs spleen, testes, thymus, thyroid/parathyroid glands, uterus, urinary bladder, lungs, trachea, femur, sternum, spine, stifle joint, skeletal muscle, lymph nodes, spleen, thymus gland, mammary glands, ovaries, uterus, vagina, kidneys and any altered tissues/organs were separated and weighed after cleaning. A complete histological examination was performed on the tissue isolated from the animals of the control and 120 mg of RS-25,259-197/kg/day treatment groups and all of those animals which died during the study.

Results:

Observed Effects: Hypersalivation, convulsions (1/sex belonging to 120 mg/kg/day group), cold to touch and inactivity were seen in animals included in 90 and 120 mg/kg/day treatment groups. Four animals (2/sex) included in 120 mg/kg/day treatment group showed clonic convulsions and 5 males showed inactivity.

Mortality: Nine and 6 animals in 90 and 120 mg/kg/day treatment groups were found dead. All of the dead animals in 90 mg/kg/day treatment group were males. These were 4 males and 5 females in 120 mg/kg/day treatment group. Most of the animals among 120 mg/kg/day treatment group died from week 1 to 6 of the study and ataxia, inactivity and convulsions were the signs before death of these animals. None of the animals included in 60 mg/kg/day treatment groups died during the study. Two females (1 each in control and 30 mg/kg/day treatment groups) died of gavage accident during the study.

Body Weight/Food Consumption/ Water Consumption: The body weight gains in either male or female mice included in 30, 60, 90 and 120 mg/kg/day treatment groups were comparable with those control animals. No change in food intake of the animals was seen. The terminal body weights of male and female mice included in control group were in the range of 34 to 43 (mean weight=37.9±0.74) and 32 to 39 (mean weight=35.5±0.67) g respectively. Mean daily food intake on week 13 of the study, was 6.0, 5.5, 5.7, 5.0 and 5.7 g in males and 5.7, 5.5, 5.9, 5.7 and 5.5 g in females, belonging to control, 30, 60, 90 and 120 mg/kg/day treatment groups, respectively.

Hematology/Coagulation/Bone Marrow Changes: No treatment related changes in hematological parameters of clinical or statistical importance were seen in male and female animals belonging to any of the treatment groups.

Blood Chemistry/Urinalysis Changes: The blood concentrations of RS25259-197 and RS-17825-007 were increased in a dose dependent manner in animals included up to 60 mg/kg/day treatment groups among males (See following table). The dose proportionality with RS 25259-197 and RS-17825-007 was not seen in animals included in 90 mg/kg/day or higher treatment groups. The blood concentrations were not increased in males and females included in 120 mg/kg/day treatment group, as the plasma concentrations in 90 and 120 mg/kg/day treatment groups were similar. Therefore the plasma saturation of the compound reached at this dose in both the sexes. In a clinical study, an oral dose of 3 to 80 ug/ml maintained a plasma concentration proportional to the dose within 4 hr of its administration.

Table
Mean Blood Concentration (ng/ml) of RS-25259-007 and RS-17825 in Mice

Plasma Concentrations	Sex	TREATMENT GROUPS (mg/kg/day)				
		Control	30	60	90	120
RS-25259-007	F	--	802±222	1150±248	1490±614	1900±291
	M	--	864±177	1490±312	2000±360	2070±277
RS-17825-007	F	--	1040±78	1350±169	1560±550	1830±369
	M	--	898±96	1570±545	1890±387	1890±105

No changes in blood chemistry parameters were seen among animals included in treatment groups. In the text of the study, sponsor stated that a large amount of protein was excreted in the urine of female mice. This was not correct as the table on urinalysis of female animals showed

that the protein excretion in urine was only slightly increased from trace to +1 in control vs from +1 to +2 in animals included in 120 mg/kg/day treatment group. This is not of clinical importance.

Vital Signs/Physical Examination/Ophthalmic Examination: No treatment related abnormal sign or an abnormality in slit lamp examination.

Organ Weight Changes: Accessory male sex organ (epididymides, prostate and seminal vesicle) absolute weight was decreased by 8.7, 5.3, 7.2 and 25.4 % in animals included in 30, 60, 90 and 120 mg/kg/day treatment groups respectively. The relative weight of the accessory sex organs to body weight was also decreased by 1.6, 3.7, 6.5 and 21.2 % in the respective treatment groups. The significant ($p < 0.01$) difference was seen in animals included in 120 mg/kg/day treatment group.

Gross Pathology: The incidences of eye opacity were 0, 4 and 2 in males and 1, 0 and 3 in females belonging to control, 90 and 120 mg/kg/day treatment groups respectively. Lung discoloration was seen in 3 males in each of 90 and 120 mg/kg/day treatment groups, and in 1 female belonging to each of the control, 30, 90 and 120 mg/kg/day treatment groups. The incidences were not dose related, therefore not due to treatment of RS-25259-197.

Histopathological Changes: Congestion and hemorrhage in lungs of 3 males belonging to 90 and 120 mg/kg/day treatment groups were seen. Two, 1 and 1 females belonging to 0, 90 and 120 mg/kg/day treatment groups also showed congestion of lungs.

In summary, a daily dose of 120 mg/kg of RS25259-197 was lethal in both male and female mice and a dose of 90 mg/kg/day was lethal in males. It produced a decrease in absolute weights of accessory male organs, pulmonic congestion and bleeding. Therefore lungs and male sex organs were identified as the target organs of toxicity in this study. A dose of 60 mg/kg/day produced only a minor change in absolute and relative weights of accessory sex organs without any histopathological alterations, therefore this dose was considered as a 'MTD' for males in the study. A dose of 90 mg/kg/day was non-lethal in females, therefore it was identified as MTD for the females in the proposed study.

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MOUSE CARCINOGENICITY STUDY:

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FDA/CDER CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) RODENT
CARCINOGENICITY FACTSHEET

NDA 21-372

DATE:

REVIEWER: Yash M. Chopra, M.D., Ph.D.

DIVISION: Division of Gastrointestinal and Coagulation Drugs Products, HFD-180

DRUG NAME: Palonosetron

SPONSOR: Helsinn HealthCare SA,
Lugano, Switzerland

LABORATORY:

CARCINOGENICITY STUDY REPORT: March 26, 2002

PRIOR FDA DOSE SELECTION CONCURRENCE: Yes, Executive –CAC meeting on
February 21, 1995

DRUG CATEGORY: Serotonin (5-HT₃) - Receptor Antagonist

MUTAGENIC/GENOTOXIC (y/n/equivocal/na; assay): y/Palonosetron was positive in a *in vitro* chromosomal aberration test in Chinese hamster ovarian cells; negative in Ames test, mouse bone marrow micronucleus test, HGPRT test in Chinese hamster ovarian cells.

1. MOUSE CARCINOGENICITY STUDY: Two control groups and 3 treatment groups

STUDY DURATION: 104 weeks

STUDY STARTING DATE: April 7, 1999

STUDY COMPLETION DATE: March 26, 2002

GLP COMPLIANCE: Yes

QAU REPORT: Yes

DRUG LOT# AND PURITY: RS-25259-197 (lot # 1304981/Mfg. Lot#30893 P104 & 1360411/
Mfg. Lot # 30893-P106)

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Drug Purity and stability: 99.0 to 100% pure and the stability and homogeneity of the samples of the solutions was stable for up to 48 hr at room temperature and for 15 days at 5 to 10°C.

MOUSE STRAIN: CD-1 mice

ROUTE: Oral Gavage

NUMBER OF ANIMALS:

Control: Two control groups 56/sex/group

Low, Mid and High Dose groups: 56/sex/group

One Additional untreated group – Health monitoring group of 14 animals/sex

Satellite Groups: Three treatment groups of 21/sex/group

BASIS OF DOSE SELECTION: (MTD, AUC ratio, saturation, maximum feasible): MTD

The dose selection was based on 3-month oral dose ranging study in mice. This study was conducted in 5 groups of animals at the oral gavage doses of 0, 30, 60, 90 and 120 mg/kg/day palonosetron. The target Organs of toxicity were lungs (histopathological findings of congestion and clinical Signs of gasping, labored breathing and raling at 90 and 120 mg/kg/day in both Sexes), central nervous system (convulsions at 120 mg/kg/day in both sexes) and male accessory sex organs (significant decrease in testicular weights in 120 mg/kg/day group males). A dose of 60 mg/kg/day, devoid of any clinical signs of toxicity or histopathology was identified as the MTD for both sexes. Adequate information on plasma levels of drug in the mouse and man are not available for dose determinations.

PRIOR FDA DOSE CONCURRENCE: Yes

Based on the recommendations of EX-CAC meeting dated February 21, 1995, doses of 0, 10, 30 and 60 mg/kg/day were used in 104-week mouse carcinogenicity study.

