

## PHARMACOLOGY AND TOXICOLOGY SUMMARY

**Drug:** pramlintide, Symlin®

**Indication:** Adjunctive therapy to insulin to improve glycemic control in type 1 and 2 diabetics

**Doses:** 30 to 90 µg (max QID) in Type 1 diabetics, 120 µg (max TID) in Type 2 diabetics

### Introduction and Historical Background:

Amylin is a 37-amino acid peptide that is co-secreted and co-localized with insulin from the pancreatic β-cell of the islets of Langerhans. Basal plasma concentrations of amylin in normal human subjects are in the range of 4 to 8 pmol/L, which can increase to 20 to 25 pmol/L after a meal. Plasma amylin concentrations show a similar profile to those of insulin, increasing in response to nutrients, and other β-cell secretagogues. Similarly, plasma amylin concentrations are reduced in parallel with plasma insulin in patients with type 1 and advanced type 2 diabetes mellitus, where nutrient-stimulated secretion is absent or blunted. Amylin has a complimentary role with insulin in the maintenance of glucose homeostasis.

**Pramlintide** is an analogue of the naturally occurring human hormone amylin and like amylin appears to exert many of its effects via the central nervous system, functioning as a neuroendocrine peptide. Pramlintide binds to specific amylin receptor binding sites in the brain, particularly the nucleus accumbens and area postrema, with similar affinity as amylin. Subcutaneous administration of pramlintide modulates the rate of absorption of nutrients from the gastrointestinal tract so that it more closely matches insulin-stimulated disposal rates and leads to a smoothing effect on plasma concentrations of nutrients, including glucose. Several other major actions of amylin and pramlintide have been identified, they include:

- inhibition of amino acid stimulated but not hypoglycemia stimulated glucagon secretion
- regulation of the gastric emptying rate
- reduction of food intake
- attenuation of pentagastrin-stimulated gastric acid secretion
- attenuation of CCK-stimulated pancreatic amylase and lipase secretion

### Toxicology:

**General Comments:** Pramlintide (SC) rapidly enters the systemic circulation with high peak plasma levels (15 to 30 min) and a short half-life (30 to 120 min) in all species tested. Plasma concentrations increase in a dose proportional manner with no significant difference in kinetics between males and females. Very little pramlintide crossed the placental barrier of the pregnant rabbit. No pramlintide was detected in the plasma of fetal rats. The bioavailability has been estimated to be about 40% in animals after SC injection. Pramlintide is metabolized primarily to des-lys pramlintide (2-37 pramlintide). Des-lys pramlintide represents approximately 30% of pramlintide plasma immunoreactivity at steady state. Of all the metabolites, only des-lys pramlintide was active at the receptor site. Chronic administration of pramlintide was well tolerated in rodents and dogs. No significant signs of toxicity were reported in the chronic toxicity studies in rats and dogs except for occasional decrease in body weight with less frequent decreases in food consumption.

**26-Week Rat Study (REST98118R1)** evaluated subcutaneous doses of 0.2 (LD), 0.5 (MD) and 1.2 (HD) mg/kg/day (6, 23 and 56X human exposure based on AUC) pramlintide in CrI:CD®BR rats (30 /sex/group). Approximately, 10 rats/sex/group were examined at the 13 weeks, the remaining animals were evaluated at the end of the study (26 weeks). Body weight gain decreased primarily in males in a dose-related manner starting Week 5. The greatest decrease in body weight gain occurred in HD male (-18%) and female (-10%) rats. There were no significant changes in food intake. There were no effects on

organ weight at Week 13, however, a slight treatment related increase in liver (males 7%) and kidney (females 9%) weights were noted at Week 26. There were no significant changes in hematology or urinalysis throughout the study. Slight but significant changes in calcium (-5%) and urea (-14 to -22%) were observed in HD male and female rats. There were no significant changes in plasma glucose, cholesterol or triglycerides.

Both control and high dose animals had slight injection site reactions. Since both control and HD groups received similar dose volume, the finding was attributed to vehicle and method of drug administration. The histological examination of the injection sites revealed mainly fibrosis and inflammatory cell foci with occasional hemorrhage. There were no major macroscopic or microscopic findings in tissues other than the injection sites. The injection site reactions in control and HD groups were similar, however, the severity of injection site fibrosis was slightly greater at 26-week than at 13 weeks suggesting greater inflammation with repeated SC administration.

**Conclusion:** Doses up to 1.2 mg/kg/day (56X human exposure) were well tolerated in rats. There was no evidence of systemic or organ toxicity except for fibrosis at the injection site noted in all animals. The severity of injection site lesions was slightly increased at the end of the study relative to interim (13-WK) animals. The number of animals with severe fibrosis injection site was slightly greater than controls.

