CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-332

Pharmacology Review(s)

MEMORANDUM
March 15, 2005

TO: File
FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-332

I have reviewed the re-submission of the marketing application for Symlin® (pramlintide acetate) Injection. The product label is accurate and consistent with the data supplied in the marketing application. In particular, "Pregnancy Category C" is appropriate for the product label.

_____
Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director for Pharmacology and Toxicology
Office of Drug Evaluation II

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/s/

Kenneth Hastings
3/15/05 12:08:58 PM
PHARMACOLOGIST

MEMORANDUM
Dec. 10, 2003

TO: File
FROM: Kenneth L. Hastings, Dr.P.H.

SUBJECT: NDA 21-332

I have reviewed the re-submission of the marketing application for Symlin® (pramlintide acetate) Injection. No new pharmacology/toxicology information was included and no additional review is needed. However, the proposed product label specifies "[redacted]" rather than "Pregnancy Category C", which was recommended by the original pharmacology/toxicology reviewer, Dr. Fred Alavi. No additional information was provided by the Sponsor to support this labeling claim. Therefore, the Sponsor should amend the proposed product label to specify "Pregnancy Category C". There are no other pharmacology/toxicology issues.

Kenneth L. Hastings, Dr.P.H.
Associate Director for Pharmacology and Toxicology
Office of Drug Evaluation II

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/s/

Kenneth Hastings
12/10/03 11:51:53 AM
PHARMACOLOGIST

MEMO TO THE FILE

Fred Alavi, Ph.D.
Dec 10, 2003

NDA: 21-332
Drug: Symlin® (pramlintide acetate)
Indication: Diabetes
Sponsor: Amylin Pharmaceuticals, Inc.
Receipt Date: June 16, 2003
Subject: Pregnancy Labeling Category for Symlin

In the June 16, 03 labeling submission to the agency, the sponsor had failed to change the pregnancy category from — to the agency recommended category C. The agency had recommended category C due to embryotoxicity data in rats. Increases in congenital anomalies (neural tube defect, cleft palate, exencephaly) were observed in fetuses of rats treated with 0.3 and 1.0 mg/kg/day (10 and 47 times human exposure) during organogenesis. In rabbits, administration of doses up to 0.3 mg/kg/day pramlintide (9 times the maximum recommended human dose based on AUC exposure) to pregnant rabbits had no adverse effects in embryofetal development. Since the sponsor has not submitted any new data to refute the agency's recommendation and no adequate and well-controlled studies have been conducted in pregnant women, the original pregnancy category C recommendation in the original NDA review has not changed.

The agency recommended pregnancy label:
Pregnancy: Teratogenic effects: Pregnancy category C:

Conclusion:
Amylin should be advised that pregnancy labeling should be revised as in the first AE letter to category C.

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/s/

Fred Alavi
12/10/03 12:28:25 PM
PHARMACOLOGIST
Pregnancy label for Symlin is category C since no new information has been submitted to support a change to [redacted]
Pregnancy Label memo for Action letter

Jeri El Hage
12/10/03 12:31:08 PM
PHARMACOLOGIST

MEMORANDUM
Oct. 3, 2001

TO: File
FROM: Kenneth L. Hastings, Dr.P.H.

SUBJECT: NDA 21-332

I have reviewed the Pharmacology/Toxicology information and concur with the conclusions of the review staff that this application is approvable. The labeling is acceptable with one comment: based on the information provided in the Pharmacology/Toxicology review and in the product label, the product should be labeled "Pregnancy Category C". This designation would be more consistent with the findings in nonclinical reproductive toxicology studies. I have discussed this issue with the primary pharmacology/toxicology reviewer, Dr. Fred Alavi, and he concurred with this proposed revision.

_____
Kenneth L. Hastings, Dr.P.H.
Acting Associate Director for Pharmacology/Toxicology

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/s/

Kenneth Hastings
10/10/01 12:11:31 PM
PHARMACOLOGIST

Food and Drug Administration
DEPARTMENT OF HEALTH & HUMAN SERVICES
Center for Drug Evaluation and Research
Division of Metabolic and Endocrine Drug Products

Memorandum

Date: October 9, 2001

To: Dr. David Orloff, MD, Division Director
DMEDP, HFD-510, CDER

Dr John Jenkins, MD, Office Director
Office of Drug Evaluation II

From: Jeri ElHage, Ph.D., Pharmacology Supervisor
DMEDP, HFD-510, CDER

Subject: Outstanding Pharmacology and Toxicology Issues for Symlin NDA
NDA 21-332

This memorandum is written to address the outstanding pharmacology issues related to the Symlin NDA 21-332.

Carcinogenicity Labeling and ECAC Review

While it is not clearly stated in the Pharmacology MaPPs 7412.1 and 7412.2 that review of final carcinogenicity study reports by the Executive Carcinogenicity Assessment Committee (ECAC) is required, it is my understanding that it is the intended outcome of the policy. Inadvertantly, the carcinogenicity studies for Symlin have not been taken to ECAC for review. Dr Alavi has scheduled discussion of these studies at the ECAC meeting on October 30, 2001. While these studies have not been reviewed by the ECAC, it is the unanimous opinion of the primary reviewer Dr Fred Alavi, the statistical reviewer Lillian Patrician, and myself that there were no drug-related tumor findings in either the mouse or rat 2-year carcinogenicity studies conducted with Symlin. We leave it to your discretion whether the suggested carcinogenicity labeling be sent with the approvable letter or postponed until after the ECAC review.

Characterization of Impurities for Different Manufacturing Processes

It came to my attention on Friday (10/5) that the different manufacturers of Symlin were using different synthesis processes [redacted] and that the safety of the [redacted] product had not been characterized in preclinical or clinical studies. The chemist, Dr. Niu, concludes that the sponsor had adequately characterized that the drug substance manufactured by the different processes is identical. However, different impurity profiles would be expected for drug substance manufactured by different processes. Dr Moore asked what preclinical data would be required to adequately characterize the safety of the impurities associated with the [redacted] product.

An Ames test conducted by [redacted] on lots from all 3 manufacturers revealed no evidence of genotoxic potential. The ICH Q3A chemistry guidance for qualifying impurities in new drug substances specifies that for drugs administered at doses less than 2 grams

per day that the qualification threshold for impurities is 0.1% or 1 mg/day. Since the daily dose of pramlintide is 360 mcg/day and the purity of the drug substance is —, the daily administration of any impurity would be very low, namely, 360 mcg [redacted] possible impurity) = [redacted] mcg/day. Therefore, no additional preclinical characterization of impurities is warranted.

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/s/

Jeri El Hage
10/11/01 10:19:38 AM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and drug administration
Center for drug evaluation and research
Division of metabolic and endocrine drug products

M E M O R A N D U M

DATE: Oct 5, 2001

FROM: Fred K. Alavi

SUBJECT: Pregnancy label modification

TO: NDA 21-332

CC: Julie H Rhee

After a brief discussion with Dr. Jeri El Hage, the pregnancy label was corrected from - to C and the sentence referring to the post-implantation loss in rabbits was removed. The pregnancy label should read:

Pregnancy: Teratogenic effects: Pregnancy category C:

DRAFT

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/s/

Fred Alavi
10/9/01 01:43:55 PM
PHARMACOLOGIST
Addendum to Symlin pregnacy label
Please, sign off on the corrected addendum to Symlin pregnancy label

Jeri El Hage
10/10/01 02:17:46 PM
PHARMACOLOGIST

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

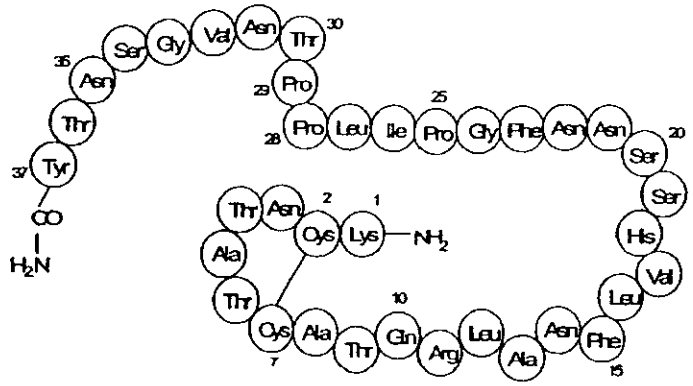
KEY WORDS: amylin, Symlin, glycemic control, antidiabetes
Reviewer Name: Fred K. Alavi, Ph.D.
Division Name: Division of Metabolic and Endocrine Drug Products (DMEDP) HFD#510
Review Completion Date: Aug 29, 2001
Review number: 1

IND/NDA NUMBER: NDA **21-332**

Serial number/date/type of submission: 000, initial stamp date Dec 13,2000.
Information to sponsor: Yes (x) No ()
Sponsor (or agent): Amylin Pharmaceuticals, Inc, 9373 Towne Centre Drive, San Diego, CA 92121, Phone: (858)-552-2200, Fax: (858)-552-2212
Manufacturer for drug substance: [ ]

DRUG

Code Name: AC-0137, Symlin ®, tripro-amylin
Chemical Name: *L-lysyl-L-cysteinyl-L-asparaginyl-L-threonyl-L-alanyl-L-threonyl-L-cysteinyl-L-alanyl-L-threonyl-L-glutaminyl-L-arginyl-L-leucyl-L-alanyl-L-asparaginyl-L-phenylalanyl-L-leucyl-L-valyl-L-histidyl-L-seryl-L-seryl-L-asparaginyl-L-asparaginyl-L-phenylalanyl-glycyl-L-prolyl-L-isoleucyl-L-leucyl-L-prolyl-L-prolyl-L-threonyl-L-asparaginyl-L-valyl-glycyl-L-seryl-L-asparaginyl-L-threonyl-L-tyrosinamide, cyclic (2→7)-disulfide, acetate (salt)*
CAS Registry Number: 196078-30-5 (CAS)
Molecular Formula/ Molecular Weight: $C_{171}H_{267}N_{51}O_{53}S_2$ / 3949.4
Structure: Pramlintide is a 37 amino acid polypeptide with proline replacement at position 25 (alanine), 28 (serine) and 29 (serine) of human amylin. Pramlintide acetate salt with a disulfide bridge between two cysteine residues is shown below:



Relevant INDs/NDAs/DMFs: IND 39,897
Drug Class: Amylin receptor agonist
Indication: Type 1 and 2 diabetes
Clinical formulation:
SYMLIN is formulated as a clear sterile solution for subcutaneous (SC) administration, containing pramlintide acetate salt, metacresol (preservative), acetic acid, sodium acetate, mannitol, and water for injection (pH 4.0). [ ]

In vials, each milliliter of SYMLIN contains 0.6 mg of pramlintide (as pramlintide acetate).

Route of administration: Subcutaneous injections (up to maximum of 4 injections/day)
Symlin doses: [ ] in type 1 diabetes and 120 mg TID in type 2 diabetes

OVERALL SUMMARY AND EVALUATION:

Introduction:

Pramlintide is a synthetic analogue of the naturally occurring human hormone amylin. It is a 37 amino acid polypeptide with proline replacement at position 25 (alanine), 28 (serine) and 29 (serine) of human amylin. Pramlintide has been shown to have the biological properties of the naturally occurring amylin hormone. The structural similarities of human amylin and pramlintide is shown in figure below:

Structure of human amylin and pramlintide---

	1 10	11 20	21 30	31 37
PRAMLINTIDE*	KCNTATCATQ	RLANFLVHSS	NNFGPILPPT	NVGSNTY-NH_2
HUMAN AMYLIN*	KCNTATCATQ	RLANFLVHSS	NNFGAILSST	NVGSNTY-NH_2

*With C-2 C-7 disulfide bond, shading indicates differences from pramlintide

Pramlintide binds to amylin receptors in specific brain areas, including the *nucleus accumbens* in the forebrain and *area postrema* in the brain stem. The *area postrema* does not possess a blood/brain barrier thus, pramlintide in blood can reach the area readily. Pramlintide, similar to amylin, may have a complimentary role with insulin in the maintenance of glucose homeostasis. Additional actions of amylin and pramlintide (physiologic and pharmacologic) include:

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

Pre-Clinical Studies:

In rats, treated subcutaneously for 6-months, the maximal dose of 1.2 mg/kg/day (0.6 mg/kg BID) pramlintide was well tolerated and caused no significant changes in clinical chemistry parameters (i.e. glucose, cholesterol, and triglycerides). A small decrease in body weight was noted in rats treated with 1.2 mg/kg/day (56X maximal human dose based on AUC). Except for injection site lesions, there were no major histological findings in rats. The injection site lesions appeared to be related to injection volume since lesions were present in both control and high dose group with similar injection volumes (1.2 ml/kg). The histological examination of the sites revealed mainly fibrosis and inflammatory cell foci with occasional hemorrhage. Since the severity of the fibrosis was slightly greater in high dose group, the impact of pramlintide in addition to dose volume can not be ruled out.

In a similar 26-WK dog study, injection site inflammatory lesions were seen in control and high dose group (0.6 mg/kg BID, 71X maximal human dose based on AUC). The severity of the lesions appeared to increase with duration of treatment (13 vs. 26 weeks). In addition, the severity of fibrosis was slightly higher in HD pramlintide group than control. Similar to 26-WK rat study, no significant toxicity except for a significant (29%) decrease in body weight was noted in male dogs treated with high pramlintide dose (0.6 mg/kg BID, 71 X maximal human dose). In the 52-WK dog study, the maximum pramlintide dose was 0.6 mg/kg/day (45X maximal human dose based on AUC). None of the doses (0.1, 0.3 and 0.6 mg/kg/day) had any effect on body weight or food intake. A significant decrease in liver, kidney (LD, MD and HD) and pituitary weights (MD and HD) were noted. These changes were considered inconsequential since there were no significant histological or functional changes. As noted in other studies, a significant increase in injection site lesions such as cellulitis/fibrosis, necrosis and occasionally vasculitis/perivasculitis occurred in HD dogs (45X human exposure). These histological findings were more severe and had greater incidence in HD group than controls, even though both groups received a similar injection volume (0.6 ml/kg). The three toxicology studies above suggest that in addition to

vehicle/injection volume, pramlintide itself may have exacerbate the severity and the incidence of injection site cellulitis and fibrosis.
In vitro and in vivo genotoxicity studies were all negative. Since sponsor had used bulk product from three suppliers, an Ames test using pramlintide from all three suppliers was performed and the findings were negative. Standard two year bioassays in mice (0, 0.2, 0.5 and 1.2 mg/kg/day) and in rats (0, 0.04, 0.2 and 0.5 mg/kg/day) were performed. The human exposure multiples relative to carcinogenicity doses in mice and rats are shown in table below:

Species	Doses, mg/kg/day	AUC_{0-t}, **ng.min/ml** (t= 120 to 300 min)	Ratio of animal to maximal human dose AUC in type I and II diabetes	
			Type I Diabetes	Type II Diabetes
104 WK Mouse Bioassay	0.2	3340	**32**	**56**
	0.5	7012	**67**	**117**
	1.2	16499	**159**	**275**
104 WK Rat Bioassay	0.04	267	**3**	**4**
	0.2	923	**9**	**15**
	0.5	2065	**20**	**34**
Human dose Type I, 360 µg/day (90 mg QID)		104		
Human dose Type II, 360 µg/day (120 mg TID)		60		

Since there were two vehicle treated control groups, control data were combined for comparative purposes. 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 both mice and rats. There was no adverse treatment-related effect on morbidity and mortality in the treated groups.

In rats, a variable incidence of sores and lesions on the back (in the subcutaneous injection area) were observed across all treated groups. 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 injection sites, the incidence and severity of microscopic non-neoplastic findings in the high dose 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 perhaps related to the smaller volume of administered test article formulation.

The spectrum of neoplastic findings in the treated rats was generally consistent with those expected in aging SD rats. No evidence of any increase in tumor incidence at the injection sites 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 that was only significant at the high dose group (0.5 mg/kg/day, 20-34X human exposure based on AUC in type I and type II diabetics) compared to control males. The biological significance of this finding is not clear since this finding was reported in males only and the overall incidence of this common tumor in rats were within historical controls. In addition, when the incidence of pituitary tumors and pituitary hyperplasia were combined, there were no statistically significant differences between controls and high dose males rats.

The mouse injection site findings were also attributed to dose and injection volume since both controls and high dose groups received a large injection volume. 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. The injection site findings in the mouse and rat bioassay corroborates the toxicology study findings noted in shorter duration studies.

The reprotoxicity of pramlintide was evaluated in both rats and rabbits. In the rat fertility study, sponsor evaluated pramlintide doses of 0.3, 1 and 3 mg/kg/day (10, 47 and 140 X maximal human exposure based on AUC). **Although, the male reproductive organ weights were measured, the sperm count or motility was not assessed.** The highest dose reduced body weight of males (-12%) which correlated with the decrease in reproductive organ weights. The numbers of gravid rats were similar and high in all dams (92 to 96%). The number of neonates in HD (140X human dose) groups was less than controls (n=4 vs. n=11-13 litters in control) due to drug-related adverse effects on parturition in HD dams. No skeletal malformation was observed at any dose. The 3 mg/kg/day (140 X human dose) was considered a maternal and embryotoxic since there were significant decreases in maternal and fetal weight, decreased number of viable embryo and delivered neonates.

In the rat teratology studies (segment II) similar doses of pramlintide (0.3, 1 and 3 mg/kg/day) were used. Most of the parameters measured were similar among groups except for a biologically significant ($p>0.05$) increase in the incidence of malformation (neural tube defect: exencephale, cleft palate, protruding tongue) in the 0.3 and 1 mg/kg/day (10 and 47 X human dose based on AUC values). Although these finding were not dose dependent, they were considered drug related. However, these malformations were not observed in fetuses of rats treated with doses up to 1.2 mg/kg/day during organogenesis in the pre- and postnatal toxicity study.

In the rabbit teratology study, sponsor used pramlintide doses of 0.3, 1 and 3 mg/kg/day (12, 42 and 89X human AUC exposure) that may have been too high. This study was considered invalid, since the pregnancy rate was too low (44 to 66%) and insufficient to do an appropriate analysis. A second study with pramlintide at reduced doses of 0.03, 0.1 and 0.3 mg/kg/day (1,4, and 9 X human AUC exposure) was performed. The pregnancy rate was within normal range. There were no statistically significant teratogenic or embryotoxic effects at doses up to 0.3 mg/kg/day (9X human AUC exposure). However, gall bladder hypoplasia was observed more frequently in fetuses of HD rabbits. The incidence of this finding was significantly higher than in concurrent or historical controls.

In the final pre- and postnatal development study, rats were injected SC with doses of 0.2, 0.5 and 1.2 mg/kg/day (6, 24 and 57X human dose based on AUC values). Pramlintide did not induce statistically or biologically significant teratogenic, embryotoxic or developmental effect at a dose of 0.2 mg/kg/day (6X human AUC exposure).

Safety evaluation:

Species	NOAEL mg/kg/d	AUC_{0-t}, **ng.min/ml** t= 240 to 300 min	Ratio of animal to maximal human dose AUC in type I and II diabetes	
			Type I Diabetes	Type II Diabetes
26-Week Rat study (0.25 mg/kg BID)	0.5	1715	16	28
26-Week Dog (0.25 mg/kg BID)	0.5	4443	45	74
52-Week Dog	0.3	6036 M 3578 F	58 34	101 60
Human dose Type I, 360 µg/day (90 mg QID)		104		
Human dose Type II, 360 µg/day (120 mg TID)		60		

Other Safety related issues:

A subcutaneous irritation study was performed in rabbits. Following a single subcutaneous injection, none of the formulations caused significant subcutaneous irritation. However, this study had evaluated the effect of single dose, thus conclusion can not be extended to multiple SC injections. This reviewer still believes that the pramlintide itself also contributed to injection site lesions noted in toxicology studies. Another study was also conducted in mice to determine if pramlintide is immunogenic as indicated by a delayed-type hypersensitivity (DTH) response. Pramlintide did not induce a DTH response in the mouse

Conclusions:
Overall, the toxicity studies did not find a significant target organ toxicity signal except for injection site lesions. As noted in the safety evaluation table, the NOAEL in rats (0.5 mg/kg/day) was at minimum 16 fold greater than the maximal dose of pramlintide type 1 diabetic subjects will receive on a daily basis. In type II patients, the safety margin is higher (28X maximal human based on AUC). The maximum daily human exposure in type 1 diabetic subjects was at least 32 fold less than NOAEL dose in dogs (0.3 mg/kg/day). The reprotoxicity studies also found no significant reproductive abnormalities in rats at 1 mg/kg/day (47X maximal human dose based on AUC) and no teratogenicity or developmental abnormalities in rabbits at 0.3 mg/kg/day (9X maximal human dose based on AUC). In a teratology study in rats, multiple malformations were seen at 0.3 and 1 mg/kg/day (10 and 47X maximal human dose) but not at 3 mg/kg/day (147X maximal human dose). Although the findings at 0.3 and 1 mg/kg in rats were not statistically different from concurrent controls, the incidence was higher than historical control rats and are considered biologically important. In a follow up pre- and postnatal development study in rats, pramlintide at 0.2 mg/kg/day (6X maximal human dose based on AUC) was not teratogenic or embryotoxic in rats.

Animal data appear to show a consistent development of lesions at the injection sites. This was true in toxicity studies in rats and dogs as well as in the 2-year mouse and rat bioassays. Subcutaneous injection associated lesions and tumors are not a cause for concern in humans. These studies certainly expose the effect of chronic irritation of the skin with repeated SC injection in animals and subsequent tumor development. In the mouse and rats bioassay, repeated injections appear to increase the incidence of tumors at the injection site. In addition the highest dose in rats (0.5 mg/kg/day, 20-34X human exposure based on AUC in type 1 and type II diabetics) significantly increased incidence of pituitary tumors in males. The biological significance of this finding is not clear since this finding was reported in males only and the overall incidence of this common tumor in rats were within historical controls.

Communication review:
Labeling review:

Carcinogenesis, Mutagenesis, impairment of fertility:

Carcinogenicity: A two-year bioassay was performed in CD-1 mice evaluating doses of 0.2, 0.5 and 1.2 mg/kg/day (32, 67 and 159 X maximal human dose based on AUC, respectively). No drug-induced tumors were observed in any organ. A two year carcinogenicity study was conducted in Sprague-Dawley rats with doses of 0.04, 0.2 and 0.5 mg/kg/day (3, 9 and 20X maximal human dose based on AUC, respectively). No drug-induced tumors were observed in any organ.

Mutagenesis: SYMLIN was not mutagenic in the Ames test and did not increase chromosomal aberration in the human lymphocyte assay. Symlin was not clastogenic in the in vivo mouse micronucleus test or in the chromosomal aberration assay utilizing Chinese hamster ovary cells.

Impairment of Fertility: Administration of 0.3, 1 or 3 mg/kg/day of pramlintide (8, 27 and 82 times maximal human exposure based upon body surface area) had no significant effects on fertility in male or female rats. The highest dose of 3 mg/kg/day resulted in dystocia in 8/12 dams secondary to significant decreases in serum calcium levels.

Pregnancy: Teratogenic effects: Pregnancy category C Reproduction studies have been performed in rats and rabbits at doses up to 10 times human dose and have revealed increase evidence of harm to fetus due to pramlintide. Pramlintide increased post-implantation loss in rabbits at doses ≥3 m/kg/day (3 times human

doses based up on body surface area). Increases in congenital anomalies (neural tube defect, cleft palate, exencephaly) were observed in fetuses of rats treated with 0.3 and 1.0 mg/kg/day (10 and 47 times human exposure) during organogenesis. No adequate and well-controlled studies have been conducted in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

RECOMMENDATIONS: approval

Internal comments: Pharmacology recommends NDA approval
There were no significant pharmacological or toxicological findings in any of the toxicity studies except for injection site lesions. The greater incidence of pituitary tumor in rats was dose-dependent and significantly higher in the rats treated with 0.5 mg/kg/day pramlintide (20X human exposure based on AUC). Since pituitary tumors in rats are common and the incidence of pramlintide related pituitary tumors are within historical range, the significance of this tumor finding is not clear.

External recommendations (to sponsor): approval. There are no pharm/tox issues.

Draft letter content for sponsor (if not same as above): See labeling recommendations.

Future development or issues: No issues

/S/

Fred K. Alavi, Ph.D.
Pharmacology Reviewer

/S/

Jeri El Hage, Ph.D.
Pharmacology Supervisor

cc: NDA Arch
HFD510/Alavi/Elhage/Misbin/Rhee
Review Code: AP
Filename: NDA 21-332.000.doc

Studies reviewed within this submission: Please see table of contents below for list of studies reviewed for this NDA.

Studies <u>not</u> reviewed within this submission: None GLP pharmacology studies

<u>Table of Contents</u>

Introduction and drug history:

Introduction:
Amylin is a 37-amino acid peptide that is co-secreted and co-localized with insulin from the pancreatic ß –cell of islets of Langerhans. Pramlintide has been shown to have the biological properties of the naturally occurring amylin hormone and can be classified as an amylin agonist or an amylinomimetic. Basal plasma concentrations of amylin in normal human subjects are in the ranged 4 to 8 pmol/L, which can increase to 20 to 25 pmol/L after a meal. In type I diabetic patients, amylin concentrations are reduced along with the decrease in insulin levels. Based on encouraging published data, sponsor has proposed that pramlintide injection can restore role of endogenous amylin in type I as well as type II diabetic patients to enhance the effects of insulin.

Structure of rat, mouse, human amylin and pramlintide

	1 10	11 20	21 30	31 37
RAT AMYLIN*	KCNTATCATQ	RLANFLVRSS	NNLGPVLPPT	NVGSNTY-NH_2
MOUSE AMYLIN*	KCNTATCATQ	RLANFLVRSS	NNLGPVLPPT	NVGSNTY-NH_2

	1 10	11 20	21 30	31 37
PRAMLINTIDE*	KCNTATCATQ	RLANFLVHSS	NNFGPILPPT	NVGSNTY-NH_2
HUMAN AMYLIN*	KCNTATCATQ	RLANFLVHSS	NNFGAILSST	NVGSNTY-NH_2

*With C-2:C-7 disulfide bond, shading indicates differences from pramlintide.

A high density of amylin receptors is present in specific brain areas, including the nucleus accumbens in the forebrain and area postrema in the brain stem. The area postrema does not possess a blood/brain barrier and is accessible to circulating amylin (and pramlintide). It is likely that many of amylin's physiologic actions to regulate metabolism are mediated via the area postrema. 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. 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.

Using subcutaneous administration (the clinical route) of pramlintide to modulate the rate of absorption of nutrients from the gastrointestinal tract so that it more closely matches insulin-stimulated disposal rates, leads to a smoothing effect on plasma concentrations of nutrients, including glucose.

PHARMACOLOGY:

Primary pharmacodynamics:

Mechanism of action:

Amylin is co-localized and co-secreted with insulin from β-cells. It binds amylin receptors in specific brain areas, including the *nucleus accumbens* in the forebrain and *area postrema* in the brain stem. The *area postrema* does not possess a blood/brain barrier and is accessible to circulating amylin (and pramlintide). Amylin has a complimentary role with insulin in the maintenance of glucose homeostasis. Several other major actions of amylin and pramlintide (physiologic and pharmacologic) have been identified, they are:

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

Actions of pramlintide involved in glucose regulation:

PARAMETER	SPECIES	ROUTE	ED_{50} (μg/kg)[a]	EC_{60} (pmol/L)
glucagon secretion	rat	IV infusion	-	30.4
gastric emptying	rat	SC	0.33	~15
food intake	mouse	IP	10[b]	-
gastric acid secretion	rat	SC	0.2	-
amylase secretion	rat	SC	0.26	-
lipase secretion	rat	SC	0.04	-

[a] calculated for rat weighing 333g
[b] estimated

Binding studies have shown that receptors with a high binding affinity for amylin, consistent with its low picomolar circulating concentrations, are present in the brain. These receptors have a characteristic binding specificity and distribution in several species. Amylin binding sites are highly localized to circumventricular organs, notably the area postrema, which have access to amylin circulating in the blood. Saturation binding isotherms showed that the *area postrema* contained receptors with high binding affinity for [^{125}I]-amylin (Kd = 25 ± 4 pmol/L), present at high density (87 ± 20 fmol/mg protein). The binding affinity for a number of structurally related peptides was also investigated with the results shown in Table below:

Binding IC_{50s} for structurally related peptides (mean ± SD; n=3-5) to "amylin" receptors in area postrema:

Peptide	IC50 (nmol/L)
Salmon calcitonin	0.022 ± 0.009
Human amylin	0.32 ± 0.20
Rat amylin	0.31 ± 0.22
Rat amylin	0.80 ± 0.23
Pramlintide (Symlin®)	0.61 ± 0.16
Human βCGRP	3.52 ± 0.46
Pig calcitonin	37.2 ± 8.56
Human α CGRP	99.7 ± 38.8
Rat calcitonin	>1000
Human calcitonin	>1000
$^{8-32}$ Salmon calcitonin	17.1 ± 15.5

Salmon calcitonin, rat, and human amylin were the most potent peptides in competing for [^{125}I]-rat amylin; the calcitonin gene related peptide (CGRP) and pig calcitonin had lower potencies and rat and human calcitonin were relatively inactive.

Pharmacology summary:

Summary of the individual studies examining the effect of pramlintide on gastric emptying:

Source	Test System	Test article	Results
Amylin Sponsored	Type I diabetic men, post-prandial glucose profiles	Pramlintide	Post-prandial glucose profiles were lowered in subjects on day infused with pramlintide at 15, 50 or 150 μg/hour, or injected as iv boluses of 30, 100 or 300 μg compared to placebo-infused and injected controls (n=6/group).
Amylin Sponsored	Type I diabetic men, post-prandial glucose profiles	pramlintide	Intravenous infusion of pramlintide (AC137) yielding steady state plasma concentrations of 225±15 pmol/L reduced postprandial plasma glucose concentrations after a standardized Sustacal meal challenge (ΔAUC -1869±5562 mg/dL.min during placebo vs. -28872±4812 mg/dL.min during pramlintide, P = 0.0015). A pramlintide infusion (plasma concentration 234± 16 pmol/L had no effect on plasma glucose profile after an intravenous glucose load.
Amylin Sponsored	Type I diabetic men, post-prandial glucose profiles	pramlintide	Subcutaneous dosing of pramlintide 30, 100, 300 μg pramlintide before meals for 14 days dose-dependently reduced post-meal glucose AUC
Amylin Research	Sprague Dawley rats, gastric emptying, Phenol Red technique	pramlintide	Subcutaneous doses of 0.03, 0.3, 1, 3, 10, 30 and 300μg/kg inhibited gastric emptying over 20 min by up to 100%. EC50 0.33μg/kg ± O. 17 log. Subcutaneous dose of 1μg slowed rate of increase of post-prandial glucose.
Amylin Sponsored	Type I diabetic men, gastric emptying, scintigraphy	pramlintide	Compared to placebo, pramlintide infused at 25μg/hour significantly delayed emptying of both liquid (median lag time 69 vs. 7.5 min) and solid (median lag time 150 vs. 44.5 rain) components of the meal. Pramlintide delayed gastric emptying so much that t(50) values could not be calculated for solid or liquid.
Amylin Research	Sprague Dawley rats, i.v. pramlintide, hypoglycemia, gastric emptying	pramlintide	Insulin-induced hypoglycemia reversed pramlintide inhibition of gastric emptying in rats
Amylin Sponsored	Beagle dogs, iv continuous infusion gamma scintigraphy	amylin	Post-prandial glucose elevation was reduced in dogs drinking 1 g/kg glucose during infusions of 1, 10, 50, 100 pmol/kg/min (plasma conc. 44, 527, 2542, 4509 pM). ED50 4.3 pmol/kg/min. During 10 pmol/kg/min infusion, t50 for emptying was delayed from 35 to 84 min.
Amylin Research	SD rats, BB rats, Phenol Red emptying method, tritiated glucose method	Rat amylin	Accelerated emptying in BB (amylin deficient) rats. Amylin s.c. slowed gastric emptying with an ED50 of ~0.4μg/rat
Amylin Research	SD rats, Phenol red gastric emptying	H. amylin Rat amylin & Pramlintide	Human amylin, rat amylin and pramlintide equipotent at slowing release of Phenol Red from stomach. ED50's 0.13 μg, 0.18μg, 0.11μg, respectively.
Amylin Research	SD rats, Phenol Red method	Pramlintide, Human amylin Rat amylin, Salmon calcitonin Rat calcitonin, Rat CGRP GLP-I, Secretin CCK-8, GRP	Pramlintide, Human amylin Rat amylin, Salmon calcitonin, Rat calcitonin, Rat CGRP. GLP-1, Secretin, CCK-8, GRP inhibited gastric emptying with ED50's (nmol/kg) of 0.09, 0.19, 0.23, 0.28, 0.94, 2.13, 2.76, 3.09, 12.8, 49.9 respectively
Amylin Research	Sprague Dawley rats, Phenol Red method	Rat amylin	Amylin, GLP-I, and CCK-8 fully inhibited gastric emptying, with ED50's of 0.42±0.07, 6.1±0.12, and 8.5±0.20 nmol/kg ± SE of log, respectively.
Amylin Research	Sprague Dawley rats, BB rats, Phenol Red method	Rat amylin (endogenous)	Accelerated gastric emptying in insulin-treated BB/W (amylin deficient IDDM) rats
Amylin Research	Sprague Dawley rats, tritiated glucose method	Rat amylin (endogenous) AC 187	The rate of appearance in plasma of tritium from gavaged [3³H]glucose in non-fasted (amylin-secreting) rats with or without intravenous pre-injection of 3mg of the selective amylin antagonist, AC 187 was 1.7-fold faster, approaching the 3.3-fold increase seen in amylin-deficient BB rats.
Amylin Research	SD rats, tritiated glucose method	Rat amylin	Gastric slowing greatest when delivered into 4" ventricle, then lateral ventricle and peripherally
Amylin Research	SD rats, tritiated glucose method	Rat amylin	Absence of amylin response after vagomoty indicated that amylin modulation of gastric emptying in rats depends upon an intact vagus nerve
Amylin Sponsored	SD rats, tritiated glucose method	Rat amylin	Area postrema (AP)-lesions block the regulation of gastric emptying by amylin
Amylin	Sprague Dawley	Rat amylin	Insulin-induced hypoglycemia reverses amylin-inhibition of gastric emptying in rats
Amylin Research	SD rats, tritiated glucose method, continuous i.v. amylin infusion. Phenol red method	Rat amylin	Insulin-induced hypoglycemia reverses amylin-inhibition of gastric emptying in rats. Hyperinsulinemia had no effect on gastric emptying during euglycemia.
Amylin Research	*SD* rats, tritiated glucose method	Rat amylin	*Amylin-inhibition of gastric* emptying reversed by *insulin-induced* hypoglycemia
Amylin Research	SD rats, exteriorized perfused gut loop	Rat amylin	Amylin infused i.v. did not affect rate of glucose uptake from a perfused loop of duodenum
Amylin Research	Fatty Zucker rats, tritiated glucose method	Rat amylin	The EC50 for inhibition of gastric emptying in obese Zucker rats was 8.3-fold higher than in lean controls (23.9pM ±0.039 log *vs.* 2.89pM ±0.092 log; P<O.0001), showing "amylin resistance". Fasting plasma concentrations of amylin in obese Zucker rats were 5.5-fold higher than in lean controls (15.7±0.93pM vs. 2.83±0.50pM; P<0.0001).