MOUSE CARCINOGENICITY STUDY (Conclusive; negative; positive; MF; M; F): Orally administered palonosetron in mice did not produce increase in the incidences of treatment related tumors in animals.

MOUSE STUDY COMMENTS: Palonosetron produced a dose related dose proportional plasma concentrations in the mouse and male animals plasma concentrations were higher than females of the study. Palonosetron treatment did not produce tumor in the study animals.

COVERSHEET FOR CARCINOGENICITY STUDY IN MOUSE

1. Report No.: PALO-99-108 (one study)
2. Name of Laboratory: _____
3. Strain: Crl:CD-1 (ICR)B
4. No./sex/group: 56/sex/group in 2 vehicle control and 3 treatment groups; 3 satellite groups of 7 mice/sex/group and 1 health monitoring group consisting of 14/sex untreated animals.
5. Interim Sacrifice: No
6. Basis for dose selection stated: Yes
7. Interim sacrifice: No
8. Total duration (weeks):
9. No. alive at termination:

<u>Treat</u> <u>Grs.</u>	<u>Male</u>	<u>% Survival</u>	<u>Female</u>	<u>% Survival</u>
O	25	44.6	21	37.5
O2	29	51.8	24	42.9
L	25	44.7	26	46.4
M	28	50	18	32.1
H	28	50	13	23.2

10. Statistical Methods Used: 1. Survival data analyzed by 2-tailed log rank trend test for an increase in mortality and 2-tailed pairwise test for each treatment group against control (PETO, R. et al 1980), 2. Log Rank Method for Mortality less than 10, 3. Incidence Tumor rates analyzed in 2 sexes separately by Life table method (PETO, R. et al 1980), for fatal tumors the life-table time strata will be the weeks the death occurred, the tumor incidences analyzed by the method of PETO, R. et al 1980, trend test will be used, 4. The incidental and fatal information will be combined in to single test statistics (PETO, R. et al 1980) and trend test applied.

11. week/site for first tumor:

Male		Female	
Gr	Week/Tumor Occurrence	Gr	Week/Tumor Occurrence
C1	41/Malignant Lymphoma H'poitic Tumor	39/Malignant Lymphoma H'poitic Tumor	
C2	30/Malignant Lymphoma H'poitic Tumor	30/Malignant Lymphoma H'poitic Tumor	
L	34/Benign Harderian Gland adenoma	22/Malignant Lymphoma H'poitic Tumor	
M	26/Malignant Lymphoma H'poitic Tumor	30/Malignant Lymphoma H'poitic Tumor	
H	17/Malignant Undifferentiated Sarcoma	25/Malignant Lymphoma H'poitic Tumor	

12. Attach Tumor and Non-tumor Data For each Tissue: See Appendix I and II.

Palonosetron Hydrochloride Carcinogenicity Study by Oral Gavage Administration to CD-1 Mice for 104 Weeks

Key Study Findings: Palonosetron treatment produced dose related and dose proportional plasma concentrations. The palonosetron treatment did not produce the tumor incidences greater than study control groups mice.

Study Number: PALO-99-18

Sponsor or contract lab: Helsinn Healthcare SA
Lugano (Switzerland)

Test Facility: _____

Date of Initiation: May 18, 1999

Date of Completion: March 26, 2002

GLP Compliance: Statement of compliance with GLP and QAU were attached

QA Report: Yes (X) No ()

DRUG LOT#: RS-25259-197 (lot # 1304981/Mfg. Lot#30893 P104 & 1360411 (Mfg. Lot # 30893-P106)

Drug Purity & stability: 99.0 to 100% pure and the stability and homogeneity of the samples of the solutions was stable for up to 48 hr at room temperature and for 15 days at 5 to 10°C.

Study Characteristics: Based on the recommendations of E-CAC and Division letter dated March 9, 1995 sponsor initiated the study. Palonosetron was administered in 5 groups of Crl:CD-1 mice at oral gavage doses of 0, 0 10, 30 and 60 mg/kg/day. Reference to Dose-range-finding study (appendix): 3-Month oral dose ranging study in mice

STUDY PROTOCOL DESIGN AND METHODS

Doses: 0, 0, 10, 30 and 60 mg/kg/day

Route of Administration: Gavage (X)

Dual control applied: yes

Species/strain of animals used: Crl:CD-1(ICR)BR(CD-1) mice

Number of animals per group: Main Study 5 groups – 56/sex/group and 3 satellite groups - 7/sex/treatment group

(additionally 8/sex animals were included in high dose treatment group and Group #6 consisted of 14/sex untreated animals for health monitoring)

Age and Mean Body weight at the initiation of Treatment: 35 to 42 days old, 28.8 to 30.3 g (males) and 22.9 to 23.4 g (females)

The animals were housed individually or group of 2 animals of same sex/cage.

Basis of Dose Selection: 3-month toxicity study in mice (a GLP and QA report)

CAC Concurrence: yes

Dose selection based on E-CAC recommendations (a copy E-CAC report attached as an appendix)

Frequency of Drug Administration: single daily dose

Controls Employed: 2 identical vehicle treated control groups and 1 health check group (7/sex). The number of animals, dose schedule and different groups' composition is shown below:

Composition and identity of treatment groups

Animals were assigned to the groups as follows:

Group	Treatment	Dosage (mg/kg/day)	Main Study			
			Cage numbers		Animal numbers	
			Male	Female	Male	Female
1	Control	0	1-28	152-179	1-56	303-358
2	Control	0	29-56	180-207	57-112	359-414
3	Palonosetron	10	57-84	208-235	113-168	415-470
4	Palonosetron	30	85-112	236-263	169-224	471-526
5	Palonosetron	60	113-144	264-295	225-288	527-590
6	Health check		145-151	296-302	289-302	591-604

Group	Treatment	Dosage (mg/kg/day)	Satellite Study +			
			Cage numbers		Animal numbers	
			Male	Female	Male	Female
3	Palonosetron	10	303-309	324-330	605-625	668-688
4	Palonosetron	30	310-316	331-337	626-646	689-709
5	Palonosetron	60	317-323	338-344	647-667	710-730

+ Satellite study animals were used for toxicokinetic sampling only.

Interim Sacrifices: Not done

Satellite PK or Special Study Group(s): Blood samples (0.3 ml) from retro-orbital sinus from 3/sex/group were collected on day 1, week 26, 52, 78 and 104 for assessing TK of the compound and metabolite RS-17825-007 by _____

Unscheduled Sacrifices or Deaths: animals in extremis were killed, examined externally for lesions and tumors, dissected and organs separated for microscopic examination and, their blood samples collected for hematology parameters.

Clinical Observations: The study animals were examined twice daily for the adverse effects during the study. Tabular or graphical presentation by dose group and time of observation were prepared for: Week 1 daily, week 2 and 4 twice weekly, week 5 to 13 once each week and week 14 onward once each 2 weeks. Animals weighed before the start of treatment (week 0), once a week for 14 weeks, then once every 4 weeks thereafter and on week 104

before necropsy. Absolute body weights and relative (percent difference from control) body weight gains were estimated. Graphical presentation of group mean body weight gain for each group over the course of the study was given. Graphical presentations of average food consumption for each group over the course of the study were constructed. The mortality/# of deaths during course of study was estimated daily and debilitated animals separated and animals in extremis killed and full necropsy done. At time of terminal sacrifice complete necropsy performed. Tabular and graphic presentation of cumulative group mortality and graph for percent survival of all animals of the study were prepared over the course of the study.

Ophthalmoscopy examination was not done.

Hematology parameters were estimated on the blood samples collected at week 104, from retro-orbital sinus collected under anesthesia for hematology parameters. Relative changes by dose and sex per group at different timings determined.

At necropsy, tissues of all the animals of the study were dissected and cleaned. The following tissues were separated and weighed: brain, epididymides, heart, kidneys, liver, lungs including bronchi, ovaries, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes, thymus and uterus and cervix. Tabular presentations for absolute and relative organ weights were given. Organ weights were not routinely recorded for animals killed or dying prematurely. At sacrifice the organs of all main study group animals were evaluated for an abnormality. The histopathological examination of tissues used for the estimation of organ weight (described above) and other tissues like adrenals, brain, femur, heart, kidneys, liver, lungs including bronchi, mammary area, spinal cord, sternum stomach, thyroid and uterus was performed.