**52-Week Dog Study (REST 98127R1)** evaluated subcutaneous doses of 0.1 (LD), 0.3 (MD) and 0.6 (HD) mg/kg/day pramlintide (22,34 and 42X human exposure based on AUC). No significant changes in body weight or food intake of male and female dogs were noted at the end of the study. Mean liver and kidney (LD, MD and HD), brain, pituitary (MD and HD), testes and epididymides (HD) and uterus and ovary weights (LD and HD) weights were lower than the controls. The pituitary weight relative to body weight of MD (-27%) and HD (-24.3%) males were significantly lower than controls. Prostate weight was actually higher in HD males than controls. These changes were not associated with any structural or functional changes. There were no significant changes in hematology, clinical chemistry (glucose, AST, cholesterol and triglycerides) or urine analyses parameters throughout the study. Both control and treated animals showed signs of inflammation at the injection sites but the severity was slightly higher in treated groups.

There were no macroscopic or microscopic findings in tissues other than the injection sites. The histological examination of the injection sites found cellulitis/fibrosis, necrosis and occasionally vasculitis/perivasculitis in both control and treated dogs. However, the treated animals appeared to have higher incidence of vasculitis/perivasculitis than controls. Pharmacokinetic analysis at weeks 1, 13, 26 and 52 found a significantly lower plasma pramlintide concentrations during Week 1. The plasma concentration of pramlintide increased in a dose-proportional manner except for the decreased AUC values in the HD group during Week 52.

**Conclusion:** Doses up to 0.6 mg/kg/day (42 X human exposure based on AUC with maximum recommended therapeutic dose of 90 µg QID) were well-tolerated in dogs. With exception of small decrease in body weight in females and some organ weights (liver, kidney, pituitary, testes, ovary) no significant toxicological finding was noted. There appeared to be an increase in the incidence vasculitis/perivasculitis at the injection sites in treated dogs.

## **Reproductive Toxicity:**

### **Fertility Study in Rats (REST98130R1)** Performed by Pharmakon Research, PA:

Male (13/dose) and females (26/dose) SD rats were treated with 0, 0.3, 1.0 and 3.0 mg/kg/day pramlintide (10, 47 and 140X human exposure based on AUC) once daily by SC route before (males 80 d, Females 15 d) and after mating. One male was mated with two females in the same dose group. At the end of the mating period, males were sacrificed and reproductive tissue samples were collected. Treatment continued

in females. One half of the females were sacrificed on gestation Day 13 and the number and location of viable and nonviable embryos, early resorption, total implantations and corpora lutea were recorded. The remaining female rats were allowed to deliver and necropsied after weaning (lactation Day 21). The mortality rate, body weight, sex and abnormality in neonates were determined.

**Male:** None of the male rats died during the study. There was a significant decrease in body weight of males (~12.5%) treated with 3 mg/kg/day (>140X human dose). The decrease in body weight correlated with the decrease in reproductive organ weights in 3mg/kg/d male rats.

**Dams:** There were no changes in body weight of female rats prior to mating. A significant decrease in body weight gain of dams treated with 3 mg/kg/d were noted (12%). Eight of the females in 3 mg/kg/d died during gestation. The fetuses from 7/8 dams appeared normal. Fetuses (3/19) from one dam (#5290) had neurotube defects and protruding tongues. Total numbers of gravid rats were similar and high in all groups (92 to 96%). There were no significant differences in the number of gravid rats, pre-implantation loss or mean length of gestation. None of the dams aborted or delivered early during the study. Although, there were no statistically significant difference in the number of litters delivered and a biologically significant decrease in the number of litters delivered in the 3 mg/kg/d dams was observed.

**Neonates:** There was a dose-dependent decrease in neonate weight. The fetal weights from 3 mg/kg/d dams were significantly less than controls. Although there were no statistical differences in the mean number of neonates between treated and controls, the total number of neonates delivered by groups was biologically significantly less in the 3 mg/kg/d dose group (n=4 litters) than other treated or control group (n=11-13 litters), secondary to the maternal toxicity (deaths, body weight decrements) observed in dams treated with 3 mg/kg/d pramlintide. There were no differences in survival or sex ratio of neonate among groups. No skeletal malformation was detected in any of the treated groups.

**Conclusion:** Animals treated with 3 mg/kg/day pramlintide had significantly lower body weight than concurrent controls. The decrease in reproductive organ weights in males corresponded to decrease in body weights at high dose. Although the total numbers of gravid rats were similar and high in all groups (92 to 96%), the number of neonates appeared to be lower in the high dose group (n=4 vs. n=11-13 litters in control). No skeletal malformation in neonates were noted at any dose. The 3 mg/kg/day pramlintide (>140 X human dose based on AUC) was considered a maternal and embryotoxic dose since there were significant decreases in maternal and fetal body weights, decreased number of viable embryo and delivered neonates.