Summary of the individual studies examining the effects of pramlintide on glucagon secretion:

Source	Test System	Test Article	Results
Amylin Research	Anesthetized HSD rat, arginine stimulated of glucagon secretion Clinical study	Pramlintide iv infusion 0.1, 1, 10µg/hr	Dose-dependent inhibition of glucagon secretion stimulated by 2mmol L-arginine. Glucagon secretion inhibited by 56±5% with EC50 of 30.4 pM ±0.38 log.
Amylin Sponsored	Clinical study type 1 diabetic patients, Sustacal challenge, plasma glucagon	pramlintide iv infusion PBO, 25µg/hr or 50µg/hr	The mean + SEM change in fasting glucagon post infusion was -0.69 ±1.10 pg/mL for placebo compared to -3.80±1.31 pg/mL and -4.79± 0.80 pg/mL for the 25µg/hr and 50 pg/hr treatments, respectively (p=-0.11 and p=0.09, crossover ANOVA). Pooled pramlintide results vs. placebo were statistically different (p=0.02).
Amylin Sponsored	Clinical study, 13 type 1 diabetic patients, responses during standard breakfast on last day	Pramlintide 120 µg/day (30 µg qid) or PBO for 4 weeks	Plasma glucagon (49.4 ± 6.6 vs. 65.4 ± 7.5 ng/L) were lower after pramlintide vs. PBO (p<0.05), and area under the curve for both plasma glucose and glucagon were reduced by 26 %
Amylin Sponsored	Clinical study, 13 type 1 diabetic patients, responses during standard breakfast	Pramlintide 120 µg/day (30 µg qid) or PBO for 4 weeks	Increment in plasma glucose and isotopically measured endogenous glucose production in response to exogenous glucagon injection was unaltered by prior pramlintide treatment
Amylin Sponsored	Clinical study, 12 type 2 diabetic patients, glucagon response to Sustacal meal	Pramlintide iv 100µg/hr for 4 hours	pramlintide suppressed glucagon secretion in patients with type 2 diabetes
Amylin Sponsored	Clinical study, euglycemic hyperinsulinemia and hypoglycemic step	Pramlintide iv 50 µg/hr or PBO in crossover	No difference in plasma glucagon concentration with or without pramlintide during hypoglycemic period
Amylin Research	Anesthetized HSD rat, euglycemic clamp, arginine stimulation of glucagon secretion	Rat amylin iv 50µg/hr	Incremental glucagon response suppressed by 47%
Amylin Research	Anesthetized HSD rat, euglycemic clamp. arginine stimulated of glucagon secretion	Rat amylin iv 0.1, 0.2, 1, 3, 10µg/hr	Dose-dependent suppression of arginine-stimulated glucagon secretion by 47-67%, with EC50 of 18pM±0.3 log.
Independent	Isolated perfused rat pancreas	AC66 (sCT[8-32]) 10 µM	No effect of amylin antagonist on basal glucagon secretion in isolated preparation
Amylin Research	Euglycemic clamp, arginine stimulated glucagon responses	Specific neutralizing anti-amylin mAb (25-27) AC 187	In animals administered specific (25-27) anti-amylin antibody, plasma glucagon levels were 61% higher throughout the clamp procedure than after non-specific (40-6) antibody (93±14 vs. 58±5pM, P<0.03, t-test, Welch correction for different SD's). After AC 187, plasma glucagon concentrations were similar before the clamp 82±9 vs. 80±7pµ, respectively, P=0.9), but after 60 min had doubled in the AC187-infused group (160±30pM, P<0.03 vs. t=0) and were higher than in the saline-infused group (103±9pM, P<0.02 between groups).
Amylin Research	Anesth HSD rat, glucagon sampling during insulin induced hypoglycemia	Rat amylin iv 50 pmol/kg/min	Amylin does not suppress hypoglycemia-induced secretion of glucagon in rats or modify the glucose : glucagon relationship
Independent	Isolated perfused rat pancreas	Rat amylin 1 nM	No effect in isolated preparation of amylin on glucagon secreted in response to low glucose, arginine, carbachol or VIP
Amylin Research	Anesth HSD rats, hyperinsulinemic, euglycemia, arginine stimulated for glucagon secretion, then insulin-induced hypoglycemia in same rat	Rat amylin iv 50 pmol/kg/min	Suppression by 45% of arginine-stimulated glucagon secretion, but no suppression of subsequent hypoglycemia-stimulated glucagon secretion

Studies of the individual studies examining the effects of other amylinomimetic agents on food intake:

Source	Test System	Test Article	Results
Amylin Research REST00149	Mice NIH(SW) ip injections, fasted, food intake	Pramlintide	Mice given intraperitoneal pramlintide of 0.1, 1, 3, 10, 30 and 100 µg/kg. Dose-dependent reduction in food intake of up to 55% measured at 30, 60, 90 min after injection.
Independent	Schedule-fed Sprague Dawley rats, intrahypothalamic injection, food intake	Rat amylin	Dose-dependent inhibition of food intake observed with all doses (0.2, 1 and 2 µg/rat i.h. in 2 µL CSF; weight 300-400g). ED50 1µg. Amylin [8-37] C-terminal fragment did not antagonize amylin action.
Independent	Sprague Dawley rats, conditioned taste aversion	Rat amylin	Co-administration of rat amylin 11µg intrahypothalamic with saccharin in drinking water did not reduce subsequent preference for saccharin (taste aversion), conclusion is that rat amylin does not cause anorexia by inducing malaise.
Independent	Sprague Dawley rats, food intake	Rat amylin	Intravenous rat amylin 100 µg/kg inhibited food intake at 1 hour, and increased plasma glucose. Changes in plasma glucose were proposed as causing satiogenic effect.
Independent	Fisher 344 Norway rats, ip injections of 50, 75, 100 µg/kg, food intake	Amylin (unspecified from ——	Amylin administered ip, decreased food intake in 4-month-old rats at doses of 50, 75, and 100 µg/kg. Amylin was slightly more potent at suppressing food intake at 13 months of age and less potent at decreasing food intake in 21 – and 25-month-old rats, but the difference was not significant.
Independent	Mice, TAC(SW), ob/ob, ob/c, db/db, db/c, C57BL/6Nnia, ip injection, food intake	Amylin (unspecified from ——	The effects of amylin on reducing food intake were not attenuated by the cholecystokinin antagonist L-364718, suggesting effects were not mediated via cholecystokinin. Vagotomy did not prevent amylin from inhibiting food intake. Amylin was equally effective at reducing food intake in genetically obese (ob/ob) and lean (ob/c) mice and in diabetic (db/db) and lean (db/c) mice. Amylin effectively suppressed food intake in mice over the age of 4-22 mo.

Independent Independent	Rats, ages 7-9 weeks, 3 months, 15-18 months, vagotomy, i.p. injection, food intake Rats, vagotomy, ip injection, food intake	Rat amylin (from Rat amylin from	In 12-h food-deprived old rats, food intake was decreased significantly by amylin (1-10µg /kg), was most marked at 2 h, and was compensated over 24 h, at doses of 1 and 5 µg/kg did not reduce food intake in undeprived old rats, but at doses of 0.1- 1 µg/kg in young rats, dose-dependently reduced food intake (food-deprived for 24 h, but not 12 h). Vagotomy did not block anorectic effects which tended to last longer in vagotomized rats. Anorectic effects of amylin did not require dietary carbohydrates in the diet. Amylin injected ip at 0.5-2.5 µg/kg reduced food intake for 2-4 h in subdiaphragmatically vagotomized rats with similar magnitude of anorectic effect, but shorter duration of effect but shorter duration of effect with the low dose. Concluded that amylin has a central site of anorectic action.
Independent	Rats Sprague Dawley IP injection, food intake	Rat amylin (from	Intraperitoneal injection 1 µg/kg amylin in food deprived rats reduced the size of the first post-deprivation meal without affecting intra-meal feeding rate or the size or timing of subsequent meals. Amylin increased latency to first post injection meal in undeprived rats. No conditioned taste aversion. Amylin 'inhibits' feeding by facilitating meal-ending satiety processes.
Independent	Rats Sprague Dawley ip injection, food intake	(from	norectic effect of ip amylin (1 µg/kg) abolished by simultaneous IP injection of **the amylin receptor antagonist CGRP(8-37)**, 10 µg/kg. Anorectic effects of 400 µg/kg ip glucagon (which stimulates amylin secretion) were totally blocked with calcitonin gene related peptide, CGRP(8-37). Anorectic effects of CCK (0.25 µg/kg) and bombesin (2 µg/kg) (also stimulate β-cell secretion) were partly neutralized by CGRP(8-37), while anorectic effect of vasopressin was not. At least part of anorectic effect of glucagon, CCK and bombesin mediated via stimulation of amylin release.
Independent	Rats Sprague Dawley ip. Injection, histamine and serotonin blockers, Food intake	Rat amylin (from	Pretreatment of rats with agents blocking histaminergic system transmission, attenuated amylin's. (1 tg/kg)anorectic effect in 24-h food-deprived rats. There was no effect of serotonergic blockers, suggesting involvement of histaminergic, but not serotonergic systems, in transduction of ip amylin's anorectic effect in rats.
Independent	Rats Sprague Dawley i.c.v. injection, amylin blockers, food intake	Rat amylin AC187 (from	ICV injection of the CGRP and amylin receptor antagonist CGRP(8-37) did not block anorectic effects of peripherally injected CGRP and amylin. Similar results with more specific amylin receptor antagonists amylin (8-37) and AC 187. Receptors outside blood-brain-barrier are likely to mediate the anorectic effects of peripherally administered amylin and CGRP.
Amylin Research	Sprague Dawley Rat amylin rats, fasted, chronic cannula, AC 187, ip. amylin		Selective amylin antagonist, AC187 (3mg) injected via cannulated jugular vein blocked effect of 10µg/kg amylin suppression of food intake in 18-hr fasted rats. Anorectic effect of amylin is mediated via receptors that interact with AC 187 (likely amylin receptors).
Amylin Research	Sprague Dawley rats. i.c.v. and i.p. amylin	Rat amylin	Comparison of inhibition of food intake following intraperitoneal (ip) or intracerebroventricular (ICV) administration of rat amylin. EDS0 for ICV amylin was 0.19 µg/rat vs. 10.2 µg/rat after ip amylin. Amylin may regulate food intake via a central structure in rats.
Independent	Wistar rats, pumps, food intake	Rat amylin	Amylin infused s.c. via osmotic mimipump at 0, 2, 7, and 25 pmol/kg/min for 8 days resulted in plasma concentrations of 10, 35, 78 and 237 pM, respectively (ie near physiologic for low dose). The minimal effective dose was 2 pmol/kg/min., decreases in food intake and body weight gain being dose dependent.
Independent	Wistar rats, aortic sampling, venous infusion, food intake	Rat amylin.	Food intake in rats with aortic catheters increased plasma amylin levels from a fasting levels of 11pM to a peak level of 19 pM at 2.2±0.5 h. The threshold intravenous dose for amylin inhibition of feeding was between 1 and 3 pmol/kg/min (decreasing 4-h intake by ~25%) and elevating plasma amylin by ~24 pM. Postprandial plasma amylin levels were nearly (but not quite) sufficient to independently produce satiety.

Summary of the individual studies examining the effects of pramlintide on digestive secretions:

Amylin Research REST98083,	Sprague Dawley rats, chronic gastric cannula, pentagastrin-stimulated acid secretion	Pramlintide subcutaneous injection 0.1, 1, 10µg/rat	In rats whose gastric acid secretion was measured by flushing intragastric cannula, subcutaneously injected pramlintide inhibited pentagastrin-stimulated (125µg/kg s.c.) acid secretion by up to 93.7±2.0% (P<0.001) with an ED50 of 0.066[± 0.2 log units.
Amylin Research	Sprague Dawley rats, anesthetized, cannulated at bile duct, stimulated with 1 µg CCK	Pramlintide subcutaneous injection 0.01, 0.03, 0. 1, 0.3t µg/rat	Pramlintide reduced by 15.8% (n.s.) 60-rain CCK-stimulated flow of pancreatic juice, measured over 60 min. Significant reduction in amylase activity seen with doses of 0.03, 0.1 and 0.3 µg/rat, and lipase activity reductions with doses of 0.01, 0.03, 0.1 and 0.3 µg/rat. Total amylase enzyme secreted in 60 rain, compared to CCK-injected controls, was decreased by up to 30%; lipase secretion reduced by up to 49%.

Summary of the individual studies examining the effects of Other amylinomimetic agents on digestive secretions:

Independent	Sprague Dawley rats, Shay test (pylorus ligated, drug injected, stomach removed after 3h for titration)	Rat amylin 2.5, 5, 10, 40, 100, or 160 µg/kg, sc or icy 1.5, 2.7, or 5 µg/rat	Dose-dependent decrease in acid secretion, significant at 1 µg/rat sc, ~100x lower with icy dose
Independent	Sprague Dawley rats, Shay test (pylorus ligated)	Rat amylin, CGRP	Rat amylin more potent than CGRP in suppression of gastric acid secretion
Amylin Research	Sprague Dawley rats, chronic gastric cannulae, pentagastrin -stimulated acid secretion	Rat amylin sc injection 0.01, 0.1, 1, 10, 100 µg/rat	In rats whose gastric acid secretion was measured by flushing intragastric cannulae, subcutaneously injected rat amylin inhibited pentagastrin-stimulated (125 µg/kg s.c.) acid secretion by up to 93.4±.6% (P<O. O01) with an ED50 of 0.05 µg/rat ± 0. 15 log units.
Independent	Isolated stomach strips from mouse	Rat amylin	Somatostatin-dependent action of rat amylin to inhibit acid secretion from mouse stomach. Blocked with AC 187, not with CGRP[8-37].
Independent	Sprague Dawley rats, Shay test), pylorus ligated)	Rat amylin	An 87% inhibition of acid secretion with sc amylin was reduced to a 27% inhibition following insulin overdose (1 U).
independent	Sprague Dawley rats, gastritis induced by ethanol and indomethacin	Rat amylin icy and sc	Gastroprotective effect only when given centrally, not when given peripherally
Independent	Sprague Dawley rats, gastritis induced by reserpine and serotonin	Rat amylin sc	Gastroprotective effect at supraphysiologic amylin doses (plasma concentrations ~ lnM)
Amylin Research	Sprague Dawley rats gavaged 1 ml absolute EtOH, blinded scaling of mucosal damage	Rat amylin sc 0.001-10µg/rat	Injury score reduced by up to 67%. ED50 0.036lag/rat ± 0.4 log, estimated peak [amylin] 7.7pM Gastroprotective effect of rat amylin blocked with AC187
Amylin Research	Anesthetized HSD rats, cannulated pancreatic duct	Rat amylin 0.1, 0.3, 1 µg/rat sc, CCK-8 1µg/rat sc	Up to 58% inhibition of amylase secretion, 67% inhibition of lipase secretion. ED50's 0.21 µg/rat ±0.18 log, 0.11 µg/rat ± 0.05 log, respectively. Estimated peak [amylin] 15-26pM
Amylin Research	Ar42j cell line, Phospholipase C activation	Rat amylin 1 µM	No direct effect of amylin on Ar42j (pancreatic acinar) cell line. Responsive to PACAP as positive control
Amylin Research	Isolated acini and AR42j cells on general activation measured in micro physiometer	Rat amylin 1 nM and I µM, respectively	No direct effect of amylin on isolated acini or Ar42) cell line. Responsive to PACAP as positive control
Independent	Isolated rat pancreas, isolated acini	CGRP	Inhibition of protein and volume output by CGRP was dose related and maximal at 10n M. CGRP 10nM failed to inhibit amylase secretion from isolated pancreatic acini, stimulated by graded concentrations of CCK8 0.1 pM-0.1µM. Tetradotoxin and atropine but not hexamethonium prevented the inhibition of volume and protein secretion by CGRP. All agents were without effect on exocrine secretion stimulated by CCK8 and secretin (controls). These results indicate that CGRP inhibits pancreatic exocrine secretion by an indirect, neutrally mediated mechanism involving cholinergic-muscarinic transmission
Amylin Research	Mice injected ip with caerulein 0.01µg, rat amylin 0.01, 0.1, µ g 3 times 2-hourly	Rat amylin	Inhibition of plasma amylase (index of severity of pancreatitis) at 0.1, 1 µg amylin injections
Amylin Research REST98'05656	HSD rats, anesthetized exteriorized gut loop	Rat amylin iv 100 µg/hr	Intravenous amylin infusion had no effect on rate of labeled glucose uptake from perfused gut lumen. Phlordzin (positive control) inhibited uptake 91%.

Pharmacology conclusion:

The activities of pramlintide at near physiological concentrations (pmol/L) on gastric emptying to those at supraphysiological concentrations (nmol/L) such as vasodilation have been examined in rats. Pramlintide dose-dependently inhibits gastric emptying in normal rats. This effect is thought to be via area postrema since lesions to this area block the gastric emptying effects of amylin. In addition, pramlintide also reportedly inhibits pentagastrin-stimulated acid secretion and food intake. Thus, pramlintide appears to regulate nutrition assimilation by collectively inhibiting gastric emptying, food intake and gastric acid inhibition. The effect of pramlintide on plasma glucose has been variable. At low concentration it may suppress post-prandial plasma glucose and increase plasma glucose at high concentration perhaps as consequence of gluconeogenesis from lactate.

SAFETY PHARMACOLOGY:

Safety pharmacology studies were conducted to assess the potential effects of pramlintide administration on the cardiovascular and central nervous systems. The potential to induce histamine release following intradermal administration of pramlintide was also investigated

Cardiovascular Effects:

Pramlintide did not induce biologically significant changes in blood pressure or heart rate when administered as a single subcutaneous dose of 0.3 mg/kg (300 µg/kg) to normotensive male rats. The heart rate response of the pramlintide-treated rats to an intra-arterial 2 µg/kg dose of epinephrine was transiently reduced without an effect on blood pressure. Blood pressure and heart rate responses to intra-arterially administered norepinephrine, isoproterenol, acetylcholine and histamine were not affected by pramlintide. Single subcutaneous doses of pramlintide of up to 0.3 mg/kg (300 µg/kg) administered to anesthetized beagle dogs had no significant effects on arterial blood pressure, heart rate, left ventricular pressure, left ventricular end diastolic pressure, +dP/dt, cardiac output, contractile force and Lead II ECG. The intravenous administration of a single dose of 0.3 mg/kg (300 µg/kg) of pramlintide to dogs decreased blood pressure through 25 minutes post-administration, but had no significant effect on the other cardiovascular parameters evaluated.

Neuropharmacology:

In the male rat, no adverse neuropharmacological signs or evidence of convulsant or anticonvulsant activity in response to a 0.2 second transcorneal electroshock occurred following single subcutaneous doses of up to 0.3 mg/kg pramlintide (300 µg/kg). In the acute toxicity studies, pramlintide decreased locomotor activity after a single intravenous dose of 10 mg/kg or subcutaneous dose of 250 mg/kg in acute studies in rats. **Pramlintide decreased locomotor activity in dogs after a single intravenous dose of 0.3 mg/kg**. In the reproductive toxicity studies in rats (repeat administration), pramlintide decreased locomotor activity in the Segment I study at a subcutaneous dose of 1.0 mg/kg/day and in the Segment III study at a subcutaneous dose of 0.5 mg/kg/day. These observations are consistent with those for amylin where an injection of amylin intracerebroventricularly in rats reduced locomotor activity dose-dependently.

Food Intake Effect:

Pramlintide reduced food intake in fasted mice with an estimated ED_{50} of 10 µg/kg. Pramlintide reduced food intake in male but not female rats in the 26-week repeat dose study when administered subcutaneously twice daily at doses of 0.2, 0.5 or 1.2 mg/kg/day. Mean food intake was lower at all doses and significantly reduced at 1.2 mg/kg/day. Pramlintide reduced food intake in male but not female dogs administered doses of 0.5 or 1.2 mg/kg/d subcutaneously twice daily for 26 weeks. Inhibition of food intake by amylin has been demonstrated in the mouse and rat following intraperitoneal or intravenous administration. The site of the effect appears to be located in the *area postrema* of the brain. Consistently reduced food intake induced by pramlintide in male rats over 26 weeks produced lower terminal body weights in males at all doses (0.2, 0.5 and 1.2 mg/kg/day). Consistent reduction of food intake by male dogs over 26 weeks produced lower terminal body weights in males at 0.2, 0.5, and 1.2 mg/kg/day.

Dermal Irritation and Immunogenic Reaction Studies

The potential local dermal irritations of different pramlintide formulations were tested in rabbits. The formulations varied in their pH (range 4.0 to 4.7) and in their concentrations of pramlintide (0.05 mg or 0.5 mg), metacresol, acetate and mannitol. Following a single subcutaneous injection, none of the formulations caused significant subcutaneous irritation. A study was also conducted in mice to determine if pramlintide is immunogenic as indicated by a delayed-type hypersensitivity (DTH) response. The mice were sensitized by three intradermal injections of pramlintide in sterile water, with or without complete Freund's adjuvant (CFA), ovalbumin (OVA) with CFA or sterile

water with CFA administered every third week. The mice were challenged one week after the last sensitization by injection of 30 µL of either 0.5% pramlintide solution, OVA or sterile water. The DTH response was determined by the difference in the thickness of the left and right ear of each mouse. Pramlintide did not induce a DTH response in the mouse. The wheal reaction induced by intradermal administration of pramlintide at doses of 0.06 or 0.6 mg/kg (60 or 600 µg/kg) was not due to the release of histamine.

Safety Summary:

The effects of pramlintide on locomotor activity, food intake, gastric emptying and body weights are consistent with those induced by amylin. Although acute dermal injection of pramlintide formulations did not cause dermal irritation in rabbits, repeated SC injections (chronic tox studies) showed significant injection site lesions that were more pronounced than vehicle treated control animals. Pramlintide did not induced delayed-type hypersensitivity reaction in mice. Intravenous administration, but not subcutaneous administration, of high doses of pramlintide reduced locomotor activity in the dogs. Reduced gastric emptying occurred after either subcutaneous or intravenous administration. In chronic studies with subcutaneous administration, pramlintide reduced food intake and lowered terminal body weights in male rats and dogs. The wheal reaction induced by pramlintide was not due to histamine release.

PHARMACOKINETICS/TOXICOKINETICS:

Dose vs. systemic exposure study in **CD-1 mice** after single SC dose and after 28 consecutive daily SC dose of AC-0137 (REST99045R1): The formulated drug and dose concentrations and strain of mice were the same as the 2-year carcinogenicity study. The AUC data from males and females and Day 1 and Day 28 were combined and used determine human exposure ratios.

- The entire dose was cleared before the next dose.
- $AUC_{0\text{-}120\ min}$ values were proportional to dose
- AUC values in males were slightly higher than in females
- AUC on Day 1 and Day 28 were similar

$AUC_{0-120\ min}$ values in ng min/mL for mice administered 1 or 28 daily subcutaneous doses of pramlintide.

Dose mg/kg	Males			Females			Combined Male & Female, Day 1 & Day 28
	1 Dose	28 Doses	Combined	1 Dose	28 Doses	Combined	Combined
0.2	4,539	3,489	4,052	2,297	2,862	2,626	3,340
0.5	6,891	8,300	7,799	6,358	6,250	6,291	7,012
1.2	20,231	17,818	19,024	14,269	13,331	13,743	16,499

Dose vs. systemic exposure study in SD rats after single SC dose and after 28 consecutive daily SC dose of AC-0137 (REST99046R1): The formulated drug and dose concentrations and strain of rats were the same as the 2-year carcinogenicity study. In the 26-WK rat study, doses were divided into two daily injections. The AUC data from males and females and Day 1 and Day 28 were combined and used to determine human exposure ratios.

- $AUC_{0\text{-}120\ min}$ values were proportional to SC doses.
- AUC values for Day 1 and Day 28 were similar
- Systemic exposure of females were higher than males.

$AUC_{0-120\ min}$ values in ng min/mL for rats administered 1 or 29 daily subcutaneous doses of pramlintide.

Dose mg/kg	Males			Females			Combined Male & Female, Day 1 & Day 29
	1 Dose	29 Doses	Combined	1 Dose	29 Doses	Combined	Combined
0.04	230	164	198	226	433	330	267
0.1	348	670	461	621	429	533	497
0.2	982	820	901	1064	832	946	923
0.6	2428	1865	2118	2350	3320	2835	2478

26 Week pramlintide TK study in rats (1186/2, REST98067R1):

Five SD rats/sex/dose were treated with daily subcutaneous injection of 0.2, 0.5 or1.2 mg/kg/d pramlintide for 26 weeks (BID). Two rats/sex/group were bled 15, 30, 60 and 120 minutes after each dosing on day 1 and in weeks 18 and 26. The data from first (morning) and second (afternoon) dose of the day were pooled to calculate the daily exposures:

- Some of the data from day 1 were missing because of testing failures or below limits of quantification.
- The plasma concentration was generally similar between week 18 and 26.

- Mean values for Cmax and AUC_{0-2} were generally proportional to dose. The values for Cmax and AUC_{0-2} appear to be more variable in females than males.
- Pramlintide is rapidly absorbed.
- In the males, Cmax and AUC_{0-2h} values for all doses at weeks 18 and 26 were higher than the values at day 1.
- All raw values were in pmol/L. The molecular weight of pramlintide is 3905 daltons. All AUC values were converted to ng.min/ml using the following formula:
  Value in pmol.hr/L÷1000 X3.905X60= value in ng.min/ml values

Pharmacokinetic Parameters[1] after Subcutaneous Pramlintide Administration to Rats at Day 1, Week 18, and Week 26

Sex	Dose (mg/kg b.i.d.)	Time of Sample	n[3]	t_{max} hrs	C_{max} ng/ml (pmol/L)	AUC_{0-2} ng·hr/ml (pmol·hr/L)
Males	0.1	Day 1[4]	8	0.25	0.88 (224.84)	0.41 (104.38)
		Week 18	12	0.25	8.12 (2080.15)	4.26 (1091.24)
		Week 26[2]	12	0.25	17.10 (4379.00)	7.43 (1902.69)
	0.25	Day 1	8	0.25	4.14 (1059.41)	1.61 (412.32)
		Week 18	14	0.25	18.94 (4850.19)	11.64 (2980.03)
		Week 26[2]	8	0.25	24.99 (6399.49)	13.84 (3544.17)
	0.6	Day 1	8	0.25	35.60 (9116.77)	13.53 (3463.56)
		Week 18	15	0.25	50.92 (13039.18)	45.03 (11530.27)
		Week 26[2]	11	0.25	65.42 (16752.88)	53.53 (13708.07)
Females	0.1	Day 1		NA	NA	NA
		Week 18	14	0.5	27.36 (7007.43)	19.68 (5039.69)
		Week 26[2]	14	0.5	38.21 (9784.89)	26.16 (6699.10)
	0.25	Day 1		NA	NA	NA
		Week 18	14	0.25	32.04 (8204.87)	14.74 (3775.90)
		Week 26[2]	16	0.25	23.47 (6010.24)	14.18 (3631.24)
	0.6	Day 1		NA	NA	NA
		Week 18	16	0.25	108.19 (27704.48)	71.63 (18343.69)
		Week 26[2]	16	0.25	80.06 (20501.92)	44.66 (11436.62)

NA = not able to determine.
[1] Mean plasma concentration was combined from each daily dose to calculate pharmacokinetic parameters for each sex at each dose (0.1, 0.25, and 0.6 mg/kg b.i.d.).
[2] In the samples taken at all time points after the first daily dose in week 26, a sample preparation error was made; thus, new blood samples were taken after the first daily dose in week 27 and the samples were analyzed for pramlintide concentration.
[3] n = number of values used to calculate parameter.
[4] The values given for Day 1, 0.1 mg/kg/day have limited reliability due to the lack of data.

<u>26 Week pramlintide TK study in dogs (1186/3, REST9806R1):</u>

Four beagle dogs/sex/dose were treated with daily subcutaneous injection of 0.2, 0.5 or1.2 mg/kg/day pramlintide for 16 and 26 weeks. Dose was divided into two daily injections. Blood samples (~1 mL) were collected from all dogs/sex/group prior to dose initiation on day 1 and at 15 and 30 minutes, and 1, 2 and 4 hours after the first and second dose on one day only during Weeks 16 and 26. There were no differences in plasma concentrations of first and second daily doses. The data from first and second dose were pooled for each sex and dose group (table below):

- Cmax and AUC_{0-4h} values were dose-related
- There were no sex differences in AUC or Cmax

Mean Pharmacokinetic Parameters after Subcutaneous Pramlintide Administration to Dogs after 16 and 26 Weeks

Sex	Dose (mg/kg)	n	Week	Mean ± SD[1]		
				t_{max} hr	C_{max} ng/ml (pmol/L)	AUC_{0-t} ng·hr/ml (pmol·hr/ml)
Male	0.1 mg/kg b.i.d. (0.2 mg/kg/day)	3[2]	16	0.25 ± 0	33.04 ± 7.88 (8461.43 ± 2018.79)	26.33 ± 6.87 (6742.15 ± 1759.53)
		4	26	0.38 ± 0.14	26.67 ± 8.61 (6829.50 ± 2205.99)	29.87 ± 14.57 (7650.07 ± 3732.12)
	0.25 mg/kg b.i.d. (0.5 mg/kg/day)	4	16	0.25 ± 0	70.81 ± 32.34 (18131.91 ± 8282.92)	55.46 ± 23.02 (14201.25 ± 5895.99)
			26	0.31 ± 0.13	64.61 ± 12.97 (16544.65 ± 3320.94)	72.35 ± 12.37 (18526.97 ± 3167.42)
	0.6 mg/kg b.i.d. (1.2 mg/kg/day)	7	16	0.29 ± 0.09	220.70 ± 154.20 (56518.18 ± 39488.88)	257.87 ± 360.78 (66036.41 ± 92390.32)
			26	0.32 ± 0.12	127.41 ± 68.45 (32627.94 ± 17528.90)	120.88 ± 83.70 (30955.58 ± 21434.74)
Female	0.1 mg/kg b.i.d. (0.2 mg/kg/day)	3[2]	16	0.25 ± 0	30.11 ± 6.30 (7711.19 ± 1613.91)	20.11 ± 2.48 (5149.93 ± 634.51)
		4	26	0.31 ± 0.13	26.04 ± 4.08 (6668.14 ± 1045.19)	22.82 ± 8.85 (5842.91 ± 2265.51)
	0.25 mg/kg b.i.d. (0.5 mg/kg/day)	4	16	0.31 ± 0.13	76.12 ± 28.28 (19493.48 ± 7242.35)	70.54 ± 26.04 (18064.61 ± 6667.66)
			26	0.31 ± 0.13	59.87 ± 16.59 (15332.88 ± 4248.64)	75.74 ± 23.21 (19394.78 ± 5943.70)
	0.6 mg/kg b.i.d. (1.2 mg/kg/day)	7	16	0.25 ± 0	167.68 ± 52.92 (42938.93 ± 13551.46)	167.00 ± 107.96 (42764.48 ± 27646.51)
			26	0.32 ± 0.12	150.69 ± 55.13 (38588.54 ± 14118.31)	124.71 ± 57.58 (31936.55 ± 14743.95)

[1] Plasma concentrations from the two daily doses were combined to calculate pharmacokinetic parameters for each sex at each of the different doses (0.2, 0.5, and 1.2 mg/kg/day).
[2] Data was insufficient for pharmacokinetic analysis in one female and one male dog in the 0.1 mg/kg b.i.d group at week 16.

52 Week pramlintide TK study in dogs (1186/9, REST98105R1):

Four beagle dogs/sex/dose were treated with once daily subcutaneous injection of 0.1, 0.25 or 0.6 mg/kg/day pramlintide for 52 weeks. Blood was collected from one dog/sex/group prior to dosing and at 15, 30 and 120 minutes postdose on day 1 and during weeks 13 and 26. On the last dose day (week 52), blood was collected prior to dosing, immediately after dosing, and at 5, 15, 30, 45, 60, 120, 180, 240 and 360 minutes after dosing (see table below):

- Pramlintide was rapidly absorbed (Tmax = 15 min) in a generally dose-related manner.
- In general, PK parameters from WK1 to WK52 and between male and female dogs were similar.
- The increases in Cmax and AUC_{0-2hr} were dose-related, with no sex-related differences.
- Cmax and AUC_{0-2h} values at weeks 13 and 26 were higher than values at week 1 (except for the male dosed at 0.1 mg/kg).

Pharmacokinetic Parameters of Pramlintide in Male Dogs
Following Daily Subcutaneous Injections for 52 Weeks

Dose (mg/kg)	Week of Sample	t_{max} (min)	C_{max} ng/ml (pmole/L)	AUC_{0-2} ng·min/ml (pmol·min/L)	AUC_{0-t} ng·min/ml (pmol·min/L)
0.1	1	15	19 (4769)	1191 (305100)	
	13	15	21 (5461)	1277 (327057)	
	26	15	16 (3998)	731 (187204)	
	52	15	26 (6635)	1136 (290908)	1222 (312958)
0.3	1	15	38 (9850)	1560 (399594)	
	13	15	59 (15206)	3850 (985950)	
	26	30	234 (60030)	23584 (6039389)	
	52	5	107 (27438)	4331 (1109189)	6036 (1545608)
0.6	1	15	119 (30593)	8917 (2283449)	
	13	15	145 (37068)	10051 (2573997)	
	26	15	181 (46391)	10057 (2575456)	
	52	5	37 (9372)	1303 (333793)	1651 (422906)

Pharmacokinetic Parameters of Pramlintide in Female Dogs
Following Daily Subcutaneous Injections for 52 Weeks

Dose (mg/kg)	Week of Sample	t_{max} (min)	C_{max} ng/ml (pmole/L)	AUC_{0-t} ng·min/ml (pmole·min/L)	$AUC_{0-\infty}$ ng·min/ml (pmole·min/L)
0.1	1	15	14 (3613)	609 (156013)	
	13	30	16 (4112)	1301 (333119)	
	26	15	27 (6948)	2029 (519542)	
	52	15	31 (8051)	1859 (475930)	2289 (586136)
0.3	1	15	46 (11718)	2154 (551658)	
	13	15	75 (19128)	4719 (1208351)	
	26	15	46 (11652)	3166 (810829)	
	52	15	46 (11892)	2787 (713775)	3578 (916306)
0.6	1	15	73 (18612)	3779 (967801)	
	13	15	151 (38652)	9675 (2477687)	
	26	15	113 (28827)	5680 (1454660)	
	52	15	104 (26606)	4137 (1059427)	4424 (1132992)

Single dose PK in female rabbits (RESR98102):

Increases in Cmax and AUC_{0-24} were dose-proportional.

Mean ± SD Pharmacokinetic Parameters after a Single Subcutaneous Dose of Pramlintide to Non-pregnant Rabbits

Dose (mg/kg)	t_{max} hr	C_{max} ng/ml (pM)	AUC_{0-24} ng·hr/ml (pM·hr)	$t_{1/2}$ hr	CL/F ml·hr/kg
0.03	0.29 ± 0.1	20.2 ± 11.3 (5183.4 ± 2893.8)	12.3 ± 3.9 (3138.8 ± 990.2)	0.28 ± 0.05[a]	2268.0 ± 439.9[a]
0.1	0.29 ± 0.10	60.1 ± 27.3 (15390.4 ± 6996.5)	41.6 ± 18.4 (10640.3 ± 4718.2)	0.47 ± 0.15[a]	2383.7 ± 692.2[a]
0.3	0.38 ± 0.31	178.87 ± 98.86 (45806.3 ± 25315.7)	124.36 ± 70.40 (31847.0 ± 18027.2)	0.52 ± 0.12[a]	6019.9 ± 9422.6

n=6 except where indicated
[a] n=5

Pharmacokinetic parameters in female rabbits after repeated SC pramlintide (REST98106):

Three non-pregnant female rabbits (six/group) were given a single subcutaneous dose of either 0.03, 0.1 or 0.3 mg/kg of pramlintide. After artificial insemination (days 6-27 post-insemination) they were treated with 0.03, 0.1 or 0.3 mg/kg pramlintide BID. Blood samples were collected from the pregnant rabbits prior to dosing and at 0.5, 1, 2 and 6 hours post dose on days 6, 12 and 18 (study days 1, 6 and 12, respectively). On day 27, blood samples were collected prior to and at 5 minutes after the first (and only) dose.

Mean ± SD Pharmacokinetic Parameters of Pramlintide in Pregnant Rabbits following b.i.d. Subcutaneous Injections from Day 6 to Day 27 Post-insemination, (Combining the 2 Daily Doses)

Dose	Day of Sample	n[a]	t_{max} hr	C_{max} ng/ml (pM)	AUC_{0-t} ng·hr/ml (pM·hr)	$t_{1/2}$ hr	CL/F ml·hr/kg
0.03 mg/kg b.i.d.	6	8[f]	0.32 ± 0.16	20.6 ± 12.8 (5281.1 ± 3284.4)	11.1 ± 4.7 (2848.5 ± 1212.4)	0.82 ± 1.02	3009.0 ± 1354.5
	12	9[f]	0.24 ± 0.12	22.1 ± 6.4 (5671.2 ± 1632.5)	12.1 ± 4.0 (3104.2 ± 1035.4)	0.89 ± 0.24	2996.1 ± 1243.6
	18	5[b]	0.27 ± 0.15	29.1 ± 9.1 (7441.0 ± 2318.0)	22.5 ± 9.0 (5765.2 ± 2295.4)	0.86 ± 0.07	1547.8 ± 655.9
0.1 mg/kg b.i.d.	6	6	0.42 ± 0.13	87.3 ± 26.9 (22345.9 ± 6890.9)	59.3 ± 11.9 (15183.2 ± 3059.4)	0.65 ± 0.16	1756.4 ± 438.8
	12	8[b]	0.66 ± 0.95	61.3 ± 34.4 (15685.4 ± 8818.7)	54.7 ± 33.4 (14010.8 ± 8548.9)	1.98	233.4
	18	6	0.38 ± 0.14	110.1 ± 25.1 (28198.2 ± 6439.3)	92.8 ± 4.1 (23766.7 ± 1056.6)	NA	1077.6
0.3 mg/kg b.i.d.	6	7[e]	0.30 ± 0.15	179.8 ± 120.9 (46054.1 ± 30960.9)	110.5 ± 80.3 (28290.5 ± 20570.1)	1.04 ± 0.81	27593.4 ± 55446.6
	12	6[c]	0.29 ± 0.10	228.7 ± 26.8 (58564.2 ± 6851.8)	131.7 ± 68.6 (33729.4 ± 17565.8)	1.65 ± 0.08	350.5 ± 219.6
	18	5[b]	0.30 ± 0.11	269.2 ± 148.2 (68944.7 ± 37945.3)	198.5 ± 116.5 (50822.4 ± 29842.0)	1.29	954.7

NA=not able to determine
[a] n=number of data points used to calculate mean parameter; [b] n=1 for $t_{1/2}$; [c] n=2 for $t_{1/2}$, [d] n=3 for $t_{1/2}$;
[e] n=5 for $t_{1/2}$, [f] n=7 for $t_{1/2}$

Mean ± SD Pharmacokinetic Parameters of Pramlintide in Pregnant Rabbits following b.i.d. Subcutaneous Injections from Day 6 to Day 27 Post-insemination

Dose	Day of Sample	Daily Dose	n[a]	t_{max} hr	C_{max} ng/ml (pM)	AUC_{0-4} ng·hr/ml (pM·hr)	$t_{1/2}$ hr	Cl/F ml·hr/kg
0.03 mg/kg b.i.d.	6	AM	4[d]	0.38 ± 0.14	11.9 ± 3.7 (3050.1 ± 958.1)	7.0 ± 1.1 (1788.0 ± 289.9)	1.35 ± 1.55	4364.3 ± 750.2
		PM	4	0.27 ± 0.17	29.3 ± 12.9 (7512.0 ± 3313.7)	15.3 ± 2.3 (3909.1 ± 588.8)	0.43 ± 0.09	1992.6 ± 282.2
	12	AM	6[f]	0.19 ± 0.09	22.2 ± 7.5 (5688.6 ± 1926.6)	11.6 ± 3.5 (2969.9 ± 889.7)	0.90 ± 0.29	2939.4 ± 1157.6
		PM	3[e]	0.33 ± 0.14	22.0 ± 4.6 (5636.3 ± 1173.8)	13.2 ± 5.7 (3372.9 ± 1465.2)	0.86	3137.8
	18	AM	2[b]	0.17	25.1 (6428.9)	17.6 (4511.3)	0.93	1490.5
		PM	3[e]	0.33 ± 0.14	31.7 ± 11.6 (8115.8 ± 2973.4)	25.8 ± 10.7 (6601.1 ± 2741.3)	0.83	1576.4
0.1 mg/kg b.i.d.	6	AM	2	0.38	78.8 (20187.9)	53.2 (13623.1)	0.59	2030.1
		PM	4	0.44 ± 0.13	91.5 ± 32.8 (23424.9 ± 8405.5)	62.3 ± 7.4 (15963.2 ± 1893.3)	0.68 ± 0.14	1619.6 ± 191.6
	12	AM	6[b]	0.79 ± 1.09	43.8 ± 11.6 (11205.5 ± 2959.5)	48.8 ± 37.3 (12493.0 ± 9541.5)	1.98	233.4
		PM	2	0.25	113.7 (29125.0)	72.5 (18564.4)	NA	1379.3
	18	AM		NA	NA	NA	NA	NA
		PM	6	0.38 ± 0.14	110.1 ± 25.1 (28198.2 ± 6439.3)	92.8 ± 4.1 (23766.7 ± 1056.6)	NA	1077.6
0.3 mg/kg b.i.d.	6	AM	5[e]	0.32 ± 0.18	197.2 ± 107.6 (50493.3 ± 27560.8)	108.2 ± 56.4 (27707.1 ± 14432.2)	0.84 ± 0.78	2803.4 ± 1463.6
		PM	2[b]	0.25	136.5 (34956.1)	116.2 (29749.2)	1.85	126753.3
	12	AM	6[c]	0.29 ± 0.10	228.7 ± 26.8 (58564.2 ± 6851.8)	131.7 ± 68.6 (33729.4 ± 17565.8)	1.65 ± 0.08	350.5 ± 219.6
		PM		NA	NA	NA	NA	NA
	18	AM		NA	NA	NA	NA	NA
		PM	5[b]	0.30 ± 0.11	269.2 ± 148.2 (68944.7 ± 37945.3)	198.5 ± 116.5 (50822.4 ± 29842.0)	1.29	954.7

NA=not able to determine

[a] n=number of data points used to calculate mean parameter; [b] n=1 for $t_{1/2}$; [c] n=2 for $t_{1/2}$, [d] n=3 for $t_{1/2}$, [e] n=4 for $t_{1/2}$; [f] n=3 for $t_{1/2}$

There were no difference between morning and evening plasma pramlintide concentrations (pooled).