Samples of the following tissues were preserved in 10% neutral buffered formalin with the exception of testes and epididymides, which were initially placed in Bouin's fluid and subsequently retained in 70% industrial methylated spirit, and eyes, which were placed in Davidson's fluid. These tissues were adrenals, optic nerves, aorta - thoracic, ovaries, brain, pancreas, cecum, pituitary, colon, prostate, duodenum, rectum, epididymides, salivary gland, one eye only, sciatic nerve - one only, femur, seminal vesicles, gall bladder, skeletal muscle - thigh, harderian glands, spinal cord, heart, spleen, ileum, sternum, jejunum, stomach, kidneys, testes, lacrimal glands, thymus, liver, thyroid with parathyroid, lungs with main stem bronchi, tongue, lymph nodes - mandibular and mesenteric, trachea, urinary bladder, mammary area - caudal, uterus with cervix, esophagus, vagina. Samples of other abnormal tissues were also retained for histopathological examination. In addition to any masses, the lymph nodes drains and tissues adjacent to those masses were also preserved.

The tissues and organs of animals of all main study groups were examined. The tables of the incidence of non-neoplastic and neoplastic findings were prepared.

The mean body weight and food consumption/animal/week of the study were calculated; homogeneity of variance using Bartlett's test was tested. The significance of the test was calculated by using Fisher's Exact test. The treatment group findings were compared using Mantel test for a trend in proportions and also pairwise Fisher's exact test for each dose with control group. If Bartlett's test for variance was not significant at 1% level, then parametric analysis was used. Tabular presentation of significant non-neoplastic and neoplastic findings

For dual identical controls, comparison of treated groups to each separate control, and to pooled controls was conducted. Sponsor in the study, provided the background range (minimum to maximum) of the tumors of the laboratory but did not provide the time period of obtaining the range and table of historical controls were not submitted. Sponsor was asked to send the historical control data of the laboratory from the year 1998-2002. The present review is based on the data used by sponsor in their summary tables of tumors.

RESULTS:

1. **Clinical Observations:** The hair loss, reduced body temperature, pallor and, increased incidences of opaque eyes and noisy respiration were seen in animals included in treatment groups. The incidences of opaque eyes were 1, 4, 4, 2 and 8 males and, 1, 1, 6, 5 and 5 females out of 56/sex animals in 0, 0, 10, 30 and 60 mg/kg/day treatment groups. The noisy respiration was in 5, 2, 3, 7 and 9 males and, 2, 2, 5, 1 and 3 females of the study groups.

2. **Mortality:** The mortality rates during the study were 55.4, 48.2, 55.3, 50 and 50% among males and, 62.5, 57.1, 57, 67.9 and 76.8% among females included in 0, 0, low, mid and high dose treatment groups (Data shown in the following table).

No. & % Dead at Study Termination:

<u>Treat</u> <u>Grs.</u>	<u>Male</u>	<u>% Mortality</u>	<u>Female</u>	<u>% Mortality</u>
01	31	55.4	35	62.5
02	27	48.2	32	57.1
L	31	55.3	30	53.8
M	28	50	38	67.9
H	28	50	43	76.8

Sponsor did not describe/determine the cause of death of these animals but the animals were generally debilitated. The incidences of distended duodenum, jejunum, ileum and cecum were more in treated than in the control animals which died during the study. More than 50% of the animals in high dose treatment group were survived on week 90 indicating that the sufficient number of animals were exposed to the drug during the study.

3. **Body Weight and Food Consumption:** On study week 104, the body weights among males included in treatment groups were similar to control groups animals. The body weight of females included in the study was also similar. The data has been shown in the table below. On week 104, the body weights of treated animals of the study were more than control group animals, i.e., by 0, 2.2 and 0.2% in males and, 3.1, 2.6 and 8.2% in females belonging to low, mid and high dose treatment groups animals, respectively. The food consumption was similar in the treated and control animals, i.e., 5.6, 5.1, 6.1, 6 and 6 g/male and, 5, 5, 4.9, 5.3 and 5.3 g/female included in 0, 0, 10, 30 and 60 mg/kg/day treatment groups.

TABLE
Table for Mean Weight (g) of mice

	Control 1	Control 2	Low Dose	Mid Dose	High Dose
Males					
Initial	30.3	30.0	28.8	29.5	29.7
Week 14	41.9	42.8	40.7	41.7	40.8
<i>Percent of Control</i>	100	102.3	97.1	99.5	97.3
Week 26	44.9	44.8	44.2	43.3	42.1
Week 54	46.7	46.6	46.5	46.4	43.8
<i>Percent of Control</i>	100	99.8	99.6	99.8	93.8
Week 74	47.9	48.0	47.6	46.7	45.7
Week 104	44.6	44.5	44.4	45.6	44.7
<i>Percent (of Control)</i>	100.0	99.6	99.55	102.2	100.16
Females					
Initial	23.4	23.0	22.9	22.9	23.3
Week 14	32.6	32.4	32.1	33.7	33.2
<i>Percent of Control</i>	100	99.4	98.5	103.4	101.8
Week 26	34.9	34.7	34.7	36.1	35.9
Week 54	37.8	37.4	38.2	38.1	39.0
<i>Percent of Control</i>	100	98.9	101.0	100.8	103.2
Week 74	40.1	40.1	39.2	40.3	41.1
Week 104	38.0	38.1	39.2	39.0	41.1
<i>Percent of Control</i>	100.0	100.3	103.2	102.6	108.2

FIGURE 3A

Group mean bodyweight versus period of treatment - males

Group	:	1	2	3	4	5
Compound	:	Control	Control	Palonosetron Hydrochloride		
Dosage(mg/kg/day)	:	0	0	10	30	60

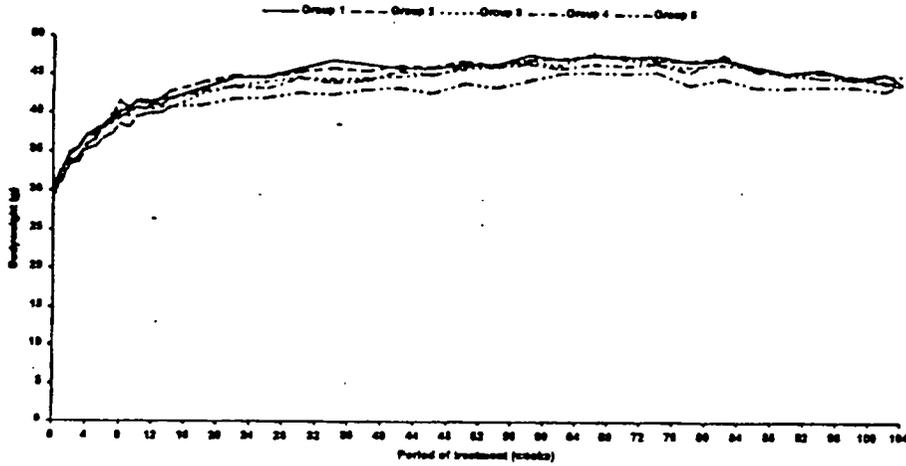
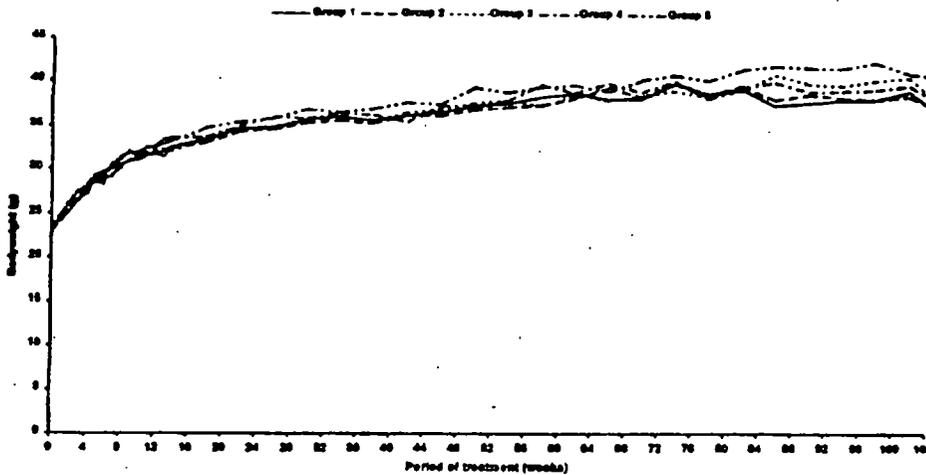


FIGURE 3B

Group mean bodyweight versus period of treatment - females

Group	:	1	2	3	4	5
Compound	:	Control	Control	Palonosetron Hydrochloride		
Dosage(mg/kg/day)	:	0	0	10	30	60



4. **Hematology Changes:** At week 104, the observed changes in the hematology parameters of the animals in high dose treatment groups were only of statistical significance and not of clinical importance or significance.