**Teratology Study in Rats, Segment II (REST98111) Performed by Pharmakon Research, PA:**

Females SD rats (27-28/dose) were treated with 0, 0.3, 1.0 and 3.0 mg/kg/day pramlintide (10, 47 and 140X human exposure based on AUC) once daily by SC injection from gestation Day 6 through 15. Animals were terminated on gestation Day 20 and the uterus of each female was excised and weighed. Early or late resorptions, the number of viable and non-viable fetuses, the total number of corpora lutea, and the sex of fetuses were determined. Approximately one half of each litter was examined for skeletal anomalies and the remaining fetuses were examined for soft tissue anomalies.

All treated animals exhibited some or all of the following test article-related signs post-dose during the treatment period: vasodilation of pinna, forepaws, hind paws, tail and nasal area. No animals died during this study. None of the dams aborted or delivered early. The body weight gain and food intake of high dose group were significantly less (-8%) than concurrent controls at gestation Day 20. There were no significant differences in the number of gravid females 92.6, 92.6, 96.3 and 96.4% for control, LD, MD and high dose group. There were no statistically or biologically significant differences observed in the group mean number of implantations, viable and non-viable fetuses, corpora lutea, number of early or late resorptions, number and percentage of pre- and post-implantation losses, fetal body weight or fetal sex distribution.

Eighteen fetuses with external, visceral or skeletal malformations were detected during the study. Malformations were observed in one control fetus from one litter (4%), 10 low dose fetuses from four litters (16%), five mid-dose fetuses from five litters (19%) and two high dose fetuses from two litters (7%). Although the malformations were not statistically different, the number of low dose fetuses exhibiting malformations (10) when compared to the concurrent control group (1) were biologically different. Although not dose-dependent, the malformations detected in the 0.3 and 1.0 mg/kg/day dose groups, primarily the neural tube defects (exencephale, craniorachischisis and hydrocephalus) were considered indicative of teratogenicity.

**Conclusion:** There appeared to be a biological increase in the incidence of malformation (primarily neural tube defects) in the 0.3 and 1.0 mg/kg/day dose groups. The maternal and developmental no-observed-effect levels (NOEL) were not established (based on AUC data, the lowest dose, 0.3 mg/kg/day is 10X human exposure).

**Teratology Study in Rabbits, Segment II (REST98110)** Performed by Pharmakon Research, PA:

Females (18/dose) white rabbits were treated with 0, 0.3, 1.0 and 3.0 mg/kg/day pramlintide (12, 42, 89X human AUC exposure) once daily by SC route from gestation **Day 6 through 18** to induce congenital malformation in offspring. Animals were terminated on gestation Day 29 and the uterus of each female was excised and weighed. Early or late resorptions, the number of viable and non-viable fetuses, the total number of corpora lutea, the sex of fetuses were determined. Approximately one half of each litter was examined for skeletal anomalies and the remaining fetuses were examined for soft tissue anomalies.

Two moribund high dose rabbits were sacrificed on Day 18 and 20 (mottled kidney, reddish discharge). The high dose females had lower (52%, 0.118 kg vs. 0.226 kg) body weight gain than controls (Day6-18). Numbers of gravid animals were 66.7, 55.6, 44.4 and 55.6% for control, 0.3, 1 and 3 mg/kg/day, respectively. Two of the moribund does in the high dose group were sacrificed (Day 18 and 20) and one rabbit was not treated with chorionic gonadotropin. There were no statistically or biologically significant differences observed in the total number of implantation sites, total number of viable fetuses, non-viable fetuses, corpora lutea, early or late resorptions, fetal sex distribution, fetal body weight, or the number and percentage of pre- or post-implantation losses.

There were no differences in the total number of fetuses or litters with malformation among treatments. Four fetuses with skeletal malformations were detected during the study: two fetuses (2.6%) from two litters (20%) in the 0.3 mg/kg/day dose group and two fetuses (3.3%) from two litters (25%) the 1.0 mg/kg/day dose group. There were no visceral variations in any dose groups during the study.

**Conclusion:** This study was not considered valid due to the abnormally low pregnancy rates in all groups, including controls. Therefore the study was repeated (Study # REST98125, page 5).

**Pre- and Postnatal Development in Rats, (REST98129R1)** Performed by Pharmakon Research, PA:

Female CrI:CD®BR rats (48/dose) were treated with 0, 0.2, 0.5 and 1.2 mg/kg/day pramlintide (6, 24 and 57 X human dose based on AUC) once daily by SC route from **gestation Day 6 through postpartum Day 21**. Half of the animals (24/dose) were terminated on gestation Day 20 (gestation dams). The second half (F0) were allowed to deliver (lactation dams). Two neonates from each F1 litter were retained as parent for the F2 generation. The total number of gravid animals in the F1 pregnancy were 40/48, 42/48, 45/48 and 42/48 for control, low, mid and high dose, respectively.