- In the pregnant rabbits the Cmax and AUC (0-6 hr) were dose-related.
- Cmax and AUC_{0-6h} values at day 18 were higher than those at day 6 in each dose group.
- **The ratio of pramlintide in fetal to parent plasma levels was low, indicating that very little pramlintide crossed the placental barrier.**

Mean ± SD Pramlintide Plasma Concentration after the First Daily Dose in Pregnant Rabbits and their Fetuses on Day 27 Post-insemination

Dose (mg/kg b.i.d.)[a]		Time of Sample[b]	n	Mean ± SD Plasma Concentration ng/ml (pM/L)	Ratio (fetus/parent)
0.03	Pregnant Rabbit	Pre-dose	6	0.15 ± 0.08 (37.63 ± 20.83)	0.006
		5 min	6	9.49 ± 7.30 (2430.28 ± 1869.14)	
	Fetus	~ 5 min	11	0.06 ± 0.04 (14.38 ± 10.36)	
0.1	Pregnant Rabbit	Pre-dose	5	0.14 ± 0.06 (36.43 ± 14.82)	0.006
		5 min	5	33.82 ± 5.07 (8660.40 ± 1298.04)	
	Fetus	~ 5 min	6	0.21 ± 0.11 (53.49 ± 28.96)	
0.3	Pregnant Rabbit	Pre-dose	6	0.12 ± 0.20 (29.89 ± 51.76)	0.003
		5 min	6	72.83 ± 44.81 (18649.33 ± 11475.60)	
	Fetus	~ 5 min	8	0.25 ± 0.07 (63.39 ± 18.76)	

[a] On day 27, the rabbits were given the first daily dose and sacrificed after the 5 min blood sample.
[b] Immediately following the maternal sacrifice, a blood sample was obtained by cardiocentesis from at least 2 fetuses per pregnant rabbit.

Pharmacokinetics summary table for pramlintide in humans:

PK parameters of pramlintide in type 1 diabetic patients on Day 1 and Day 5 (study protocol 137-143):

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide, Day 1

	Plasma Pramlintide					
	Treatment A N=11		Treatment B N=11		Treatment C N=11	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax(pmol/L)	41.9	22.9	64.5	23.7	99.4	31.4
Tmax(hr)	0.273	0.0753	0.365	0.237	0.321	0.115
AUC(0-t)(pmol*hr/L)	32.86	28.40	74.14	34.26	118.5	65.37
AUC(0-inf)(pmol*hr/L)	81.37	22.17	102.6	37.24	144.2	65.74
T1/2(hr)	1.12	1.01	0.970	0.337	0.774	0.281
Kel(1/hr)	0.887	0.374	0.796	0.278	1.03	0.423
AUCR	0.738	0.155	0.784	0.0650	0.879	0.0308

Treatment A = 1 x 30 µg Pramlintide TID
Treatment B = 1 x 60 µg Pramlintide TID
Treatment C = 1 x 90 µg Pramlintide TID

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide, Day 5

	Plasma Pramlintide					
	Treatment A N=10		Treatment B N=11		Treatment C N=10	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax(pmol/L)	37.6	22.8	74.4	20.0	92.7	26.7
Tmax(hr)	0.328	0.120	0.334	0.113	0.288	0.0827
AUC(0-t)(pmol*hr/L)	26.45	21.93	79.68	48.98	104.8	63.53
AUC(0-inf)(pmol*hr/L)	64.96	9.629	117.2	44.39	136.3	66.70
T1/2(hr)	0.726	0.239	0.789	0.326	0.722	0.230
Kel(1/hr)	1.02	0.282	1.00	0.384	1.03	0.281
AUCR	0.800	0.0873	0.835	0.0707	0.867	0.0455

Treatment A = 1 x 30 µg Pramlintide TID
Treatment B = 1 x 60 µg Pramlintide TID
Treatment C = 1 x 90 µg Pramlintide TID

PK parameters of pramlintide in type 2 diabetic patients (study protocol 137-144)

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide, Day 1

	Plasma Pramlintide					
	Treatment D N=12		Treatment E N=11		Treatment F N=12	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax pmol/L	36.4	18.5	55.7	25.7	74.0	24.6
Cmax/BMI pmol/L	1.3	0.8	1.9	1.2	2.6	1.1
Tmax hr	0.290	0.0723	0.253	0.00808	0.260	0.0303
AUC(0-t) pmol*hr/L	32.24	46.23	53.93	52.15	76.24	54.37
AUC(0-t)/BMI pmol*hr/L	1.164	1.697	1.924	2.032	2.693	2.095
AUC(0-inf) pmol*hr/L	201.5	111.6	124.9	85.32	119.2	79.45
AUC(0-inf)/BMI pmol*hr/L	7.532	3.259	4.592	3.145	4.231	2.974
T1/2 hr	2.72	1.49	1.38	1.20	1.10	0.778
Kel 1/hr	0.300	0.165	0.725	0.338	0.811	0.338
AUCR	0.620	0.0558	0.757	0.0832	0.788	0.0700

Treatment D = 1 x 60g Pramlintide TID
Treatment E = 1 x 90g Pramlintide TID
Treatment F = 1 x 120g Pramlintide TID

Summary of the Pharmacokinetic Parameters of Plasma Pramlintide, Day 5

	Plasma Pramlintide					
	Treatment D N=12		Treatment E N=11		Treatment F N=12	
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	Arithmetic Mean	SD
Cmax pmol/L	42.2	20.2	60.1	24.4	77.2	28.2
Cmax/BMI pmol/L	1.4	0.8	2.0	1.0	2.7	1.3
Tmax hr	0.255	0.00862	0.301	0.105	0.275	0.0712
AUC(0-t) pmol*hr/L	35.06	38.05	59.66	46.52	91.19	77.00
AUC(0-t)/BMI pmol*hr/L	1.235	1.471	2.070	1.784	3.252	2.942
AUC(0-inf) pmol*hr/L	107.7	67.12	129.2	63.29	152.4	102.7
AUC(0-inf)/BMI pmol*hr/L	3.813	2.682	4.550	2.519	5.532	3.852
T1/2 hr	1.42	1.40	1.43	0.991	1.15	0.543
Kel 1/hr	0.791	0.425	0.615	0.243	0.686	0.231
AUCR	0.755	0.100	0.760	0.0943	0.783	0.0527

Treatment D = 1 x 60g Pramlintide TID
Treatment E = 1 x 90g Pramlintide TID
Treatment F = 1 x 120g Pramlintide TID

For the purpose of exposure comparisons, the AUC values were converted from pmol/L to ng.min/L. Sponsor had reported AUC values in type I and II diabetics on day 1 and day 5. To assess total human exposure, the total AUC was calculated by multiplying the AUC values by the number of daily injections in type 1 and 2 patients. **In addition, the mean AUC from Day 1 and Day 5 were calculated for comparison with animal AUC data.** The maximal dose in type I and type II diabetic subjects are 90 µg QID and 120 µg TID, respectively.

Example: After single 90 µg dose of pramlintide in type 1 patient (118.5+104.8)/2 ≈**112 pmol.hr/L**
112 pmol.hr/L➔ (112/1000)*3.949*60= 26.54 ng.min/ml per dose
26.54 ng.min/ml X 4 = **106 ng.min/ml total AUC**

Dose, µg	N	AUC, pmol.hr/L	AUC, ng.min/ml
90 µg in type 1 diabetes (QID)	10	112 (448)	26 (26x4=106)
120 µg in type 2 diabetes (TID)	12	84 (252)	20 (20x3=60)

Dose proportionality of pramlintide was demonstrated when either 60 µg or 120 µg was delivered in a constant volume of 0.2 mL indicating that the concentration of pramlintide does not affect bioavailability. It was also shown that the pharmacokinetic parameters Cmax, AUC_{0-t} and AUC_{0-inf} were dose proportional across the range 30 to 120 µg, and that Tmax and the elimination half-life stayed approximately constant.

In addition, the pramlintide pharmacokinetic parameters Cmax and AUC_{0-t} were fairly consistent in healthy volunteers and subjects with type 1 diabetes at doses of 30 µg and 60 µg. Due to its short terminal half-life, pramlintide does not accumulate following multiple dose administration within the therapeutic dose range, and multiple dose kinetics are the same as those following single dose.

Mean Pharmacokinetic Parameters Following Administration of Single SC Doses of Pramlintide (Study 137-126)

Subcutaneous Dose (µg)	$AUC_{(0-300min)}$ pmol·min/L	$AUC_{(0-inf)}$ pmol·min/L	C_{max} (pmol/L)	T_{max} (min)	$t_{1/2}$ (min)
30	3215	3750	39	21	55
60	6261	6778	79	20	49
90	7939	8507	102	19	51
120	11380	11970	147	21	48

Cross-reference: 137-126 PK Addendum Page 2

Excretion: The direct excretion of pramlintide in the urine of rats was minimal. Following the intravenous infusion of 0.2 mg pramlintide, the pramlintide and des-lys pramlintide excreted in the urine were <0.003% of the total pramlintide infused from renal-ligated and sham-operated rats demonstrated a 3.7-fold higher concentration of total circulating immunoreactive pramlintide (IEMA) associated with renal ligation. **This indicates that the kidney is important in pramlintide metabolism and clearance.**

Metabolism of Pramlintide:
Pramlintide and the major metabolite, des-lys pramlintide, were readily detected in the plasma of rats following intravenous infusion of 10 mg pramlintide/hr for one hour. **Des-lys pramlintide was the primary metabolite** representing approximately 30% of pramlintide plasma immunoreactivity. Four minor metabolites were also identified; carboxy-terminal pramlintide fragments 15-37, 16-37, 17-37 and 24-37. The metabolic profile was similar following subcutaneous administration of 1 mg pramlintide to rats. Pramlintide and des-lys pramlintide were similar in potency when tested in the rat nucleus accumbens receptor binding assay for amylin and exhibited similar ED_{50s} in the rat

gastric emptying assay, inhibiting gastric emptying in a dose-dependent manner. In the dog, des-lys pramlintide was also identified as the major metabolite. The des-lys pramlintide concentration relative to that of total immunoreactive pramlintide increased with time from 19% at 15 minutes postdose to 48% at 120 minutes postdose.

Protein Binding: The binding of pramlintide to plasma components, evaluated in the mouse, rat, rabbit, dog and man, was low in all species tested. The mean percent of pramlintide tracer bound to soluble plasma components ranged from 11% in the dog to **33% in man.** The pharmacokinetic and pharmacodynamic activity of pramlintide and des-lys pramlintide were similar in rats.

PK/TK conclusions:

In rats, the toxicokinetics of different lots of pramlintide from three different manufacturers of bulk drug were similar. After 18 or 26 weeks of daily subcutaneous doses of 0, 0.2, 0.5 or 1.2 mg/kg/day of pramlintide to rats (BID), the Cmax and $AUC_{0\text{-}2\ hr}$ values at Weeks 18 and 26 were higher than the values on Day 1. The mean Cmax and $AUC_{0\text{-}2\ hr}$ were dose-related but were more variable in the females than in the males.

In the 52-week dog toxicity study, pramlintide (0, 0.1, 0.3 or 0.6 mg/kg/day) was rapidly absorbed after SC administration. In the 52-week study, Cmax and $AUC_{0\text{-}2\ hr}$ values at Weeks 13 and 26 were higher than values at Week 1. Values for the 52-week samples tended to be lower than values for the samples taken at Week 26. The AUC data from 52-WK and 26-WK PK studies were not consistent (i.e. the AUC for 0.6 mkd in 52-WK study for males (in WK 13) was **167.5** ng.hr/ml, where as the AUC for 1.2 mkd was 257.8 or **128.5** ng.hr/ml for 0.6 mkd). The plasma Symlin levels decreased with time in both 26-WK and 52-WK dog study. Whether, there was an antibody related neutralization of Symlin in dogs is unknown.

In rabbits, after single pramlintide dose, the increases in Cmax and $AUC_{0\text{-}24\ hr}$ were dose-proportional and the plasma clearance was fairly constant across all doses. Following 21 days of subcutaneous administration (BID at 0.03, 0.1 or 0.3 mg/kg/dose) in pregnant rabbits, the Cmax and $AUC_{0\text{-}6\ hr}$ values increased in a dose-related manner. The fetal plasma concentrations of pramlintide were low compared to the parent plasma concentration (fetal-to-dam ratios of 0.003 to 0.006) in the rabbit. Pramlintide and human amylin did not cross the placental barrier in pregnant rats.

The plasma metabolites of pramlintide were 2-37 pramlintide (des-lys 1 pramlintide), 15-37 pramlintide, 16-37 pramlintide, 17-37 pramlintide and 24-37 pramlintide. Only des-lys 1 pramlintide was active in the receptor-binding assay. The pharmacodynamic activity of pramlintide and des-lys 1 pramlintide were similar in rats following a 10 µg intravenous infusion of pramlintide or des-lys 1 pramlintide. Both compounds increased arterial plasma glucose and lactate concentrations, decreased blood pressure, and ionic calcium concentrations. The binding of pramlintide to plasma components in the mouse, rat, rabbit, dog, and man was low in all species tested. The mean percent of pramlintide tracer bound to soluble plasma components ranged from 11% in the dog to 33% in man.

TOXICOLOGY:

Several acute and repeated dose studies were carried out in rats and dogs. Since these studies were not reviewed in detail, only a summary of general findings will be discussed. A detailed review of the 26-WK in rat and dog and the 52-WK dog study were made and reported.

Acute Toxicity Studies:
After a single subcutaneous dose of 500 mg/kg (rat) or 2.0 mg/kg (dog) or an intravenous dose of 10.0 mg/kg (rat and dog), no drug-related mortality occurred. At subcutaneous doses of 250 mg/kg or higher, an abnormal gait and an abnormal stance were observed. These signs occurred shortly after dosing and were transient, disappeared within 30 minutes to 4 hours after dosing. Clinical signs in dogs treated with 1 mg/kg or higher (SC) included diarrhea. When given intravenously, diarrhea, decreased activity and emesis occurred at doses of 0.3, 1.0 and 10.0 mg/kg or greater, respectively.

Repeat Dose Toxicity Studies
In a 28 day acute study, pramlintide doses up to 2 mg/kg/day (SC) was well tolerated by mice. Small but significant changes in some erythrocyte parameters were noted. Following subcutaneous injection, higher erythrocyte counts and hemoglobin and hematocrit values, as compared to control, occurred in the 2.0 mg/kg dosed group. Serum glucose (≥0.3 mg/kg) and lactate (2.0 mg/kg) values were lower and phosphorus values (2.0 mg/kg) were higher than the concurrent control values. In both studies injection site irritation, observed in all groups including the vehicle controls, was characterized by hemorrhage and cellulitis or epidermatitis. In the 28-day study in rats, pramlintide was administered BID at total daily doses of 0.2, 0.5 and 1.2 mg/kg. Vasodilation of the extremities was observed at ≥0.2 mg/kg. Serum glucose (≥0.2 mg/kg), total protein (≥0.5 mg/kg) and albumin (≥1.2 mg/kg) values were lower compared to control. Heart weights were lower than control values in the 1.2 mg/kg-dosed males; however, there were no changes in the heart histology.
A second 28-day subcutaneous study in rats compared the toxicologic effects of pramlintide lots manufactured by three different bulk drug manufacturers [ ]. Plasma concentrations were similar for the three products. Irritation induced at the injection site was similar across the three products. There were no signs of systemic toxicity.
In dogs administered pramlintide subcutaneously at doses up to 2.0 mg/kg for 14 days, irritation at the injection site was considered to be treatment-related. Diarrhea occurred intermittently in females at 0.3 mg/kg and in males and females at 1.0 and 2.0 mg/kg. Dogs administered 0.3 mg/kg pramlintide by intravenous injection for 14 days, experienced emesis, quivering and decreased activity. Diarrhea occurred at all doses with a dose-related increase in frequency. In the 28-day study, the principal findings were postdose vasodilation (≥0.2 mg/kg), and lower body weight gains (≥0.5 mg/kg), shorter prothrombin times (≥0.5 mg/kg), lower serum phosphorus and mean urine specific gravity levels (≥0.2 mg/kg) as compared to the controls. The injection sites were discolored in treated groups as well as the vehicle control.

Study Title: AC-0137: 26-Week Subcutaneous Administration Chronic Toxicity Study in Rats with a 13-Week Interim Kill

Key study findings: There were no major findings except for injection site fibrosis in all high dose and control rats. There were no significant changes in clinical chemistry and food intake, However, the body weight gains in high dose animals were lower than controls.
Study no: 1186/2-1050 (REST98118R1)

Volume #, and page #: 1.46-1.47 and pages 1-778
Conducting laboratory and location: [redacted]
Date of study initiation: Aug 18, 1994/completed on Feb 22, 1994
GLP compliance: yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: 94-0501CB, 94-0603CB, 94-0903CB, 95-0106EB, 94-0604CB. The purity was [redacted]
Formulation/vehicle: Vehicle was provided by the sponsor to the [redacted] It contained metacresol (preservative), acetic acid, sodium acetate, mannitol, and water for injection.
Methods (unique aspects): The toxicity of AC-0137 was evaluated in rats after SC injections at interim 13 weeks (10/sex/dose) and at the end of the study, week 26 (15 /sex/dose). Rats were housed 5/sex/cage.

Dosing:
Species/strain: — CDBR rat, [redacted]
#/sex/group or time point (main study): 30 rats/sex/group was used at the start of the study. 10/sex/dose were terminated at the interim 13-week evaluation, the rest were sacrificed at week 26.
Satellite groups used for toxicokinetics or recovery: 5 animals/sex/group
Age: 28 days
Weight: 262 to 302 for males, 177 to 226 for females
Doses in administered units: 0, 0.2, 0.5 and 1.2 mg/kg. Drug solution was prepared daily and samples were taken for future analysis. The doses were divided into two SC injections 12 hours apart. The drug levels in the administered solutions were equal to the target solution concentrations.
Route, form, volume: subcutaneous, solution, ≤1.2 ml/kg. The test article was in a clear liquid form.

Observations and times:

Clinical signs:	Once a day up to WK4 and once a week thereafter
Body weights:	weekly
Food consumption:	Weekly
Ophthalmoscopy:	Prior to and on Week 12 and 25
EKG:	NA

Hematology: Standard Hematology test, WK 13, 26: Leukocyte, Erythrocyte, Hemoglobin, Hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet, Prothrombin Time, Activated Partial Thromboplastin Time (APTT), Differential Leukocyte Count Percent and Absolute Neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophil, Platelet, Red Cell Morphology. Insufficient blood was collected from some interim animals, so they were bled again on WK14.

Clinical chemistry: Standard tests, WK 13, 26: Albumin, Total Protein, Globulin, A/G Ratio, Total Bilirubin, Urea Nitrogen, Creatinine, Alkaline Phosphatase, Alanine Aminotransferase, Aspartate Aminotransferase, Gamma Glutamyltransferase, Glucose, Total Cholesterol, Calcium, Chloride, Phosphorus, Potassium, Sodium, Triglycerides, Immunoglobulin E. Microscopy of Sediment. Insufficient blood was collected from some interim animals, so they were bled again on WK14.

Urinalysis: Specific Gravity, pH, Urobilinogen, Total Volume, Color, Appearance, Protein, Glucose, Ketones (KET), Bilirubin (BIL), Occult Blood (BLD), Leukocytes (LEU), Nitrites (NIT).

Gross pathology: Gross lesions from all animals were examined. See addendum page 38
Organs weighed: See addendum for list page 38
Histopathology: Control and HD animals were evaluated. All tissues from animals killed before scheduled time were also evaluated. Animals were euthanized with sodium pentobarbitone. See standard tissue histopath, see addendum for list, page 38

Toxicokinetics: Blood samples (caudal vein) taken from 2 satellite rats/group/sex 15 min, 30 min, 1hr and 2 hrs after both doses on Day 1, and in WK18 ad 26. The PK/TK data are also shown in PK section of the review.

Results:

Mortality: There were 11 mortalities. The cause of demise was not related to the drug. The cause of death in 1 control and 1HD was not determined. Most had damaged eye related to blood sampling (1 C, 3LD, 2 MD, 1HD). **Two animals had liver necrosis (1 MD and 1 HD) due to perhaps torsion of a liver lobe.** Five of the dead animals were from the interim group (1C, 2 LD, 1MD, 1HD). Six of the dead animals were from the terminal groups (1C, 2LD, 2MD, 2HD).

Clinical signs:

- Red extremities were noted from Week 1 onward in most pramlintide treated animals. Control males also displayed similar red extremities but only during Week 2.
- AT Wk 26, the injection site reactions were more severe than at WK 13. However, the reaction at the injection sites in HD groups was similar to controls.

Body weights: There was slight dose-dependent decrease in body weight gain primarily in males (-20%, $p<0.05$) from WK 5 until the end of the study. Body weight of HD males at WK 13 and WK 26 were lower than controls (-7% and –11.5%).

Body weight gain,	0.2 mg/kg		1.2 mg/kg	
	M	F	M	F
Body weight gain, % change, WK 13-26			-29%	
Body weight gain, % change, WK 0-26	-10.2%		-20%	

Food consumption: No significant effect on food consumption was noted.
Ophthalmoscopy: No significant treatment related finding.
Hematology: No overall changes in hematology although at WK 26 except for a significant dose-dependent increase in platelet counts in pramlintide treated females (max 11%). Some of the hematology findings at WK 13 were not found at WK 26.
Clinical chemistry:

- There were no significant changes in plasma glucose , cholesterol
- There were significant decreases (-5%) in calcium levels of MD and HD males.
- BUN and creatinine decreased in HD females by 22 and 16%, respectively.
- Immunoglobulin E level in LD males was lower (26.8 %) relative to controls at WK13. The dose-response test was significant for the increase in immunoglobulin E levels in males at WK 26.

Urinalysis: No significant finding.
Organ weights: No changes in absolute and relative organ weights were noted at WK 13. However, at WK 26, the relative liver and kidney weights were elevated ($p<0.05$, see table).

Body weight adjusted Organ weights compared to controls		0.5 mg/kg/d		1.2 mg/kg/d	
		M	F	M	F
	Week				
Liver , % Δ	26	6.8%			
Kidney, % Δ	26				9%

Gross pathology:

- Injection site reactions were seen in both controls and treated animals.
- There were no macroscopic or microscopic findings at WK 13 or WK26.
- One HD female had large pituitary.
- The ovaries of 6/20 LD females were large.

Histopathology at WK 13:

- Pancreatic fatty atrophy, 1/10, HD male
- Liver lobular necrosis 1/10 HD male and 1/9 HD female
- Optic nerve neuropathy, 1/10 HD male
- Focal nephropathy in 1/9 HD female

Injection site histopathology in rats killed WK13

Tissue	Sex	Dose, mg/kg/day			
		0	0.2	0.5	1.2
Left Shoulder	M	10/10	0/9	0/10	6/10
Fibrosis	F	6/9	0/9	0/9	7/9
	M	0/10	0/9	0/10	1/10
Myositis	F	1/9	0/9	0/9	1/9
Right Shoulder	M	9/10	0/9	0/10	7/10
Fibrosis	F	7/9	0/9	0/9	7/9
	M	2/10	0/9	0/10	0/10
Myositis	F	1/9	0/9	0/9	1/9
Left Thigh	M	7/10	0/9	0/10	9/10
Fibrosis	F	8/9	0/9	0/9	8/9
	M	0/10	0/9	0/10	0/10
Myositis	F	2/9	0/9	0/9	1/9
Right Thigh	M	9/10	1/9	4/10	0/10
Fibrosis	F	8/9	0/9	0/9	8/9
	M	0/10	0/9	1/10	0/10
Myositis	F	1/9	0/9	0/9	1/9

Histopathology at WK 26:

	Pramlintide, mg/kg/day							
	Females, n =19-20/dose				Males, n=19-20/dose			
	0	0.2	0.5	1.2	0	100	300	500
Optic Neuropathy	3/20	0	0	4/19	0	0	0	5/19
Liver Inflammatory cell foci	19/20	1/19	0	18/19	19/19	1/20	0	19/19
Pancreas, inflammatory cell foci	1/20	0	0	5/19	4/19	0	0	2/19
Kidney, Focal nephropathy	7/20	0	0	3/19	0	0	0	1/19
Pituitary adenoma	0	0	0	1/19	0	0	0	0

Injection site histopathology in rats killed WK26

Tissue	Sex	Pramlintide, mg/kg/day			
		0	0.2	0.5	1.2
Left Shoulder	M	20/20	0	0	9/19
Fibrosis	F	19/19	0	1/19	19/19
	M	0	0	0	1/19
Myositis	F	3/19	0	0	6/19
Right Shoulder	M	17/20	0	0	19/19
Fibrosis	F	19/19	1/20	1/19	17/19
	M	3/20	0	0	5/19
Myositis	F	3/19	0	0	1/19
Left Thigh	M	20/20	0	0	18/19
Fibrosis	F	17/19	0	0	18/19
	M	0	0	0	1/19
Myositis	F	0	0	0	1/19
Right Thigh	M	19/20	0	0	19/19
Fibrosis	F	18/19	0	1/19	19/19
	M	0	0	0	1/19
Myositis	F	0	0	0	4/19

PK data:

- No data were available for day 1, dose 1 because of testing failures.
- Plasma concentration of pramlintide for the 0.1, 0.25 and 0.6 mg/kg bid, groups were erratic or below the level of detection limits.
- The plasma concentration between week 18 and 26 was generally similar.
- Mean values for Cmax and AUC_{0-2} were generally proportional to dose. The values for Cmax and AUC_{0-2} appear to be more variable in females than males.
- Pramlintide is rapidly absorbed.
- In the males, Cmax and AUC_{0-2h} values for all doses at weeks 18 and 26 were higher than the values at day 1.

Pharmacokinetic Parameters[1] after Subcutaneous Pramlintide Administration to Rats at Day 1, Week 18, and Week 26

Sex	Dose (mg/kg b.i.d.)	Time of Sample	n[3]	t_{max} hrs	C_{max} ng/ml (pmol/L)	AUC_{0-2} ng·hr/ml (pmol·hr/L)
Males	0.1	Day 1[4]	8	0.25	0.88 (224.84)	0.41 (104.38)
		Week 18	12	0.25	8.12 (2080.15)	4.26 (1091.24)
		Week 26[2]	12	0.25	17.10 (4379.00)	7.43 (1902.69)
	0.25	Day 1	8	0.25	4.14 (1059.41)	1.61 (412.32)
		Week 18	14	0.25	18.94 (4850.19)	11.64 (2980.03)
		Week 26[2]	8	0.25	24.99 (6399.49)	13.84 (3544.17)
	0.6	Day 1	8	0.25	35.60 (9116.77)	13.53 (3463.56)
		Week 18	15	0.25	50.92 (13039.18)	45.03 (11530.27)
		Week 26[2]	11	0.25	65.42 (16752.88)	53.53 (13708.07)
Females	0.1	Day 1		NA	NA	NA
		Week 18	14	0.5	27.36 (7007.43)	19.68 (5039.69)
		Week 26[2]	14	0.5	38.21 (9784.89)	26.16 (6699.10)
	0.25	Day 1		NA	NA	NA
		Week 18	14	0.25	32.04 (8204.87)	14.74 (3775.90)
		Week 26[2]	16	0.25	23.47 (6010.24)	14.18 (3631.24)
	0.6	Day 1		NA	NA	NA
		Week 18	16	0.25	108.19 (27704.48)	71.63 (18343.69)
		Week 26[2]	16	0.25	80.06 (20501.92)	44.66 (11436.62)

NA = not able to determine.
[1] Mean plasma concentration was combined from each daily dose to calculate pharmacokinetic parameters for each sex at each dose (0.1, 0.25, and 0.6 mg/kg b.i.d.).
[2] In the samples taken at all time points after the first daily dose in week 26, a sample preparation error was made; thus, new blood samples were taken after the first daily dose in week 27 and the samples were analyzed for pramlintide concentration.
[3] n = number of values used to calculate parameter.
[4] The values given for Day 1, 0.1 mg/kg/day have limited reliability due to the lack of data.

Summary:

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 ⟍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 starting at Week 5 in a dose-related manner. 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 at the injection site was slightly greater than controls.

Study Title: AC-0137: 26-Week Subcutaneous Administration Chronic Toxicity Study in the Dog

Key study findings: There were no significant pramlintide related effects on ECG, blood pressure, clinical pathology evaluation or organ weights. Only notable finding was the inflammatory lesions at the injection sites attributed to dose volume. There was no evidence of any systemic toxicity at doses up to 1.2 mg/kg/day (84X human dose based on AUC).

Study no: 1186/3 (REST98123R1)
Volume #, and page #: 1.48 and pages 1-445
Conducting laboratory and location: [ ]
Date of study initiation: Aug 23, 1994
GLP compliance: yes
QA report: yes (X) no ()
Drug, lot #, radiolabel, and % purity: 94-0501CB, 94-0603CB, 94-0903CB, 95-0106EB, 94-604C. The purity was [ ]
Formulation/vehicle: Vehicle was provided by the sponsor to the [ ]. It contained metacresol (preservative), acetic acid, sodium acetate, mannitol, and water for injection.
Methods (unique aspects): The toxicity of AC-0137 was evaluated in dogs after SC injections at 13 and at the end of the study, week 26. Drug was injected twice daily (12 hrs apart) subcutaneously in a rotating manner between shoulder.
Dosing:
Species/strain: Beagle dogs, [ ]
#/sex/group or time point: See table below. Interim animals were killed on WK13. The recovery animals for Control and HD were allowed to recover for 4 WK and terminated on

Group number	Group description	Dose level (mg/kg/day)	Number of animals					
			Main study		Interim study●		Treatment-free	
			Male	Female	Male	Female	Male	Female
1	control	0	4	4	2	2	3	3
2	low	0.2	4	4	2	2		
3	intermediate	0.5	4	4	2	2		
4	high	1.2	4	4	2	2	3	3

WK30.
Satellite groups used for toxicokinetics or recovery: None
Age: 4 to 6 month old
Weight: 5.4 to 10.2 kg males and 5.5 to 8.1 kg females

Doses in administered units: 0, 0.2, 0.5 and 1.2mg/kg/day. Drug solution was prepared daily and divided into two SC injections.

Route, form, volume: The volume of SC administrations were 0.6, 0.1, 0.25 and 0.6 ml/kg/day for control, LD, MD and HD animals.

Observations and times:

Clinical signs:	Daily plus complete biweekly examination.
Body weights:	weekly
Food consumption:	daily
Ophthalmoscopy:	Prior to and on Week 13 and 26
EKG and BP:	Prior to treatment and on Wk 13 and 26

Hematology: Standard Hematology test, prior to and during WK 13 and 26

Clinical chemistry: Standard tests, prior to and during WK 13 and 26

Urinalysis: Standard urinalysis prior to and during WK13 and 26

Gross pathology: Gross lesions from all animals were examined. See addendum page 38

Organs weighed: See addendum for list page 38

Histopathology: Control and HD animals were evaluated. All tissues from animals killed before scheduled time were also evaluated. Animals were euthanized with sodium pentobarbitone. See standard tissue histopath, see addendum for list, page 38

Toxicokinetics: Blood samples (caudal vein) were taken from all animals at 15, 30 min and 1, 2, and 4 hrs post dose on Day 1, WK 17 and 26.

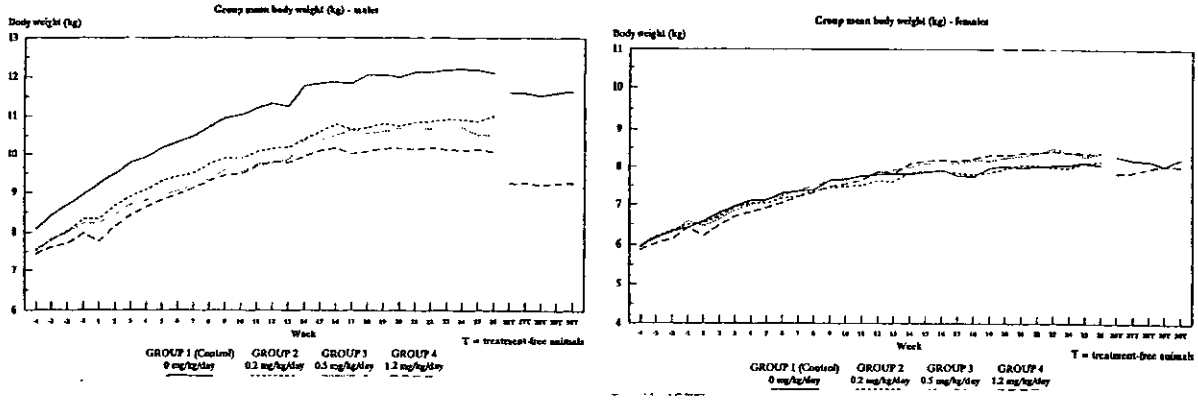
Results:

Mortality: There were no deaths.

Clinical signs: Some animals had soft/loose feces and incidence of vomiting. The incidences may have been higher in the treated groups.

Body weights:

- Both male and female treated dogs gained less weight than respective controls during the first 4 weeks but only MD and HD groups were significant at WK4.
- Body weight gain was dose-dependently decreased with pramlintide treatment.
- HD males had significantly lower body weight gain on WK 13 (-21%) and WK 26 (-29.8%) than controls.



Food consumption: The food intake of all pramlintide treated males decreased by WK 4. The drop in food consumption persisted until the end of the treatment (WK 26). The food intake of LD, MD and HD males at WK were 2.7, 5.9 and 7.7% lower than control ($p<0.05$). No significant change in food intake of females was noted which corresponded with no changes in body weights (see figure above).

Ophthalmoscopy: No significant treatment related finding.

ECG and BP: Heart rate in HD group was below controls. No change in ECG or BP

Hematology: No notable finding

Clinical chemistry: No notable change
Urinalysis: No notable change
Organ weights: No notable change in absolute or relative organ weight.
Gross pathology: No treatment related macroscopic finding

Histopathology:

There were no drug-related histopathology finding in any of the organs examined except for injection site reactions that included changes in subcutis. The injection site findings were: increased hemorrhage, cellulitis/fibrosis, necrosis and occasionally vasculitis/perivasculitis (see next page for incidence). Since these findings were seen in all treated animals and especially with larger injection volumes in control and HD group, the findings were attributed to method of drug delivery and volume and vehicle used in this study.

The injection site reactions in the recovery animals were less severe than in animals from interim (WK 13) or terminal kill (WK26). The findings consisted mainly of fibrosis with very little inflammation.

Terminal kill: Incidence of selected findings at injection sites

		Group and sex							
Tissue and finding		1M	2M	3M	4M	1F	2F	3F	4F
Left shoulder	Number examined	4	4	4	4	4	4	4	4
Vasculitis/perivasculitis	Grade -	4	1	0	0	4	1	0	1
	1	0	3	1	3	0	2	0	2
	2	0	0	2	0	0	1	3	1
	3	0	0	1	1	0	0	1	0
Cellulitis/fibrosis	*Grade -*	*0*	*1*	*0*	*0*	*0*	*0*	0	0
	1	0	2	0	0	2	3	0	0
	2	2	1	0	1	1	1	2	1
	3	2	0	2	0	1	0	2	3
	4	0	0	2	3	0	0	0	0
Necrosis	Grade -	2	3	2	0	2	4	3	3
	1	2	1	1	2	2	0	1	1
	2	0	0	1	2	0	0	0	0
Right shoulder	Number examined	4	4	4	4	4	4	4	4
Vasculitis/perivasculitis	Grade -	4	2	0	0	4	3	0	0
	1	0	1	0	2	0	1	2	0
	2	0	1	0	1	0	0	1	3
	3	0	0	4	1	0	0	1	1
Cellulitis/fibrosis	Grade -	0	1	0	0	0	0	0	0
	1	1	2	0	0	2	3	0	0
	2	1	1	1	0	1	1	1	0
	3	2	0	2	2	1	0	3	3
	4	0	0	1	2	0	0	0	1
Necrosis	Grade -	2	3	2	0	2	4	2	1
	1	1	1	2	1	0	0	2	1
	2	1	0	0	2	2	0	0	2
	3	0	0	0	1	0	0	0	0

Key: Grade - = finding not present, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe.