5. **Toxicokinetic/Plasma concentration Estimation:** On week 26, the steady state plasma concentrations (AUC) were 1360, 3939 and 8620 ng.hr/ml in male and, 535, 1988 and 4462 in female mice. The peak concentrations were reported within 1 hr of the administration. The increase in plasma concentrations were dose proportional on week 26, 52, 78 of the study. The plasma concentrations in males were greater than the females as shown in the following text table 4 (sponsor submission vol 48.381, pp 0031). Its half-life varied from 1.7 to 3.5 hr in males and, 1.5 to 2.4 hr in females on study week 26 to 104. The compound was not cumulated and not detected in blood samples tested after 24 hr of its administration.

TABLE 31

Pharmacokinetic parameters of palonosetron on Day 1 and during Weeks 26, 52, 78 and 104 of 104-Weeks of daily oral administration of palonosetron to male mice

a. **C_{max}**

Sex	Doses (mg/kg/day)	C _{max}				
		Day 1	Wk26	Wk 52	Wk 78	WK 104
M	10	159.83	470.7	310.3	305.5	352.7
	30	638.11	878.5	652.0	1138.1	1093.2
	60	1534.5	1620.5	2071.5	1956.9	1502.4
F	10	198.5	227.9	166.9	139.2	242.0
	30	814.0	665.6	610.8	817.7	681.6
	60	1728.6	1467.1	2550.8	1423.0	1036.2

b. **AUC_(0-24hr) (ng·h/ml)**

Sex	Doses (mg/kg/day)	Day 1	AUC _(0-24hr) (ng·h/ml)			
			Wk26	Wk 52	Wk 78	WK 104
M	10	645	1360	1167	1250 ^a	1680
	30	1985 ^b	3963	3411	5754 ^b	05621
	60	5475	8620	10460	9058 ^a	10889
F	10	404	535	521	556	931
	30	2035 ^a	1988	2307	2410	2069
	60	4623	4462	6052	6443 ^a	5619 ^c

C_{max} values calculated from 3 animals; except ^a2 animals; AUC_(0-24hr) values calculated from 15 samples, except ^a14 samples, ^b13 samples, ^c12 samples

During the study, the chief metabolite RS-17825-007 (M9; N-oxide) in plasma was detected from 15 min to 1 hr of the administration of the compound in male and female mice. Its half life varied from 2.5 to 3.5 hr in both sexes. The pharmacokinetic profile of the metabolite estimated in the study were tabulated and are given below:

TABLE Pharmacokinetic parameters of RS-17825-007 on Day 1, during Week 26, 52, 78 and 104 of 104 weeks of daily oral administration of palonosetron to male mice

a. C _{max} :						
Sex	Doses		C _{max} (ng/ml)			
M	(mg/kg/day)	Day 1	Wk26	Wk 52	Wk 78	WK 104
	10	204.13	236.2	163.97	178.2	175.0
	30	531.71	421.3	339.85	389.6	364.0
	60	1035.16	761.3	596.4	695.8	459.12
F	10	49.44	201.6	216.5	131.7	165.4
	30	521.79	310.4	346.3	288.77	190.9
	60	783.60	678.0	475.9	681.6	552.5

b. AUC _(0-24hr) (ng.h/ml)						
Sex	Doses		AUC _(0-24hr) (ng.h/ml)			
M	(mg/kg/day)	Day 1	Wk26	Wk 52	Wk 78	WK 104
	10	708	786	605	710 ^b	931
	30	1944 ^c	2168	1984	2025 ^d	2015
	60	5398	4881	3797	3530 ^b	4361
F	10	381	410	329	342	578
	30	1201 ^b	914	969	1021	714
	60	3028	2436	2552	2925 ^c	2944 ^d

C_{max} values calculated from 3 animals; except ^a2 animals; AUC_(0-24hr) values calculated from 15 samples, except ^b14 samples, ^c13 samples, ^d12 samples, ^e11 samples

Orally administered palonosetron produced a dose related dose proportional plasma concentration in mice. On day 1, the estimated half lives of the compound was 0.9 to 1 hr in both males and females and these were shorter than at any other time interval, indicated that the uniform peak blood concentrations were not reached on day 1. The study indicated that the plasma concentrations in male animals were higher and for a longer period than in female animals.

6. **Organ Weights Changes:** Slight increases of 12.6 and 21.6% in the absolute weights of kidneys and liver of male rats included in 60 mg/kg/day treatment group were reported. Mean absolute weight of spleen was increased by 2.0, 0 and 46.0% among males and 62.3, 42.0 and 111.6% among females included in 10, 30 and 60 mg/kg/day treatment groups, respectively. The mean absolute weight of uterus + cervix was decreased in a dose related manner, i.e., 39.0, 43.8 and 57.2% in animals of 10, 30 and 60 mg/kg/day treatment groups. The relative weight (in relation to body weight) of uterus + cervix was also reduced and were 1.7, 2.32 and 2.5 times less than the weight of the tissues in control group animals.

7. **Physical Examination/Ophthalmoscopic Changes:** These tests were not conducted.

8. **Gross Pathology Changes:** The incidences of opaque eye were 2, 4, 2, 2 and 7 in males and, 2, 2, 5, 2 and 7 in females included in control 1, control 2, low, mid and high dose treatment groups. Distended ileum was in 0, 1, 2, 5 and 4 males and, 0, 1, 0, 4 females of the study groups. Enlarged lymph node (mesenteric) was in 11, 5, 17, 8 and 12 males and, 6, 8, 11, 9 and 16 males

of the 5 study groups. Small testes in 1 animal in mid and high dose treatment group animals and, uterine cyst in 37, 41, 42, 3 and 46 females of the study groups were reported.

9. Histopathological Changes:

b. Non-Neoplastic Changes: Interstitial testicular cell hyperplasia was increased in mid and high dose treatment groups in a slightly higher number than the control groups. The incidences of acinar hyperplasia of mammary gland were slightly more than the control group animals.

TABLE

Incidences of Non-Neoplastic Lesions in Control and Treatment groups Mice

Histopathological Lesion	Treatment Groups				
	Control 1	Control 2	Low Dose	Mid Dose	High Dose
Male Reproductive Organs:					
Test. Inter. Cell Hyperplasia.	8	9	4	14	16
Rete Tubular Hyperplasia	0	0	0	0	1
Female Mammary Gland					
Acinar Hyperplasia F	11	15	12	18	22
Skin					
Inflammation M	1	2	4	1	7
F	0	7	4	4	5

b. Neoplastic Changes:

No incidences of tumors were seen in animals included in the study and the observed incidences were within the background range (minimum to maximum) of the tumor incidences of the historical control data of sponsor's lab (attached as Appendix VI of the present review).

In summary, palonosetron administered at oral gavage doses of 0, 0, 10, 30 and 60 mg/kg/day for 104 weeks produced a dose related dose proportional plasma concentrations of the parent compound and the metabolite RS-17825 (major human metabolite). The concentrations of the compound were more in males than females during the study. On week 26, the plasma concentrations of the compound were 289.3 and 149.7 times the plasma concentration (AUC values) reported in man with the suggested clinical dose of 5 ug/kg. Palonosetron up to a dose of 60 mg/kg/day produced histopathological changes of interstitial testicular cell hyperplasia in males and hyperplasia of mammary glands in females but did not show any trend to produce tumor. It was considered as non-tumorigenic in mice.

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RAT CARCINOGENICITY STUDY:

APPEARS THIS WAY
ON ORIGINAL

1. Three Months Oral Dose Ranging Study with RS-25259-007 in Rats
(Report No. AT 6665)

Testing Laboratories: Syntex
Palo Alto, CA

Study Started: September 8, 1993

Study Completed: May 24, 1994

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

Animals: Crl:CD[®]BR VAF/Plus Sprague Dawley Rats; 8-9 weeks of age; weight; males: 279-348 g, females: 188-248 g.

Lot No./Drug Batch Nos.: # PA16505-69S

Methods: Five groups of rats (15/sex/group) were orally administered RS-25259-007 (by gavage) at doses of 0 (vehicle), 18, 60, 120, and 180 mg/kg/day for 3 months in a total dose volume of 5 ml/kg. The vehicle used in the present study consisted of 13.2 mg/ml Glacial acetic acid USP and 53.08 mg/ml sodium acetate USP in purified water, USP adjusted to pH 5.0 ± 0.2. Dose selection was based on a 1-month oral toxicity study in rats in which RS-25259-007 (180 mg/kg) produced reduced body weights and histopathologic changes in the liver, testes and thymus, while doses of 6, 18, 60 had no histopathologic effects. All rats were examined daily for mortality and clinical signs of toxicity. Body weights were determined at predose, weekly and terminally, whereas food consumption measurements were recorded weekly. Ophthalmoscopic examinations were conducted on all animals at predose and in the last week of treatment. Blood samples were collected at 1 hr (5 lowest numbered rats/sex/ group), 3 hours (5 middle numbered rats/sex/group) and at 5 hrs (5 highest numbered rats/group) after dosing on Day 1 (0.1 to 0.5 ml) and during the last week of treatment (0.2 to 1.0 ml) for determination of plasma drug levels. Additional blood samples (1.0 ml) were collected from all animals immediately before euthanasia by cardiocentesis for determination of hematology and clinical chemistry. Attempts were also made to collect urine samples for urinalysis near the end of the last week of dosing. After 60 days of treatment surviving males in control, 18, 60 and 120 mg/kg dose groups were transferred to study 41-R-94 in order to evaluate fertility. These males were returned to the study at the end of the breeding period with no interruption of dosing at any time. Rats in all groups underwent complete gross examinations, with determination of organ weights for heart, liver, adrenal glands, pituitary glands, spleen, thymus, brain, ovaries, uterus/cervix, prostate gland/seminal vesicles, testes, and kidneys. Tissues from control and high dose animals and from rats which died or were killed in extremis, as well as any tissue which showed gross alterations were examined microscopically.