**F0 Gestation Dams:** None of the F0 dams died during the study. Some of the clinical signs in high dose dams included ptosis, abnormal stance and gait, tremors, decreased activity, and labored respiration. There were no biologically significant differences in the group mean body weights or body weight changes for the F0 dams during gestation. There were no biologically or statistically significant differences observed in the total number of implantation sites, total number of viable fetuses, non-viable fetuses, corpora lutea,

early or late resorptions, fetal sex distribution, fetal body weight, or the number and percentage of pre- or post-implantation losses for the F0 dams in the test article treated groups at cesarean section when compared to controls. Seventy five percent of controls, 91.7% of low, mid and high dose were gravid ( $p < 0.05$ ). There were no significant incidences of fetal malformation or developmental variation in pups of treated animals.

**F0 Lactation Dams:** Significant decreases in body weight gains were observed in the mid and high dose groups at different time intervals but at the end of lactation period (Day 0-21) only the body weight of HD group was significantly (23%) less than concurrent controls. The duration of gestation was slightly increased in low and mid dose group. The slightly lower number of delivered neonates in high dose groups was not significantly different than concurrent controls. There were no significant difference in the number of litters delivered or weaned or litters with stillborns. The increase in length of gestation in low and mid dose dams relative to controls was not statistically significant.

**Neonates:** The total number of neonates were low but similar among groups. The numbers of litters were 14/22(63.3%), 13/19(68.4%), 19/24 (79.2%) and 16/19 (84.2%) for control, 0.2, 0.5 and 1.2 mg/kg/day, respectively. A significant decrease in body weight of mid and high dose male and female neonates were noted on Day 7 and for all doses on Day 14 and 21. There were no notable differences in the number, viability and sex distribution of neonates. There were no skeletal malformations, or behavioral/physical development effects (surface righting test, negative geotaxis, air drop, grasp/holding, Galton whistle, pinna detachment, incisor eruption, fur growth, eye opening, etc). There were no significant difference in neonate survival or sex ratio. However, the body weight of male and females in mid and high dose group were significantly lower than concurrent controls Days 22 and 29 post-partum.

**F1 Generation:** No clinical signs of toxicity in F1 dams during gestation were noted. Eighty three F1 dams were mated with no significant difference in body weight or in the number of gravid dams (90.5, 94.4, 86.4 and 88% for control, 0.2, 0.5 and 1.2 mg/kg/d, respectively). All fetuses (F2) except for two in mid dose were normal. There were no difference in the number of viable fetuses, nonviable fetuses, early resorptions, late resorptions, total number of implantations, corpora lutea, fetal sex distribution, mean fetal body weight, post implantation loss or the percentage of the pre and post implantation losses. Other variations were considered incidental.

**Conclusion:** In this study, pramlintide did not induce biologically or statistically significant teratogenic or embryotoxic effects at a dose of 0.2 mg/kg/day (6 X human AUC exposure). The maternal and developmental NOEL was established as 0.5 mg/kg/day (24X human exposure).

**Embryo-Fetal Development in Rabbits (REST98125) Performed by Pharmakon Research, PA:**

Females (18/dose) white rabbits were treated with 0, 0.03, 0.1 and 0.3 mg/kg/day pramlintide (1, 4 and 9X human exposure based on AUC) once daily by SC route from gestation Day 6 through 19 to determine effects on organogenesis. Animals were terminated on gestation Day 29 and the uterus of each female was excised and weighed. Early or late resorptions, the number of viable and non-viable fetuses, the total number of corpora lutea, the sex of fetuses were determined. Approximately one half of each litter was examined for skeletal anomalies and the remaining fetuses were examined for soft tissue anomalies.

None of the rabbits died during the study. Fifty-nine of the 72 does on study were gravid (81.9%). From control to high dose groups 14/18 (77.8%), 16/18 (88.9%), 14/18 (77.8%) and 15/18 (83.3%) were gravid. There was no significant overall drug effect on body weight gain. There were no biologically or statistically significant differences observed in the total number of implantation sites, total number of viable fetuses, non-viable fetuses, corpora lutea, early or late resorptions, fetal sex distribution, fetal body weight, or the number and percentage of pre- or post-implantation losses when compared to the concurrent control.

There were no statistically or biologically significant differences in the number of fetuses or litters exhibiting malformations in any of the test article-treated groups when compared to the concurrent placebo control group. The preponderance of skeletal variations were observed in the sternbrae and ribs. These skeletal variations were noted across all dose groups. A few statistically significant differences were noted in the number of fetuses exhibiting skeletal variations in the test article-treated groups when compared to the control group. Statistically significant decrease in the number of fetuses exhibiting incomplete ossification of the 5<sup>th</sup> sternbrae in the mid and high dose and 13 full pair of ribs in the low and mid dose groups were noted. Visceral variations were primarily confined to the gall bladder and kidneys.