Treatment-free kill: incidence of selected findings at injection sites

Tissue and finding		Group and sex 1M	2M	3M	4M	1F	2F	3F	4F
Left shoulder	Number examined	3	0	0	3	3	0	0	3
Vasculitis/perivasculitis	Grade -	3	0	0	2	3	0	0	1
	1	0	0	0	1	0	0	0	2
Cellulitis/fibrosis	Grade -	1	0	0	0	1	0	0	0
	1	1	0	0	1	2	0	0	1
	2	1	0	0	2	0	0	0	2
Necrosis	Grade -	3	0	0	3	3	0	0	3
Right shoulder	Number examined	3	0	0	3	3	0	0	3
Vasculitis/perivasculitis	Grade -	3	0	0	2	3	0	0	1
	1	0	0	0	1	0	0	0	2
Cellulitis/fibrosis	Grade 1	3	0	0	0	3	0	0	1
	2	0	0	0	3	0	0	0	2
Necrosis	Grade -	3	0	0	3	3	0	0	3

Key: Grade - = finding not present, 1 = minimal, 2 = slight

Toxicokinetics: for more detail see PK/TK section

Mean Pharmacokinetic Parameters after Subcutaneous Pramlintide Administration to Dogs after 16 and 26 Weeks

Sex	Dose (mg/kg)	n	Week	Mean ± SD[1] t_{max} hr	C_{max} ng/ml (pmol/L)	AUC_{0-t} ng·hr/ml (pmol·hr/ml)
Male	0.1 mg/kg b.i.d. (0.2 mg/kg/day)	3[2]	16	0.25 ± 0	33.04 ± 7.88 (8461.43 ± 2018.79)	26.33 ± 6.87 (6742.15 ± 1759.53)
		4	26	0.38 ± 0.14	26.67 ± 8.61 (6829.50 ± 2205.99)	29.87 ± 14.57 (7650.07 ± 3732.12)
	0.25 mg/kg b.i.d. (0.5 mg/kg/day)	4	16	0.25 ± 0	70.81 ± 32.34 (18131.91 ± 8282.92)	55.46 ± 23.02 (14201.25 ± 5895.99)
			26	0.31 ± 0.13	64.61 ± 12.97 (16544.65 ± 3320.94)	72.35 ± 12.37 (18526.97 ± 3167.42)
	0.6 mg/kg b.i.d. (1.2 mg/kg/day)	7	16	0.29 ± 0.09	220.70 ± 154.20 (56518.18 ± 39488.88)	257.87 ± 360.78 (66036.41 ± 92390.32)
			26	0.32 ± 0.12	127.41 ± 68.45 (32627.94 ± 17528.90)	120.88 ± 83.70 (30955.58 ± 21434.74)
Female	0.1 mg/kg b.i.d. (0.2 mg/kg/day)	3[2]	16	0.25 ± 0	30.11 ± 6.30 (7711.19 ± 1613.91)	20.11 ± 2.48 (5149.93 ± 634.51)
		4	26	0.31 ± 0.13	26.04 ± 4.08 (6668.14 ± 1045.19)	22.82 ± 8.85 (5842.91 ± 2265.51)
	0.25 mg/kg b.i.d. (0.5 mg/kg/day)	4	16	0.31 ± 0.13	76.12 ± 28.28 (19493.48 ± 7242.35)	70.54 ± 26.04 (18064.61 ± 6667.66)
			26	0.31 ± 0.13	59.87 ± 16.59 (15332.88 ± 4248.64)	75.74 ± 23.21 (19394.78 ± 5943.70)
	0.6 mg/kg b.i.d. (1.2 mg/kg/day)	7	16	0.25 ± 0	167.68 ± 52.92 (42938.93 ± 13551.46)	167.00 ± 107.96 (42764.48 ± 27646.51)
			26	0.32 ± 0.12	150.69 ± 55.13 (38588.54 ± 14118.31)	124.71 ± 57.58 (31936.55 ± 14743.95)

[1] Plasma concentrations from the two daily doses were combined to calculate pharmacokinetic parameters for each sex at each of the different doses (0.2, 0.5, and 1.2 mg/kg/day).
[2] Data was insufficient for pharmacokinetic analysis in one female and one male dog in the 0.1 mg/kg b.i.d group at week 16.

Multiple of human exposure based on maximum dose of 360 µg/day and AUC data from dogs treated with 0.2, 0.5 and 1.2 mg/kg/day

Dose, mg/kg/d	Sex	AUC, ng.min/ml	Multiple of human exposure (max dose, 360 µg/day)	
			Type I diabetes (AUC 106 ng.min/ml)	Type II diabetes (AUC 60 ng.min/ml)
0.2	M	1792	**17 X**	**30 X**
	F	1369	**13 X**	**23 X**
0.5	M	4341	**41 X**	**72 X**
	F	4544	**43 X**	**77 X**
1.2	M	7253	**68 X**	**121 X**
	F	7482	**71 X**	**125 X**

Summary:
Dogs were treated with 0, 0.2, 0.5 and 1.2 mg/kg/day pramlintide for 26 weeks. The dose was divided into two daily injections 12 hrs apart. The volume of injections for control and HD were 0.6 ml/kg/day. There were no mortalities. Pramlintide treatment appeared to increase the incidence of vomiting which may have contributed to decrease in body weight of dogs since HD males had significantly lower body weight at Week 13 (-20%) and Week 26 (29%). The effect was not apparent in females at any dose levels. There were no significant pramlintide related effects on ophthalmology, ECG, blood pressure or clinical chemistry evaluation. There were no changes in organ weights. Only notable finding was the inflammatory lesions at the injection sites. Since the severity of the lesions increased with injection volume (control and HD group) and increases in study duration (13 WK vs. 26 WK) and resolved during the recovery, the injection site findings were not attributed to the drug substance. There was no evidence of any systemic toxicity at doses up to 1.2 mg/kg/day (70X human dose based on AUC). The minor elevations in adjusted liver and kidney weights were not accompanied by any histological lesions and pramlintide was considered not to produce any organ toxicity.

Study Title: AC-0137: 52-Week Subcutaneous Administration Chronic Toxicity Study in the Dog

Key study findings: 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. No significant toxicological finding was noted. There was an increase in the incidence vasculitis/perivasculitis at the injection sites in treated dogs.

Study no: 1186/9-1050 (REST98127R1)
Volume #, and page #: 1.49 and pages 1-387
Conducting laboratory and location: [redacted]

Date of study initiation: Jan 26, 1995/completed on July 22,1996
GLP compliance: yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: 94-1202GB, 94-0604CE, 94-0806 CB, 95-0303GB, 94-1101GB. The purity was [redacted]
Formulation/vehicle: Vehicle was provided by the sponsor to the [redacted] It contained metacresol (preservative), acetic acid, sodium acetate, mannitol, and water for injection.
Methods (unique aspects): The toxicity of AC-0137 was evaluated in dogs after SC injections at 13, 26, 38 week and at the end of the study, week 52. Drug was injected subcutaneously in a rotating manner from left to right shoulders to left to right thigh. The volume of administration was 0.6 ml/kg.

Dosing:
Species/strain: Beagle dogs, —
#/sex/group or time point (main study): 4 animals/sex/group
Satellite groups used for toxicokinetics or recovery: None
Age: 4 to 6 months old
Weight: 5.4 to 10.2 kg males and 5.5 to 8.1 kg females
Doses in administered units: 0, 0.1, 0.3 and 0.6 mg/kg/day. Drug solution was prepared daily and samples were taken for future analysis. The drug levels in the administered solutions were equal to the target solution concentrations.
Route, form, volume: subcutaneous, solution volume 0.6 ml/kg

Observations and times:

Clinical signs:	daily and with detailed weekly examination
Body weights:	weekly
Food consumption:	weekly
Ophthalmoscopy:	Prior to and on Week 12, 25, 38 and 51
EKG:	Prior to treatment and on Wk 25 and 51

Hematology: Standard Hematology test, WK 13, 26, 39 and 52 after 18 hr fast. Bone marrow smears were also prepared at necropsy but not examined.
Clinical chemistry: Standard tests, WK 13, 26, 39 and 52 after 18 hr fast (ad lib water, no food).
Urinalysis: Standard urinalysis at the same time schedule as clinical chemistry.
Gross pathology: Gross lesions from all animals were examined. See addendum page 38
Organs weighed: See addendum for list page 38
Histopathology: All animals were evaluated. Animals were euthanized with sodium thiopentone. See standard tissue histopath, see addendum for list, page 38
Toxicokinetics: Blood samples (caudal vein) taken on Day 1 and in WK 13 and 28 (-5, 15, 30 and 120 min post doing). Blood samples were also take on WK 52 at –5, 5, 15, 30, 45, 60, 120, 180, 240 and 360 min. The plasma from WK 28 pre-dose was also analyzed for antibody presence. PK/TK data is also located in PK section.

Results:
Mortality: No mortalities
Clinical signs:
- Soft or loose stool was noted in all treated and controls animals.
- Occasional vomiting was also noted during the study.
- Incidence of observation at the injection site was limited.

Body weights:
- There was no statistically significant treatment effect among males.
- The 28, 31 and 47% decrease in body weight gains in respective LD, MD and HD females were not statistically lower than concurrent controls.

Food consumption:
- The food intakes among males were similar.
- The food consumption of HD females (--23%) during the first 4WKs were lower than controls. The overall 52 WK food consumption was lower but not statistically different.

Ophthalmoscopy: No significant treatment related finding.
EKG: The heart rate of all animals decreased during the study relative to basal values. No treatment effect on ECG was noted.
Hematology: No significant dose related change or trend in hematology parameters was noted.
Clinical chemistry:
- No significant dose-related trends in clinical chemistry parameters indicative of drug toxicity were noted.

- No change in glucose, cholesterol and AST levels were observed except for a minor increase in glucose levels in HD females (13%) at WK52.
- ALT levels in males tend to be lower than controls.
- The HD females had lower total bilirubin (-63%) than controls.

Urinalysis: Minor unremarkable variations in urine parameters were noted.
Organ weights:

- Pituitary weight in MD and HD males were significantly lower (~27%) than controls.
- Liver weight of treated males (~10%) and females (~14%) appeared to be lower than controls.
- The ovarian weight of all treated animals appeared to be lower than controls by as much as 42%.
- The kidney weight of all females were lower than controls by as much as ~20%.

Gross pathology:

- No notable macroscopic findings except for red appearance of the injection site were noted in all animals.
- Dark areas were frequently found in treated animals at the injection sites.

Histopathology:
There were no notable histopathological findings except for injection site reactions.
Injection Site histopathology findings were observed in dogs treated with vehicle, 0.1, 0.3 and 0.6 mg/kg/day pramlintide for 52 WKs. In general, the injection site findings (vasculitis/perivasculitis) in treated animals were slightly more severe than controls. Since, the injection volume was the same for all animals (0.6 ml/kg), the slight increase in severity may have been due to drug substance. In previous studies, the injection volumes in low and mid dose were lower than controls or HD group.

Incidence of selected histopathology findings at injection sites

Tissue and finding		Group and sex 1M	2M	3M	4M	1F	2F	3F	4F
Left shoulder	Number examined	4	4	4	4	4	4	4	4
Vasculitis/perivasculitis	Grade -	4	0	1	1	3	1	0	0
	1	0	1	1	1	1	1	2	0
	2	0	3	2	1	0	1	1	3
	3	0	0	0	1	0	1	1	1
Cellulitis/fibrosis	Grade -	0	0	0	0	1	0	0	0
	1	3	1	1	0	3	1	2	0
	2	1	3	3	2	0	2	0	3
	3	0	0	0	2	0	1	1	1
	4	0	0	0	0	0	0	1	0
Right shoulder	Number examined	4	4	4	4	4	4	4	4
Vasculitis/perivasculitis	Grade -	4	0	0	1	3	1	0	0
	1	0	1	1	1	1	1	1	1
	2	0	2	3	0	0	1	2	2
	3	0	1	0	2	0	1	1	1
Cellulitis/fibrosis	Grade 1	1	2	0	0	1	2	0	0
	2	3	1	2	1	3	1	3	1
	3	0	1	2	3	0	1	1	3
Left thigh	Number examined	4	4	4	4	4	4	4	4
Vasculitis/perivasculitis	Grade -	4	0	0	1	4	2	0	0
	1	0	0	2	0	0	1	1	1
	2	0	3	2	3	0	1	1	1
	3	0	1	0	0	0	0	2	2
Cellulitis/fibrosis	Grade 1	1	0	1	0	2	3	0	1
	2	3	2	3	2	2	0	2	2
	3	0	2	0	2	0	1	2	1
Right thigh	Number examined	4	4	4	4	4	4	4	4
Vasculitis/perivasculitis	Grade -	3	0	0	1	4	2	0	0
	1	1	1	0	1	0	0	2	0
	2	0	3	4	1	0	1	2	3
	3	0	0	0	1	0	1	0	1
Cellulitis/fibrosis	Grade -	0	0	0	1	0	0	0	0
	1	1	1	0	0	2	3	2	0
	2	3	1	3	3	1	0	2	4
	3	0	2	1	0	1	1	0	0

Key: Grade - = finding not present, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe.

Toxicokinetics:

Pramlintide was rapidly absorbed (Tmax = 15 min) in a generally dose-related manner.

- In general, PK parameters from WK1 to WK52 and between male ad female dogs were similar.
- The increases in Cmax and $AUC_{0\text{-}2hr}$ were dose-related, with no sex-related differences.
- Cmax and $AUC_{0\text{-}2h}$ values at weeks 13 and 26 were higher than values at week 1 (except for the male dosed at 0.1 mg/kg).

Pharmacokinetic Parameters of Pramlintide in Male Dogs
Following Daily Subcutaneous Injections for 52 Weeks

Dose (mg/kg)	Week of Sample	t_{max} (min)	C_{max} ng/ml (pmole/L)	$AUC_{0\text{-}2}$ ng·min/ml (pmol·min/L)	$AUC_{0\text{-}6}$ ng·min/ml (pmol·min/L)
0.1	1	15	19 (4769)	1191 (305100)	
	13	15	21 (5461)	1277 (327057)	
	26	15	16 (3998)	731 (187204)	
	52	15	26 (6635)	1136 (290908)	1222 (312958)
0.3	1	15	38 (9850)	1560 (399594)	
	13	15	59 (15206)	3850 (985950)	
	26	30	234 (60030)	23584 (6039389)	
	52	5	107 (27438)	4331 (1109189)	6036 (1545608)
0.6	1	15	119 (30593)	8917 (2283449)	
	13	15	145 (37068)	10051 (2573997)	
	26	15	181 (46391)	10057 (2575456)	
	52	5	37 (9372)	1303 (333793)	1651 (422906)

Pharmacokinetic Parameters of Pramlintide in Female Dogs
Following Daily Subcutaneous Injections for 52 Weeks

Dose (mg/kg)	Week of Sample	t_{max} (min)	C_{max} ng/ml (pmole/L)	AUC_{0-3} ng·min/ml (pmole·min/L)	AUC_{0-4} ng·min/ml (pmole·min/L)
0.1	1	15	14 (3613)	609 (156013)	
	13	30	16 (4112)	1301 (333119)	
	26	15	27 (6948)	2029 (519542)	
	52	15	31 (8051)	1859 (475930)	2289 (586136)
0.3	1	15	46 (11718)	2154 (551658)	
	13	15	75 (19128)	4719 (1208351)	
	26	15	46 (11652)	3166 (810829)	
	52	15	46 (11892)	2787 (713775)	3578 (916306)
0.6	1	15	73 (18612)	3779 (967801)	
	13	15	151 (38652)	9675 (2477687)	
	26	15	113 (28827)	5680 (1454660)	
	52	15	104 (26606)	4137 (1059427)	4424 (1132992)

Multiple of human exposure based on maximum dose of 360 µg/day and AUC data from dogs treated with 0.1, 0.3 and 0.6 mg/kg/day for 52 WKs. The NOAEL was considered 0.6 mg/kg/day in dogs. PK data in male dogs were erratic.

Dose, mg/kg/d	Sex	AUC, ng.min/ml	Multiple of human exposure (max dose, 360 µg/day)	
			Type I diabetes (AUC 106 ng.min/ml)	Type II diabetes (AUC60 ng.min/ml)
0.1	M	1222	12 X	20 X
	F	2289	22 X	38 X
0.3	M	6036	57 X	101 X
	F	3578	34 X	60 X
0.6	M	1651	16 X mean~30	27 X
	F	4424	42 X	74 X

Summary:

52-Week Dog Study (REST 98127R1) evaluated subcutaneous doses of 0.1 (LD), 0.3 (MD) and 0.6 (HD) mg/kg/day pramlintide. 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 (>30 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.

Addendum

Histopathology Inventory for pramlintide, NDA # 21-332

Study	1186/2	1186/3	1186/9	1186/5	1186/4	
Species	26WK Rat	26WK Dog	52WK Dog	2 Y Carci Mouse	2 Y Carci rat	
Adrenals	X*	X*	X*	X	X	
Aorta	X	X	X	X#	X#	
Bone Marrow smear		X	X	X	X	
Bone (femur)	X	X	X	X#	X#	
Brain	X*	X*	X*	X	X	
Cecum	X	X	X	X	X	
Cervix						
Colon	X	X	X	X	X	
Duodenum	X	X	X	X	X	
Epididymis	X*	X*	X*	X	X	
Esophagus		X	X	X	X	
Eye	X	X	X	X	X	
Fallopian tube						
Gall bladder		X	X	X	X	
Gross lesions	X	X	X	X	X	
Harderian gland	X	X	X	X	X	
Heart	X*	X*	X*	X	X	
Ileum	X	X	X	X	X	
Injection site	X	X	X	X	X	
Jejunum	X	X	X	X	X	
Kidneys	X*	X*	X*	X	X	
Lacrimal gland	X	X	X	X	X	
Larynx				X		
Liver	X*	X*	X*	X	X	
Lungs	X	X	X	X	X	
Lymph nodes, cervical						
Lymph nodes mandibular	X	X	X	X	X	
Lymph nodes, mesenteric	X	X	X	X	X	
Mammary Gland	X	X	X	X	X	
Nasal cavity	X			X#	X#	
Optic nerves	X	X	X	X	X	
Ovaries	X*	X*	X*	X	X	
Pancreas	X	X	X	X	X	
Parathyroid	X*	X*	X*	X	X	
Peripheral nerve	X	X	X	X	X	
Pharynx				X		
Pituitary	X*	X*	X*	X	X	
Prostate	X*	X*	X*	X	X	
Rectum	X	X	X	X#	X#	
Salivary gland	X	X	X	X	X	
Sciatic nerve	X	X	X	X#	X#	
Seminal vesicles	X	X	X	X#	X#	
Skeletal muscle	X	X	X	X#	X#	
Skin	X	X	X	X	X	
Spinal cord	X	X	X	X	X	
Spleen	X	X*	X*	X	X	
Sternum	X	X	X	X	X	
Stomach	X	X	X	X	X	
Testes	X*	X*	X*	X	X	
Thymus	X	X*	X*	X	X	
Thyroid	X*	X*	X*	X	X	
Tongue	X	X	X	X#	X#	
Trachea	X	X	X	X	X	
Urinary bladder	X	X	X	X	X	
Uterus	X	X*	X*	X	X	
Vagina	X	X	X	X#	X#	
Zymbal gland	X			X#	X#	

Tissues not examined histologicaly (the remaining tissues from control and HD group were evaluated).
*Organ weight obtained

GENETIC TOXICOLOGY:

Study title: Ames/Salmonella-E.coli liquid pre-incubation Assay on AC-0137

Key findings: Ames assay was negative
Study no: REST98132
Study type (if not reflected in title)**:** mutation (base-pair substitution and frameshift)

Volume #, and page #: 1.68 1-69
Conducting laboratory and location: [illegible]

Date of study initiation: Sep 30, 1992 and completed Nov 1, 1992
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # B232C03F ([illegible])
Formulation/vehicle: solvent control, 100 µl /plate di-water

METHODOLOGY:
Strains/Species/Cell line:

Strain	Genotype	Type of mutation
Salmonella typhimurium		
TA98	HisD3052, rfa, uvrB, pKM101	Frame shift
TA100	HisG46, rfa, uvrB, pKM101	Base-pair substitution
TA1535	HisG46, rfa, uvrB	Base-pair substitution
TA1537	HisC3076, rfa, uvrB	Frame shift
TA1538	HisD , rfa, uvrB	Frame shift
Eschericia coli WP2 uvrA	trp, uvrA	Base-pair substitution

The preliminary toxicity test was carried out with Strains of TA 1538, TA100 and WP2 with 5 doses (50, 167, 500, 1670 and 5000 µg/plate) of AC-0137 in the absence of S9. Each test was run in duplicate. The main mutagenicity study was carried out in triplicates.

Dose Selection Criteria: Limit dose of 5000 µg/plate or toxicity

Basis of dose selection: Concentration of 5 mg/plate is generally considered to be limit dose for this assay. A dose greater than 5 mg/plate test substance may prevent bacterial growth or become crystallized. In the toxicity prescreening test, doses greater than 500 µg/plate precipitated out of solution.

Range finding studies: From the pre-screening toxicity study, the doses for the Salmonella (5, 16.7, 50, 167, 500 and 1000 µg/plate) and E.coli (16.7, 50, 167, 500, 1000 and 1670 µg/plate) in the presence and absence of S9 were evaluated in the first assay. In the confirmatory assay, the concentrations were increased to 10,000µg/plate in the presence of S9.

Test Agent Stability: AC-0137 was stable.

Metabolic Activation System: S9 liver fraction of rats treated with Aroclor 1254. One ml of S9 mixture was made up of 0.3 ml of S9 fraction and 0.7 ml of co-factor solution.

CONTROLS:

Vehicle: bidistilled water

Exposure Conditions: Approximately 0.1 ml of the test AC-0137 solution, plus 0.1 ml of bacteria culture and 0.5 ml of S9mix or 0.1 M sodium-potassium phosphate buffer (pH 7.4 in metabolically activated tests) were transferred to 20 ml of minimum agar and incubated for 48 hrs at 37 ± 1.5 °C.

Incubation and sampling times: 2 days

Doses used in definitive study: 5, 16.7, 50, 167, 500, 1000, and 1670 µg/plate in the absence of S9 and 50, 167, 500, 1670, 5000 and 10,000 µg/plate in presence of S9.

STUDY DESIGN:
ANALYSIS:
No. slides/plates/replicates/animals analyzed: 3 plate/ dose
Counting method: —— electronic colony counter interfaced with personal computer.
Cytotoxic endpoints: prevention of normal growth of bacteria (antibacterial toxic effect)
Genetic toxicity endpoints/results: revertant mutation
Statistical methods: Statistical analysis was performed using program developed by Snee and Irr (1981). Statistics analysis was performed only when a 50% increase in revertant frequency, relative to the concurrent controls was observed.
Criteria for Positive Results:
The test substance is considered positive in this test system if one or both of the following conditions are met:

- A statistically significant, dose-dependent increase in the number of histidine- or tryptophan-independent revertants with at least one dose level inducing a revertant frequency that is two fold the spontaneous solvent control value.
- If the test article does not induce a statistically significant, dose-dependent increase in revertant frequency but does induce a revertant frequency at one dose levels that is two fold the spontaneous control value, the results was considered equivocal.
- A negative result is defined as the absence of a statistically significant, dose-dependent increase in the number f independent revertants.

RESULTS:
Study Validity: Test validity was examined by use of several positive controls and a negative control (vehicle). The tester stain characteristics were checked for genotype (requirement of amino acid, ability of DNA repair and ampicillin resistance). A test is considered acceptable if the mean colony counts of the control values of all strains are within the acceptable ranges and if the results of the positive controls meet the criteria for a positive response. In either case, the final decision has to be based on scientific judgement.

Historical Data - Spontaneous Revertants*

Strain	S9	n	Average(± 1SD)	Range($\bar{X}$ ± 2SD)
TA1535	-	261	9.64 ± 2.82	4.01 - 15.3
	+	260	10.1 ± 2.81	4.47 - 15.7
TA1537	-	263	7.88 ± 2.63	2.61 - 13.1
	+	258	9.19 ± 2.80	3.60 - 14.8
TA1538	-	272	5.19 ± 2.43	0.325 - 10.1
	+	276	12.4 ± 3.82	4.73 - 20.0
TA98	-	273	19.3 ± 5.19	8.91 - 29.7
	+	285	27.5 ± 6.72	14.1 - 41.0
TA100	-	277	86.7 ± 17.9	50.8 - 123
	+	280	98.9 ± 18.1	62.7 - 135
WP2 uvrA	-	163	6.95 ± 2.91	1.13 - 12.8
	+	159	7.77 ± 3.13	1.52 - 14.0

*January 1, 1990 - September 30, 1992

Toxicity Prescreen on AC 0137

Dose (µg/plate)	Background Growth[1] TA1538	TA100	uvrA
0.00[2]	+	+	+
50.0	+	+	+
167	±[a]	+	+
500	±[c]	±[b/c]	+
1670	±[c]	±[c]	±[c]
5000	±[c]	±[c]	±[c]

[1]Evaluated in the absence of S9 only. Background growth evaluated for normal (+), inhibited (±) or no growth (-).
[2]Solvent control (100 µL/plate di-H_2O).
[a]Slight toxicity.
[b]Moderate toxicity.
[c]Severe toxicity.

Original Assay

CONTROLS

			AVERAGE REVERTANTS/PLATE					
SOLVENT CONTROLS		S9	TA1535	TA1537	TA1538	TA98	TA100	UVR A
DI-H2O (100 UL)		(-)	6 (2)	8 (2)	2 (1)	13 (5)	95 (14)	3 (2)
DI-H2O (100 UL)		(+)	8 (4)	5 (3)	5 (1)	28 (10)	89 (10)	2 (2)
POSITIVE CONTROLS (UG/PL)								
SODIUM AZIDE	10.0	(-)	742*(64)	--- (---)	--- (---)	--- (---)	649*(62)	--- (---)
9-AMINOACRIDINE	150	(-)	--- (---)	1104*(36)	--- (---)	--- (---)	--- (---)	--- (---)
2-NITROFLUORENE	5.00	(-)	--- (---)	--- (---)	150*(3)	175*(26)	--- (---)	--- (---)
2-ANTHRAMINE	2.50	(+)	1058*(41)	497*(123)	1689*(71)	2123*(138)	2154*(318)	--- (---)
ENNG	2.00	(-)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	930*(31)
2-ANTHRAMINE	80.0	(+)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	524*(124)

TEST ARTICLE: AC 0137

		AVERAGE REVERTANTS/PLATE					
DOSE LEVEL (UG/PL)	S9	TA1535	TA1537	TA1538	TA98	TA100	UVR A
5.00	(-)	6 (2)	8 (5)	1 (1)	12 (5)	93 (5)	--- (---)
16.7	(-)	6 (2)	7 (2)	2 (2)	14 (4)	92 (16)	3 (1)
50.0	(-)	4 (3)	6 (1)	1 (0)	15 (1)	83 (12)	3 (2)
167	(-)	6 (1)	8 (6)a	1 (0)a	9 (2)	90 (21)a	2 (1)
500	(-)	6 (5)a/b	10 (2)a/b	2 (1)a/b	12 (3)a/b	69 (11)b/c	2 (2)a/b
1000	(-)	8 (4)c	6 (3)c	1 (0)c	15 (5)c	72 (9)c	2 (1)b
1670	(-)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	3 (2)c
5.00	(+)	5 (3)	5 (2)	6 (2)	20 (5)	90 (14)	--- (---)
16.7	(+)	6 (1)	7 (4)	4 (2)	16 (1)	100 (21)	2 (1)
50.0	(+)	4 (3)	4 (2)	6 (2)	18 (3)	88 (8)	3 (1)
167	(+)	9 (2)	10*(2)	5 (2)	21 (6)	77 (15)	2 (2)
500	(+)	8 (0)	9 (2)	8 (3)	21 (3)	85 (4)	3 (2)
1000	(+)	10 (3)	8 (2)	5 (4)	18 (5)	58 (12)	2 (1)
1670	(+)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	3 (2)

*Positive response: ≥2X solvent (TA1535, TA1537, TA1538, TA98, TA100, UVR A).
Data reported as: mean (standard deviation).
a/b/c = slight/moderate/severe toxicity.
Test article precipitate at ≥167 ug/plate -S9 only.

Confirmatory Assay

CONTROLS

SOLVENT CONTROLS		S9	AVERAGE REVERTANTS/PLATE TA1535	TA1537	TA1538	TA98	TA100	UVR A
DI-H2O (100 UL)		(-)	10 (2)	3 (1)	3 (2)	12 (5)	97 (10)	2 (1)
DI-H2O (100 UL)		(+)	8 (2)	4 (1)	7 (2)	17 (8)	112 (10)	2 (1)
POSITIVE CONTROLS (UG/PL)								
SODIUM AZIDE	10.0	(-)	755*(12)	--- (---)	--- (---)	--- (---)	567*(97)	--- (---)
9-AMINOACRIDINE	150	(-)	--- (---)	1186*(80)	--- (---)	--- (---)	--- (---)	--- (---)
2-NITROFLUORENE	5.00	(-)	--- (---)	--- (---)	172*(15)	158*(16)	--- (---)	--- (---)
2-ANTHRAMINE	2.50	(+)	164*(28)	392*(77)	1662*(147)	1780*(422)	2450*(109)	--- (---)
ENNG	2.00	(-)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	818*(42)
2-ANTHRAMINE	80.0	(+)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	507*(71)

TEST ARTICLE: AC 0137

DOSE LEVEL (UG/PL)	S9	AVERAGE REVERTANTS/PLATE TA1535	TA1537	TA1538	TA98	TA100	UVR A
5.00	(-)	4 (2)	5 (1)	6*(5)	14 (1)	112 (15)	--- (---)
16.7	(-)	4 (3)	2 (2)	2 (1)	7 (2)	98 (12)	3 (1)
50.0	(-)	4 (3)	4 (3)	3 (2)	8 (6)a	108 (14)	1 (2)
167	(-)	6 (3)a/b	3 (1)a/b	2 (2)a	10 (6)a/b	100 (10)	3 (2)
500	(-)	6 (5)b/c	5 (2)b/c	4 (1)c	12 (4)b/c	105 (7)a/b	2 (2)
1000	(-)	5 (2)c	3 (3)c	3 (3)c	8 (3)b/c	85 (10)b	1 (1)a/b
1670	(-)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	2 (1)a/b
50.0	(+)	7 (2)	3 (1)	7 (3)	14 (6)	100 (10)	3 (1)
167	(+)	5 (1)	5 (4)	8 (2)	17 (4)	107 (24)	2 (1)
500	(+)	6 (3)	5 (3)	8 (5)	21 (4)	113 (4)	1 (1)
1670	(+)	5 (3)a	4 (2)a	9 (4)	17 (8)a	112 (13)a	2 (1)a
5000	(+)	2 (2)c	0 (0)c	0 (0)c	10 (5)c	21 (6)c	1 (1)c
10000	(+)	2 (1)c	2 (1)b/c	0 (1)c	17 (7)b/c	41 (10)b/c	0 (1)c

*Positive response: ≥2X solvent (TA1535, TA1537, TA1538, TA98, TA100, UVR A).
Data reported as: mean (standard deviation).
a/b/c = slight/moderate/severe toxicity.
Test article precipitate at ≥500 ug/plate -S9, and ≥5000 ug/plate +S9.

SUMMARY:

In the toxicity prescreening test using the pramlintide (AC-0137) product of [redacted] doses above 500 µg/plate precipitated and at ≥167 µg/plate AC-0137 reduced background lawn in TA1538 and TA100 and yet sponsor chose to use doses much higher than that in the original and confirmatory assays in addition to several lower concentrations. Since the toxicity test was carried out in only three stains, it can be argued that the article may not be toxic to the same extent to other strains.

In the original assay, a statistically significant increase in revertant frequency (2.2 fold) was observed in strain TA1537 at a dose of 167 µg/plate with S9. In the confirmatory assay, the slight increase in TA1538 without S9 at 5 µg/plate was considered statistical aberration due to random fluctuations since they were not dose dependent and occurred only at very low dose. In the final analysis, under the condition of this Ames assay, AC-0137 was considered negative.

Study title: Ames/Salmonella-E.coli liquid pre-incubation Assay on AC-0137

Key findings: Ames assay was negative
Study no: REST98133
Study type (if not reflected in title): mutation (base-pair substitution and frameshift)
Volume #, and page #: 1.68 1-79
Conducting laboratory and location: [redacted]

Date of study initiation: Dec 6, 94 and completed Dec 8, 1994
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 94-0802 BB [redacted] [redacted]%
Formulation/vehicle: solvent control, 100 µl /plate di-water

METHODOLOGY:
Strains/Species/Cell line:

Strain	Genotype	Type of mutation
Salmonella typhimurium		
TA98	HisD3052, rfa, uvrB, pKM101	Frame shift
TA100	HisG46, rfa, uvrB, pKM101	Base-pair substitution
TA1535	HisG46, rfa, uvrB	Base-pair substitution
TA1537	HisC3076, rfa, uvrB	Frame shift
TA1538	HisD , rfa, uvrB	Frame shift
Eschericia coli WP2 uvrA	trp, uvrA	Base-pair substitution

The preliminary toxicity test was carried out with Strains of TA 1538, TA100 and WP2 with 5 doses (50, 167, 500, 1670 and 5000 µg/plate) of AC-0137 in the absence of S9. Each test was run in duplicate. The main mutagenicity study was carried out in triplicates.

Dose Selection Criteria: Limit dose of 5000 µg/plate or toxicity

Basis of dose selection: Concentration of 5 mg/plate is generally considered to be limit dose for this assay. A dose greater than 5 mg/plate test substance may prevent bacterial growth or become crystallized. In the toxicity prescreening test, doses greater than 500 µg/plate precipitated out of solution.

Range finding studies: From the pre-screening toxicity study, the doses for the Salmonella and E.coli (5, 16.7, 50, 167, 500 and 1000 µg/plate) in the presence and absence of S9 were evaluated in the original assay. In the confirmatory assay concentration were increased to 5,000µg/plate in the presence of S9.

Test Agent Stability: AC-0137 was stable.

Metabolic Activation System: S9 liver fraction of rats treated with Aroclor 1254. One ml of S9 mixture was made up of 0.3 ml of S9 fraction and 0.7 ml of co-factor solution.

CONTROLS:

Vehicle: bidistilled water

Exposure Conditions: Approximately 0.1 ml of the test AC-0137 solution, plus 0.1 ml of bacteria culture and 0.5 ml of S9mix or 0.1 M sodium-potassium phosphate buffer (pH 7.4 in metabolically activated tests) were transferred to 20 ml of minimum agar and incubated for 48 hrs at 37 ± 1.5 °C.

Incubation and sampling times: 2 days

Doses used in definitive study: 5, 16.7, 50, 167, 500 and1000 µg/plate in the absence of S9 and 5, 16.7, 50, 167, 500, 1670 and 5000 µg/plate in presence of S9.

STUDY DESIGN:
ANALYSIS:
No. slides/plates/replicates/animals analyzed: 3 plate/ dose
Counting method: —— electronic colony counter interfaced with personal computer.
Cytotoxic endpoints: prevention of normal growth of bacteria (antibacterial toxic effect)
Genetic toxicity endpoints/results: revertant mutation
Statistical methods: Statistical analysis was performed using program developed by Snee and Irr (1981). Statistics analysis was performed only when a 50% increase in revertant frequency, relative to the concurrent controls was observed.
Criteria for Positive Results:
The test substance is considered positive in this test system if one or both of the following conditions are met:

- A statistically significant a, dose-dependent increase in the number of histidine- or tryptophan-independent revertants with at least one dose level inducing a revertant frequency that is two fold the spontaneous solvent control value.
- If the test article does not induce a statistically significant, dose-dependent increase in revertant frequency but does induce a revertant frequency at one dose levels that is two fold the spontaneous control value, the results was considered equivocal.
- A negative result is defined as the absence of a statistically significant, dose-dependent increase in the number f independent revertants.

RESULTS:
Study Validity: Test validity was examined by use of several positive controls and a negative control (vehicle). The tester stain characteristics were checked for genotype (requirement of amino acid, ability of DNA repair and ampicillin resistance). A test is considered acceptable if the mean colony counts of the control values of all strains are within the acceptable ranges and if the results of the positive controls meet the criteria for a positive response. In either case, the final decision has to be based on scientific judgement.

Historical Data - Spontaneous Revertants*

Strain	S9	n	Average(± 1SD)	Range($\bar{x}$ ± 2SD)
TA1535	-	392	9.51 ± 2.81	3.90 - 15.1
	+	394	10.2 ± 2.74	4.71 - 15.7
TA1537	-	399	7.99 ± 2.56	2.87 - 13.1
	+	397	9.32 ± 2.82	3.66 - 15.0
TA1538	-	406	5.77 ± 2.61	0.555 - 11.0
	+	411	12.9 ± 3.91	5.10 - 20.7
TA98	-	408	20.9 ± 5.45	10.0 - 31.8
	+	431	28.9 ± 6.81	15.3 - 42.5
TA100	-	414	87.6 ± 19.0	49.6 - 126
	+	424	102 ± 18.2	65.6 - 138
WP2 uvrA	-	264	6.13 ± 2.75	0.625 - 11.6
	+	259	6.73 ± 3.01	0.714 - 12.7

*January 1, 1990 - December 31, 1994

Toxicity Prescreen

Dose (µg/plate)	Background Growth[1] TA1538	TA100	WP2 uvrA
0.00[2]	+	+	+
50.0	+	+	+
167	+	+	+
500	±[b]	±[a/b]	±[a/b]
1670_{ppt}	±[c]	±[c]	±[c]
5000_{ppt}	±[c]	±[c]	±[c]

[1]Evaluated in the absence of S9 only. Background growth evaluated for normal (+), inhibited (±) or no growth (-).
[2]Solvent control (100 µL/plate di-H_2O).
[a]Slight toxicity.
[b]Moderate toxicity.
[c]Severe toxicity.
[ppt]Test article incompletely soluble.