Results:

1. **Observed Effects:** Sporadic clinical signs including railing, wasting and unthriftiness were observed at doses ≥ 60 mg/kg, whereas at the 180 mg/kg tremors and convulsions were observed.

2. **Mortality:** Treatment related death occurred in 12 of 15 males and 6 of 15 females dosed at the 180-mg/kg dose. The majority (72%) of the aforementioned deaths occurred from week 4 through 8, with 27% occurring during week 8. One female rat each of the vehicle, 18, 60, and 120 mg/kg/day groups also died during the study. The cause of death in each of the said instances was not described, with the exception of the 120 mg/kg dose female, which died of complications due to bacteremia/septicemia.

3. **Body Weight/Food Consumption:** Body weights in male and female vehicle control rats were increased by 68.7% and 34.8% to final body weights of 519.9 ± 45.76 g and 287.0 ± 23.42 g, respectively, at the end of the study. In comparison, treatment of males with RS-25259-007 produced dose-dependent suppression of body weight gains (8.5%, 13.7%, 30.6%, and 54.5% at the 18, 60, 120, and 180 mg/kg doses, respectively). In females the 18 and 60 mg/kg doses were associated with increased body weight gains (22 and 13%, respectively, relative to control gains), whereas the 120 and 180 mg/kg resulted in suppressed body weight gains of 12 and 11%, respectively, at the end of the treatment period. Although males at the 120 and 180 mg/kg dose groups and females at the 180 mg/kg dose groups showed significant reductions in food consumption (7-30%, mostly during weeks 3-8) no treatment-related differences were apparent by the end of the study. Mean daily food consumption values for control males and females during last week of dosing (week 13) were 5.6, 5.1, 6.1, 5.2 and 5.2 g in males and, 5.0, 5.0, 4.6 and 5.3 g, respectively.

4. **Hematology:** Mild decreases in erythrocytes (8-14%), hemoglobin (7-24%) and hematocrit (6-25%) were evident in males at doses ≥ 60 mg/kg and in females at the 180 mg/kg doses. Males at the 120 mg/kg dose and females at doses ≥ 120 mg/kg also had lower platelet counts (8-21%). Finally higher total counts for leukocytes (60-204%), neutrophils (49-286%), and lymphocytes (55-189%) were seen for males at the 120 mg/kg doses and for females at 120 and 180 mg/kg doses. Effects in males at the 180 mg/kg dose were difficult to determine, since data from only three surviving males were available at this dose. In general, increases in leukocytes, neutrophils, and lymphocytes were more pronounced in females, compared to males.

5. **Blood Chemistry:** Changes in blood chemistry which were limited to surviving males (n=3) at the 180 mg/kg dose included: increased BUN (58%) and creatinine (135%). Increased bilirubin concentrations were also seen in surviving males at the 180 mg/kg dose (from control values of 0.13 mg/dl to 0.77 mg/dl) and in females at the 180 mg/kg dose (from control values of 0.26 mg/dl to 1.0 mg/dl). However, the increases in bilirubin in males were attributed to assay interference by lipemic serum. Other treatment-related effects on blood chemistry included: increased phosphorus (13-34%, at 120 and 180 mg/kg in males and at 180 mg/kg in females) and decreased values for aspartate aminotransferase (23 to 69% at 60, 120, and 180 mg/kg in males and at all doses in females); alanine aminotransferase (30 to 43%, at 120 and 180 mg/kg in males) and alkaline phosphatase (31 to 38%, at 60, 120, and 180 mg/kg in males). RS-25259-007 also produced dose-dependent increases in triglyceride levels (from a control value of 54.3

mg/dl up to 79.3, 79.3, 95.8, and 1289 mg/dl at the 18, 60, 120, and 180 mg/kg doses, respectively) and in females (from a control value of 59.1 mg/dl, up to 126 and 1741 mg/dl at the 120 and 180 mg/kg doses, respectively). The relationship of observed differences in cholesterol levels observed in males at the 60 and 120 mg/kg doses (decreased 16-28%) and at the 180 mg/kg dose (increased 475% in males and 92% in females) is unclear. Finally, lower values for total protein (9-19%), sodium (2-4%) and albumin (7-44%) were observed in males and/or females at the 120 and 180 mg/kg doses.

6. **Urinalysis:** Rats (both sexes) at the 180 mg/kg doses showed increased protein content in the urine compared to controls, with no other treatment-related changes observed.

7. **Ophthalmic Examinations:** No treatment-related ocular effects were observed.

8. **Organ Weights:** Increased absolute and relative weights for adrenal glands (16 to 70% and 33 to 118%); kidneys (10 to 27% and 9 to 58%) and pituitary glands (36% and 25 to 76%) were observed in one or both sexes at doses of 120 and 180 mg/kg, respectively. Males at 180 mg/kg and females at 60 to 180 mg/kg also showed increased absolute weights for liver (13 to 74%), with increased relative weights for liver (6 to 106.5%) also seen in males at all doses and in females at 60 to 180 mg/kg doses. Males at 120 and 180 mg/kg doses and females at all doses showed increased absolute weights for spleen (10 to 55%) with increased relative spleen weights (12 to 81%) seen in both sexes at doses ≥ 60 mg/kg. Reduced thymus weights (32%) were also seen in males at the 180 mg/kg doses. Finally, males at the 120 and 180 mg/kg doses showed reduced absolute and relative weights (47 to 58%) for testes and reduced accessory sex organ weights (16 to 42%).

9. **Gross Pathology:** Gross alterations in male sex organs occurred mainly at doses of 120 and 180 mg/kg and included: small epididymides (5 of 15 and 6 of 15 rats at the 120 and 180 mg/kg doses respectively); small prostate (6 of 15 males at the 180 mg/kg dose); small seminal vesicles (1 of 15 and 11 of 15 rats at the 120 and 180 mg/kg doses, respectively) and small testes (1 of 15, 15 of 15, and 14 of 15 rats each at the 60, 120, and 180 mg/kg doses, respectively). Other gross findings included a small or indistinguishable thymus (4 of 15 females at the 180 mg/kg dose); increased incidence of discolored lungs (7 of 15 males and 4 of 15 females at the 180 mg/kg doses). Finally, 9 of 15 males at the 180 mg/kg dose showed one or more alterations in the GI tract, the most common being that of a distended small intestine and/or stomach 7 of the 9 aforementioned males. Two of the latter 7 rats also had either a nodule and/or fluid filled pocket in the stomach or a thickened stomach. Finally, two other males had either a thickened and discolored stomach or an erosion/ulcer of the stomach, with no other GI lesions. None of the aforementioned gross observations were observed in control animals, with the exception of a discolored lung in 1 of 15 females.