**Conclusion:** In this study, pramlintide did not induce any statistically or biologically significant teratogenic or embryotoxic effects at a dose of 0.3 mg/kg/day (9 X human dose based on AUC) or less. A maternal NOEL was established at 0.03 mg/kg/day (1 X human dose). The developmental NOEL was 0.3 mg/kg/day (9 X human dose).

### **Genotoxicity**

The mutagenic and clastogenic potentials of pramlintide were evaluated in six in vitro and in vivo studies. Pramlintide was not mutagenic in the Ames assay and was not clastogenic in the in vitro chromosomal aberration assay in human lymphocytes, the AS52/XPRT mammalian cell forward mutation assay or the in vivo micronucleus test. Pramlintide is manufactured by three suppliers: Bachem, Mallinckrodt and UCB. Specific tests and findings are described:

1. Ames tests conducted on product from 3 different manufacturers (Mallinckrodt, Bachem and UCB) of pramlintide were negative.
2. In vitro chromosomal aberration test using human lymphocytes: Since pramlintide was cytotoxic at concentrations higher than 1670 µg/ml (no scoreable metaphase cell) in the initial study, pramlintide concentrations between 100 and 1670 µg/ml were tested. Pramlintide did not cause significant changes in the number of aberrations /cell at any concentration. However, in the confirmatory test (167, 1000 and 1670 µg/ml), there was a slight increase in aberrations/cell at concentration of 1000 µg/plate. This was considered a statistical aberration since it was within the acceptable negative control value. The overall test was considered negative.
3. AS52/XPRT Mammalian Cell Forward Gene Mutation Assay. The ability of pramlintide to induce mutation at xanthine-guanine phosphoribosyl transferase was assessed in AS52 Chinese hamster ovary (CHO) cells. Pramlintide concentrations of 16.7, 50, 167, 500, 1670, 2000, 2500, 3000, 3500, 4000 and 5000 µg/plate were used in presence or absence of S9. Pramlintide was toxic at ≥3500 µg/plate with S9 and ≥ 2500 µg/plate without S9. Pramlintide tested negative in the AS52/XPRT Mammalian Cell Forward Gene Mutation Assay.
4. In Vivo Micronucleus Test in Mouse Bone Marrow Erythropoietic Cells. The potential of pramlintide to induce micronuclei in the newly formed polychromatic erythrocytes (PCEs) from mouse bone marrow was tested. Based on the results of the preliminary toxicity test, nine groups of mice (5/sex/dose) were treated with single dose of 25, 125 and 250 mg/kg pramlintide with sacrifice times of 24, 48 and 72 hrs.  
There were no significant increases in the number of micronucleated PCE at any dose from bone marrow smears from male mice. A statistically significant increase in micronucleated PCE frequency (7 fold) over control values was noted in female mice treated with 25 mg/kg pramlintide at 72 hr harvest time. When the data from male and female mice were combined, there were no statistically significant increase in micronucleated PCE at any dose. Since the increase in female mice at 25 mg/kg was not dose-dependent and negative controls levels in the assay were very low, the finding was considered a statistical aberration. In final analysis, the ability of pramlintide to induce micronuclei under the conditions of this assay was considered negative.

**Carcinogenicity**

**104-Week Subcutaneous Oncology Study in the Mouse (Report # REST98108):** CrI:CD-1@(ICR)BR mice were treated daily with 0, 0.2, 0.5 and 1.2 mg/kg/day subcutaneously (32, 67 and 159 times human exposure based on AUC). The dose volumes were 12, 2, 5 and 12 ml/kg for control, low, mid and high dose, respectively. Because of high number of deaths in male animals, all surviving males were terminated during Week 97, earlier than females (Week 104). The two vehicle treated controls were combined before statistical analysis.

There were no clinical signs or drug related toxicity. There was no drug-related effect on survival. Minor changes in body weight were not consistent. Percent survival of male and female mice are shown at several time intervals in the 104 week bioassay (n=51/sex/dose at WK 1).

Week	Percent survival, in male mice					Percent survival, female mice				
	Control 1	0.2 mg/kg/d	0.5 mg/kg/d	1.2 mg/kg/d	Control 2	Control 1	0.2 mg/kg/d	0.5 mg/kg/d	1.2 mg/kg/d	Control 2
14	100 %	98 %	100 %	96 %	96 %	98 %	100 %	98 %	96 %	100 %
28	98 %	94 %	94 %	96 %	94 %	94 %	100 %	96 %	96 %	100 %
52	94 %	90 %	88 %	88 %	88 %	80 %	94 %	82 %	90 %	98 %
80	71 %	75 %	69 %	59 %	63 %	69 %	76 %	53 %	66 %	75 %
96	29 %	45 %	43 %	37 %	27 %	43 %	47 %	45 %	42 %	55 %
104						29 %	29 %	37 %	30 %	33 %

In addition to injection site lesions (masses), necropsy findings noted included pancreatic masses in 1LD and 2 HD males and 1LD,1 MD, 2HD females mice. The masses at the injection sites were subdivided into small movable, small stationary, large movable and large stationary masses, which were observed in both controls and high dose mice.