Summary Data - Original Assay

CONTROLS

SOLVENT CONTROLS		S9	AVERAGE REVERTANTS/PLATE TA1535	TA1537	TA1538	TA98	TA100	UVR A
DI-H2O (100 UL)		(-)	14 (6)	8 (5)	10 (2)	21 (4)	109 (6)	6 (4)
DI-H2O (100 UL)		(+)	13 (6)	12 (3)	20 (4)	38 (7)	120 (17)	7 (4)
POSITIVE CONTROLS (UG/PL)								
SODIUM AZIDE	10.0	(-)	1296*(47)	--- (---)	--- (---)	--- (---)	1170*(72)	--- (---)
9-AMINOACRIDINE	150	(-)	--- (---)	1346*(191)	--- (---)	--- (---)	--- (---)	--- (---)
2-NITROFLUORENE	5.00	(-)	--- (---)	--- (---)	601*(29)	549*(44)	--- (---)	--- (---)
2-ANTHRAMINE	2.50	(+)	63*(21)	410*(129)	1472*(226)	2556*(115)	1908*(358)	--- (---)
ENNG	2.00	(-)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	467*(33)
2-ANTHRAMINE	80.0	(+)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	577*(47)

TEST ARTICLE: AC-0137

DOSE LEVEL (UG/PL)	S9	AVERAGE REVERTANTS/PLATE TA1535	TA1537	TA1538	TA98	TA100	UVR A
5.00	(-)	13 (2)	13 (1)	17 (2)	25 (9)	123 (4)	6 (1)
16.7	(-)	12 (3)	15 (2)	15 (4)	28 (4)	101 (21)	4 (3)
50.0	(-)	14 (2)	10 (3)	14 (2)	22 (5)	113 (7)	4 (2)
167	(-)	9 (1)	8 (1)	13 (6)a	21 (5)	119 (18)	4 (1)
500	(-)	12 (2)a/b	10 (3)a	12 (4)a/b	23 (4)a/b	101 (13)a/b	5 (3)a
1000	(-)	10 (2)b/c	11 (7)b/c	9 (3)c	21 (1)c	86 (7)c	4 (1)b
5.00	(+)	9 (3)	14 (7)	33 (4)	31 (4)	125 (23)	6 (4)
16.7	(+)	11 (2)	8 (1)	23 (9)	34 (6)	130 (20)	7 (3)
50.0	(+)	11 (4)	13 (1)	20 (8)	33 (4)	131 (14)	6 (2)
167	(+)	10 (3)	9 (3)	26 (1)	37 (14)	125 (12)	6 (3)
500	(+)	14 (4)	11 (5)	29 (7)a	35 (5)a	119 (6)	6 (1)
1000	(+)	17 (2)a/b	13 (5)a	32 (5)b	44 (1)a/b	100 (4)a/b	5 (3)

*Positive Response: ≥2X Solvent (TA1535, TA1537, TA1538, TA98, TA100, UVR A).
Data Reported as: Mean (Standard Deviation).
a/b/c = slight/moderate/severe toxicity.
Test article insoluble at 1000 µg/plate -S9 only.

Summary Data - Confirmatory Assay

CONTROLS

SOLVENT CONTROLS		S9	AVERAGE REVERTANTS/PLATE TA1535	TA1537	TA1538	TA98	TA100	UVR A
DI-H2O (100 UL)		(-)	12 (8)	9 (5)	9 (5)	27 (9)	112 (13)	4 (0)
DI-H2O (100 UL)		(+)	13 (6)	13 (4)	20 (1)	39 (8)	126 (15)	4 (3)
POSITIVE CONTROLS (UG/PL)								
SODIUM AZIDE	10.0	(-)	1488*(105)	--- (---)	--- (---)	--- (---)	1411*(80)	--- (---)
9-AMINOACRIDINE	150	(-)	--- (---)	1972*(193)	--- (---)	--- (---)	--- (---)	--- (---)
2-NITROFLUORENE	5.00	(-)	--- (---)	--- (---)	838*(160)	610*(123)	--- (---)	--- (---)
2-ANTHRAMINE	2.50	(+)	54*(13)	417*(88)	1409*(136)	2080*(165)	1987*(259)	--- (---)
ENNG	2.00	(-)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	495*(112)
2-ANTHRAMINE	80.0	(+)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	775*(89)

TEST ARTICLE: AC-0137

DOSE LEVEL (UG/PL)	S9	AVERAGE REVERTANTS/PLATE TA1535	TA1537	TA1538	TA98	TA100	UVR A
5.00	(-)	16 (4)	13 (3)	8 (6)	31 (6)	111 (7)	8*(1)
16.7	(-)	13 (6)	11 (6)	7 (1)	30 (2)	106 (9)	5 (1)
50.0	(-)	11 (4)	11 (4)	10 (4)	26 (6)	106 (22)	3 (1)
167	(-)	9 (3)	12 (2)a/b	12 (1)a/b	27 (9)	94 (16)	5 (2)
500	(-)	12 (6)a/b	11 (4)b/c	9 (4)c	25 (4)a/b	119 (5)b	2 (1)a
1000	(-)	12 (7)c	8 (3)c	9 (1)c	23 (6)c	96 (10)c	3 (1)c
5.00	(+)	18 (7)	12 (5)	21 (4)	39 (3)	115 (8)	--- (---)
16.7	(+)	16 (5)	9 (1)	18 (3)	35 (7)	119 (16)	--- (---)
50.0	(+)	16 (3)	13 (4)	18 (8)	37 (5)	139 (38)	6 (3)
167	(+)	15 (4)	13 (3)	15 (3)	45 (9)	112 (8)	3 (2)
500	(+)	13 (1)a	8 (3)a	23 (5)a/b	41 (2)a/b	125 (6)a	3 (1)
1000	(+)	17 (9)a/b	10 (7)b	20 (0)b/c	44 (11)b	105 (10)b	8 (3)
1670	(+)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	5 (2)a/b
5000	(+)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	2 (1)c

*Positive Response: ≥2X Solvent (TA1535, TA1537, TA1538, TA98, TA100, UVR A).
Data Reported as: Mean (Standard Deviation).
a/b/c = slight/moderate/severe toxicity.
Test article insoluble at ≥1670 µg/plate +S9 and at 1000 µg/plate -S9.

SUMMARY:

This Ames assay was carried out with product from [redacted] AC-0137 was toxic at concentrations greater than 500 µg/plate in TA 1538, tA100 and WP2 strains. In the original assay, a slight increase in revertant frequency (1.6 to 1.8 fold) were observed in strain TA1538 at doses of 5 and 1000 µg/plate with S9 and in strain TA1537 at a dose of 16.7µg/plate without S9. In the confirmatory assay, the slight increase (2 fold) in UVR A was observed at 5 µg/plate without S9. This strain was retested again without S9. There were no significant changes in revertant frequencies at any of doses in the retest. Each assay had at least three concentrations where the drug was not cytotoxic. Most of the increase in revertant frequencies were slight, non-dose dependent and not confirmed in when tests were repeated. In the final analysis, under the condition of this Ames assay, AC-0137 was considered negative.

Study title: Mutagenicity test with AC-0137 in the Salmonella-E.coli / mammalian-microsomal reverse mutation assay of AC-0137 provided by three suppliers.

Key findings: Ames assay was negative
Study no: REST98134
Study type (if not reflected in title): mutation (base-pair substitution and frameshift)

Volume #, and page #: 1.68 1-67
Conducting laboratory and location: [redacted]

Date of study initiation: Nov 11, 97 and completed Feb 17, 98
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 94-0402 AB [redacted] 96-0401BB [redacted] 96-1102LB [redacted]
Formulation/vehicle: solvent control, 100 µl /plate di-water

METHODOLOGY:
Strains/Species/Cell line:

Strain	Genotype	Type of mutation
Salmonella typhimurium		
TA98	HisD3052, rfa, uvrB, pKM101	Frame shift
TA100	HisG46, rfa, uvrB, pKM101	Base-pair substitution
TA1535	HisG46, rfa, uvrB	Base-pair substitution
TA1537	HisC3076, rfa, uvrB	Frame shift
Eschericia coli WP2 uvrA	trp, uvrA	Base-pair substitution

The preliminary cytotoxicity test was carried out with Strains of TA 98, TA100 and WP2uvrA with 10 doses ranging from 6.7 to 5000µg/plate using — product in the presence and absence of S9. Each test was run in singleton. No cytotoxicity was observed at any dose in presence or absence of S9. The main mutagenicity study was carried out in triplicates. Test article from all suppliers was soluble in water.

Dose Selection Criteria: Limit dose of 5000 µg/plate or toxicity

Basis of dose selection: Concentration of 5 mg/plate is generally considered to be limit dose for this assay. A dose greater than 5 mg/plate test substance may prevent bacterial growth or become crystallized.

Range finding studies: From the pre-screening toxicity study, the doses for the Salmonella and E.coli (5, 16.7, 50, 167, 500 and 1000 µg/plate) in the presence and absence of S9 were evaluated in the original assay. In the confirmatory assay concentration were increased to 5,000µg/plate in the presence of S9.

Test Agent Stability: AC-0137 was stable.

Metabolic Activation System: S9 liver fraction of rats treated with Aroclor 1254. One ml of S9 mixture was made up of 0.3 ml of S9 fraction and 0.7 ml of co-factor solution.

CONTROLS:

Vehicle: bidistilled water

Exposure Conditions:

For plate incorporation method, approximately 200µl of the test AC-0137 solution, plus 0.1 ml of bacteria culture were added to 2.5 of molten selective top agar and maintained 45±2 C. When S9 mix was required, 500 µl of S9, 100µl of tester strain, 200 µl of vehicle or test article were added to 2 ml of agar and incubate for 48 hours at 37 degree.

For Treat and Plate Exposure method, For S9 mix test, 2.5 ml of S9mix, 500 µ of tester strain, 1000 µl of vehicle or test article were added to 15 ml tube. For assays without S9 mix, 2.5 ml of 0.1M phosphate buffer was substituted for S9 mix. The tubes were incubated for 60 min at 37 C. The bacteria was centrifuged down and resuspended in 500 µl of 0.1M phosphate buffer. A 110µl aliquot of the suspension was added to 2.5 ml of molten agar and overlaid onto the surface of 25 ml of minimal bottom agar in a petri dish. The plates were incubated for 48 hrs at 37 C.

Incubation and sampling times: 2 days

Doses used in definitive study: 33.3, 100, 333, 1000, 3330 and 5000 µg/plate used for plate incorporation method with or without S9. For treat and plate method, 4.16, 12.5, 41.6, 125, 416 and 625 µg/plate with S9 and without S9

STUDY DESIGN:

ANALYSIS:

No. slides/plates/replicates/animals analyzed: 3 plate/ dose

Counting method: Manual counting was used for test article and vehicle. Automated counter was used for positive controls.

Cytotoxic endpoints: prevention of normal growth of bacteria (antibacterial toxic effect)

Genetic toxicity endpoints/results: revertant mutation

Statistical methods: Mean and standard deviation were calculated. No other statistic method

Criteria for Positive Results:

- For test article to be positive, it had to produce at least a 2-fold increase in the mean number of revertants per plate of at least one of these tester strains relative to vehicle.
- There had to be dose-response with increasing concentrations of the test article.
- For tester strain, TA1535 and TA1537 there had to be at least a 3-fold increase in the mean number of revertants per plate accompanied with a dose-response.

RESULTS:

Study Validity: Test validity was examined by use of several positive controls and a negative control (vehicle). The tester stain characteristics were checked for genotype (requirement of amino acid, ability of DNA repair and ampicillin resistance). A test is considered acceptable if the mean colony counts of the control values of all strains are within the acceptable ranges and if the results of the positive controls meet the criteria for a positive response. In either case, the final decision has to be based on scientific judgement.

Dose-range finding study: The cytotoxicity assay found no significant toxicity in the presence or absence of S9 at any of the concentrations ranging from 6.67 to 5000 µg/plate in tester stains TA98, TA100 and WP2uvrA.

Plate Incorporation Exposure Method:

- AC-0137 product from — A 2.1 fold increase in tester strain TA98 (5000µg/plate) and 2.2 fold increase in TA100 (3330 µg/plate) in presence of S9 was noted. The1.9 fold increase in TA1535 (5000µg/plate) in presence of S9 did not meat the 3 fold increase in test criteria.
- AC-0137 product from [ ] No positive increases in the mean number of revertants per plate were observed with any of the tester strains in presence or absence of S9 mix.
- AC-0137 from [ ] No positive increases in the mean number of revertants per plate were observed with any of the tester strains in presence or absence of S9 mix.

In the initial experiments, low levels increase in the mean number of revertants per plate were noted with tester strains TA98, TA100 and TA1535 in the presence of S9. Sponsor had noted that test article contained histidine, the selective agent for the *Salmonella* strain. In the conformity tests, treat and plate exposure method allowed to test article to be separated from the tester stains.

PLATE INCORPORATION METHOD
SUMMARY

TEST ARTICLE ID: AC-0137 (Supplier: —

EXPERIMENT ID: 18977-B1

DATE PLATED: 20-Nov-97 VEHICLE: Deionized Water

DATE COUNTED: 24-Nov-97,25-Nov-97 PLATING ALIQUOT: 200µl

	DOSE/PLATE		MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION								BACKGROUND LAWN*
			TA98		TA100		TA1535		TA1537		WP2uvrA		
			MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	
MICROSOMES: RAT LIVER													
VEHICLE CONTROL			21	4	70	13	10	2	8	2	13	5	1
TEST ARTICLE	33.3	µg	23	6	75	13	7	3	6	4	10	2	1
	100	µg	25	5	93	10	9	3	6	3	12	2	1
	333	µg	32	4	91	13	11	4	7	4	11	3	1
	1000	µg	37	6	109	6	10	1	8	2	18	1	1
	3330	µg	36	3	153	2	16	3	6	1	13	4	1
	5000	µg	44	4	136	18	19	10	8	3	14	2	1
POSITIVE CONTROL**			1063	30	896	263	142	19	145	13	241	32	1
MICROSOMES: NONE													
VEHICLE CONTROL			13	5	63	9	13	3	7	3	14	2	1
TEST ARTICLE	33.3	µg	15	3	83	4	13	3	4	2	18	5	1
	100	µg	16	6	66	3	10	5	5	1	18	8	1
	333	µg	15	6	90	13	13	1	5	2	19	3	1
	1000	µg	16	8	90	11	10	4	5	2	16	3	1
	3330	µg	13	1	74	10	12	4	6	2	12	8	1
	5000	µg	11	2	46	13	8	2	2	1	10	6	1
POSITIVE CONTROL***			116	26	316	8	417	33	617	40	480	61	1

** TA98 2-aminoanthracene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2 aminoanthracene 2.5 µg/plate
WP2uvrA 2 aminoanthracene 25.0 µg/plate

*** TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate
WP2uvrA 4-nitroquinoline-N-oxide 1.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal
2 = slightly reduced
3 = moderately reduced
4 = extremely reduced
5 = absent
6 = obscured by precipitate
sp = slight precipitate
mp = moderate precipitate (requires hand count)
hp = heavy precipitate (requires hand count)

Treat and Plate Exposure Method:

- AC-0137 from — Concentrations ≥1250/ml tended to gel during preincubation phase, which prohibited pellet formation. For this reason tester strain WP2uvrA was retested over a lower range of doses (41.6, 125 and 416 µg/ml of reaction mixture). No positive increase in the mean number of revertant with tester stains TA98, TA1537 and WP2uvrA in presence or absence of S9 was observed
- AC-0137 from [ ] Similar to — product, no positive increase in the mean number of revertant with tester stains TA98, TA1537 and WP2uvrA in presence or absence of S9 was observed.
- AC-0137 from [ ] Similar to — product, no positive increase in the mean number of revertant with tester stains TA98, TA1537 and WP2uvrA in presence or absence of S9 was observed.

Summary:

Plate Incorporation Exposure

The results of the *Salmonella - Eschericia coli/Mammalian-Microsome* Reverse Mutation Assay of AC-0137 Provided by Three Suppliers, indicate that under the conditions of this study using the

plate incorporation exposure, test articles identified as AC-0137 (Supplier: [redacted]) and AC-0137 (Supplier: [redacted]) did not cause positive increases in the mean number of revertants per plate of any of the tester strains in either the presence or absence of microsomal enzymes prepared from Aroclor®-induced rat liver (S9). The test article identified as AC-0137 (Supplier: [redacted]) did cause positive increases in the mean number of revertants per plate with tester strains *TA98* (2.1-fold) and TA 100 (2.2-fold) in the presence of S9 mix. No positive increases were observed with any of the remaining tester strain/activation condition combinations.

Treat and Plate Exposure
The results of the *Salmonella - Eschericia coli/Mammalian-Microsome* Reverse Mutation Assay of AC-0137 Provided by Three Suppliers, indicate that under the conditions of this study using the treat and plate exposure, the three test articles identified as AC-0137 (Supplier: [redacted]) AC-0137 (Supplier: [redacted]), and AC-0137 (Supplier: [redacted]), did not cause positive increases in the mean number of revertants per plate of any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor®induced rat liver (S9).

Study title: In vitro chromosomal aberration analysis of AC-0137 in human lymphocytes

Key findings: Chromosomal aberration test was negative.
Study no: REST98135
Study type (if not reflected in title): Chromosomal aberration
Volume #, and page #: 1.69, 1-370
Conducting laboratory and location: [redacted]
Date of study initiation: Oct 94
GLP compliance: Yes
QA reports: yes (X) no ()
Drug, lot #, radiolabel, and % purity: Lot # 94-0802BB and [redacted], purity
Formulation/vehicle: di-water
Methods:
Strains/species/cell line: human lymphocytes
Dose selection criteria: cytotoxicity (mitotic index)
Basis of dose selection: Cytotoxicity study showed that concentrations as high as 5000 µg/ml were not toxic. So three concentrations levels 167, 1000 and 1670 µg/ml were used in the main study.
Range finding studies: In the initial studies, doses 0.167, 0.5, 1.67, 5, 16.7, 50, 167, 500, 1670 and 5000 were tested for cytotoxicity.
Test agent stability: determined by the sponsor to be stable for the duration of the study
Metabolic activation system: Aroclor 1254-induced male SD rat liver homogenate was used in the study. The S9 mix contained 10 mM MgCl2, 10 mM CaCl2, 30 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP, 50 mM sodium phosphate buffer (pH 7.4) and 10% (v/v) liver homogenate. Due to technical error, excessive NADP (5.33 µM) was used in the preparation S9 mix but this should not have altered the study since insufficient NADP is more critical.
Controls:
Vehicle: Sterile water
Negative controls: Sterile water
Positive controls: Mitomycin C (MMC, at 0.100 or 0.250 µg/ml) for tests without activation and cyclophosphamide (40 µg/ml) for S9 activation
Exposure conditions:
Incubation and sampling times:

Doses used in definitive study: 167, 1000 and 1670 µg/ml

Study design: Since AC-0137 was found to be cytotoxic at ≥1670 µg/ml without S9 and ≥5000µg/ml with S9. Minimum of 3 concentrations without cytotoxicity are needed for Chromosomal Aberration test.

Analysis:

No. of replicates: Each concentration was run in duplicate cultures.

Counting method: Approximately 1000 cells per culture were examined for mitotic index. 200 well-spread metaphases were scored for the presence of chromosomal aberration for each concentration of AC-0137.

Criteria for positive results:

a) it induces a statistically significant, dose-dependent increase in the frequency of aberrations/cell or in the proportion of aberrant metaphases; or

2) it induces a reproducible, statistically significant increase in either endpoint for at least one test article concentration at the same treatment/sampling interval.

Summary of individual study findings:

Study validity: Study is considered valid if a) the number of aberrant metaphases for the negative (solvent) is less than 9 and B) both positive controls induce significant increase ($p>0.05$) in the frequency of aberration/cell and in the proportion of aberrant metaphases, as compared to the negative control. All these parameters were met in the study.

Study outcome:

- AC-0137 was cytotoxic at ≥1670 µg/ml without S9 and ≥5000 µg/ml with S9.
- The AC-0137 did not have a deleterious effect upon pH or osmolality at concentrations up to 5000 µg/ml.
- Results of the first assay indicated that AC-0137 did not induce any statistically significant or dose-dependent increases in the proportion of aberrant metaphases, or in the frequency of aberrations/cell, at any concentration using any treatment schedule.
- In the confirmatory assay, AC-0137 induced a statistically significant increase in aberration/cell in cultures treated for 48 hours at concentration of 1000 µg/ml without S9. Since this was within the acceptable range of negative control value, and the negative control was unusually low, it was considered a statistical aberration. Furthermore, it was not dose-dependent. Thus, it was considered negative.

Proportion of Aberrant Cells and Aberrations per Cell (-S9, Assay 2)[a]

Compound	Dose (µg/mL)	Aberrant Cells		Aberrations/cell ($\bar{X}$ ± SD)
		Number	%	
Schedule III[b]				
Untreated	0.00	2	1.0	0.010 ± 0.100
di-H_2O	10.0[c]	3	1.5	0.015 ± 0.122
AC-0137	100	2	1.0	0.010 ± 0.100
AC-0137	500	0	0.0	0.000 ± 0.000
AC-0137	1000	3	1.5	0.015 ± 0.122
Schedule IV[d]				
Untreated	0.00	2	1.0	0.010 ± 0.100
di-H_2O	10.0[c]	0	0.0	0.000 ± 0.000
AC-0137	100	2	1.0	0.010 ± 0.100
AC-0137	500	2	1.0	0.010 ± 0.100
AC-0137	1000	6	3.0*	0.030 ± 0.171*
MMC	0.250	30	30.0**	0.630 ± 1.134**

[a]As described in the text, 200 metaphase cells were scored per dose group, except for the MMC positive control (100 metaphase cells).
[b]treated 24 hours after stimulation for 24 hours.
[c]µL/mL.
[d]treated 48 hours after stimulation for 25 hours.
*,**Statistically significant increase (ps 0.05 and ps 0.01, respectively) as determined by Chi-square (% aberrant cells) or Dunnett's analysis (aberrations/cell).

Proportion of Aberrant Cells and Aberrations per Cell (+S9, Assay 2)[a]

Compound	Dose (µg/mL)	Aberrant Cells		Aberrations/cell ($\bar{X}$ ± SD)
		Number	%	
Schedule I[b]				
Untreated	0.00	2	1.0	0.010 ± 0.100
di-H_2O	10.0[c]	1	0.5	0.005 ± 0.071
AC-0137	167	2	1.0	0.010 ± 0.100
AC-0137	1000	2	1.0	0.010 ± 0.100
AC-0137	1670	5	2.5	0.035 ± 0.232
Schedule II[d]				
Untreated	0.00	1	0.5	0.005 ± 0.071
di-H_2O	10.0[c]	5	2.5	0.025 ± 0.157
AC-0137	167	6	3.0	0.035 ± 0.210
AC-0137	1000	3	1.5	0.015 ± 0.122
AC-0137	1670	1	0.5	0.005 ± 0.071
CP	40.0	52	52.0**	0.780 ± 0.883**

[a]As described in the text, 200 metaphase cells were scored per dose group, except for the CP positive control (100 metaphase cells).
[b]treated 24 hours after stimulation for 5 hours.
[c]µL/mL.
[d]treated 48 hours after stimulation for 5 hours.
**Statistically significant increase (ps 0.01) as determined by Chi-square (% aberrant cells) or Dunnett's analysis (aberrations/cell).

Conclusion: AC-0137 did not cause chromosomal aberration in human lymphocytes under the test conditions and according to the criteria of the test protocol.

Study Title: In vivo micronucleus Test with AC-0137 in mouse bone marrow erythropoietic cells.

Study No: REST98137
Study Type: clastogenicity study using micronucleated polychromatic erythrocytes (MNPCE)
Amendment #000, Volume #1.70 Page # 1-136
Conducting Laboratory: [redacted]

Date of Study Initiation/completion: Dec 6, 94
GLP Compliance: Yes
QA- Report: Yes (X) No ( )
Drug Lot Number: 94-0802BB (purity — %)
Study Endpoint: Presence or absence of micronucleated polychromatic erythrocytes (MNPCE).

METHODOLOGY:
Strains/Species/Cell line: CD-1 Albino mice [redacted]
Dose Selection Criteria: In the preliminary study, maximal doses of 2000 and lower doses of 100, 125, 250, 500, 1000 and 2000 mg/kg were injected intraperitoneally to male and female mice (2/dose/sex). In the definitive study, doses of 25, 125 and 250 mg/kg were used (5/sex/doso, IP). Animals were sacrificed at 24, 48 and 72 hours post dose.
Basis of dose selection: The sponsor tested up to test limit (2000 mg/kg). Symlin caused mortality at doses greater than 500 mg/kg/day with severe clinical signs observed at 500 mg/kg/day.
Range finding studies: Findings of the preliminary study was used to determine the doses in the main study.

Test Agent Stability: Previous data and stability study showed that AC-0137 was stable. The sponsor used fresh preparation of the test agent in the studies.
Metabolic Activation System: Metabolic activation was not performed (an in vivo system).

CONTROLS:
Vehicle: distilled water (vehicle)
Negative Controls: vehicle
Positive Controls: Mitomycin C (MMC, 4 mg/kg)
Exposure Conditions: Mice were given either a single dose of vehicle or several doses of AC-0137. 24, 48 and 72 hours after the dose, animals were sacrificed and bone marrow was collected.
Incubation and sampling times: Bone marrow sample and other data were collected 24 hrs after administration of test substance.
Doses used in definitive study: 0, 25, 125, 250 mg/kg based on the toxicity study

STUDY DESIGN:
Thirteen groups of CD-1 mice about 11 weeks of age were used in the definitive study.
Each group comprised of 10 animals (five/sex). The dosing volume for control was10 ml/kg.
15 groups with 2 animals/dose/sampling time were used. Bone marrow samples were collected at 24, 48 and 72 hrs post dose.
ANALYSIS:
No. slides/plates/replicates/animals analyzed: 2 slides/animal
Counting method: After slides were stained with modified Weight's Stain Pak containing polychrome methylene blue-eosin in [redacted] automatic slide stainer. One thousand (1000) polychromatic erythrocytes per individual and the frequency of appearance of micronucleated polychromatic erythrocytes were measured. They also examined 200 or more erythrocytes total (polychromatic erythrocytes + normochromatic erythrocytes) per individual and obtained the rate (%) of polychromatic erythrocytes relative to total erythrocytes.

Cytotoxic endpoints: Maximal dose limit/animal toxicity (Maximal dose used was 2000 mg/kg).
Genetic toxicity endpoints/results: Increased frequency of micronucleated polychromatic erythrocytes.
Statistical methods: Student's t-test
Criteria for Positive Results: The positive control, Mitomycin C (MMC) caused an increase in frequency of MNPCE.
Criteria for valid test: The results of an assay are considered to be acceptable if: 1) the individual frequency of MPCEs in each mouse is M6 per 1000, and the average MPCE frequency is M2.5 per 1000, in the vehicle control groups; 2) the positive control produces a statistically significant increase in the average MPCE frequency (p M0.05); and 3) a minimum of seven mice per group are alive at the time of sacrifice. If any of these criteria are not met, the assay is repeated.

If the test article induced a statistically significant or dose-dependent increase in MPCE frequency, it is considered to have produced an equivocal response. A test article producing neither type of increase is considered to be negative (non-clastogenic).

RESULTS:

In the preliminary toxicity assay, 500, 1000 and 2000 mg/kg dose were found toxic. Both 1000 and 2000 mg/kg caused mortality (2/2) with in 48 hrs. The 500 mg/kg/d decrease activity and body tone in all animals but there were no deaths. Sponsor chose 250 mg/kg/d as the maximal dose the main study. Since animals in the 500 mg/kg/d survived, using 500 mg/kg/d should have been the maximal dose for this study in mice. MPCE frequencies for all negative control groups were within acceptable range and the control positive (CP) produced a statistically significant increase in MPCE frequency ($p< 0.01$). AC-0137 did not induce any statistically significant or dose-dependent increases in MPCE frequencies, at any harvest time evaluated, as compared to the concurrent negative controls. Similar results were observed for male mice treated with AC-0137. In contrast, a statistically significant increase in MPCE frequency, to approximately 7-fold control values, was observed in female mice treated with AC-0137 at a dose of 25 mg/kg and sacrificed 72 hours after treatment ($p<0.05$). However, this increase was not dose-dependent, and appeared to be due primarily to a concurrent negative control frequency that was lower than usual historical data.

MPCE Frequencies (by sex)[a]

Compound	Dose (mg/kg)	Time (hr)	Sex	Total MPCEs (range)	MPCEs (%) $\bar{x} \pm$ 1SD
di-H_2O	0.00	24	M	5	0.100 ± 0.100
AC-0137	25.0	24	M	4	0.080 ± 0.045
AC-0137	125	24	M	4	0.080 ± 0.110
AC-0137	250	24	M	6	0.120 ± 0.084
CP	60.0	24	M	92	1.840 ± 0.103**
di-H_2O	0.00	24	F	5	0.100 ± 0.071
AC-0137	25.0	24	F	2	0.040 ± 0.089
AC-0137	125	24	F	3	0.060 ± 0.114
AC-0137	250	24	F	3	0.060 ± 0.055
CP	60.0	24	F	79	1.580 ± 0.626**
di-H_2O	0.00	48	M	6	0.120 ± 0.164
AC-0137	25.0	48	M	5	0.100 ± 0.000
AC-0137	125	48	M	5	0.100 ± 0.173
AC-0137	250	48	M	2	0.040 ± 0.055
di-H_2O	0.00	48	F	2	0.040 ± 0.055
AC-0137	25.0	48	F	6	0.120 ± 0.164
AC-0137	125	48	F	1	0.020 ± 0.045
AC-0137	250	48	F	6	0.120 ± 0.110
di-H_2O	0.00	72	M	3	0.060 ± 0.055
AC-0137	25.0	72	M	3	0.060 ± 0.089
AC-0137	125	72	M	3	0.060 ± 0.089
AC-0137	250[b]	72	M	6	0.040 ± 0.058
di-H_2O	0.00	72	F	1	0.020 ± 0.045
AC-0137	25.0	72	F	7	0.140 ± 0.114*
AC-0137	125	72	F	4	0.080 ± 0.084
AC-0137	250	72	F	6	0.120 ± 0.130

[a]Except as noted below, by-sex data are for 5000 PCEs/group (1000 PCEs/mouse; five/group).
[b]Data reported for this group are for 4000 PCEs (1000 PCEs/mouse; one animal died prior to sacrifice).
*,**Statistically significant increase at $p\leq0.05$ and $p\leq0.01$, respectively.

MPCE frequencies in all other groups of females, for all other doses and harvest times, approximated the control values. There were no statistically significant depressions in PCE/NCE ratios observed for any dose of AC-0137 at any sampling time, or in the animals treated with the positive control.

Summary

No data except, for female mice treated with AC-0137 at a dose of 25 mg/kg at the 72-hour sacrifice were significantly higher than control. This was considered to be a statistical aberration due to random fluctuation of the spontaneous MPCE frequency (i.e., a low negative control value) and not biologically significant (all observed MPCE frequencies approximated historical negative control values). Therefore, AC-0137 is considered to be negative (non-clastogenic) in the In *Vivo* Micronucleus Test in Mouse Bone Marrow Erythropoietic Cells, at the doses and harvest times evaluated, under the conditions and according to the criteria of the test protocol.

Study Title: AS52/XPRT Mammalian cell forward mutation assay AC-0137.

Study No: REST98136
Study Type: Ability to induce mutation in genetically engineered AS52 cell line
Amendment #000, Volume #1.70 Page # 1-136
Conducting Laboratory: [redacted]

Date of Study Initiation/completion: Oct 31, 94
GLP Compliance: Yes
QA- Report: Yes (X) No ( )
Drug Lot Number: 94-0802BB (purity ·—%)
Study Endpoint: mutation in AS52/XPRT cell line
METHODOLOGY:
The mutation assay was performed using duplicate cultures for each concentration. The relative survival was determined for each culture. In the confirmatory assay, AC-0137 concentrations of 16.7, 50, 167, 500, 1670, 2000, 2500 and 3000µg/ml with S9 and 16.7, 50, 167, 500, 1670 and 2000 µg/ml without S9 were evaluated.
Strains/Species/Cell line: AS52/XPRT Mammalian cell
Dose Selection Criteria: Dose selection was based on cell growth inhibition tests and the type and duration of test to be performed. The confirmatory test article concentrations were based on cytotoxicity and original assay.
Basis of dose selection: Dose selection was based on reduction in colony forming ability of the cells following 5 hour treatment with AC-0137 in presence or absence of S9.
Test Agent Stability: Certified to be stable for the duration of the test (analyzed at the end of studies).
Metabolic Activation System: S9 liver fraction of SD rats was used as metabolic activator.

CONTROLS:
Vehicle: distilled deionized water
Negative Controls: di-water
Positive Controls: In direct method assay the positive control was N-Methyl-N'-nitrosoguanidine (MNNG). For metabolic activation assay, the positive control was benzo[a]pyrene.
Mutation assay
The mutation assay was performed using duplicate cultures for each test article concentration, as well as positive and negative controls. Following treatment, relative survival was determined for each culture, cells that have undergone mutation to XPRT form colonies in the presence of 10 µM

TG. After growth for a period of 8 days to allow expression of the mutant phenotype, 106 cells from each culture were plated in medium containing TG to select for mutant cells.

Estimation of initial survival and subculturing for expression of mutation
Following the overnight incubation after test article treatment, (i.e., on Day 1) the cells were harvested and a cell number was determined for each culture as described above (the *apparent* deviation from the 19-hour incubation stated in the protocol, occurring here and in the toxicity prescreen, had no adverse effect upon the integrity of the study). An aliquot of each culture was diluted in Saline G to a density of 1000 cells per mL and 0.2 mL were then added to each of three 60-mm plates containing 5 mL F12CM5 (200 cells/plate). These plates were used to determine the relative initial cell survival following treatment, and were incubated for 7 days before the colonies were fixed, stained and counted as previously described. An additional aliquot yielding 1 x 10^6 (or all remaining) cells was subcultured for phenotypic expression into a 100-mm dish containing 10 mL F12CM5. Subcultures were again performed on Days 3 and 6 (or 4 and 6) with selection on Day 8. The subcultures were performed by harvesting the cells as described above, determining the cell density and adding an aliquot of 1 x 10^6 cells to a 100-mm plate containing 10 mL F12CM5, or "re-feeding" all remaining cells with 10 mL F12CM5 (in the same dish).

Selection of Mutant AS52 Cells On Day 8 the cells were harvested and, after the cell density was determined, an aliquot was diluted to approximately 1 x 10^5 cells/mL in HX'F12CM5 (hypoxanthine-free F12CM5). Two mL of this suspension were added to each of five 100-mm plates containing 8 mL HXF12CM5 supplemented with 12.5 µM TG (approximately 2 x 105 cells/plate). An additional aliquot was diluted to a density of 1000 cells/mL in Saline G and 0.2 mL were added to each of three 60-mm plates containing 5 mL HX'F12CM5 (approximately 200 cells/plate). The 60-mm plates were cloning efficiency plates used to correct the mutant frequencies observed on the selection plates. All plates were incubated, and the colonies were fixed, stained and counted.

RESULTS:

Mutagenicity Data - Control Cultures ±S9

Compound	µg/mL	S9 (±)	Relative Survival(%)[a]	Total No. of Mutants (5 plates)	Cloning Effic'y(%)	Mutant Frequency (mutants/10^6 clonable cells)	Average Mutant Frequency
Untreated	0.00	-	96.49	12	89.50	13.41	
Untreated	0.00	-	94.08	12	89.67	13.38	13.40
Untreated	0.00	+	107.88	19	91.00	20.88	
Untreated	0.00	+	119.96	13	87.33	14.89	17.88
di-H_2O[b]	100	-	93.90	10	91.00	10.99	
di-H_2O	100	-	81.68	15	86.17	17.41	14.20
di-H_2O	100	+	98.30	17	91.00	18.68	
di-H_2O	100	+	107.72	12	73.00	16.44	17.56
EMS	200	-	9.91	99	86.33	114.67	
EMS	200	-	13.35	100	62.83	159.15	136.91**
DMN	100	+	11.96	123	59.17	207.89	
DMN	100	+	18.78	111	48.33	229.66	218.77**

[a]Viable cells per culture = 4.00 x 10^6 (relative survival calculated as in Table 1).
[b]µL/mL.
**Significant increase ($p < 0.01$; Snee and Irr, 1981).
Pooled negative control cultures: $\bar{x}$ = 15.76 ± 3.22 (1SD)
$2\bar{x}$ = 31.52
$\bar{x}$ + 30 = 45.76
95% confidence interval = 22.20

Mutagenicity Data - Treated Cultures +S9

Compound	µg/mL	Relative Survival(%)[a]	Total No. of Mutants (5 plates)	Cloning Effic'y(%)	Mutant Frequency (mutants/10^6 clonable cells)	Average Mutant Frequency
AC-0137	16.7	84.92	19	90.33	21.03	
AC-0137	16.7	94.48	22	101.50	21.67	21.35
AC-0137	50.0	76.53	18	72.33	24.88	
AC-0137	50.0	96.17	11	73.83	14.90	19.89
AC-0137	167	81.57	24	99.00	24.24	
AC-0137	167	67.01	14	80.00	17.50	20.87
AC-0137	500	65.48	23	79.17	29.05	
AC-0137	500	57.04	24	77.83	30.84	29.94*
AC-0137	1670	61.88	16	83.17	19.24	
AC-0137	1670	65.55	16	64.17	24.94	22.09
AC-0137	2000	61.52	16	67.33	23.76	
AC-0137	2000	71.12	14	89.83	15.58	19.67
AC-0137	2500	19.77	23	87.83	26.19	
AC-0137	2500	24.90	13	74.50	17.45	21.82
AC-0137	3000	11.05	13	53.67	24.22	
AC-0137	3000	2.35	12	47.83	25.09	24.66

[a]Viable cells per culture = 3.49×10^6 (relative survival calculated as in Table 1).

Mutagenicity Data - Treated Cultures -S9

Compound	µg/mL	Relative Survival(%)[a]	Total No. of Mutants (5 plates)	Cloning Effic'y(%)	Mutant Frequency (mutants/10^6 clonable cells)	Average Mutant Frequency
AC-0137	16.7	93.66	16	85.33	18.75	
AC-0137	16.7	87.44	11	95.00	11.58	15.16
AC-0137	50.0	78.37	16	91.67	17.45	
AC-0137	50.0	68.54	10	79.50	12.58	15.02
AC-0137	167	70.25	9	92.17	9.76	
AC-0137	167	62.51	17	82.33	20.65	15.21
AC-0137	500	77.21	13	83.33	15.60	
AC-0137	500	57.94	14	77.67	18.03	16.81
AC-0137	1670	3.33	13	82.17	15.82	
AC-0137	1670	4.61	15	80.83	18.56	17.19
AC-0137	2000	4.24	13	85.00	15.29	
AC-0137	2000	11.71	15	84.83	17.68	16.49
AC-0137	2500	Not determined - discarded due to extreme cytotoxicity.				
AC-0137	2500	Not determined - discarded due to extreme cytotoxicity.				