10. **Histopathology:** Table 1 (page 7) shows the incidence of treatment-related histological effects observed in male and female rats following 3 months of oral treatment with RS-25259-007. Briefly, males at doses ≥ 60 mg/kg showed diffuse testicular tubular atrophy. Associated aspermatogenesis and decreased spermatozoa in the epididymis were also seen at the 120 and 180 mg/kg doses. Some males at the 180 mg/kg doses also showed decreased prostate gland and

Adrenal Glands		
Increased cytoplasm in glomerulosa cells....	0 0 0 0 5	0 0 0 0 6
Thyroid Gland		
Increased height of Follicular epithelium.	0 0 0 0 1	0 0 0 0 7
Bone Marrow	0 0 0 0 11	
Decreased cellularity..		0 0 0 0 5
Spleen;		
Lymphoid Atrophy:		
Marginal Zone Lymphoid Sheath.....	0* 0* 0* 6* 13	1 0 0 0 14
Lymphoid sheath itself	0* 0* 0* 0* 0	0 0 0 0 5
Lymphoid Necrosis.....	0 0 0 0 11	0 0 0 0 5
Liver; Congestion.....	0 0 0 0 2	0 1 1 1 6
Hepatocytic Vacuolation	7 0 0 9 11	1 0 0 0 2
Thymus; Atrophy.....		2 0 1 4 12 [□]
Femur; Decreased Trabecular metaphysis..	12* 12* 13* 13*14	
Slight.....	12 12 10 7 2	0 0 0 0 4
Moderate.....	-- 3 5 3	-- -- -- 3
Marked.....	-- -- 1 9	-- -- -- 1
Epididymides		
Decreased Spermatozoa..	0 0 0 11**15	-- -- -- --
	0 0 0 0 10	-- -- -- --
Prostate Gland	0 0 ^{□□} 0 4 12	-- -- -- --
Decreased Secretions...		
Seminal Vesicles	0* 0* 0* 15* 12	-- -- -- --
Decreased Secretions...		
Testes	0* 0* 2* 15* 14	-- -- -- --
Aspermatogenesis.....	0 0 0 0 11	-- -- -- --
Diffuse tubular atrophy.....	3* 3* 2* 3* 9	0 1 1 1 5
Lung; Congestion.....		
Kidney; Chronic Nephrosis syndrome....		0* 1* 0* 4* 5

Group # 1 = Vehicle, 2 = 18 mg/kg, 3 = 60 mg/kg, 4 = 120 mg/kg, 5 = 180 mg/kg RS-25259-007

Note: The number of rats examined for each parameter is as indicated in Number examined: except as noted below:

* and ** = 15 and 11 rats/group, respectively

□ and □□ = 14 and 1 rats/group, respectively

11. Plasma Levels of the Drug: Plasma concentrations measured in blood samples obtained at 1, 3, and 5 hours post dosing on day 1 and during the last week of scheduled dosing are presented in Table 1 below.

Briefly, the data in Table 2 shows that increasing oral doses of RS-25259-007 in the rat produced

TABLE 1
MEAN PLASMA CONCENTRATIONS (ng/mL) AND AUC VALUES
OF RS-25259-007 FOLLOWING SINGLE DAILY ORAL DOSES
OF RS-25259-007 TO RATS
STUDY 42-R-94-25259-007-PO-TX

Day	Gender	Time (hr) After Dosing	Dose (mg/kg/d)				
			0	18	60	120	180
Day 1	Females	1	0	128	655	647	1680
		3	0	193	380	671	1160
		5	0	0	446	1230	842
0-5 hr AUC (ng-hr/mL)			0	578	2190	3540	5660
Day 1	Males	1	0	11.0	174	319	501
		3	0	0 ^a	134	273	528
		5	0	0	70.5	392	717 ^a
0-5 hr AUC (ng-hr/mL)			0	16.5	600	1420	2220
Last Day	Females	1	0	227	896	1320	3190 ^b
		3	0 ^a	36.2	512	836	1610
		5	0	20.5 ^a	417	1070	1470 ^c
0-5 hr AUC (ng-hr/mL)			0	433	2790	4720	9480
Last Day	Males	1	0	29.4	431	943	1820 ^c
		3	0	14.7	348	1030	10900 ^b
		5	0	1.62	144	617	NS
0-5 hr AUC (ng-hr/mL)			0	75.1	1490	4090	ND

Mean values calculated from data report IAR-B-2047. N=5 except. ^a N=4, ^b N=1, ^c N=3.
ND is not determined. NS denotes no samples submitted. AUC is the area under the
concentration-time curve calculated by the linear trapezoid rule.

linear increases in mean plasma concentrations and AUC values. In females, increases in plasma concentrations were generally proportional to dose and similar on day 1 and during the last week of dosing. However, in males increases in plasma levels and AUC values were disproportional to doses and values during the last week of the study were much greater than those seen on day 1. On day 1, females showed greater plasma concentrations compared to levels in male rats in samples collected similar times at comparable doses. Similar though less dramatic differences were still evident during the last week of the study. In a previously submitted metabolism study in rats (Report # AT 6264), at least 8 plasma metabolites have been isolated following oral administration. However, only the most abundant metabolite #1, was identified (6-hydroxy-

RS-25259). Currently, the Sponsor provided pharmacokinetic information on an additional N-oxide metabolite (RS-17825-007) in rats. Both the 6-hydroxy-RS-25259 and the N-oxide metabolites have been identified in human plasma following i.v. dosing (Report No. CL-6753, submitted in amendment No. 33 dated 7/22/94). Levels of RS-17825-007 detected currently were less than 6% of the mean levels of RS-25259, tended to increase with increasing dose, and were higher in females compared to males on both sampling days. Additional comparisons of the metabolic profiles in rats versus humans are not possible, since the other 7 metabolites isolated in rats have not been identified.

In conclusion, oral administration of RS-25259-007 to rats, at doses of 18, 60, 120, and 160 mg/kg, for a period of 3 months produced dose-dependent suppression of body weight gains in males (9-55%, at doses \geq 18 mg/kg) and in females at doses of 120 and 180 mg/kg doses (12 and 11%, respectively). Mortality occurred at the 180 mg/kg dose in both sexes. Target organs of toxicity included: the bone marrow [decreased cellularity (both sexes at 180 mg/kg) and reduced femoral trabeculae (increased severity in males at doses \geq 60 mg/kg and in females at the 180 mg/kg dose)], male sex organs (testes with diffuse testicular atrophy at doses \geq 60 mg/kg, with aspermatogenesis at the 120 and 180 mg/kg doses) and possibly the kidney (increased incidence and severity of chronic progressive nephrosis at the 180 mg/kg dose in both sexes) and liver (increased weights, reduced liver enzymes and histological correlates of congestion and vacuolation at the 180 mg/kg dose). The 18 and the 60 mg/kg doses could be considered the no effect doses in males and females, respectively. The maximum tolerated dose (MTD) in males was the 60 mg/kg dose, based on only slight suppression (14%) of body weight gains and a limited incidence of diffuse testicular tubular atrophy (2 of 15 rats). Treatment-related effects in males at the next higher dose (120 mg/kg) included: suppressed body weight gains (31%) alterations in hematology and blood chemistry, reduced weights for testes and accessory sex organs, with histological findings including: testicular tubular atrophy (100%), testicular aspermatogenesis (100%), lymphoid atrophy of the spleen (60%) and increased severity of reductions in femoral metaphyseal trabecular bone. The MTD in females was the 120 mg/kg dose, based on only slight suppression of body weight gains (12%), lower platelet counts, mild increases in total counts for leukocytes, neutrophils, and lymphocytes, increased triglycerides, increased weights for liver and spleen, and histological lesions limited to an increased incidence of chronic nephrosis syndrome (36% versus no controls). The next higher dose in females (180 mg/kg) produced tremors, convulsions, and mortality in 6 of 15 animals tested. Effects in surviving females at the 180 mg/kg dose included alterations in hematology and clinical chemistry and histopathological lesions including: bone marrow toxicity (hypocellularity and reductions in femoral metaphyseal trabecular bone), splenic lymphoid atrophy and necrosis, increased height of the follicular epithelium of the thyroid and hypertrophy of the adrenal glomerulosa cells, increased severity of chronic progressive nephrosis syndrome and congestion of the liver.

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RAT CARCINOGENICITY STUDY:

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FDA/CDER CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) RODENT
CARCINOGENICITY FACTSHEET

REVIEWER: Yash M. Chopra

NDA 21-372

DIVISION: Division of Gastrointestinal and Coagulation Drug Products, HFD-180

DRUG NAME: Palonosetron

SPONSOR: Helsinn HealthCare SA,
Lugano, Switzerland

LABORATORY: 

REVIEW DATE:

CARCINOGENICITY STUDY REPORT: March 29, 2002

PRIOR FDA DOSE SELECTION CONCURRENCE: Yes, Executive -CAC meeting on
October 4, 1994

DRUG CATEGORY: Serotonin (5-HT₃) - Receptor Antagonist

MUTAGENIC/GENOTOX: (Y/N/EQUIVAOCAL/NA): Palonosetron was not mutagenic in
Ames test, in vivo mouse bone marrow micronucleus test, HGPRT test in Chinese hamster
ovarian cells but it was clastogenic in an *in vitro* chromosomal aberration test in Chinese hamster
ovarian cells.

RAT CARCINOGENICITY STUDY: Two control groups and 3 treatment groups

STUDY DURATION: 104 weeks

STUDY STARTING DATE: April 21, 1999

STUDY COMPLETION DATE: March 29, 2002

GLP COMPLIANCE: YES

QAU REPORT: Yes

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DRUG LOT#: RS-25259-197 (lot # 1304981/Mfg. Lot#30893 P104 & 1360411 (Mfg. Lot # 30893-P106)

DRUG PURITY AND STABILITY: 99.0 to 100% pure and the stability and homogeneity of the samples of the solutions was stable for up to 48 hr at room temperature and for 15 days at 5 to 10°C.