Histopathology: Majority of injection sites showed evidence of chronic inflammation. They were more pronounced in control and HD groups and more common in males than females.

**Non-neoplastic:**

- Chronic inflammation manifested as dermatitis/ folliculitis, panniculitis / myopathy and fibrosis
- Fibrosis in the dermis or subcutis incidence and severity was related to dose volume of the test material injected rather than the active compound.
- No other histopathological finding suggesting systemic toxicity in mice.

Sex	Incidence of selected injection site findings by grade*									
	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Number examined	51	51	51	50	51	51	51	51	51	51
Fibrosis										
normal	0	0	0	0	0	0	0	1	0	0
minimal	0	9	7	1	1	3	15	18	3	1
slight	19	40	36	21	17	38	33	31	27	35
moderate	27	2	7	27	31	9	3	1	19	15
marked	5	0	1	1	2	1	0	0	2	0

\* Grade score represents maximum at any site for an animal

**Neoplastic:**

- There was an increased incidence of tumors at the injection sites, particularly in control and high dose males. The incidence of injection site tumors was small in low and intermediate dose males and in all female groups.
- In the majority of cases, the sarcomas were composed of spindle or fusiform cells with a high mitotic rate and were locally invasive.
- In a few animals, there was evidence of differentiation towards rhabdomyosarcoma (with the presence of giant cells) or the histological appearance resembled that of a malignant fibro-histiocytoma.
- The sarcomas appeared during the second year of the study. They were rapidly growing and in many cases led to the removal of the animal from study on welfare grounds, but there was no evidence of metastases. The incidence was statistically lower in Group 2 ( $p < 0.001$ ) and Group 3 ( $p < 0.05$ ) males compared to the control groups.
- The spectrum and incidence of other tumors was similar in treated and control groups and therefore appeared to be secondary to injection of a large volume, rather than to test compound.
- There was a statistically significant decrease in liver tumor incidence in high dose males ( $p=0.024$ ) compared to the controls. However, this was not considered to be biologically significant. There was no evidence of any systemic carcinogenic effect of the test article.

Incidence of tumors in male and female mice

Sex	male mice					female mice				
	Dose, mg/kg/day	Control1	0.2	0.5	1.2	Control 2	Control 1	0.2	0.5	1.2
Injection site sarcoma	14/51	2/34	5/34	16/51	14/51	2/51	3/39	1/38	0/51	3/51
Hepatocellular adenoma	11/51	10/41	8/39	3/51	7/51	0/50	0/44	1/35	1/51	1/51
Hepatocellular carcinoma	3/50	2/41	0/39	1/50	2/51	0/50	0/44	0/35	0/51	1/51

**Conclusion:** The highest dose used in mice was greater than 159 times human exposure based on AUC. Both vehicle controls and pramlintide increased the incidence of injection site sarcomas, however, there were no significant differences between controls and treated groups. The statistically similar increase in mortality in control and high dose male mice were attributed to injection site masses.

**104 Week Subcutaneous Administration Carcinogenicity Study in the Rat (Report # REST98109:**

Sprague Dawley rats were treated daily with 0, 0.04, 0.2 and 0.5 mg/kg/day subcutaneously (4, 6 and 24 times human exposure based on AUC). The dose volumes were 2.5, 0.4, 1.0 and 2.5ml/kg for control, low, mid and high dose, respectively. The two vehicle (mannitol, metacresol, glacial acetic acid, sodium acetate trihydrate and water, pH4.0) treated controls were combined for analysis. Terminal necropsies in females were carried out during WK 101, earlier than stated 2-year study (104 WK males), due to higher mortality in females.

The mortality rate among controls and treated females rats were similar. No significant changes in body weight, food intake or hematology were observed. The findings from the physical examination of the animals are noted below:

- Sores observed on the back of both control and treated groups were attributed to injections (procedures, volume).
- Incidence of palpable subcutaneous masses categorized as “small movable”, “small stationary”, “large stationary” and “large movable” are shown in tables below. With the exception of small stationary palpable masses, all other types of subcutaneous masses were more common in females than males.
- There were no apparent difference among treated and controls regarding the incidence of subcutaneous masses.