[a]Viable cells per culture = 4.00×10^6 (relative survival calculated as in Table 1).

Results of the prescreen indicated AC-0137 was cytotoxic in the presence or absence of S9. Relative survivals at a concentration of 1670 µg/mL were 65.35% and 30.22% with and without S9, respectively. Based upon these findings, AC-0137 was evaluated in duplicate cultures in the initial mutation assay at concentrations of 16.7, 50.0, 167, 500, 1670, 2000, 2500, 3000, 3500, 4000, 4500 and 5000 µg/mL with S9, and concentrations of 16.7, 50.0, 167, 500, 1670, 2000, 2500, 3000, 3500 and 4000 µg/mL without S9. Those cultures treated at concentrations ~3500 µg/mL with S9, and ~2500 µg/mL without S9, were discarded on the day after treatment due to extreme immediate cytotoxicity.

The average mutant frequencies of the negative control cultures ranged from **13.40 to 17.88 TG^r mutants/10^6 clonable cells**, while those of the remaining cultures treated with AC-0137 ranged from 12.55 to 27.14 TG^r mutants/10^6 clonable cells. A statistically significant increase in average mutant frequency was observed at a concentration of 500 µg/mL with S9 ($p < 0.05$).

However, this increase was not dose dependent, it did not represent an increase of 30 TG^r mutants/10^6 clonable cells over concurrent negative control values, and it was within acceptable negative control values.

AC-0137 was re-evaluated in the confirmatory assay at concentrations of 16.7, 50.0, 167, 500, 1670, 2000, 2500 and 3000 µg/mL with S9, and concentrations of 16.7, 50.0, 167, 500, 1000, 1330, 1670 and 2000 µg/mL without S9 (doses evaluated in the confirmatory assay were adjusted based upon cytotoxicity observations made in the original assay).

A statistically significant increase in average mutant frequency again was observed at a concentration of 500 µg/mL with S9 ($p< 0.05$). However, this increase was not dose dependent, it did not represent an increase of 30 TG^r mutants/10^6 *clonable* cells *over concurrent* negative *control* values, and it was within acceptable negative control values. All positive and negative control values were within acceptable ranges. Thus, the slight increase observed at a concentration of 500 µg/mL with S9 is considered to be a statistical aberration due to random fluctuation of the spontaneous mutant frequency. Therefore, AC-0137 is considered to be negative in the presence of metabolic activation in the ASSZ/XPRT Mammalian Cell Forward Gene Mutation Assay under the conditions, and according to the criteria, of the test protocol.

Conclusion:
Slight increase observed at a concentration of 500µg/ml with S9 was considered a statistical aberration and was not dose-dependent. AC-0137 was considered negative in the presence of metabolic activation in the AS52/XPRT Mammalian Cell Forward Gene Mutation Assay under the conditions and criteria of the test protocol.

Genotoxicity Summary:
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: [ ]. Specific tests and findings are described:

1. Ames tests conducted on product from 3 different manufacturers ([ ] of pramlintide were negative.
2. In vitro chromosomal aberration test using human lymphocytes: Since pramlintide was cytotoxic at concentrations higher than 1670µg/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. In the preliminary test, the 1000 and 2000 mg/kg

dose caused mortality in all groups with in 48 hrs. 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 increases in micronucleated PCE at any dose. Since the increase in female mice at 25 mg/kg was not dose-dependent and negative control 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.

Labeling recommendations:

Mutagenesis: SYMLIN was not mutagenic in the Ames test and did not increase chromosomal aberration in the human lymphocyte assay. Symlin was not clastogenic in the in vivo mouse micronucleus test or in the chromosomal aberration assay utilizing Chinese hamster ovary cells.

CARCINOGENICITY:

Study title: AC-0137: 104 Week Subcutaneous Oncology Study in the Mouse

Key study findings: Due to higher mortality in males, all surviving males were terminated during Week 97, earlier than females (Week 104). 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.

Study number: — 1186/5 (Report # REST98108)
Volume #, and page #: 50-56, 1-2038
Conducting laboratory and location: [redacted]
Date of study initiation: Jan 10, 1995
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: AC0137 (1 mg/mL): 94-0604CB, 95-0303GB, 94-0806CB, 94-1202GB, 94-1101GB and AC0137 (0.1 mg/mL): 94-0607FB, 94-0905FB, Drug products were manufactured by [redacted] Purity ranges between [redacted]
CAC concurrence: No
Study Type: 2 year bioassay in mice
Species/strain: —.CD-1®(ICR)BR mice
Number/sex/group: 51/group/sex, Males: 26-38 g, Females: 20-33 g
Age at start of study: Mice were less than 8 WK old at the start of the study
Animal housing: 3/cage
Formulation/vehicle: Not specified (assumed to be the vehicle used in final product)
Drug stability/homogeneity: Product samples were collected and tested every three month. Product was stable.
Methods: Doses: 0, 0.2, 0.5, 1.2 and 0 mg/kg/day, with dose volumes of 12, 2, 5, 12 and 12 ml/kg, respectively. The exposure ratios were based on calculated from 28-Day mouse study.

Species	Doses, mg/kg/day	$AUC_{0-t,\ (t=120\text{-}300m)}$ ng.min/ml	Ratio of animal to maximal human dose AUC in type I and II diabetes	
			Type I Diabetes	Type II Diabetes
104 WK Mouse Bioassay	0.2	3340	32	56
	0.5	7012	66	117
	1.2	16499	156	275
Human dose Type I, 360 µg/day (90 mg QID)		106		
Human dose Type II, 360 µg/day (120 mg TID)		60		

Basis of dose selection: AUC multiple of maximal human dose (≥ 25 times)
Restriction paradigm for dietary restriction studies:
Route of administration: single daily subcutaneous dose
Frequency of drug administration: once daily
Dual controls employed: Both controls received formulation vehicle (placebo) at 12 ml/kg/day SC.
Interim sacrifices: No
Satellite PK or special study group(s): TK not assessed.
Deviations from original study protocol: Some of the terminal necropsies in males were carried out in WK 97 due, earlier than stated 2-year design because of higher mortality rate. From Day 1 to Day 3 of WK 9, the administered test article was the

0.1 mg/ml (stock) preparation received from the sponsor. The rest of the study was carried out with test article diluted to 0.1 mg/ml with vehicle at the site from stock solution provided by the sponsor (1 mg/ml).

Statistical methods: Standard statistical methods were used to compare treated groups to combined control groups. For survival analysis, the male and female animals were analyzed separately. Survival probability functions were estimated by the Kaplan-Meier technique. Survival curves were compared by the log-rank procedure. One directional tests for an increasing and a decreasing dose-response in mortality across all groups and one-directional pairwise tests of the combined control groups against groups 2, 3 and 4 were performed in accordance with the IARC annex. Where the test for a dose response was not significant, a Bonferroni adjustment was used for the pairwise tests against combined controls. The survival curves were compared to the start of the terminal kill phases (week 97 males, week 105 females). Tissues from all animals were examined in control groups 1 and 5 and in the high dose group (group 4). In addition, the injection sites were examined in all animals. The number of tumor bearing animals were analyzed separately for males and females, for tumor types found in at least 3 animals of the given sex within groups 1, 4 and 5. For all other tumor types, one directional pairwise tests for both an increasing and a decreasing dose response were performed between group 4 and the combined controls, in accordance with the IARC annex. Non-fatal tumors were analyzed broadly in accordance with the IARC annex; fixed intervals of 1 to 50 weeks, 51 to 80 weeks, 81 to 96 weeks (males) or 81 to 104 weeks (females) and the terminal kill phase were used in place of the "ad-hoc" runs methods. Permutation tests were used to establish the significance of findings wherever fatal or non-fatal tumors were observed with a total incidence of at least 3 but less than 10, or wherever the total combined incidence of both fatal and non-fatal tumors was at least 3 but less than 10. The exact (permutational) sampling distributions of the test statistics were then obtained. The fatal and non-fatal results were combined in accordance with the IARC annex. Only if this combined test gave a significant result ($P<0.05$) are the separate fatal and non-fatal results also reported.

Observations and times:

Clinical signs: Observe daily with full examination once/week.

Body weights: Weekly for until WK16, once every 4WKs there after until necropsy.

Food consumption: Weekly for until WK16, and one WK every 4WKs.

Hematology: 0.5 ml blood from all animals at terminal necropsy (males WK97, females WK105) after an overnight fasting. RBC and WBC counts were determined,

Clinical chemistry: Not done

Organ weights: please see addendum on page 25 for list organs

Gross pathology: Performed on all animals. Please see addendum on page 25 for list of tissue.

Histopathology: Tissue samples from control and high dose animals were examined. Injection site histology was performed on low and mid dose animals as well.

Toxicokinetics: Not done

Results:

Mortality: Survival was comparable across all groups ($p>0.05$). There was however, an increase in mortality in control and HD males because of sarcoma at one or more injection sites.

Incidence of selected cause of demise:

Sex	Male					Female				
Dose (mg/kg)	0	0.2	0.5	1.2	0	0	0.2	0.5	1.2	0
Number examined	36	28	29	32	37	36	36	32	36	34
Injection site sarcoma	13	1	4	12	11	2	1	0	0	2

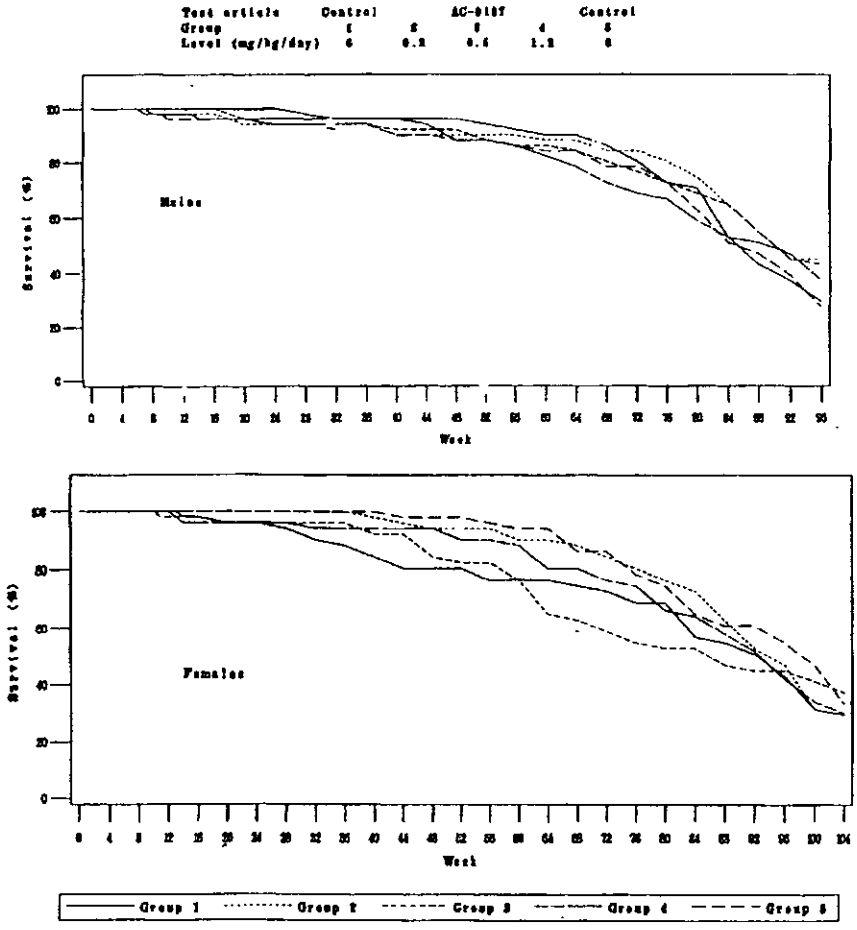
Number of mice alive at the end of 52, 80 and at the termination of the 2-year bioassay study in

	Male (n=51/dose)					Female (n=51/dose)				
Dose, mg/kg/day	0	0.2	0.5	1.2	0	0	0.2	0.5	1.2	0
# animals alive, WK52	49	46	47	45	45	41	48	42	46	50
# animals alive, WK80	36	38	36	31	32	35	39	27	33	38
# animals alive, at WK96 (M), Wk104 (F)	15	23	22	19	14	15	15	15	17	

Percent survival at different time intervals:

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 %

Graphical representation of Percent survival



Pramlintide - 104-Week Subcutaneous Oncogenicity Study in the Mouse-Results: Causes of Death

AC-0137: 104-Week Subcutaneous Oncogenicity Study in the Mouse [REST98108R1]

Causes of Death

	Pramlintide (mg/kg)									
	Male					**Female**				
	0.0 (1)	**0.0 (2)**	**0.2**	**0.5**	**1.2**	**0.0 (1)**	**0.0 (2)**	**0.2**	**0.5**	**1.2**
No. Examined	36	37	28	29	32	36	34	36	32	36
Injection site sarcoma	12	11	1	4	12	2	2	1	0	0
Amyloidosis	4	5	7	5	4	3	1	5	0	4
Urogenital tract lesion	3	4	10	5	4	1	3	3	1	0
Hemolymp. tumor	1	2	2	3	1	4	8	2	4	5
Neurological lesion*	0	1	0	1	3	4	2	3	7	10

* No clinical signs, no gross observations and no microscopic findings recorded in the individual animal data that support this description (most deaths appeared due to mechanical trauma to the spinal cord)

Group incidence: cause of morbidity and mortality in mice (group1=control vehicle, group2=0.2 mkd, group3=0.5 mkd, group 4=1.2 mkd and group 5= control vehicle)

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=D,M;FIND=ALL;SUBSET=T

	--- NUMBER-OF-ANIMALS-AFFECTED									
SEX:	----------MALE----------					---------FEMALE---------				
GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION — NUMBER:	36	28	29	32	37	36	36	32	36	34
** TOP OF LIST **										
CAUSE OF DEMISE — NUMBER EXAMINED:	36	28	29	32	37	36	36	32	36	34
--SKIN/APPENDAGE LESION	2	2	4	1	4	4	3	1	2	6
--ORAL CAVITY LESION	0	0	0	0	0	0	0	0	0	1
--NEUROLOGICAL LESION	0	0	1	3	1	4	3	7	10	2
--GASTROINTESTINAL TRACT LESION	0	1	0	0	0	0	0	0	0	0
--GLOMERULONEPHROPATHY	0	1	0	1	2	2	2	4	1	1
--UROGENITAL TRACT LESION	3	10	5	4	4	1	3	1	0	3
--HAEMORRHAGIC OVARIAN CYST	0	0	0	0	0	0	2	2	5	1
--CARDIOVASCULAR LESION	3	1	3	2	2	0	1	0	0	0
--AMYLOIDOSIS	4	7	5	4	5	3	5	0	4	1
--HAEMORRHAGE	0	0	1	0	0	5	0	0	0	0
--OTHER LESIONS	0	0	1	0	2	0	3	1	2	0
--SKIN/SUBCUTIS TUMOUR	1	0	0	0	0	0	1	1	0	4
--CONNECTIVE TISSUE TUMOUR	1	0	0	1	0	0	1	0	0	0
--HAEMOLYMPHORETICULAR TUMOUR	1	2	3	1	2	4	2	4	5	8
--BRAIN/SPINAL CORD TUMOUR	0	0	0	0	0	0	1	0	0	0
--MAMMARY TUMOUR	0	0	0	0	0	0	1	3	1	1
--LIVER TUMOUR	1	0	0	0	0	0	0	0	0	0
--UTERINE TUMOUR	0	0	0	0	0	4	3	4	1	3
--LUNG TUMOUR	5	3	0	1	2	0	3	1	1	0
--BLOOD VESSEL TUMOUR	0	0	0	0	0	0	0	0	0	1
--MULTIPLE ORGAN TUMOURS	0	0	0	1	0	0	0	0	0	0
--VAGINAL TUMOUR	0	0	0	0	0	1	0	0	0	0
--INJECTION SITE SARCOMA	12	1	4	12	11	2	1	0	0	2
--OTHER TUMOUR	0	0	0	0	0	1	0	1	0	0
--NOT DETERMINED	3	0	2	1	2	5	1	2	4	0
** END OF LIST **										

Clinical signs: Many of the clinical signs were of aging mice. No drug related toxicity. Incidence of lesions on the back appeared to be drug dose volume related since it was noted primarily in controls and HD group (12 ml/kg). There were also palpable subcutaneous masses, movable and stationary:

INCIDENCE OF SMALL STATIONARY SUBCUTANEOUS TISSUE MASSES [% = (NUMBER OF ANIMALS OBSERVED WITH MASS IN DESIGNATED WEEK + NUMBER OF ANIMALS ENTERING DESIGNATED WEEK) X 100]

Time	Vehicle		0.2 mg/kg		0.5 mg/kg		1.2 mg/kg		Vehicle	
	M	F	M	F	M	F	M	F	M	F
Wk 13	4	0	0	0	2	0	2	0	4	0
Wk 26	6	0	10	0	8	0	4	0	4	0
Wk 39	10	0	17	0	23	0	18	0	15	0
Wk 52	18	0	26	0	33	0	22	2	16	0
Wk 65	20	0	42	0	23	3	30	2	28	0
Wk 78	35	0	31	0	14	4	12	3	32	0
Last Wk	47	7	87	0	36	5	21	12	56	0

INCIDENCE OF SMALL MOVABLE SUBCUTANEOUS TISSUE MASSES [% = (NUMBER OF ANIMALS OBSERVED WITH MASS IN DESIGNATED WEEK + NUMBER OF ANIMALS ENTERING DESIGNATED WEEK) X 100]

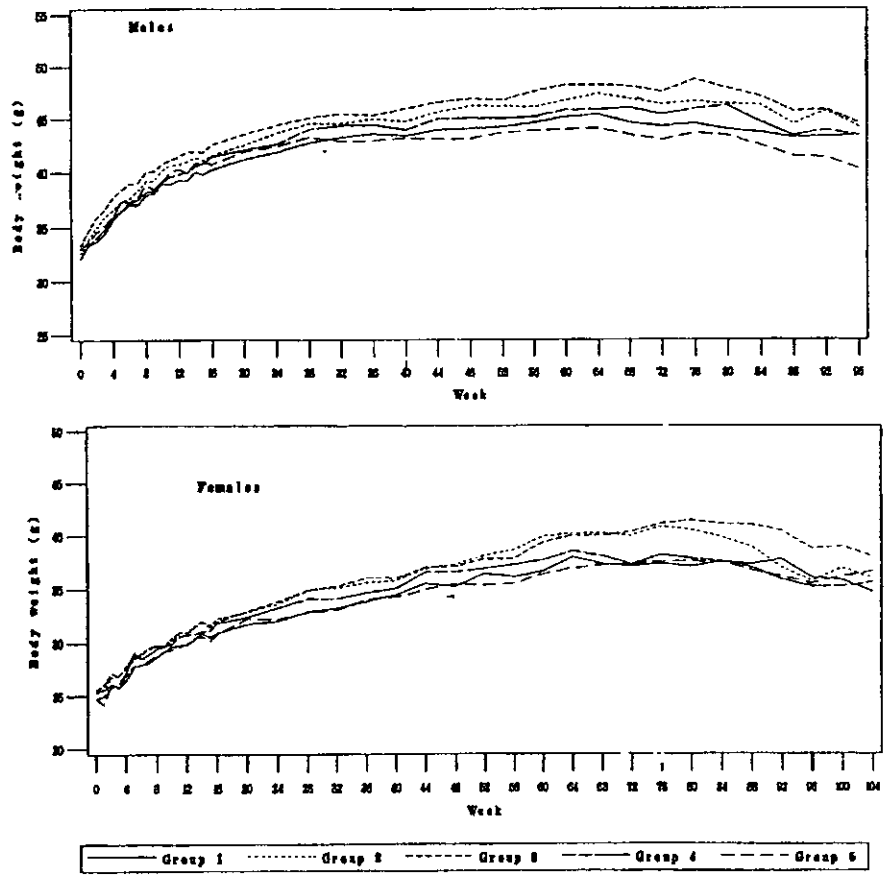
Time	Vehicle		0.2 mg/kg		0.5 mg/kg		1.2 mg/kg		Vehicle	
	M	F	M	F	M	F	M	F	M	F
Wk 13	0	0	0	0	0	0	0	0	0	0
Wk 26	0	0	0	0	4	0	0	0	0	0
Wk 39	2	0	0	0	0	0	0	0	0	0
Wk 52	8	0	0	0	0	0	0	0	0	0
Wk 65	2	0	0	0	2	0	2	0	0	0
Wk 78	5	0	0	0	3	0	3	0	6	0
Last Wk	0	0	13	0	9	0	5	0	0	0

Large stationary masses were noted in control and treated groups (see table):

INCIDENCE OF LARGE STATIONARY SUBCUTANEOUS TISSUE MASSES
[% = (NUMBER OF ANIMALS OBSERVED WITH MASS IN DESIGNATED WEEK ÷ NUMBER OF ANIMALS ENTERING DESIGNATED WEEK) X 100]

Time	Vehicle		0.2 mg/kg		0.5 mg/kg		1.2 mg/kg		Vehicle	
	M	F	M	F	M	F	M	F	M	F
Wk 13	0	0	0	0	0	0	0	0	0	0
Wk 26	0	0	0	0	0	0	0	0	0	0
Wk 39	0	0	0	0	0	0	2	0	0	2
Wk 52	0	0	0	0	2	2	0	0	0	0
Wk 65	0	0	0	0	2	0	0	0	0	0
Wk 78	0	0	3	0	5	0	9	0	3	3
Last Wk	0	0	4	0	0	5	0	0	0	0

Body weights: Minor changes in body weight at particular intervals were not considered significant.



Food consumption: No treatment related effect on food consumption (see table in the next page).

Pramlintide - 104-Week Subcutaneous Oncogenicity Study in the Mouse-Results: Body Weight Gain and Food Consumption

AC-0137: 104-Week Subcutaneous Oncogenicity Study in the Mouse [REST98108R1] Body Weight Gains and Food Consumption										
	Pramlintide (mg/kg)									
	Male					Female				
	0.0 (1)	0.0 (2)	0.2	0.5	1.2	0.0 (1)	0.0 (2)	0.2	0.5	1.2
Body Weight Gain (g) (mean)										
Weeks 0-28	10.6	10.4	12.1*	11.9*	11.9*	7.4	8.2	9.6**	9.3*	9.4**
Weeks 28-52	1.3	0.5	1.8	1.5	1.1	3.2	2.4	3.1	2.7	2.8
Weeks 52-104[a]	-2.4	-2.0	-2.0	-1.7	-1.5	-2.0	0.3	-1.5	-1.1	-0.1
Weeks 0-104[a]	10.4	8.2	12.0	11.1	11.9	9.2	11.8	10.5	12.4	11.3
Food Consumption (g) (mean)										
Weeks 0-28	36.9	38.7	39.0	39.5	37.6	35.9	36.2	37.8	35.7	36.2
Weeks 28-52	34.4	35.9	35.6	37.5	36.4	34.6	34.4	33.8	34.4	35.5
Weeks 52-104[a]	37.1	39.0	37.0	40.4	38.3	34.6	35.2	34.8	35.2	37.0
Weeks 0-104[a]	36.4	37.8	37.7	39.7*	37.3	34.2	35.5	35.4	35.2	36.2
[a] All surviving male mice sacrificed at week 97; *p<0.05 (compared to combined control groups); **p<0.01 (compared to combined control groups)										

Hematology: No treatment related effect

Gross pathology: Aside from injection site lesions and masses in control and HD groups, necropsy findings were similar among groups (see table above). Pancreatic mass was noted in 1 LD male, 2 HD male and 1 LD, 1MD and 2HD female mouse.

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 by dermatitis/ foliculitis, 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.

Incidence of selected injection site findings by grade*

Sex	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.
- 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	Control2
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

Chronological listing of tumor data, in control males group 1

GROUP 1 MALES

Week of necropsy	Week tumour discovered	Animal number	Mode of death	Tissue/organ	Tumour type	Malignancy
55	55	25	U	LIVER	M-HEPATOCELLULAR CARCINOMA	M
57	57	20	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
69	69	45	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
71	69	15	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
72	72	48	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
74	74	24	U	MESENTERIC LN	B-HAEMANGIOMA	B
75	52	49	U	HAEM/LYMPH/RETIC	M-GRANULOCYTIC LEUKAEMIA	M
80	80	31	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
81	77	19	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
82	78	8	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
82	82	13	U	ADRENAL	B-BENIGN PHAEOCHROMOCYTOMA	B
83	83	4	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
84	81	3	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
84	84	3	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
84	82	6	U	HARDERIAN GLAND	B-ADENOMA	B
84	84	6	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
84	81	6	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
84	84	7	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
84	82	7	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
84	84	30	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
84	83	30	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
86	86	2	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
86	75	2	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
87	87	9	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
88	83	37	U	SKIN + SUBCUTIS	M-BASAL CELL CARCINOMA	M
88	88	43	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
89	89	18	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
91	90	10	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
91	91	10	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
92	92	42	U	SKIN + SUBCUTIS	B-FIBROMA	B
95	95	36	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
95	94	38	U	CONNECTIVE TISS	M-HISTIOCYTIC SARCOMA	M
95	90	38	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
96	96	32	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
96	96	39	U	SPLEEN	B-HAEMANGIOMA	B
96	96	39	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M

GROUP 1 MALES

Week of necropsy	Week tumour discovered	Animal number	Mode of death	Tissue/organ	Tumour type	Malignancy
97	97	11	T	ABDOMINAL CAVITY	M-SARCOMA	M
97	97	11	T	LIVER	M-HEPATOCELLULAR CARCINOMA	M
97	97	14	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	16	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	17	T	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
97	97	17	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	22	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	29	T	LIVER	B-HAEMANGIOMA	B
97	97	33	T	LIVER	M-HEPATOCELLULAR CARCINOMA	M
97	97	33	T	KIDNEY	B-TUBULAR CELL ADENOMA	B
97	97	40	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	46	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	47	T	LUNG	B-BRONCHIOLO-ALVEOLAR	B
97	97	50	T	LIVER	B-HEPATOCELLULAR ADENOMA	B

Chronological listing of tumor data for male mice treated with 0.2 mg/kg/day Symlin

GROUP 2 MALES

Week of necropsy	Week tumour discovered	Animal number	Mode of death	Tissue/organ	Tumour type	Malignanc
66	66	91	U	HAEM/LYMPH/RETIC	M-GRANULOCYTIC LEUKAEMIA	M
67	66	84	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
80	80	68	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
82	82	67	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
82	74	95	U	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
83	83	82	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
83	83	94	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
85	85	88	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
87	87	87	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
87	87	87	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
88	88	92	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
90	90	58	U	LIVER	M-HEPATOCELLULAR CARCINOMA	M
90	90	70	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
90	90	102	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
91	91	63	U	LIVER	M-HEPATOCELLULAR CARCINOMA	M
97	97	56	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	60	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	64	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	65	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	69	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	75	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	78	T	TESTIS	B-INTERSTITIAL CELL ADENOMA	B
97	97	85	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	86	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	89	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	93	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	96	T	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
97	97	98	T	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
97	97	101	T	LIVER	B-HEPATOCELLULAR ADENOMA	B

Chronological listing of tumors in males treated with 0.5 mg/kg/day Symlin (group3)

GROUP 3 MALES

Week of necropsy	Week tumour discovered	Animal number	Mode of death	Tissue/organ	Tumour type	Malignancy
22	20	148	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
40	40	142	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
72	67	110	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
72	72	110	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
75	75	139	U	SKIN + SUBCUTIS	M-HAEMANGIOSARCOMA	M
75	62	139	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
80	80	143	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
82	81	134	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
82	82	134	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
84	75	132	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
87	87	114	U	HAEM/LYMPH/RETIC	M-GRANULOCYTIC LEUKAEMIA	M
87	87	128	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
88	88	131	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
90	90	117	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
90	90	122	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
92	92	146	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
94	94	144	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	104	T	JEJUNUM	M-CARCINOMA	M
97	97	105	T	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
97	97	107	T	SPLEEN	M-HAEMANGIOSARCOMA	M
97	97	107	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	123	T	CONNECTIVE TISS	M-HISTIOCYTIC SARCOMA	M
97	97	123	T	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
97	97	127	T	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
97	97	127	T	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
97	97	135	T	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
97	97	135	T	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
97	97	138	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	149	T	LIVER	B-HEPATOCELLULAR ADENOMA	B

T = Terminal necropsy, U = Unscheduled death, B = Benign, M = Malignant

Chronological listing of tumors in male mice treated with 1.2 mg/kg/day Symlin (group 4)

GROUP 4 MALES

Week of necropsy	Week tumour discovered	Animal number	Mode of death	Tissue/organ	Tumour type	Maligna
43	43	171	U	STERNUM + MARROW	M-FIBROSARCOMA	M
45	45	195	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
56	55	169	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
60	57	193	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
64	63	159	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
64	64	159	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
64	61	185	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
67	63	168	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
72	69	158	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
74	74	200	U	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
77	74	191	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
79	76	164	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
79	76	202	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
80	80	204	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
80	80	204	U	CONNECTIVE TISS	M-HAEMANGIOSARCOMA	M
80	80	204	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
82	82	177	U	TESTIS	B-INTERSTITIAL CELL ADENOMA	B
82	78	177	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
83	83	192	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
83	83	192	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
94	94	181	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
94	94	183	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
94	94	184	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
95	93	161	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
97	97	156	T	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
97	97	173	T	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
97	97	173	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	173	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	174	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	176	T	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
97	97	186	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	186	T	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
97	97	196	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	196	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	197	T	LIVER	M-HEPATOCELLULAR CARCINOMA	M
97	97	197	T	STOMACH	B-KERATOACANTHOMA	B
97	97	197	T	KIDNEY	B-TUBULAR CELL ADENOMA	B

T = Terminal necropsy, U = Unscheduled death, B = Benign, M = Malignant

Chronological listing of tumors in second group of control male mice (group 5)

GROUP 5 MALES

Week of necropsy	Week tumour discovered	Animal number	Mode of death	Tissue/organ	Tumour type	Malignancy
54	54	214	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
60	56	222	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
60	60	222	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
66	66	221	U	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
74	70	249	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
76	76	205	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
77	75	208	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
77	77	208	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
77	77	233	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
79	78	241	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
79	79	241	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
82	80	232	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
82	82	235	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
82	82	235	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
83	80	230	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
83	83	239	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
83	80	239	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
84	81	216	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
86	84	213	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
93	93	243	U	LIVER	B-HEPATOCELLULAR ADENOMA	B
93	93	243	U	LIVER	M-HEPATOCELLULAR CARCINOMA	M
94	94	217	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
94	94	228	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
96	92	209	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
96	73	209	U	HARDERIAN GLAND	B-ADENOMA	B
96	96	209	U	ADRENAL	B-SUBCAPSULAR CELL TUMOUR	B
97	97	210	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	215	T	LIVER	M-HEPATOCELLULAR CARCINOMA	M
97	97	215	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	215	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	218	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	223	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	224	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	236	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
97	97	236	T	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
97	97	240	T	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
97	97	252	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
97	97	253	T	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
97	97	253	T	ADRENAL	B-CORTICAL ADENOMA	B
97	97	254	T	TESTIS	B-INTERSTITIAL CELL ADENOMA	B
97	97	254	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B

T = Terminal necropsy, U = Unscheduled death, B = Benign, M = Malignant

Chronological listing of tumors in first control females (group 1)

GROUP 1 FEMALES

Week of necropsy	Week tumour discovered	Animal number	Mode of death	Tissue/organ	Tumour type	Malignancy
34	34	290	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
39	39	281	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
43	43	299	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
52	52	274	U	UTERUS	M-HISTIOCYTIC SARCOMA	M
69	69	302	U	HAEM/LYMPH/RETIC	M-LYMPHOMA	M
74	74	282	U	UTERUS	M-LEIOMYOSARCOMA	M
82	82	285	U	CONNECTIVE TISS	M-HISTIOCYTIC SARCOMA	M
84	84	292	U	ADRENAL	B-CORTICAL ADENOMA	B
84	79	292	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
87	87	287	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
92	92	259	U	VAGINA	B-LEIOMYOMA	B
92	92	264	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
95	95	283	U	OVARY	M-LEIOMYOSARCOMA	M
95	95	283	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
95	95	283	U	UTERUS	B-POLYP	B
98	96	297	U	UTERUS	M-CARCINOMA	M
98	98	305	U	UTERUS	B-HAEMANGIOMA	B
98	98	305	U	UTERUS	B-LEIOMYOMA	B
99	98	278	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
101	101	275	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
105	105	261	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
105	105	263	T	UTERUS	B-POLYP	B
105	105	270	T	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
105	105	271	T	ADRENAL	B-BENIGN PHAEOCHROMOCYTOMA	B
105	105	271	T	UTERUS	B-LEIOMYOMA	B
105	105	273	T	UTERUS	B-POLYP	B
105	105	276	T	UTERUS	B-LEIOMYOMA	B
105	105	293	T	LUNG	B-BRONCHIOLO-ALVEOLAR	B

T = Terminal necropsy, U = Unscheduled death, B = Benign, M = Malignant

Chronological listing of tumors in females treated with 0.2 mg/kg/day Symlin (group2)

GROUP 2 FEMALES

Week of necropsy	Week tumour discovered	Animal number	Mode of death	Tissue/organ	Tumour type	Malignancy
59	59	341	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
72	72	333	U	BRAIN	M-MIXED GLIOMA	M
72	72	333	U	UTERUS	B-LEIOMYOMA	B
76	76	335	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
79	79	310	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
82	80	316	U	UTERUS	M-HISTIOCYTIC SARCOMA	M
88	86	334	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
88	85	337	U	SKIN + SUBCUTIS	M-HAEMANGIOSARCOMA	M
90	82	312	U	UTERUS	M-HISTIOCYTIC SARCOMA	M
91	91	318	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
91	91	346	U	UTERUS	M-HISTIOCYTIC SARCOMA	M
92	92	336	U	OVARY	B-BENIGN LUTEOMA	B
93	93	322	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
93	93	322	U	OVARY	B-CYSTADENOMA	B
94	94	328	U	BONE	B-OSSIFYING FIBROMA	B
94	84	328	U	MAMMARY GLAND	M-CARCINOMA	M
94	94	328	U	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
97	97	327	U	OVARY	B-CYSTADENOMA	B
97	97	327	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
97	97	327	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
97	97	344	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
98	98	339	U	UTERUS	M-HISTIOCYTIC SARCOMA	M
99	99	308	U	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
99	99	326	U	OVARY	B-BENIGN GRANULOSA TUMOUR	B
100	100	309	U	CONNECTIVE TISS	M-HISTIOCYTIC SARCOMA	M
105	105	317	T	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
105	105	317	T	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
105	105	317	T	PITUITARY	B-ADENOMA	B
105	105	317	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
105	105	317	T	MAMMARY GLAND	B-ADENOMA	B
105	105	321	T	UTERUS	B-LEIOMYOMA	B
105	105	345	T	HAEM/LYMPH/RETIC	M-HISTIOCYTIC SARCOMA	M
105	105	345	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
105	105	348	T	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
105	105	354	T	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M

T = Terminal necropsy, U = Unscheduled death, B = Benign, M = Malignant

Chronological listing of tumors in female mice treated with 0.5 mg/kg/day Symlin (group3)

GROUP 3 FEMALES

Week of necropsy	Week tumour discovered	Animal number	Mode of death	Tissue/organ	Tumour type	Malignancy
39	39	358	U	HAEM/LYMPH/RETIC	M-LYMPHOMA	M
45	45	363	U	HAEM/LYMPH/RETIC	M-LYMPHOMA	M
46	46	362	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
58	58	365	U	UTERUS	B-HAEMANGIOMA	B
62	62	369	U	SKIN + SUBCUTIS	B-HAEMANGIOMA	B
62	62	369	U	ADRENAL	B-BENIGN PHAEOCHROMOCYTOMA	B
62	48	372	U	MAMMARY GLAND	M-CARCINOMA	M
63	63	406	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
71	71	373	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
74	68	382	U	MAMMARY GLAND	M-CARCINOMA	M
76	76	359	U	UTERUS	M-STROMAL SARCOMA	M
76	76	359	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
79	79	402	U	UTERUS	B-POLYP	B
86	86	396	U	UTERUS	B-BENIGN GRANULAR CELL TUMOU	B
86	86	396	U	OVARY	B-BENIGN GRANULOSA TUMOUR	B
87	86	385	U	UTERUS	M-HISTIOCYTIC SARCOMA	M
88	88	405	U	UTERUS	B-BENIGN GRANULAR CELL TUMOU	B
92	82	364	U	UTERUS	M-LEIOMYOSARCOMA	M
100	88	376	U	VULVA	B-HISTIOCYTOMA	B
100	100	376	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
100	96	393	U	MAMMARY GLAND	M-CARCINOMA	M
100	100	393	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
101	101	398	U	UTERUS	M-HISTIOCYTIC SARCOMA	M
105	105	370	T	UTERUS	B-POLYP	B
105	105	374	T	UTERUS	B-HISTIOCYTOMA	B
105	105	379	T	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
105	105	379	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
105	105	380	T	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
105	105	380	T	FEMUR + MARROW	B-OSTEOMA	B
105	101	380	T	UTERUS	B-LEIOMYOMA	B
105	105	383	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
105	88	387	T	SKIN + SUBCUTIS	B-SQUAMOUS CELL PAPILLOMA	B
105	105	391	T	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
105	105	392	T	MAMMARY GLAND	B-ADENOMA	B
105	105	397	T	UTERUS	B-LEIOMYOMA	B
105	105	399	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
105	105	399	T	OVARY	B-BENIGN LUTEOMA	B
105	98	400	T	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
105	105	400	T	OVARY	B-TUBULOSTROMAL ADENOMA	B
105	105	404	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
105	105	407	T	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M

T = Terminal necropsy, U = Unscheduled death, B = Benign, M = Malignant

Chronological listing of tumors in female mice treated with 1.2 mg/kg/day Symlin (group 4)

GROUP 4 FEMALES

Week of necropsy	Week tumour discovered	Animal number	Mode of death	Tissue/organ	Tumour type	Malignancy
32	32	411	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
49	49	422	U	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
63	63	430	U	TONGUE	B-POLYP	B
70	70	412	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
72	72	434	U	BRAIN	M-OLIGODENDROCYTIC GLIOMA	M
79	79	437	U	UTERUS	M-STROMAL SARCOMA	M
79	78	441	U	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
87	86	447	U	UTERUS	B-HAEMANGIOMA	B
89	89	409	U	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
90	90	436	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
94	94	413	U	LIVER	B-HAEMANGIOMA	B
96	88	433	U	MAMMARY GLAND	M-CARCINOMA	M
97	89	420	U	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
100	100	443	U	UTERUS	B-HISTIOCYTOMA	B
102	102	423	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
105	105	417	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
105	105	417	T	UTERUS	B-POLYP	B
105	105	421	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
105	105	429	T	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
105	105	432	T	OVARY	B-BENIGN LUTEOMA	B
105	105	432	T	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
105	105	435	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
105	105	435	U	UTERUS	B-LEIOMYOMA	B
105	105	439	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
105	105	444	T	UTERUS	B-LEIOMYOMA	B
105	105	449	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
105	105	457	T	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
105	105	457	T	LUNG	B-BRONCHIOLO-ALVEOLAR	B

T = Terminal necropsy, U = Unscheduled death, B = Benign, M = Malignant

Chronological listing of tumors in second control female mice (group 5)

GROUP 5 FEMALES

Week of necropsy	Week tumour discovered	Animal number	Mode of death	Tissue/organ	Tumour type	Malignancy
41	39	464	U	SKIN + SUBCUTIS	M-OSTEOSARCOMA	M
65	65	463	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
65	65	490	U	UTERUS	B-POLYP	B
67	67	503	U	HAEM/LYMPH/RETIC	M-LYMPHOMA	M
68	67	461	U	UTERUS	M-HISTIOCYTIC SARCOMA	M
76	76	492	U	HAEM/LYMPH/RETIC	M-LYMPHOMA	M
77	77	499	U	HAEM/LYMPH/RETIC	M-LYMPHOMA	M
79	79	478	U	ADRENAL	M-MALIGNANT PHAEO.	M
79	78	478	U	UTERUS	B-HAEMANGIOMA	B
81	74	494	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
81	80	510	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
86	82	507	U	MAMMARY GLAND	M-CARCINOMA	M
88	88	491	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
88	88	491	U	UTERUS	B-POLYP	B
88	87	491	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
94	88	482	U	LIVER	B-HAEMANGIOMA	B
94	88	482	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
94	88	482	U	SPLEEN	M-HAEMANGIOSARCOMA	M
95	95	474	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
95	95	474	U	SKIN + SUBCUTIS	M-HAEMANGIOSARCOMA	M
95	95	474	U	SPLEEN	M-HAEMANGIOSARCOMA	M
97	97	460	U	OVARY	B-HAEMANGIOMA	B
97	94	460	U	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
97	94	460	U	UTERUS	M-HISTIOCYTIC SARCOMA	M
99	99	486	U	UTERUS	B-HAEMANGIOMA	B
100	98	489	U	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
100	100	489	U	UTERUS	B-LEIOMYOMA	B
100	100	500	U	BONE	B-HAEMANGIOMA	B
101	101	466	U	PANCREAS	B-ISLET CELL ADENOMA	B
101	101	470	U	UTERUS	B-LEIOMYOMA	B
101	101	470	U	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
101	101	470	U	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
101	101	483	U	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M
102	100	481	U	UTERUS	M-CARCINOMA	M
102	102	481	U	OVARY	B-BENIGN GRANULOSA TUMOUR	B
103	103	498	U	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
104	103	465	U	UTERUS	M-HISTIOCYTIC SARCOMA	M
105	105	462	T	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
105	105	467	T	LIVER	B-HEPATOCELLULAR ADENOMA	B
105	105	468	T	ADRENAL	B-SUBCAPSULAR CELL TUMOUR	B
105	105	473	T	UTERUS	M-HISTIOCYTIC SARCOMA	M
105	103	475	T	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
105	100	484	T	UTERUS	B-POLYP	B
105	105	484	T	HARDERIAN GLAND	B-ADENOMA	B
105	105	496	T	UTERUS	M-HISTIOCYTIC SARCOMA	M
105	105	497	T	HAEM/LYMPH/RETIC	M-LYMPHOCYTIC LYMPHOMA	M
105	105	497	T	LIVER	M-HEPATOCELLULAR CARCINOMA	M
105	105	502	T	LUNG	M-BRONCHIOLO-ALVEOLAR CARC.	M
105	105	502	T	SKIN + SUBCUTIS	M-INJECTION SITE SARCOMA	M
105	105	504	T	LUNG	B-BRONCHIOLO-ALVEOLAR ADENO.	B
105	105	508	T	HAEM/LYMPH/RETIC	M-MIXED LYMPHOMA	M

T = Terminal necropsy, U = Unscheduled death, B = Benign, M = Malignant

Summary:

104-Week Subcutaneous Oncology Study in the Mouse (Report # REST98108): ⁻.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).