RAT STRAIN: SD strain

ROUTE: Oral Gavage

NUMBER OF ANIMALS:

Control: Two control groups 65/sex/group

Low, Mid and High Dose groups: 65ex/group

One Additional untreated group – health monitoring group - 15/sex

Satellite Groups: Three treatment groups of 15/sex/group

(The toxicokinetic of the compound was determined in 3 satellite groups of 3 animals/sex/group.)

BASIS OF DOSE SELECTION: (MTD, AUC ratio, saturation, maximum feasible dose): MTD

The dose selection was based on 3-month oral dose ranging study in rats. In this study palonosetron was administered at the oral gavage doses of 0, 18, 60, 120 and 160 mg/kg/day. A dose of 160 mg/kg/day was lethal and males included in 120 and 160 mg/kg/day treatment groups showed significant body weight retardation. The dose of 60 mg/kg/day was MTD in males. In females, a dose of 120 mg/kg/day was MTD but it was very close to lethal dose, therefore, a dose of 90 mg/kg/day was considered as the highest dose in females.

PRIOR FDA DOSE CONCURRENCE: Yes

CAC-EC meeting on October 4, 1994 (final report dated October 7, 1994) recommended the doses of 0, 15, 30 and 60 mg/kg/day in males and, 0, 15, 45 and 90 mg/kg/day in females of 104-week rat carcinogenicity study. (These doses are equivalent to 90, 180 and 360 mg/mm²/day in males and 90, 270 and 540 mg/mm² in female animals)

RAT CARCINOGENICITY STUDY: 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.

COVERSHEET FOR 2-YEAR CARCINOGENICITY STUDY IN RAT

1. Report No.: PALO-98-03
2. Name of Laboratory: _____
3. Strain: Sprague-Dawley CrI:CD®BR strain Rats
4. No./sex/group: 65/sex/group in 2 vehicle control and 3 treatment groups; 3 satellite groups of 7 rats/sex/group and 1 health monitoring group consisting of 14/sex untreated animals.
5. Doses (0, 0, L, M, H): 0, 0, 15, 30 and 60 mg/kg/day (0, 0, 90, 180 and 360 mg/mm²)- Males and 0, 0, 15, 45 and 90 mg/kg/day (0, 0, 90, 270 and 540 mg/mm²) -Females
6. Basis for dose selection stated: Yes
7. Interim sacrifice: No
8. Total duration (weeks): 104
9. Week/site for first tumor:

<u>Male</u>	<u>Female</u>
C1 47/Malignant Squamous Carcinoma	49/Malignant Lymphoma H'poetic Tumor
C2 56/Benign Harderian Gland Adenoma	56/ Benign Par Distalis Pituitary Adenoma
L 57/Benign Par Distalis Pituitary Adenoma	49/Benign Mammary Gland Fibroadenoma
M 29/Malignant Renal Nephroblastoma	50/ Benign Par Distalis Pituitary Adenoma & Benign Mammary Gland Fibroadenoma
H 12/Malignant Lymphoma H'Poietic	23/Malignant Lymphoma H'poetic Tumor

10. No. Survived at termination:

<u>Treat</u> <u>Grs.</u>	<u>Male</u>	<u>% Survival</u>	<u>Female</u>	<u>% Survival</u>
C1	30	46.1	31	47.7
C2	35	53.8	23	35.4
L	33	50.8	24	36.9
M	27	41.5	22	33.8
H	18	27.7	12	18.5

Statistical Methods Used: 1. Survival data was used to determine mortality rate. The mean body weight and food consumption/week changes were calculated throughout the study. The organ weight and body weight changes were determined by using Bartlett's test and the significant test was used to perform pairwise comparison. In other cases, a Dunnett's test was used. Intergroup differences in pathology and histopathology were assessed using Fisher's Exact test. The tumor incidences analysis and trend test were done and, 4. The incidental and fatal information were combined in to single test statistics (PETO, R. et al 1980) and trend test applied.

12. Tumor and Non-tumor Data For each Tissue: See Appendix I.

Palonosetron Hydrochloride Carcinogenicity Study by Oral Gavage Administration to CD-1 Rats for 104 Weeks

Key Study Findings: Increased occurrences of 1) benign pheochromocytoma in male and females, 2) benign adenoma of par distalis (common tumor), combined benign and malignant pheochromocytoma, pancreatic acinar cell adenoma alone and combined incidences of acinar cell adenoma and carcinoma in males and, 3) hepatocellular adenoma and thyroid C-cell adenoma in females of high dose treatment group were seen.

Study Number: PALO-98-03

Sponsor or contract lab: Helsinn Healthcare SA
Lugano (Switzerland)

Test Facility: _____

Date of Initiation: April 7, 1999

Date of Submission: March 29, 2002

GLP Compliance: Statement of compliance with GLP and QAU were attached

QA Report: Yes (X) No ()

Study Characteristics: Based on the recommendations of E-CAC and Division letter dated March 9, 1995 sponsor initiated the study. Palonosetron was administered in 5 groups of Crl:CD-1 rats at oral gavage doses of 0, 0 10, 30 and 60 mg/kg/day in males and 0, 0, 15, 45 and 90 mg/kg/day in females.

Reference to Dose-range-finding study (appendix at the end of the study): 3-Month oral dose ranging study in rats

STUDY PROTOCOL DESIGN AND METHODS

DRUG LOT# AND PURITY: RS-25259-197 (lot # 1304981/Mfg. Lot#30893 P104 & 1360411 (Mfg. Lot # 30893-P106)

DRUG PURITY AND STABILITY: 99.0 to 100% pure and the stability and homogeneity of the samples of the solutions was stable for up to 48 hr at room temperature and for 15 days at 5 to 10°C.

Study Type: Gavage (X)

Species/strain of animals used: SD rats

Number of animals per group: Main Study 5 groups – 65/sex/group and 3 satellite groups (15/sex/treatment group). Group #6 consisted of 14/sex untreated animals for health monitoring

Age and Mean Body weight at the initiation of Treatment: 34 to 38 days old (male) and 35 to 39 days (females) with mean body weight of 140 to 142 g (males) and 131 to 137 g (females).

The animals were housed individually or group of 5 animals of same sex/cage.

Basis of Dose Selection: 3-Month oral dose ranging study in rats (a GLP and QA report). In this study, palonosetron was administered at oral gavage doses of 0, 18, 60, 120 and 160 mg/kg/day. A dose of 160 mg/kg/day was lethal and males included in 120 and 160 mg/kg/day treatment groups showed significant body weight retardation. The doses of 60 and 120 mg/kg/day were MTDs in males and females, respectively. Dose of 120 mg/kg/day was very close to lethal dose therefore, 90 mg/kg/day was the highest dose for the females of the study.

CAC Concurrence : Yes

Dose selection based on E-CAC recommendations (report is attached at the end of the review as an appendix)

Frequency of Drug Administration: A single daily dose

Controls Employed: Two identical vehicle treated control groups (65/sex/group) and 1 health checks group (15/sex). The number of animals, dose schedule and different groups' composition is shown below:

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Composition and identity of treatment groups

Animals were assigned to the groups as follows:

Group	Treatment	Dosage		Main Study			
		(mg/kg/day)		Cage numbers		Animal numbers	
		Male	Female	Male	Female	Male	Female
1	Control	0	0	1-13	69-81	1-65	341-405
2	Control	0	0	14-26	82-94	66-130	406-470
3	Palonosetron	15	15	27-39	95-107	131-195	471-535
4	Palonosetron	30	45	40-52	108-120	196-260	536-600
5	Palonosetron	60	90	53-65	121-133	261-325	601-665
6	Health check	-	-	66-68	134-136	326-340	666-680

Group	Treatment	Dosage		Satellite Study †			
		(mg/kg/day)		Cage numbers		Animal numbers	
		Male	Female	Male	Female	Male	Female
3	Palonosetron	15	15	137-139	146-148	681-695	726-740
4	Palonosetron	30	45	140-142	149-151	696-710	741-755
5	Palonosetron	60	90	143-145	152-154	711-725	756-770

† Satellite study animals were used for toxicokinetic sampling only.

Cage labels, identifying the occupants by experiment, animal number, sex and treatment group, were colour-coded.

Interim Sacrifices: Not done. Treatment related high mortality was seen in week 103 among females of 90-mg/kg/day-treatment group, the treatment was stopped in this group and the surviving animals were retained until the end of study treatment period. The remaining animals of other groups were treated for 104 weeks.