Survival rats in male and female rats treated with pramlintide for two years

Week	Percent survival, in male rats		Percent survival, female rats	

	Control 1	0.04 mg/kg/d	0.2 mg/kg/d	0.5 mg/kg/d	Control 2	Control 1	0.04 mg/kg/d	0.2 mg/kg/d	0.5 mg/kg/d	Control 2
14	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %
28	100 %	98 %	100 %	100 %	96 %	100 %	100 %	98 %	100 %	98 %
52	92 %	96 %	98 %	98 %	92 %	98 %	96 %	98 %	98 %	98 %
80	78%	80 %	82 %	60 %	70 %	80 %	64 %	62 %	84 %	62 %
96	52 %	58 %	54 %	36 %	42 %	44 %	38 %	36 %	42 %	40 %
101	44 %	56 %	50 %	34 %	38 %	38 %	30%	36 %	34 %	36 %
104	40 %	32 %	40%	24 %	34 %					

Non-neoplastic findings:

- Incidence of non-neoplastic findings in controls were consistent with historical data in SD rats.
- Majority of injection sites showed evidence of fasciitis/fibrosis and edema with hemorrhage, myopathy, dermatitis and dermal fibrosis. It appeared that vehicle and volume of dose were irritant to subcutaneous tissue (see table below).
- Subcutaneous response in the control and highest dose were comparable.

Sex Group	Incidence of rats with fasciitis/fibrosis at injection site									
	Male					Female				
	1	2	3	4	5	1	2	3	4	5
Number examined	50	50	50	50	50	50	50	50	50	50
Fasciitis/fibrosis										
-minimal	0	0	0	0	0	0	7	2	0	1
-slight	19	46	33	12	13	29	42	43	24	26
-moderate	16	4	16	16	21	19	1	4	25	22
-marked	15	0	1	22	16	2	0	1	1	1

Neoplastic:

- The incidence of tumors in controls was consistent with historical data in aging SD rats. The incidence of tumors at the injection sites in the two groups of controls was similar.
- The incidence of benign and malignant tumor-bearing animals in the control and high dose groups were generally similar.
- The incidence of skin tumors at the injection sites in the treated groups was not different from controls.
- The incidence of tumors in other organs was comparable to control groups with the exception of pituitary adenoma in male rats.
- Incidence of pituitary adenoma in male rats appeared to be dose-dependent and the incidence in high dose groups was significantly higher than controls (p<0.001).

Sex	Incidence of tumors in male and female rats									
	male mice					female mice				
Dose, mg/kg/day	Control 1	0.04	0.2	0.5	Control 2	Control 1	0.04	0.2	0.5	Control 2
Injection site sarcoma	3/50	1/43	0/43	7/50	4/50	0/50	0/37	1/33	0/50	2/50
Skin sarcoma	3/50	0/43	0/43	2/50	3/50	0/50	0/37	0/33	1/50	0/50
Uterus polyp						1/50	1/42	4/41	8/50	4/50
Pancreas β cell adenoma	2/50	1/34	0/30	4/50	2/49	1/50	0/34	1/32	0/50	0/50
Pituitary adenoma <sup>1</sup>	28/50	28/49	33/50	36/50	25/50	43/50	37/50	38/50	40/50	34/50

<sup>1</sup> Significant increase in pituitary tumors in high dose males relative to combined controls. There was also a dose-related increase in pituitary tumors in male rats (p<0.005).

These data indicate that the number of control rats with pituitary adenomas ranged from 25% to 58% (mean~45%), pituitary carcinomas ranged from 0% to 2% (mean~0.28%) and focal hyperplasia ranged from 22 to 37% (mean~29%). The sponsor claims that the greater incidence of pituitary tumors in high dose symlin group are probably not coincidental for several reasons: a) none of the male treated rats with

0.5 mg/kg pramlintide, or at any dose level, was diagnosed with a pituitary carcinoma, b) the increased incidence of male rats with pituitary adenoma occurs only in rats receiving 0.5 mg/kg, c) there were no increases in female rats with pituitary carcinoma, pituitary adenoma or focal hyperplasia of the pituitary gland or pituitary carcinoma, adenoma and hyperplasia combined, d) the incidence of male rats with focal hyperplasia was highest in one of the control groups followed by the group receiving 0.04 mg/kg pramlintide, e) the group of male rats receiving 0.5 mg/kg pramlintide had the lowest incidence of rats with focal hyperplasia (i.e. the reverse of a treatment related effect), f) the combined number of rats with either focal pituitary hyperplasia or pituitary adenoma was essentially the same across control and treated groups, g) the number of control rats with pituitary adenoma in one of the two control groups in the current study (28/50, 56%) is close to the maximum observed in any of the historical control groups 75/130 (58%) from similar studies, conducted at the same time as this study. Pituitary adenomas are a commonly occurring neoplasm in this strain of rat. The historical pituitary adenoma in Crl:CD®(SD)BR rats is about 47% (range 1 to 70%) in males and 70% (range 26 to 92%) in females (Charles River Lab). Thus, an increase of pituitary adenoma in high dose male rats compared to control groups may be statistically significant but in the absence of an increase in rats with pituitary carcinoma or focal/multifocal hyperplastic lesions, the increase is not biologically indicative of a carcinogenic effect.