In addition to injection site lesions (masses), necropsy findings noted included pancreatic masses in 1LD and 2 HD males and 1LD, 1MD, 2HD female 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/ foliculitis, 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.

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.

Conclusion: The highest dose used in mice was greater than 159 times maximal 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.

Adequacy of the carcinogenicity study and appropriateness of the test model:

Mouse appeared to be an appropriate model since mouse does respond to pharmacological effects of pramlintide and sufficient number of animals were used in the study.

Evaluation of tumor findings:

Repeated administration of pramlintide increased the incidence of tumors at the injection sites, particularly in control and high dose males but the incidence was relatively small in low and intermediate dose males and in all female groups. Most of 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. Incidence of other tumors was similar in treated and control groups. Since these tumor findings were found in both control and high dose animals, the high incidence of injection site tumors were attributed to dosage volume and not to pramlintide itself.

Other notable tumor finding was significantly lower incidence of liver tumor in high dose males ($p=0.024$) compared to the controls. This was not considered of biological significance.

Study title: AC-0137: 104 Week Subcutaneous Administration Carcinogenicity Study in the Rat

Key study findings: Female and male rats were terminated during WK 101/102 and 105/106, respectively. There a significant increase in incidence of skin tumors at injection sites in both control and high dose rats. Other finding was a dose-dependent increase in the incidence of pituitary tumors in male rats. However, the incidence was only significant in high dose males.

Study number: — #1186/4 (Report # REST98109)
Volume #, and page #: 57-64, 1-2316
Conducting laboratory and location: [redacted]
Date of study initiation: Nov 22, 1994
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: AC0137 (1 mg/mL): 94-0604CB, 95-0303GB, 94-0806CB, 94-1202GB, 94-1101GB, Drug products were manufactured by [redacted] Purity ranges between [redacted]
CAC concurrence: No
Study Type: 2-year bioassay in rats
Species/strain: — CD(SD)BR rats obtained from [redacted] at 28 days of age
Number/sex/group: 50/group/sex
Age at start of study: Animals were less than 8 WKs old at the start of dosing
Animal housing: 5 rats/cage, Males: 195-283g, Females: 148-254 g
Formulation/vehicle: Not specified (assumed to be the vehicle used in final product)
Drug stability/homogeneity: Product samples were collected and tested every three month. Product was stable.
Methods: Doses: 0, 0.04, 02, 0.5 and 0 mg/kg/day, with dose volumes of 2.5, 0.4, 1.0, 2.5 and 2.5ml/kg, respectively.

Species	Doses, mg/kg/day	$AUC_{0\text{-}t}$, ng.min/ml	Ratio of animal to maximal human dose AUC in type I and II diabetes	
			Type I Diabetes	Type II Diabetes
104 WK Rat Bioassay	0.04	267	3	4
	0.2	923	9	15
	0.5	2065	20	34
Human dose Type I, 360 µg/day (90 mg QID)		104		
Human dose Type II, 360 µg/day (120 mg TID)		60		

t= 120 to 300 min

Basis of dose selection: AUC multiple of maximal human dose (≥25 t[redacted]
Route of administration: subcutaneous
Frequency of drug administration: once daily into alternating dorsal skin with 25G needle (left shoulder, right shoulder, right flank, and left flank)
Dual controls employed: Both controls received formulation vehicle (placebo) at 2.5 ml/kg/day SC
Interim sacrifices: No
Satellite PK or special study group(s): NO
Deviations from original study protocol: Some of the terminal necropsies in females were carried out in WK 101 because of higher mortality rate.

Statistical methods: Standard statistical methods were used to compare treated groups to combined control groups. For survival analysis, the male and female animals were analyzed separately. Survival probability functions were estimated by the Kaplan-Meier technique. Survival curves were compared by the log-rank procedure. One directional tests for an increasing and a decreasing dose-

response in mortality across all groups and one-directional pairwise tests of the combined control groups against groups 2, 3 and 4 were performed in accordance with the IARC annex. Where the test for a dose response was not significant, a Bonferroni adjustment was used for the pairwise tests against combined controls. The survival curves were compared to the start of the terminal kill phases (week 104 males, week 101 females). Tissues from all animals were examined in control groups 1 and 5 and in the high dose group (group 4). In addition, the injection sites were examined in all animals. The number of tumor bearing animals were analyzed separately for males and females, for tumor types found in at least 3 animals of the given sex within groups 1, 4 and 5. For all other tumor types, one directional pairwise tests for both an increasing and a decreasing dose response were performed between group 4 and the combined controls, in accordance with the IARC annex. Non-fatal tumors were analyzed broadly in accordance with the IARC annex; fixed intervals of 1 to 50 weeks, 51 to 80 weeks, 81 to 104 weeks (males) or 81 to 101 weeks (females) and the terminal kill phase were used in place of the "ad-hoc" runs methods. Permutation tests were used to establish the significance of findings wherever fatal or non-fatal tumors were observed with a total incidence of at least 3 but less than 10, or wherever the total combined incidence of both fatal and non-fatal tumors was at least 3 but less than 10. The exact (permutational) sampling distributions of the test statistics were then obtained. The fatal and non-fatal results were combined in accordance with the IARC annex. Only if this combined test gave a significant result ($P<0.05$) are the separate fatal and non-fatal results also reported.

Observations and times:

Clinical signs: Observe daily with full examination once/week. Animals that showed sign of ill health were isolated, killed and necropsied to prevent cannibalism and autolysis.

Body weights: Weekly until WK16, once every 4WKs there after until necropsy.

Food consumption: Weekly until WK16, and one WK every 4WKs.

Hematology: 0.5 ml blood from all animals at terminal necropsy after an overnight fasting except for females (males WK104, females WK101/102). Due to operational constraint, females were not fasted before blood collection. RBC and differential WBC counts were determined from EDTA blood samples.

Clinical chemistry: Not done

Organ weights: please see addendum on page 25 for list organs

Gross pathology: Performed on all animals. Please see addendum on page 25 for list of tissue.

Histopathology: Tissue samples from control and high dose animals were examined. Injection site histology was performed on low and mid dose animals as well.

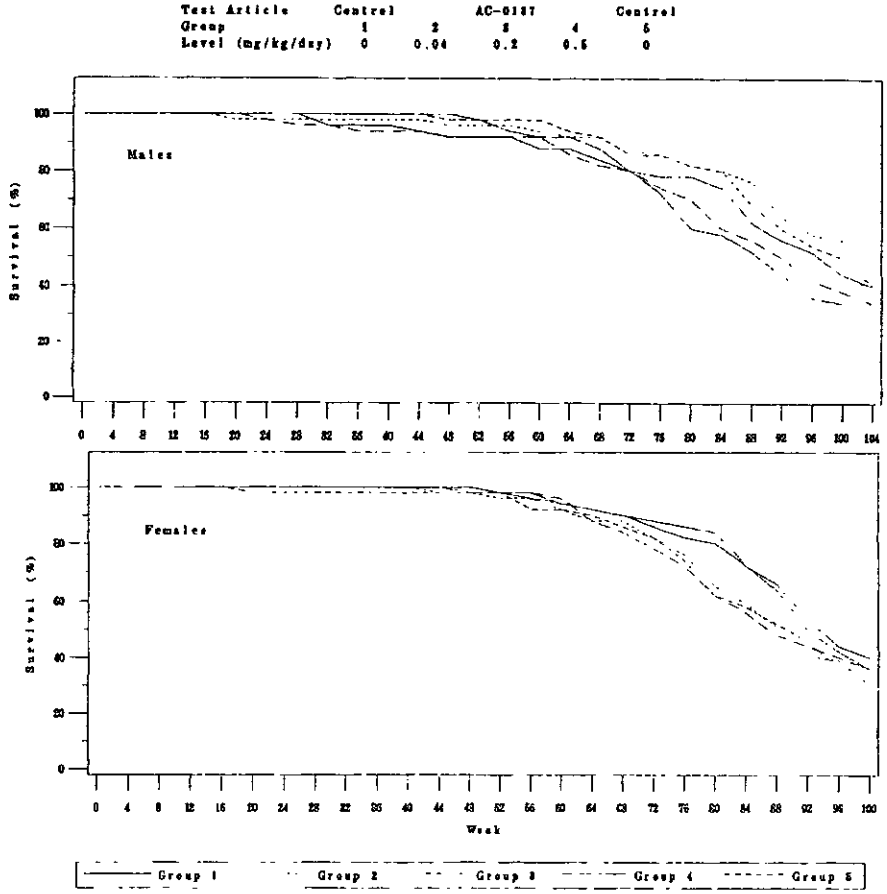
Toxicokinetics: Not done

Results:

Mortality:

- Due to high mortality rate in females, all surviving female rats were terminated during WK 101/102. Male animals were terminated during WK 105/106.
- When the control groups were combined, there were no significant difference in survival control and treated groups.
- There appeared to be higher proportion of decedents with fatal pituitary tumors in high dose males ($p<0.001$).
- In treated females, the overall incidence of tumor cause of demise was similar to controls.

Graphical presentation of survival rats in male and female rats treated with pramlintide



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 %	100 %
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 %					

Pramlintide - 104-Week Subcutaneous Administration Carcinogenicity Study in the Rat – Results: Causes of Death

AC-0137: 104-Week Subcutaneous Administration Carcinogenicity Study in the Rat [REST98109] Causes of Death										
	Pramlintide (mg/kg)									
	Male					Female				
	0.0 (1)	0.0 (2)	0.04	0.2	0.5	0.0 (1)	0.0 (2)	0.02	0.2	0.5
No. Examined	30	33	34	30	38	31	32	35	32	33
Pituitary tumors	9	8	10	12	22	14	12	19	15	21
Mammary tumors	0	0	0	0	0	17	20	10	12	10

Clinical signs:

- There were noted incidences of red extremities up to 4 hrs after dose in the treated group.
- 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.

INCIDENCE OF RED EXTREMITIES FROM POST DOSING OBSERVATIONS (% of SURVIVING ANIMALS)

Time	Vehicle		0.04 mg/kg		0.2 mg/kg		0.5 mg/kg		Vehicle	
	M	F	M	F	M	F	M	F	M	F
Wk 1	0	0	86	54	100	100	100	96	0	0
Wk 5	0	0	58	36	62	36	82	52	0	0
Wk 9	0	0	36	32	0	0	0	0	0	0
Wk 13	0	0	0	0	50	32	100	100	0	0
Wk 21	0	0	0	0	100	0	100	100	0	0
Wk 29	0	0	0	0	32	6	84	78	0	0
Wk 33	0	0	0	0	92	+	100	100	0	0

+ data excluded due to inadequate recording of time of onset

INCIDENCE OF SORES TO BACK (% OF SURVIVING ANIMALS)

Time	Vehicle		0.04 mg/kg		0.2 mg/kg		0.5 mg/kg		Vehicle	
	M	F	M	F	M	F	M	F	M	F
Wk 13	4	2	2	0	0	0	0	2	0	0
Wk 26	0	0	0	0	0	0	2	0	4	2
Wk 39	0	4	0	0	0	4	0	2	9	2
Wk 52	4	2	0	0	0	0	2	2	2	8
Wk 65	2	0	0	0	2	2	2	0	2	5
Wk 78	5	3	3	0	5	0	9	2	11	3
Last Wk	5	0	13	0	0	0	0	0	6	6

INCIDENCE OF SMALL MOVABLE SUBCUTANEOUS TISSUE MASSES [% = (NUMBER OF ANIMALS OBSERVED WITH MASS IN DESIGNATED WEEK ÷ NUMBER OF ANIMALS ENTERING DESIGNATED WEEK) X 100]

Time	Vehicle		0.04 mg/kg		0.2 mg/kg		0.5 mg/kg		Vehicle	
	M	F	M	F	M	F	M	F	M	F
Wk 13	0	0	0	0	0	0	0	0	0	0
Wk 26	0	0	0	0	0	0	0	2	0	0
Wk 39	2	4	0	4	2	2	0	2	2	2
Wk 52	0	6	0	6	2	10	2	4	7	12
Wk 65	2	20	2	11	6	33	2	15	5	25
Wk 78	0	27	3	19	14	25	11	29	8	26
Last Wk	0	29	5	31	0	28	0	22	0	67

INCIDENCE OF SMALL STATIONARY SUBCUTANEOUS TISSUE MASSES [% = (NUMBER OF ANIMALS OBSERVED WITH MASS IN DESIGNATED WEEK ÷ NUMBER OF ANIMALS ENTERING DESIGNATED WEEK) X 100]

Time	Vehicle		0.04 mg/kg		0.2 mg/kg		0.5 mg/kg		Vehicle	
	M	F	M	F	M	F	M	F	M	F
Wk 13	2	0	4	0	0	0	2	0	0	0
Wk 26	2	0	6	2	0	0	4	2	0	2
Wk 39	4	2	2	0	2	0	8	0	2	2
Wk 52	11	0	6	2	6	2	4	2	4	4
Wk 65	23	2	15	9	4	0	11	4	9	7
Wk 78	38	2	18	11	14	6	22	7	35	6
Last Wk	48	38	29	19	35	11	33	28	18	6

INCIDENCE OF LARGE MOVABLE SUBCUTANEOUS TISSUE MASSES [% = (NUMBER OF ANIMALS OBSERVED WITH MASS IN DESIGNATED WEEK ÷ NUMBER OF ANIMALS ENTERING DESIGNATED WEEK) X 100]

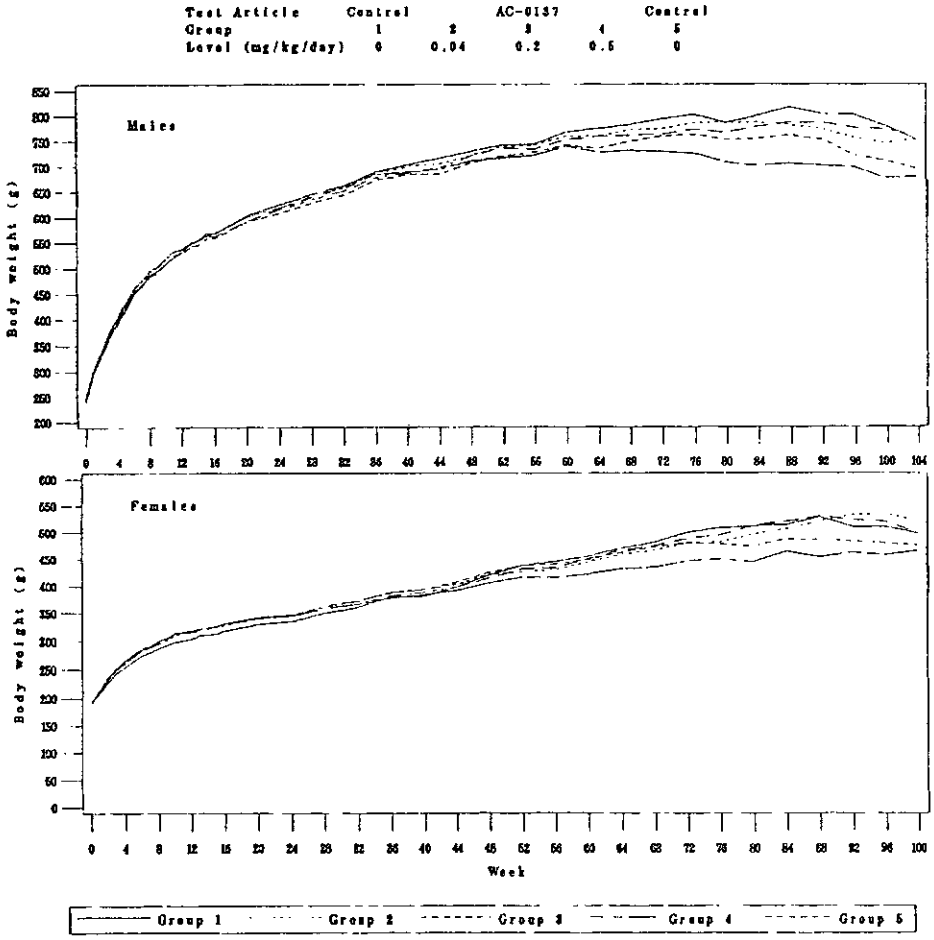
Time	Vehicle		0.04 mg/kg		0.2 mg/kg		0.5 mg/kg		Vehicle	
	M	F	M	F	M	F	M	F	M	F
Wk 13	0	0	0	0	0	0	0	0	0	0
Wk 26	0	0	0	0	0	0	0	0	0	0
Wk 39	0	6	0	2	0	0	0	0	0	0
Wk 52	0	4	0	8	0	0	0	2	0	6
Wk 65	0	15	0	5	0	4	2	4	0	11
Wk 78	0	24	3	16	0	19	0	19	0	29
Last Wk	5	14	14	13	9	28	0	22	0	33

INCIDENCE OF LARGE STATIONARY SUBCUTANEOUS TISSUE MASSES [% = (NUMBER OF ANIMALS OBSERVED WITH MASS IN DESIGNATED WEEK ÷ NUMBER OF ANIMALS ENTERING DESIGNATED WEEK) X 100]

Time	Vehicle		0.04 mg/kg		0.2 mg/kg		0.5 mg/kg		Vehicle	
	M	F	M	F	M	F	M	F	M	F
Wk 13	0	0	0	0	0	0	0	0	0	0
Wk 26	0	0	0	0	0	0	0	0	0	0
Wk 39	0	0	0	0	0	0	0	2	0	0
Wk 52	0	6	0	4	0	2	0	2	0	4
Wk 65	0	4	2	7	4	2	0	2	2	5
Wk 78	5	5	10	3	12	17	8	5	8	14
Last Wk	5	43	0	6	9	17	8	22	18	39

Body weights: There was no significant differences in group mean body weights for either sex through out the course of the study. Minor changes in body weight at particular intervals were not considered significant.

Group mean body weight in male and female rats.



Food consumption: No treatment related effect on food consumption.

Pramlintide - 104-Week Subcutaneous Administration Carcinogenicity Study in the Rat – Results: Body Weight Gains and Food Consumption

AC-0137: 104-Week Subcutaneous Administration Carcinogenicity Study in the Rat [REST96109]

Body Weight Gains and Food Consumption

	Pramlintide (mg/kg)									
	Male					Female				
	0.0 (1)	0.0 (2)	0.04	0.2	0.5	0.0 (1)	0.0 (2)	0.04	0.2	0.5
Body Weight Gain (g) (mean)										
Weeks 0-28	402.0	393.4	395.1	386.3	395.6	158.1	164.3	170.8	169.7	167.8
Weeks 28-52	102.1	103.6	101.7	92.3	78.0***	88.6	74.6	68.9	76.0	59.0**
Weeks 52-104[a]	33.0	47.6	5.6	5.0	17.7	80.2	80.0	100.4	87.6	71.4
Weeks 0-104[a]	510.7	524.5	503.8	457.4	446.3	290.9	281.6	313.4	266.3	254.7
Food Consumption (g) (mean)										
Weeks 0-28	189.5	188.5	195.6*	191.9	193.5	137.2	138.6	143.2	142.4	142.9
Weeks 28-52	192.3	189.5	193.9	187.1	185.7	146.0	145.5	147.7	147.9	142.8
Weeks 52-104[a]	189.9	189.4	188.8	180.4	179.8	156.4	156.2	156.8	153.9	144.7*
Weeks 0-104[a]	189.3	188.9	192.8	187.0	187.5	145.8	146.0	146.7	146.6	143.5

[a] All surviving female rats sacrificed at weeks 101-102; *p<0.05 (compared to combined control groups); **p<0.01 (compared to combined control groups), ***p<0.001 (compared to combined control groups)

Hematology: No treatment related effect

Gross pathology:

- Necropsy findings in the controls were consistent with the historical background for the strain of rats used.
- At the site of injection, the incidence of reddening was more common in treated group than controls.
- The number of subcutaneous masses were similar among groups and sexes (control vs. treated groups).
- The incidence of pituitary masses in the HD group (0.5 mg/kg/d) were higher than controls. See table for the incidence of pituitary masses in all groups examined.

	(Group) mg/kg AC-0137									
	Male					Female				
	(1)	(2)	(3)	(4)	(5)	(1)	(2)	(3)	(4)	(5)
	0	0.04	0.2	0.5	0	0	0.04	0.2	0.5	0
Pituitary n										
decedent	30	34	30	38	33	31	35	32	33	32
terminal	20	16	20	12	17	19	15	18	17	18
decedent + terminal	50	50	50	50	50	50	50	50	50	50
Pituitary Mass n										
decedent	9	14	15	22	8	22	23	19	25	19
terminal	2	1	1	1	1	6	6	5	4	9
decedent + terminal	11	15	16	23	9	28	29	24	29	28
Pituitary Mass %										
decedent	30.0	41.2	50.0	57.9	24.2	71.0	65.7	59.4	75.8	59.4
terminal	10.0	6.2	5.0	8.3	5.9	31.6	40.0	27.8	23.5	50.0
decedent + terminal	22.0	30.0	32.0	46.0	18.0	56.0	58.0	48.0	58.0	56.0

Histopathology:

Non-neoplastic findings:

- Incidences 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 was an irritant to subcutaneous tissue (see table below).
- Subcutaneous response in the control and highest dose were comparable.

Incidence of rats with fasciitis/fibrosis at injection site										
Sex	Male					Female				
Group	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 HD groups was significantly higher than controls ($p<0.001$).

Incidence of tumors in male and female rats

Sex	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 [1]	28/50	28/49	33/50	36/50	25/50	43/50	37/50	38/50	40/50	34/50

1 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 ($p<0.005$).

Incidence of rats with skin/subcutis and mammary tumors

Sex		Male			Female		
Group		1	4	5	1	4	5
Tissue							
Skin	Number examined	50	50	50	50	50	50
-epithelial - injection sites		2	3	3	0	0	1
-epithelial - other sites		4	3	6	0	0	0
-epithelial - injection sites and others		6	5	7	0	0	1
-non-epithelial - injection sites		17	16	14	1	1	5
-non-epithelial - other sites		10	10	7	4	3	2
-non-epithelial injection sites and others		23	21	19	5	4	6
Mammary	Number examined	4	4	3	50	50	50
-injection sites		0	0	0	10	10	17
-other sites		0	0	0	35	25	31
-injection sites and others		0	0	0	39	30	40

Incidence of rats with proliferative lesions of the pituitary gland

		Male					Female				
Group		1	2	3	4	5	1	2	3	4	5
Tissue											
Pituitary	Number examined	50	49	50	50	50	50	50	50	50	50
focal hyperplasia		14	13	9	4	12	4	6	5	8	5
adenoma		28	28	33	36	25	43	37	38	40	37
carcinoma		0	0	0	0	0	0	4	0	0	1
total focal hyperplasia/tumour		42	41	42	40	37	47	47	43	48	43

In order to ascertain whether or not this was a true treatment related effect, a complete, weight of evidence, review of all available data pertaining to the pituitary gland was undertaken.

Pituitary lesions in male rats treated with Symlin (mg/kg/day)						
Doses of Symlin, mg/kg/day		0, control1	0.04	0.2	0.5	0, control2
No of rats with pituitary adenoma (benign)	decedent	16	21	19	31	15
	Terminal	12	7	14	5	10
	All (total)	28	28	33	36	25
No. of rats with pituitary carcinoma (malignant)	decedent	0	0	0	0	0
	Terminal	0	0	0	0	0
	All (total)	0	0	0	0	0
No of rats with focal hyperplasia	decedent	7	5	4	1	7
	Terminal	7	8	5	3	5
	All (total)	14	13	9	4	12
Mean severity of focal pituitary hyperplasia (1=minimal, 2=slight)	decedent	1.86	1.6	2	1.	1.71
	Terminal	1.71	2	1.8	2	1.2
	All (total)	1.79	1.85	1.89	1.75	1.5
Pituitary tumors contributed to rats demise		9	10	12	22	8
No of rats with pituitary adenoma and hyperplasia		42	41	42	40	37

Historical control data pituitary lesions

Historical control data - pituitary lesions (November 1993 - November 1997)								
Male Rats	Study Number							
	6	9	61	62	64	65	107	Total
Number examined	60	120	130	120	60	96	120	704
Adenoma	23 (38)*	55 (46)	75 (58)	58 (48)	34 (57)	40 (42)	30 (25)	315 (45)
Carcinoma	1 (2)	-	-	-	-	1 (1)	-	2 (.28)
Focal Hyperplasia	18 (30)	31 (26)	28 (22)	34 (28)	16 (27)	30 (31)	44 (37)	201 (29)

* percent of those examined

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 increase of a few rats with pituitary adenoma over that observed in the 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.

Summary:

Sprague Dawley rats were treated daily with 0, 0.04, 0.2 and 0.5 mg/kg/day subcutaneously (3, 9 and 20 times human exposure based on AUC in type I diabetics and 4, 15 and 34 X human exposure in type II patients). 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.

Non-neoplastic findings:

- Incidences 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.

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 ($p<0.001$).

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 related to drug substance 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 —CD®(SD)BR rats is about 47% (range 1 to 70%) in males and 70% (range 26 to 92%) in females [ ] 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.

In conclusion, there were no notable effects of Symlin on body weight, food intake and survival rat in rats. Repeated subcutaneous administration of Symlin and vehicle caused a notable increase in injection site tissue masses with marked fasciitis, fibrosis and edema. This finding was noted in all injected animals, thus it was considered to be related to injection method or vehicle. Similar finding was noted in the 2-year mouse bioassay. The other major finding was significant increase in pituitary tumors in high dose male rats (20 and 34 times human dose based on AUC of type 1 and type 2 patients). There was no such finding in female rats. The biological significance of this finding is not clear since the incidence of pituitary tumor was reported in males only (not in females) and the overall incidence of this common tumor in rats were within historical parameters. 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.

Carcinogenicity Summary:

SD Rats were treated with SC injection of 0, 0.04, 0.2 and 0.5 mg/kg/day (3, 9 and 20 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 was 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.

Carcinogenicity conclusions:
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 those 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.

Recommendations for further analysis:
Labeling Recommendations:

Sponsor proposed:
Carcinogenesis:

[DRAFT]

FDA proposed:

[DRAFT]

Addendum/appendix listing:

Dose-ranging study report:
CAC report:
Alternative study protocols and CAC report:

Sponsor's incidence of histopathology findings:
List of organs and tissues examined:
Body weight changes versus dose level:
Group body weight summary:
Individual data listing:

Appears This Way
On Original

REPRODUCTIVE TOXICOLOGY:

Study title: Study of fertility and general reproductive performance in rats (segment 1)

Key study findings: 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 malformations in neonates were noted at any dose. The 3 mg/kg/day dose of 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.

Study no.: REST98130R1
Volume #, and page #: 1.65, 1-268
Conducting laboratory and location: [redacted]

Date of study initiation: Aug 3 1992
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # B106C01FMW non-radiolabeled and — purity
Formulation/vehicle: AC-0137 was in white powder form. It was reconstituted with water. Placebo was also a white powder provided by the sponsor and reconstituted with water.

Methods:

Species/strain: SD rats [redacted]
Doses employed: 0, 0.3, 1, 3 mg/kg/day
Route of administration: SC
Study design: Fifty two male and 104 female SD rats were given placebo, or 0.3, 1, and 3 mg/kg/day of AC-0137. 13 males/dose were treated for al least 80 day prior to mating until mating was completed. The 1 treated male rat was mated with 2 female rats. 26 females were treated 15 days prior to mating and some continued until Day 21 of lactation. Half of the females (13/dose) were terminated on Day 13 of gestation.

Number/sex/group: 13 male and 26 female rats/dose
Parameters and endpoints evaluated: drug effects on gonadal function and weight, estrous cycle, mating behavior, conception rate, the early and late stages of gestation, parturition and lactation.

Results:

Mortality: None of the males died during the study. Eight females rats in HD group died during gestation (3 on day 21, 5 were moribund and sacrificed on Day 20, 21 and 22). They were all gravid and fetuses appeared normal except for Dam#8290 had 3/19 fetuses with neurotube defects. The deaths appeared to have resulted from dams' inability to liter.

Clinical signs:

- Some of the control and treated males exhibited edema at scapula region, alopecia and occasionally vasodilation of pinna, forepaws.
- No clinical signs were noted in control females before mating. Vasodilation of the pinna and forepaws were observed after dose in most treated females.
- During gestation, similar clinical signs were noted in dams. Mid dose dams exhibited nasal discharges and lacrimation. HD dams exhibited decreased motor activity, tremor, and red amniotic fluid.

Body weight:

Males: The body weights of HD males were 10 to 12 % lower ($p<0.05$) than controls from Day 43 through 96.

Summary of Male Group Mean Body Weights (grams)
Prior to and During Mating

Dose (mg/kg/day)		Day of Study 1	8	15	22	29	36	43	50	57	64	71	78	85	92	96
0	$\bar{x}$	273.4	326.7	366.3	406.9	432.8	461.5	483.7	497.6	510.8	530.7	547.5	560.5	550.1	566.1	568.7
	S.D.	15.41	19.64	23.52	26.57	30.98	36.35	41.80	49.80	47.29	54.28	58.70	62.90	66.18	66.25	68.72
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
0.3	$\bar{x}$	275.6	337.6	380.9	426.6	458.3	491.1	519.6	542.5*	550.4	564.6	584.6	595.7	585.0	599.5	600.5
	S.D.	14.46	21.73	26.96	32.21	36.72	41.45	46.69	50.10	55.66	55.06	60.15	64.44	67.75	69.08	70.68
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
1.0	$\bar{x}$	274.5	331.2	367.8	406.4	433.4	457.0	474.5	493.8	502.7	516.0	532.9	545.4	539.5	554.4	557.2
	S.D.	10.32	13.44	16.92	21.62	24.58	25.78	30.75	37.41	32.06	36.06	38.80	43.24	44.10	49.84	51.54
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
3.0	$\bar{x}$	271.1	315.2	346.4	375.5**	396.6**	416.2**	433.1**	444.8**	456.2**	470.8**	483.2**	491.3**	486.3**	500.0*	503.8**
	S.D.	13.69	15.94	18.82	21.95	23.91	27.18	32.75	34.43	39.16	38.56	40.58	43.76	40.27	40.29	40.43
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13

* = $p \leq 0.05$
** = $p<0.01$

Females:

- No significant change before mating.
- Significant decrease in body weight of HD dams on gestation Days 0, 7, 13 and 20 relative to control dams. The significant decrease in body weight gain (19%) in HD dams was attributed to the AC-0137 treatment. The most marked effects on body weight were observed on gestation days 0-7(n=24) and days 13-20 (n=12).

Group Mean Dam Body Weight Change (grams)
During Gestation

Dose (mg/kg/day)		Gestational Interval (Days) 0 - 7	7 - 13	13 - 20	0 - 20
0	$\bar{x}$	43.9	32.2	84.3	158.2
	S.D.	9.29	6.59	18.54	18.23
	N	25[a]	25	13	13
0.3	$\bar{x}$	43.0	33.1	78.6	152.8
	S.D.	12.09	11.12	14.72	21.40
	N	24[b]	24	12	12
1.0	$\bar{x}$	41.0	33.2	77.5	150.6
	S.D.	6.41	6.97	18.11	21.99
	N	24[c]	24	11	11
3.0	$\bar{x}$	32.2*	30.5	69.1	127.8*
	S.D.	9.20	5.25	19.05	17.83
	N	24[d]	24	12	12

[a] = 13/13 rats designated for delivery were gravid, 12/13 rats designated for Day 13 sacrifice were gravid.
[b] = 13/13 rats designated for delivery were gravid, 12/13 rats designated for Day 13 sacrifice were gravid. Dam #8240 delivered on Day 8, gestation data not included in statistical analysis.
[c] = 11/13 rats designated for delivery were gravid, 13/13 rats designated for Day 13 sacrifice were gravid.
[d] = 12/13 rats designated for delivery were gravid, 12/13 rats designated for Day 13 sacrifice were gravid.
* = $p \leq 0.05$

Neonates:

- Statistically significant decrease in body weight in 3 mg/kg/day dose group on postpartum day 0, 4, 7, 14 and 21 was noted (see table below for details).
- A dose dependent decrease in body weight on Day 4 was observed.

Number, Viability, Sex and Body Weights (grams) of Neonates

Dose (mg/kg/day)	0	0.3	1.0	3.0
Total neonates delivered	194	195	167	51
Total viable	188	191	165	51
Total stillborn	6	2	1	0
Total dead (Day 0/born alive)	0	2	1	0
Viable neonate at delivery (%)	96.9	97.8	98.8	100.0
Number viable on Day 4[a]	187	191	161	48
Number viable on Day 4[b]	103	102	85	29
Number viable on Day 7	102	102	84	29
Number viable on Day 14	102	102	83	29
Number viable on Day 21	102	102	83	29
Total viable males delivered	83	97	88	20
Total viable females delivered	105	94	77	31
Body weight, Day 0				
$\bar{x}$	6.6	6.6	6.5	6.2*
S.D.	0.71	0.71	0.81	0.53
Body weight, Day 4[a]				
$\bar{x}$	10.6	10.5	10.3	9.2*
S.D.	1.57	1.39	1.83	1.42
Body weight, Day 7				
$\bar{x}$	17.4	17.5	17.3	14.5*
S.D.	2.79	2.10	2.90	2.16
Body weight, Day 14				
$\bar{x}$	35.3	34.8	34.5	28.8*
S.D.	5.31	3.06	4.73	5.40
Body weight, Day 21				
$\bar{x}$	56.9	55.9	55.7	48.7*
S.D.	7.63	4.90	6.76	21.08
Neonate survival (%) Day 0-4, prior to standardization	99.5	100.0	97.6	94.1
Neonate survival (%) Day 4-7, after standardization	99.0	100.0	98.8	100.0
Neonate survival (%) Day 7-14, after standardization	100.0	100.0	98.8	100.0
Neonate survival (%) Day 4-21, after standardization	99.0	100.0	97.6	100.0
Neonate survival (%) Day 14-21, after standardization	100.0	100.0	100.0	100.0

a = Number of neonates prior to standardization.
b = Number of neonates after standardization.
* = p≤0.05 End of Table 26

Food consumption: Not reported
Toxicokinetics: No TK data
Other: Serum calcium values were decreased in a dose dependent manner in dams. The serum calcium levels in HD dams were 46% less than controls which most probably contributed to the difficulty with delivery and consequent deaths in 8/12 HD dams.