Satellite PK or Special Study Group(s): Blood samples (0.5 ml) from retro-orbital sinus of 3/sex/group were collected at 0.25, 1, 4, 6, 10 and 24 hr of the administration of the compound on day 1, week 26, 52, 78 and 103/104 for assessing TK of the compound and metabolite RS-17825-007 by

The animals were discarded without necropsy. The assay sensitivity for the compound was 2 ng/ml. Unscheduled Sacrifices or Deaths: Animals in extremis killed, examined externally for lesions and tumors, dissected and organs separated for microscopic examination and, their blood samples were collected for hematology parameters.

Clinical Observations

The study animals were examined once daily for week 1, twice during week 2 and 4, once each week from week 5 to 13, and once each week each 2 weeks immediately before and after dosing, on completion of dosing in each group and between 1 and 2 hr after completion of dosing in all groups of animals. These observations were made 5day/week during the study. In addition, the palpitation, palpable masses, swellings were also noted and day of their occurrence were noted. Animals were weighed at the time of acclimatization and, week 0 (start of treatment) and once a

week for 14 weeks, once every 4 weeks thereafter and on week 104 before necropsy. Absolute body weights and relative (percent difference from control) body weight gains estimated. The food consumption was recorded for each week for first 14 weeks and once every 4th week of the study. The mortality/# of deaths during course of study was estimated daily and debilitated animals separated and animals in extremis killed and full necropsy done. At time of terminal sacrifice, a complete necropsy performed. Tabular presentation of cumulative group mortality and graph for percent survival of all animals of the study were prepared over the course of the study.

Ophthalmoscopic examination was not done. The hematology parameters were estimated on the blood samples collected from fasted animals at week 104 (from retro-orbital sinus collected under anesthesia). Relative changes by dose and sex per group at different timings were determined. The toxicokinetic of the drug was determined on the blood samples (0.5 ml) collected on day 1, during week 26, 52, 78 and 104. The samples were collected at 0.25, 1, 4, 6, 10, and 24 hr after dosing. At necropsy, tissues of all the animals of the study were dissected, separated and cleaned. The tissues weighed were: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes, thymus, thyroid, uterus and cervix. Tabular presentation for absolute and relative organ weights was given. Organ weights of animals killed or dying prematurely were not recorded. The histopathological examination of the tissues used for the estimation of organ weight and, aorta, cecum, colon, duodenum, eye, femur, Harderian glands, ileum, jejunum, lachrymal glands, bronchi, lymph nodes, mammary area, esophagus, optic nerve, pancreas, spinal cord, sternum stomach, thyroid tongue, trachea, urinary bladder, vagina, cervix and uterus were separated and preserved in 10% neutral buffered formalin with the exception of testes and epididymides, which were placed in Bouin's fluid and subsequently in 70% industrial methylated spirit. Eyes were placed in Davidson's fluid. In addition to any masses, the lymph nodes drains and tissues adjacent to those masses were also preserved. The tissues and organs of animals of all main study groups were examined. The tables of the incidence of non-neoplastic and neoplastic findings were prepared.

The mean body weight and food consumption/week of the study was calculated; homogeneity of variance using Bartlett's test was tested. The significance of the test was calculated by using Fisher's exact test. The treatment group findings were compared using Mantel test for a trend in proportions and also pairwise Fisher's exact test for each dose with control group was used. If Bartlett's test for variance was not significant at 1% level, then parametric analysis was used. Tabular presentations of significant non-neoplastic and neoplastic findings were included in the study. The tumor incidences were analyzed statistically and compared with the (minimum to maximum) range of historical control data of sponsor laboratory.

RESULTS:

1. **Clinical Observations:** The dose related incidences of ungroomed hair coat (3, 7, 4, 0 and 11 in males and, 29, 34, 44, 52 and 61 in females), incidences of brown staining at perigenital area (0, 0, 3, 8 and 20 males and, 1, 0, 1, 9 and 12 females), pale eyes (1, 2, 0, 1 and 9 males and, 4, 5,

1, 3 and 6 females of 65/sex/group) was seen in animals of 0, 0, low, mid and high dose treatment groups. Salivation in mid and high dose treatment group animals was seen from week 2 and its intensity and incidences were reduced by week 84 to the end of the study. The noisy respiration (congestion) was noted in 12, 11, 25, 34 and 42 males and, 17, 19, 18, 38 and 49 females of the of 0, 0, low, mid and high dose treatment groups. The mean number of palpable masses were 27, 34, 38, 39 and 44 in males and, 53, 38, 42, 54 and 49 in females of the of 0, 0, low, mid and high dose treatment groups. Reduced body temperature was seen in 2, 1, 1, 3 and 8 males and, 0, 2, 3, 2 and 3 females of 5 study groups.

2. **Mortality:** The mortality rates were 53.8, 46.1, 49.3, 58.5 and 72.3% among males and, 52.3, 64.6, 63.1, 66.1 and 81.5% among females included in 0, 0, low, mid and high dose treatment groups. The trend test was significant in both sexes (two-sided $p=0.005$ (males) and $p<0.001$ for females). Sponsor did not provide/determine the cause of death of the animals and stated that the animals died during study were generally debilitated.

3. **Body Weight and Food Consumption:** On week 104, the mean body weights were 738, 756, 803, 719 and 666 g in males and, 512, 491, 466, 496 and 401 g in females included in 0, 0, 15, 30/45, 60/90 mg/kg/day treatment groups. On week 104, the body weights of 3 treatment groups were 0, 2.5 and 9.7% lesser than the control males. At the same time, the body weights of 3 treatment groups (in low, mid and high dose treatment groups) females were 9.1, 3.1 and 21.7% lesser than control females respectively (as shown in the able below). On week 104, the mean daily food consumption/animal included in the 2 study control groups and treatment groups was 27.4, 27.7, 26.4, 27.6 and 29.0 g/male/day and, 23.1, 22.2, 24.0, 25.3 and 24.0 g/female/day included in 0, 0, 15, 30/45 and 60/90 mg/kg/day treatment groups, respectively indicating a slight increase to no effect of the treatment on food consumption in animals.

TABLE
Table for Mean Weight (g) of rats

	Control 1	Control 2	Low Dose	Mid Dose	High Dose
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Males					
Initial	142	142	141	141	140
Week 14	514	515	520	504	507
<i>Percent of Control</i>	100.0	100.2	101.2	98.0	98.6
Week 26	619	606	624	600	594
Week 54	734	714	742	700	684
<i>Percent of Control</i>	100.0	97.3	101.1	95.4	93.2
Week 74	777	759	782	732	698
Week 104	738	756	803	719	666
<i>Percent of Control</i>	100.0	102.4	108.8	97.4	90.2
Females					
Initial	137	134	132	131	134
Week 14	308	303	303	309	309
<i>Percent of Control</i>	100	98.3	98.3	100.3	100.3
Week 26	339	337	336	343	333
Week 54	412	411	402	414	381
<i>Percent of Control</i>	100.0	99.8	97.6	100.5	92.5
Week 74	466	463	454	461	405
Week 104	512	491	466	496	401
<i>Percent of Control</i>	100.0	95.9	91.0	96.9	78.3

FIGURE 3B

Group mean bodyweight versus period of treatment - females

Group	:	1	2	3	4	5
Compound	:	Control	Control	Palonosetron Hydrochloride		
Dosage (mg/kg/day)	:	0	0	15	30/45	60/90

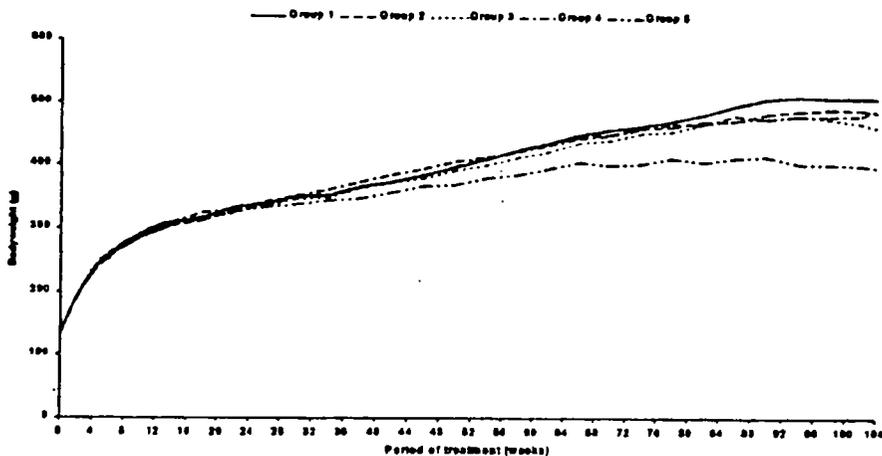


FIGURE 3A

Group mean bodyweight versus period of treatment - males

Group	:	1	2	3	4	5
Compound	:	Control	Control	Palonosetron Hydrochloride		
Dosage (mg/kg/day)	:	0	0	15	30/45	60/90