#### **Carcinogenicity Summary:**

SD Rats were treated with SC injection of 0, 0.04, 0.2 and 0.5 mg/kg/day (4, 6 and 24 times human exposure based on AUC). There were two vehicle treated control groups. The controls were combined and compared to pramlintide treated groups. The survival, body weight and food consumption of the animals dosed with pramlintide at dosages up to 0.5 mg/kg/day were unaffected by the treatment in rats. There was no adverse treatment-related effect on morbidity and mortality in the treated groups. With the exception of an infrequent and variable incidence of red extremities noted after dosing from week 4, there were no clinical signs attributable to the test article. However, a variable incidence of sores and lesions on the back (in the subcutaneous injection area) was observed across all groups. This finding was attributed to dosing method. In addition, there was a low incidence of palpable tissue masses in all groups at the injection sites, which may also relate to the dosing procedure. At the sites of injection, the incidence and severity of microscopic non-neoplastic findings in the high dose group animals were generally comparable to that in the controls, the findings appearing most severe in the males. In the low and intermediate dose groups, the response seen was less severe and related to the volume of test article formulation administered.

In the 2-year mouse bioassay, similar findings were noted. The mouse injection site findings were also attributed to dose and injection volume since both controls and high dose groups received a large volume of the vehicle. The incidence of palpable tissue masses (small stationary, large movable and stationary) were generally higher in males than females with the exception of small movable tissue masses in females. There were no significant differences between controls and pramlintide treated groups regarding the incidence of tissue masses palpated on the back of the animals near injection sites.

In conclusion, there was no evidence of carcinogenic potential of pramlintide in the 2 year rat and mouse bioassays. The spectrum of neoplastic findings in the treated animals was generally consistent with that expected in aging SD rats. No evidence of any increase in tumor incidence at the sites of injection was seen in the treated animals. In the high dose males, an increase in the incidence of pituitary tumors was observed. Although the incidence of pituitary tumors in low and mid dose males were not statistically different from controls, there appeared to be a dose-related increase in pituitary tumor but was only significant at the high dose level compared to control males. The biological significance of this finding is not clear since this finding was reported in males only (not in females) and the overall incidence of this common tumor in rats were within historical control rates. In addition, when the incidence of pituitary tumors and pituitary hyperplasia were combined, there were no significant differences between controls and high dose males rats.

### **Overall Conclusions:**

In the repeat dose toxicity studies, the main clinical signs observed in rats and dogs were cellulitis and irritation at the subcutaneous injection site. They occurred in both control and treated animals and were attributed to the injection or the vehicle or both and appeared to be related to volume of injection. Pramlintide-treated animals however, had higher incidence of fibrosis at the injection site than concurrent controls. Other clinical signs that appeared in one or more species included vasodilation of the extremities, loose stools, diarrhea, emesis, quivering, abnormal stance and gait, reduced activity, and salivation. In male rats and dogs administered high doses of pramlintide, food consumption was less than in control animals.

At necropsy, irritation and sores were seen at the injection sites of both treated and control animals. Microscopically, injection site irritation was characterized by cellulitis and/or hemorrhage. Vasculitis and perivasculitis were observed in pramlintide-dosed animals only, indicating a slight local tissue response to pramlintide. Other injection site observations included myositis, necrosis and fibrosis. The local irritation induced at the injection site was similar for drug product made from bulk pramlintide manufactured by each of the three manufacturers, and in a special toxicity study in rabbits, a single subcutaneous injection of each of four formulations of pramlintide demonstrated no significant difference in the induction of irritation.

A standard battery of in vitro and in vivo tests were conducted to assess the genotoxic potential of pramlintide. The mutagenic and clastogenic potentials of pramlintide were evaluated in six in vitro and in vivo studies. Pramlintide was not genotoxic in the Ames test, the in vitro chromosomal aberration assay in human lymphocytes, the AS52/XPRT mammalian cell forward mutation assay or the in vivo micronucleus test. The carcinogenic potential of pramlintide was evaluated in 2-year studies in the rat and the mouse with daily subcutaneous administration. Pramlintide was not considered carcinogenic at any dose in either sex of either species.

The reproductive toxicity studies with pramlintide demonstrated the compound had no effect upon the fertility or reproductive performance of the adult rat of either sex, although fertility and mating index in males were not reported. The no effect dose for the neonates was 1.0 mg/kg. Pramlintide was not teratogenic in the rat at doses up to 3 mg/kg/d (140X human exposure) or in rabbits at up to 0.3 mg/kg/d (9X human exposure). In prenatal/postnatal studies in the rat, pramlintide had no effect on the physical, neurodevelopment or reproductive performance of the first generation (F1) pups of pramlintide treated rats.

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Fred Alavi

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PHARMACOLOGIST

Pharm/Tox NDA summary for advisory committee [Symlin, NDA 21,332]  
NDA summary for AC is ready,

Jeri El Hage

6/13/01 01:17:54 PM

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