For fertility studies:

In-life observations:

- In male rats, only the weights of the reproductive organs were weighed. **No other fertility indices in males were evaluated.**
- Total of 106 females were mated. The total number of gravid rats by dose group from control to high dose group was 25/26 (96%), 25/26 (96%), 24/26 (92%) and 24/26 (92%). Of the rats designed for the gestation Day 13 sacrifice, 12/13 (92%) in control, 0.3 and 3 mg/kg/d were gravid. In the 1 mg/kg/day group all 13/13 (100%) animals were gravid. Rats that were

allowed to deliver 13/13 (100%) in control and 0.3 mg/kg/d were gravid. 11/13 (85%) in the 1 mg/kg/day and 12/13 (92%) in the 3 mg/kg/d were gravid.

Terminal and necroscopic evaluations:

- Male rats: The combined male reproductive organ weights (testes, epididymides, seminal vesicles) in HD groups was significantly less (18%) than controls. This decrease correlated with decrease in body weight, thus the relative reproductive organ weight was unchanged.
- The numbers of gravid females were similar among groups.

Pramlintide - Study of Fertility and General Reproductive Performance in Rats, Segment I – Results: Observations at the Day 13 Sacrifice

Study of Fertility and General Reproductive Performance in Rats, Segment I [REST98130R1]				
Summary of Observations at the Day 13 Sacrifice				
	Pramlintide (mg/kg/day)			
	0.0	0.3	1.0	3.0
Rats examined at:				
Day 13 sacrifice	13	13	13	13
Non-gravid	1	1	0	1
Gravid	12	12	13	12
Abortions	0	0	0	0
Resorptions	0	0	0	0
Dams with viable embryos	12	12	13	12
Viable embryos/dam	16.8	17.2	15.2	14.6*
Total implantations/dam	17.8	18.4	16.5	15.3*
Post-implantation loss/dam	1.0	1.3	1.4	0.7
Corpora Lutea/dam	18.8	19.2	18.0	16.6
Pre-implantation loss/dam (%)[a]	5.1	3.1	8.2	8.3
Post-implantation loss/dam (%)[b]	5.6	6.5	8.1	4.0

[a] % Pre-implantation loss by Dam = $\frac{\text{(Corpora Lutea – Implantations)}}{\text{Corpora Lutea}} \times 100$

[b] % Post-implantation loss by Dam = $\frac{\text{(Implantations – Viable Embryos)}}{\text{Implantations}} \times 100$

*p≤0.05

Pramlintide - Study of Fertility and General Reproductive Performance in Rats, Segment I – Results: Data for Dams Designated for Delivery

Study of Fertility and General Reproductive Performance in Rats, Segment I [REST98130R1]				
Summary of Data for Dams Designated for Delivery				
	Pramlintide (mg/kg/day)			
	0.0	0.3	1.0	3.0
Rats designated for delivery	13	13	13	13
Gravid	13	13	11	12
Non-gravid	0	0	2	1
Rats that died	0	0	0	8
Gravid	0	0	0	8
Non-gravid	0	0	0	0
Dams delivering	13	13	11	4
Dams gravid/did not deliver	0	0	0	8
Dams with stillborn	6	2	1	0
Dams with agalactia/dystocia	0	0	0	0
Length of gestation (days)	22.0	22.0[a]	22.1	21.8
Number of litters delivered	13	13	11	4
Number of litters weaned	13	13	11	4
Neonates per litter at delivery	14.9	15.0	15.2	12.8
Neonates per litter viable at delivery	14.5	14.7	15.0	12.8
Neonates per litter prior to standardization (day 4)	14.4	14.7	14.6	12.0

[a] Dam #8240 delivered on day 8 of gestation, not included in length of gestational data

For embryofetal development studies:

Terminal and necroscopic evaluations:

Dams sacrificed on gestation Day 13:

- There were no statistically significant differences in the number of early resorptions, or the number and percentage of pre/post implantation losses.
- The number of viable embryos per dam and total implantations per dams in the 3 mg/kg/d dose group were decreased (16.8 vs. 14.6) relative to controls (see table below). This effect appeared to be due to a decreased number of corpora lutea in HD females.
- There were no significant differences in the pre-implantation loss per dam.

Dams allowed to deliver:

- There was a biologically significant decrease in the number of delivered neonates perhaps due to significant decrease in serum calcium and consequent dystocia.
- None of the dams died during lactation period.
- There were no significant differences in the group mean body weight changes during lactation.
- None of the dams aborted or delivered early. There were no agalatia.
- There were no differences in the length of gestation among groups.

Summary of Group Mean Dam and Embryonic Observations at the Day 13 Sacrifice

Dose (mg/kg/day)	0		0.3		1.0		3.0	
	No. (SD)	%	No. (SD)	%	No. (SD)	%	No. (SD)	%
Rats designated for Day 13 sacrifice:	13		13		13		13	
Rats that were gravid:	12	92.3	12	92.3	13	100.0	12	92.3
Rats that died:	0	0.0	0	0.0	0	0.0	0	0.0
Non-gravid:	0	0.0	0	0.0	0	0.0	0	0.0
Gravid:	0	0.0	0	0.0	0	0.0	0	0.0
Rats that aborted/ delivered early:	0	0.0	0	0.0	0	0.0	0	0.0
Rats examined at Day 13 sacrifice:	13	100.0	13	100.0	13	100.0	13	100.0
Non-gravid:	1	7.7	1	7.7	0	0.0	1	7.7
Gravid:	12	92.3	12	92.3	13	100.0	12	92.3
Dams with resorptions only:	0	0.0	0	0.0	0	0.0	0	0.0
Dams with viable embryos:	12	100.0	12	100.0	13	100.0	12	100.0
Viable embryos/dam:	16.8(1.96)	-	17.2(2.08)	-	15.2(2.82)	-	14.6*(2.47)	-
Total implantations/ dam:	17.8(1.91)	-	18.4(2.54)	-	16.5(2.63)	-	15.3*(2.73)	-
Post-implantation loss/dam:	1.0(0.74)	-	1.3(1.06)	-	1.4(1.76)	-	0.7(0.65)	-
Corpora lutea/dam:	18.8(2.01)	-	19.2(3.33)	-	18.0(1.78)	-	16.6(2.71)	-
Pre-implantation Loss (%)[a]	5.1(6.48)	-	3.1(7.77)	-	8.2(11.34)	-	8.3(9.38)	-
Post-implantation loss/dam[b]	5.6(4.47)	-	6.5(4.90)	-	8.1(10.72)	-	4.0(3.91)	-

[a] = Group Mean % Pre-implantation by Dam = $\frac{(\text{Corpora lutea} - \text{Implantations}) \times 100}{\text{Corpora Lutea}}$

[b] = Group Mean % Post-implantation loss by Dam = $\frac{(\text{Implantations} - \text{Viable Embryos}) \times 100}{\text{Implantations}}$

- = Not applicable

* = $p \leq 0.05$

Offspring:

- The number of litters delivered and weaned were 13/13, 13/13, 11/11 and 4/4 for control, 0.3, 1 and 3 mg/kg/day, respectively. **A significant decrease in the number of litters delivered in the 3 mg/kg/day was noted perhaps due to significant decrease in serum calcium levels and consequent dystocia.**
- The total number of stillborns were not significantly different [6(46.2%), 2 (15.4%), 1 (7.7%) and 0 (0%) in control, LD, MD and HD groups, respectively].

- There was no statistically difference in sex ratio in any dose group.
- There was a biologically significant decrease in the number of neonates in the HD groups (n=12) relative to controls (n=14.8).
- There were 6, 3, 3 and 1 neonate skeletons evaluated in the 0, 0.3, 1 and 3 mg/kg/day. No skeletal malformation was detected. Since only 1 neonate in the HD groups was examined, the overall value of the result does not negate the possibility of greater malformation if higher number of neonates in the HD group were examined.

Results of Neonate Skeletal Examination

Dam #	Dose (mg/kg/day)	No. of Neonates Examined	Observations (Incidence)
8202	0	1	Incomplete ossification of the 5th sternebra
8206	0	1	Normal
8208	0	1	Incomplete ossification of the 5th sternebra
8214	0	1	Incomplete ossification of the 5th sternebra
8218	0	1	Incomplete ossification of of the 5th sternebra
8226	0	1	Incomplete ossification of the 5th sternebra
8236	0.3	1	Incomplete ossification of the 5th sternebra
8244	0.3	1	Incomplete ossification of the 5th sternebra
8248	0.3	1	Normal
8260	1.0	1	Normal
8272	1.0	1	Normal
8276	1.0	1	Incomplete ossification of the 5th sternebra
8300	3.0	1	Normal

Summary:
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 was noted (12%). Eight of the 12 females in 3 mg/kg/d died at the time of parturition. 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. 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. The number of viable embryos per dam and total implantations per dams in the 3 mg/kg/d dose group were decreased (16.8 vs. 14.6) relative to controls. This effect appeared to be due to a decreased number of corpora lutea in HD females. Although the total numbers of gravid rats were similar and high in all groups (92 to 96%), the number of neonates were lower in the high dose group (n=4 vs. n=11-13 litters in control) due to maternal deaths or moribund sacrifice in 8/12 dams at the time of parturition. HD dams had 46% decrease in serum calcium levels resulting in an inability to deliver (dystocia). In addition pup weights of litters from HD dams that did deliver were lower at birth and remained decreased over the entire 21-day lactation period. No skeletal malformations 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, maternal death at parturition, decreased number of viable embryos and delivered neonates.

Study title: Teratology Study in Rats (Segment II) with AC-0137.

Key study findings: There appeared to be a biological increase in the incidence of malformation (primarily neural tube defect: cleft palate, protruding tongue, exencephale and hydrocephalus) 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 values, the lowest dose, 0.3 mg/kg/day was 10X human exposure.

Study no.: REST98111
Volume #, and page #: 1.66, 1-86
Conducting laboratory and location: [ ]
Date of study initiation: Nov 10, 1992
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # B106C01FMW non-radiolabeled and —, purity
Formulation/vehicle: AC-0137 was in white powder form. It was reconstituted with water. Placebo was also a white powder provided by the sponsor and reconstituted with water.
Methods:
Species/strain: SD rats [ ]
Doses employed: 0, 0.3, 1, 3 mg/kg/day
Route of administration: SC

Study design: 109 female SD rats were given placebo, or 0.3, 1, and 3 mg/kg/day of AC-0137 subcutaneously from Day 6 implantation through gestation Day 15 to evaluate the incidence of congenital malformations in offspring of rats. Injection volume was 0.3 ml/kg. There were 27 animals/group except for the high dose group (3 mg/kg) which had 28 animals to ensure at least 20 litters.

Number/sex/group: 27-28 female rats/dose

Parameters and endpoints evaluated: uterine weight, number of viable and non-viable fetuses, total number of corpora lutea, fetal external, soft tissue, and skeletal abnormalities.

For embryofetal development studies:

In-life observations:

- There were no clinical signs in controls. All treated animals exhibited post dose signs of pinna, forepaw, hind paw and tail vasodilation. Some of the animals in MD and HD groups had signs of lacrimation and soft stool.
- None of the animals died during the study.
- None of the dams aborted.
- Statistically significant decrease in body weight change (22%) in HD groups on Days 0-20 was noted.
- A significant decline in food consumption (by as much as 21%) of HD dams between day 0 and 15 was noted.

Group Mean Dam Body Weight (grams) and Overall Body Weight Change (grams) During Gestation

Dose (mg/kg/day)		Day of Gestation 0	6	9	12	15	20	Corrected[a] Body Weight	Corrected Body Weight Change (0-20)
0.0	$\bar{x}$	312.5	342.8	352.7	370.7	389.0	472.2	377.2	64.4
	SD	13.09	15.37	17.59	19.44	19.44	25.04	21.22	13.38
	N	26	26	26	26	26	25[b]	25	25
0.3	$\bar{x}$	315.1	347.4	363.0*	382.5*	400.9*	483.4	387.6*	72.5*
	SD	13.28	13.23	15.75	16.78	18.14	23.76	18.08	13.87
	N	25	25	25	25	25	25	25	25
1.0	$\bar{x}$	313.5	343.8	352.3	371.3	390.3	469.0	377.6	64.1
	SD	15.12	15.58	16.80	19.50	21.85	30.63	24.52	21.83
	N	26	26	26	26	26	26	26	26
3.0	$\bar{x}$	317.2	346.1	347.2	365.2	380.6	463.7	367.1	50.0*
	SD	14.61	17.81	18.95	20.27	19.56	24.03	20.80	13.55
	N	27	27	27	27	27	27	27	27

[a] = Body weight after removal of gravid uterus and contents.
[b] = Dam #8518 was prematurely cesarean sectioned on Day 19 of gestation.
* = $p \leq 0.05$

Terminal and necroscopic evaluations:

Dams:

- 25/27 (96.2), 25/27 (92.6%), 26/27 (96.3%) and 27/28 (96.4) in control, 0.3, 1.0 and 3 mg/kg/day were gravid, respectively.
- There were no statistical or biologically significant difference in the 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 compared to controls.

Summary of dam and fetal observation in rats treated with AC-0137:

Summary of Group Mean Dam Fetal Observations at Cesarean Section

Dose (mg/kg/day)	0 No. (S.D.)	0 %	0.3 No. (S.D.)	0.3 %	1.0 No. (S.D.)	1.0 %	3.0 No. (S.D.)	3.0 %
Rats designated for cesarean section:	27[a]	-	27	-	27	-	28	-
Rats that were gravid:	25	92.6	25	92.6	26	96.3	27	96.4
Rats that died:	0	0.0	0	0.0	0	0.0	0	0.0
Non-gravid:	0	0.0	0	0.0	0	0.0	0	0.0
Gravid:	0	0.0	0	0.0	0	0.0	0	0.0
Rats that aborted/ delivered early:	0	0.0	0	0.0	0	0.0	0	0.0
Rats examined at cesarean section:	26	96.3	27	100.0	27	100.0	28	100.0
Non-gravid:	1	3.8	2	7.4	1	3.7	1	3.6
Gravid:	25	96.2	25	92.6	26	96.3	27	96.4
Dams with resorption only:	0	0.0	0	0.0	0	0.0	0	0.0
Dams with viable fetuses:	25	100.0	25	100.0	26	100.0	27	100.0
Viable fetuses/dam:	15.4(2.38)	-	15.4(2.45)	-	14.8(3.88)	-	15.4(2.87)	-
Total implantations/ dam:	16.4(1.81)	-	17.0(2.11)	-	16.2(3.80)	-	16.7(2.23)	-
Post-implantation loss/dam:	1.1(1.04)	-	1.6(1.35)	-	1.3(1.49)	-	1.3(1.29)	-
Corpora lutea/dam:	19.3(2.80)	-	19.2(2.82)	-	19.2(3.52)	-	19.3(2.76)	-
Pre-implantation loss(%)[a]	13.5(13.08)	-	10.1(10.86)	-	15.9(16.47)	-	12.3(8.08)	-
Post-implantation loss(%)[b]	6.9(6.95)	-	9.7(8.02)	-	8.0(9.54)	-	13.8(9.07)	-
Fetal sex distribution								
Male -	202	52.5	187	48.6	190	49.2	220	52.8
Female -	183	47.5	198	51.4	196	50.8	197	47.2
Mean fetal body weight (grams):	4.0(0.29)	-	4.1(0.41)	-	4.1(0.36)	-	4.1(0.27)	-

[a] = Dam #8518 was prematurely cesarean sectioned on Day 19 of gestation, data not included in statistical analysis.

[b] = Group Mean % Pre-implantation by Dam = $\frac{\text{(Corpora Lutea - Implantations)}}{\text{Corpora Lutea}} \times 100$

[c] = Group Mean % Post-implantation loss by Dam = $\frac{\text{Implantations - Viable Fetuses)}}{\text{Implantations}} \times 100$

- = Not applicable

Offspring:

- Total malformations were 1, 10, 5 and 2 in control, 0.3, 1 and 3 mg/kg/d, respectively.
- The number of malformations (10) in LD were significantly higher than concurrent control. The incidence of malformation in MD (5) was higher than control (1) but the difference was not statistically different. Since the incidence of malformation was not dose-dependent, the significance of higher incidence in LD is not clear.
- Dam #8543 had 16 fetuses (10 normal) in the LD (1.0 mg/kg/d) groups had 6 pups with abnormal skull bone, 1 craniorachischisis, 5 exencephale, 5 protruding tongue, 4 cleft palate, 2 open eye, 1 anophthalmia. Dam #8545, in LD group had 2 pups with exencephale, 1 abnormal skull bone, 1 open eye and protruding tongue and 1 misshapen brain.
- Dam #8576 in MD (1.0 mg/kg/d) group had 1 pup with slightly hydrocephalus
- Dam # 8316 in HD (3 mg/kg/d) group had a pup with ectopic esophagus and right-sided stomach.
- Umbilical herniation were seen in control (1), LD (2), MD (3) and HD (1) groups.

Incidence of fetal malformation in rats treated with AC-0137

Summary of Incidence of Fetal Malformations

Dose (mg/kg/day)	0		0.3		1.0		3.0	
No. of litters examined:	25[a]		25		26		27	
No. of fetuses examined externally:	385[b]		385		386		417[b]	
No. of fetuses examined viscerally:	197		193		198		215	
No. of fetuses examined skeletally:	187		192		188		201	
	No.	%	No.	%	No.	%	No.	%
Fetuses with soft tissue malformations:	1	0.5	3	1.6	4	2.0	2	0.9
Fetuses with skeletal malformations:	0	0.0	7	3.6	1	0.5	0	0.0
Total fetuses with malformations:	1	0.3	10*	2.6	5	1.3	2	0.5
Litters with soft tissue malformations:	1	4.0	3	12.0	4	15.4	2	7.4
Litters with skeletal malformations:	0	0.0	2	8.0	1	3.8	0	0.0
Total litters with malformations:	1	4.0	4[c]	16.0	5	19.2	2	7.4

a = One dam was inadvertently cesarean sectioned on Day 19, data not included in statistical analysis.
b = One fetus, not skeletally evaluated lost during processing.
c = One litter exhibited skeletal and visceral malformations.
* = $p \leq 0.05$

The incidences of skeletal variations in fetuses from control and AC-0137 treated groups are shown in table blew:

Type and Incidence of Skeletal Variations

Dose (mg/kg/day)	0				0.3				1.0				3.0			
Variations Observed	Fetuses		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses		Litters	
	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Sternebrae, Incomplete Ossification																
1st (Manubrium)	2	1.1	2	8.0	1	0.5	1	4.0	3	1.6	3	11.5	1	0.5	1	3.7
2nd	21	11.2	11	44.0	26	13.5	15	60.0	14	7.4	9	34.6	20	10.0	13	48.1
3rd	2	1.1	2	8.0	3	1.6	3	12.0	2	1.1	2	7.7	0	0.0	0	0.0
4th	6	3.2	4	16.0	7	3.6	6	24.0	9	4.8	8	30.8	9	4.5	8	29.6
5th	115	61.5	25	100.0	127	66.1	25	100.0	101	53.7	24	92.3	143	71.1	26	96.3
6th (Xiphoid Process)	80	42.8	25	100.0	76	39.6	22	88.0	88	46.8	24	92.3	71	35.3	23	85.2
Sternebrae, Unossified																
1st (Manubrium)	0	0.0	0	0.0	0	0.0	0	0.0	1	0.5	1	3.8	1	0.5	1	3.7
2nd	4	2.1	3	12.0	1	0.5	1	4.0	4	2.1	3	11.5	2	1.0	2	7.4
3rd	1	0.5	1	4.0	0	0.0	0	0.0	1	0.5	1	3.8	1	0.5	1	3.7
4th	1	0.5	1	4.0	1	0.5	1	4.0	2	1.1	1	3.8	1	0.5	1	3.7
5th	65	34.8	20	80.0	61	31.8	22	88.0	83	44.1	22	84.6	56	27.9	21	77.8
6th (Xiphoid Process)	26	13.9	15	60.0	25	13.0	13	52.0	29	15.4	15	57.7	30	14.9	13	48.1
Sternebrae Bipartite																
6th	1	0.5	1	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Ribs 14th Ossification																
Center, Left	2	1.1	2	8.0	1	0.5	1	4.0	1	0.5	1	3.8	3	1.5	3	11.1
Pair	0	0.0	0	0.0	0	0.0	0	0.0	2	1.1	2	7.7	1	0.5	1	3.7
Skull Incomplete Ossification	6	3.2	5	20.0	5	2.6	3	12.0	5	2.7	4	15.4	8	4.0	5	18.5
Hyoid, Unossified	12	6.4	9	36.0	13	6.8	8	32.0	4	2.1	1	3.8	17	8.5	5	18.5
Hyoid, Incomplete Ossification	0	0.0	0	0.0	0	0.0	0	0.0	1	0.5	1	3.8	0	0.0	0	0.0
Fontanelles Enlarged	1	0.5	1	4.0	1	0.5	1	4.0	0	0.0	0	0.0	2	1.0	2	7.4
Thoracic, Incomplete Ossification	12	6.4	7	28.0	11	5.7	9	36.0	6	3.2	5	19.2	5	2.5	4	14.8
Thoracic Bipartite	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.5	1	3.7
Lumbar, Incomplete Ossification	1	0.5	1	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

* = $p \leq 0.05$

Summary:

Teratology Study in Rats, Segment II (REST98111) Performed by [ ]

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 cleft palate) were considered indicative of teratogenicity.**

Conclusion: There appeared to be a biological increase in the incidence of malformation (primarily neural tube defects: cleft palate, protruding tongue, exencephale and hydrocephalus) in the 0.3 and 1.0 mg/kg/day dose groups. The abnormalities seen in fetuses from 0.3 and 1.0 mg/kg/day dose groups were considered indicative of teratogenicity. These abnormal findings will be in the label. 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).

Study title: Teratology study in rabbits, segment II

Key study findings: The pregnancy rate was about 50%, thus the study was invalid. The study was repeated (see Study #REST98125, page 103)

Study no.: REST98110
Volume #, and page #: 1.66, 1-76
Conducting laboratory and location: [redacted]

Date of study initiation: March 18, 1993
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # B288C02FMW, not radiolabeled (—)
Formulation/vehicle: The white lyophilized powder (white solids) was reconstituted with water.

Methods:
Species/strain: New Zealand white rabbits
Doses employed: 0, 0.3, 1, 3 mg/kg/day
Route of administration: SC
Study design: Four groups of female rabbits were artificially inseminated. They received a dose volume of 0.3 ml/kg of vehicle or 3 dosage levels of the drug (0.3, 1, 3 mg/kg/d) by SC

route from Day 6 to Day 18 gestation. On day 29 necropsy was performed. Body weights were measured on days 0, 6, 9, 12, 15, 18, 22, 24 and 29.

Number/sex/group: 18 female rabbits/dose

Parameters and endpoints evaluated: Evaluation included the uteri, ovaries, the number of viable embryos, total number of corpora lutea, early and late resorptions, viable and non-viable fetuses, total implantations and corpora lutea. The brain, ovaries and pituitary gland were weighed. Visceral, skeletal and external abnormalities, sex of fetuses were evaluated.

Results:

Mortality: Two does in the 3 mg/kg/day group were moribund thus sacrificed on Day 18 and 20. Necropsy revealed mottled kidneys and red lungs. One of the does had also large gallbladder.

Clinical signs: No clinical signs in controls. All treated groups exhibited some signs of soft stool, vasodilation of pinna, sclera, and conjunctiva and decreased activity.

Body weight: **A statistically non-significant decrease in body weight gain of 52% in the 3 mg/kg/day** dose group from Day 6-18 was noted (i.e. during the doing interval).

Group Mean Doe Body Weight Changes (kilograms) During Gestation

Dose (mg/kg/day)		Gestational Interval (Days) 0-6	6-9	9-12	12-15	15-18	18-22	22-24	24-29	6-18	0-29
0	$\bar{x}$	0.116	0.076	0.021	0.145	-0.015	0.092	0.017	0.120	0.226	0.571
	SD	0.075	0.065	0.042	0.057	0.081	0.076	0.040	0.091	0.067	0.131
	N	11	11	11	11	11	11	11	11	11	11
0.3	$\bar{x}$	0.126	0.012	0.066	0.142	-0.008	0.128	0.034	0.097	0.213	0.597
	SD	0.038	0.092	0.096	0.055	0.073	0.036	0.031	0.061	0.060	0.152
	N	10	10	10	10	10	10	10	10	10	10
1.0	$\bar{x}$	0.122	0.036	0.094*	-0.016	0.149	0.105	0.021	0.048	0.263	0.559
	SD	0.079	0.058	0.040	0.300	0.251	0.077	0.025	0.068	0.080	0.080
	N	8	8	8	8	8	8	8	8	8	8
3.0	$\bar{x}$	0.120	0.021	0.041*	0.049	0.008	0.107	0.048	0.062	0.118	0.546
	SD	0.028	0.061	0.089	0.173	0.085	0.100	0.035	0.046	0.309	0.063
	N	10	10	10	10	10	9[A]	9	9	10	9

[A] = Doe #1566 was moribund sacrificed on Day 18 of gestation.
* = $p \leq 0.05$

Food consumption: No difference in food consumption

Toxicokinetics: See page 20 of this review for PK data in pregnant rabbits.

For embryofetal development studies:

In-life observations:

- None of the does aborted.
- Two does in the 3 mg/kg/day group were moribund and sacrificed on Day 18 and 20.
- Out of 72 animals (18/group) 40 were gravid (55.6%) but 38 were used in evaluation. One gravid control doe was not included in the analysis (no chorionic gonadotropin injection). 11/18 (61.1%), 10/18 (55.6%), 8/18 (44.4%), 9/16 (56.3%) animals in control, 0.3, 1 and 3 mg/kg/day were gravid, respectively. The low number of pregnancies did not meet the number requirements for this type of study.

Terminal and necropsy evaluations:

Dams:

- Although there were no statistical difference between groups, the number of gravid animals in all groups were low between 44 and 61%, below the ICH guidelines recommend numbers 16 to 20 litters for evaluation.

- There were no differences in the total number of implantation sites, total number of viable fetuses, non-viable fetuses, corpora lutea or fetal sex distribution, fetal body weight and percent pre- and post implantation losses.

Summary of Group Mean Doe and Fetal Observations at Cesarean Section

Dose (mg/kg/day)	0			0.3			1.0			3.0		
	No.	(S.D.)	%	No.	(S.D.)	%	No.	(S.D.)	%	No.	(S.D.)	%
Rabbits on Study:	18			18			18			18		
Rabbits that were gravid:	12		66.7	10		55.6	8		44.4	10		55.6
Rabbits that died:	0		0.0	0		0.0	0		0.0	0		0.0
Rabbits sacrificed early:	0		0.0	0		0.0	0		0.0	2		11.1
Non-gravid:	0		0.0	0		0.0	0		0.0	1		50.0
Gravid:	0		0.0	0		0.0	0		0.0	1		50.0
Rabbits that aborted:	0		0.0	0		0.0	0		0.0	0		0.0
Rabbits delivered early:	0		0.0	0		0.0	0		0.0	0		0.0
Rabbits examined at cesarean section:	18[a]		100.0	18[a]		100.0	18[a]		100.0	16[a]		88.9
Non-gravid:	1		8.3	2		16.7	4		33.3	1		10.0
Gravid:	11		91.7	10		83.3	8		66.7	9		90.0
Does with resorptions only:	0		0.0	0		0.0	0		0.0	0		0.0
Does with non-viable fetuses only:	0		0.0	0		0.0	0		0.0	0		0.0
Does with viable fetuses:	11		100.0	10		100.0	8		100.0	9		100.0
Viable fetuses/doe:	6.6	(3.11)		7.7	(2.31)		7.5	(2.00)		7.3	(1.58)	
Total implantations/doe:	7.1	(2.91)		8.6	(2.01)		8.1	(2.10)		8.4	(1.74)	
Post-implantation loss/doe:	0.5	(0.69)	-	0.9	(0.88)	-	0.6	(0.52)	-	1.1	(1.05)	-
Corpora lutea/doe:	9.3	(1.56)	-	10.7	(3.30)	-	9.3	(2.38)	-	9.8	(0.97)	-
Pre-implantation Loss (%)[b]	23.1	(28.97)	-	18.3	(12.30)	-	11.8	(13.40)	-	13.2	(17.35)	-
Post-implantation Loss (%)[c]	7.5	(11.19)	-	12.5	(15.44)	-	7.5	(6.40)	-	12.3	(10.99)	-
Fatal sex distribution												
Male -	40		54.8	38		49.4	30		50.0	35		53.0
Female -	33		45.2	39		50.6	30		50.0	31		47.0
Mean fetal body weight (grams):	48.8	(6.48)	-	49.5	(4.89)	-	47.9	(4.09)	-	47.8	(5.07)	-

[a] = Six does were not injected with chorionic gonadotrophin, not included in statistical analysis.

[b] = Group Mean % Pre-implantation Loss by Doe = $\frac{\text{Corpora Lutea - Total Implantations}}{\text{Corpora Lutea}} \times 100$

[c] = Group Mean % Post-implantation Loss by Doe = $\frac{\text{Total Implantations - Viable Fetuses}}{\text{Total Implantations}} \times 100$

- = Not applicable

End of Table 8

Offspring:

- There were no significant differences in the number of fetus or litters with malformation among groups.
- The incidences of skeletal variations are shown in table below. There were no visceral malformations in any group. Two fetuses (2.6%) from 2 litters (20%) in the LD and 2 fetuses (3.3%) from 2 litters (25%) in MD exhibited skeletal variations fetal malformations. See table below for details.

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Type and Incidence of Skeletal Variations

Dose (mg/kg/day)	0				0.3				1.0				3.0			
	Fetuses		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses		Litters	
Variations Observed	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Skull Incomplete Ossification	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.5	1	11.1
Hyoid Unossified	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.5	1	11.1
Sternebrae Bipartite 6th (Xiphoid Process)	0	0.0	0	0.0	1	1.3	1	10.0	1	1.7	1	12.5	0	0.0	0	0.0
Sternebrae Incomplete Ossification																
2nd	0	0.0	0	0.0	2	2.6	2	20.0	1	1.7	1	12.5	0	0.0	0	0.0
4th	1	1.4	1	9.1	1	1.3	1	10.0	3	5.0	1	12.5	0	0.0	0	0.0
5th	28	38.4	10	90.9	54*	70.1	10	100.0	30	50.0	8	*100.0*	*40**	*60.6*	9	*100.0*
6th (Xiphoid Process)	10	13.7	4	36.4	37*	48.1	10	100.0	15	25.0	5	62.5	18	27.3	7	77.8
Sternebrae Unossified																
5th	9	12.3	5	45.5	14	18.2	4	40.0	21	35.0	4	50.0	2	3.0	1	11.1
6th (Xiphoid Process)	1	1.4	1	9.1	5	6.5	2	20.0	0	0.0	0	0.0	1	1.5	1	11.1
Sternebrae Asymmetrical																
3-4	0	0.0	0	0.0	1	1.3	1	10.0	0	0.0	0	0.0	0	0.0	0	0.0
3-5	0	0.0	0	0.0	1	1.3	1	10.0	0	0.0	0	0.0	0	0.0	0	0.0
Ribs Bifurcated	0	0.0	0	0.0	0	0.0	0	0.0	1	1.7	1	12.5	0	0.0	0	0.0
Ribs Bulbous	1	1.4	1	9.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Ribs 13th Extra																
Pair	10	13.7	4	36.4	9	11.7	6	60.0	16	26.7	6	75.0	11	16.7	3	33.3
Right	4	5.5	3	27.3	2	2.6	1	10.0	2	3.3	1	12.5	5	7.6	5	55.6
Left	5	6.8	4	36.4	8	10.4	5	50.0	2	3.3	2	25.0	5	7.6	5	55.6
Ribs 13th Floating																
Pair	0	0.0	0	0.0	2	2.6	2	20.0	0	0.0	0	0.0	3	4.5	2	22.2
Right	2	2.7	2	18.2	2	2.6	2	20.0	5	8.3	3	37.5	1	1.5	1	11.1
Left	5	6.8	3	27.3	8	10.4	4	40.0	1	1.7	1	12.5	3	4.5	3	33.3
Ribs 13th Rudimentary																
Pair	3	4.1	3	27.3	4	5.2	4	40.0	5	8.3	4	50.0	5	7.6	4	44.4
Right	8	11.0	6	54.5	9	11.7	6	60.0	1	1.7	1	12.5	8	12.1	4	44.4
Left	4	5.5	4	36.4	6	7.8	5	50.0	6	10.0	4	50.0	4	6.1	4	44.4
Ribs 13th Ossification Center Right	1	1.4	1	9.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	30.0

Summary:

Female (18/dose) white rabbits were treated with **0, 0.3, 1.0 and 3.0** mg/kg/day pramlintide (6, 30, 60X 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, p>NS) 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. 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).

Study title: Study for effects on embryo-fetal development in rabbits

Key study findings: 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 in this study. 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).

Study no.: REST98125
Volume #, and page #: 1.66, 1-81
Conducting laboratory and location: [redacted]

Date of study initiation: Jan 26, 1995
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 94-0806CB not radiolabeled (—), clear, colorless liquid (different than white powder that needed reconstitution with water)
Formulation/vehicle: Not reported (it was supplied by the sponsor to the contracted lab)

Methods:
Species/strain: New Zealand white rabbits
Doses employed: 0, 0.03, 0.1 and 0.3 mg/kg/day (these doses are ten fold lower than earlier study, where the number of gravid animals was only 50% of total number of rabbits).
Route of administration: SC
Study design:

Group	No. of females	No. of litters	Dose Level (mg/kg/day)
1	18	14	0
2	18	16	0.03
3	18	14	0.1
4	18	15	0.3

Injections were made from Day 6 of gestation through Day 19 of pregnancy. Body weights were measured on days 6, 19, 22, 24 and 29.
Number/sex/group: 18 female rabbits/dose
Parameters and endpoints evaluated: Evaluation included the uteri, ovaries, the number of viable embryos, total number of corpora lutea, early and late resorptions, viable and non-viable fetuses, total implantations and corpora lutea. The ovaries, uterus and vagina were preserved. Visceral, skeletal and external abnormalities, sex of fetuses were evaluated. Approximately one half of the fetuses were decapitated and the brain abnormalities were examined.

Results:

Mortality: None of the rabbits died

Clinical signs: No clinical signs in controls. All treated groups exhibited some clinical signs (i.e. scant or soft stool, vasodilation of pinna).

Body weight: There were no significant decreases in body weights of treated animals compared to controls. However, at two time points body weight gains in MD and HD group were significantly reduced by as much as 84 and 59%, respectively. The decrease in maternal weight of 57% in the LD groups was not statistically significant. Since all doses had reduced BW gain on Day 22-24, the value of this weight loss may be coincidental. In the earlier study sponsor used 10 fold higher doses and body weights were reduced at 3 mg/kg/day.

Group Mean Doe Body Weight Changes (kilograms) During Gestation

Dose (mg/kg/day)		Gestational Interval (Days) 0-3	3-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14
0	$\bar{x}$	0.053	0.034	0.021	0.012	0.013	0.025	0.028	0.024	0.035	0.045
	S.D.	0.052	0.053	0.045	0.060	0.043	0.053	0.079	0.047	0.044	0.030
	N	14	14	14	14	14	14	14	14	14	14
0.03	$\bar{x}$	0.074	0.010	0.025	0.013	0.009	0.012	0.017	0.028	0.037	0.042
	S.D.	0.115	0.119	0.024	0.025	0.026	0.019	0.018	0.037	0.029	0.030
	N	16	16	16	16	16	16	16	16	16	16
0.1	$\bar{x}$	0.041	0.066	0.009	0.015	0.011	0.025	0.016	-0.004	0.052	0.042
	S.D.	0.124	0.041	0.028	0.039	0.038	0.032	0.028	0.099	0.109	0.043
	N	14	14	14	14	14	14	14	14	14	14
0.3	$\bar{x}$	0.049	0.049	-0.027*	0.035	0.023	0.033	0.025	0.021	0.026	0.033
	S.D.	0.058	0.039	0.037	0.026	0.030	0.031	0.031	0.035	0.033	0.044
	N	15	15	15	15	15	15	15	15	15	15

* = p≤0.05

Dose (mg/kg/day)		Gestational Interval (Days) 14-15	15-16	16-17	17-18	18-19	19-22	22-24	24-29	6-19	0-29
0	$\bar{x}$	0.028	0.018	0.011	0.000	0.026	0.055	0.056	0.091	0.285	0.574
	S.D.	0.035	0.037	0.034	0.047	0.079	0.046	0.047	0.068	0.078	0.093
	N	14	14	14	14	14	14	14	14	14	14
0.03	$\bar{x}$	0.028	0.028	0.009	0.009	0.003	0.051	0.024	0.087	0.258	0.504
	S.D.	0.039	0.035	0.029	0.033	0.030	0.035	0.033	0.050	0.070	0.131
	N	16	16	16	16	16	16	16	16	16	16
0.1	$\bar{x}$	0.039	0.002	0.012	-0.005	0.024	0.077	0.009*	0.088	0.237	0.520
	S.D.	0.050	0.058	0.034	0.059	0.041	0.057	0.042	0.066	0.121	0.197
	N	14	14	14	14	14	14	14	14	14	14
0.3	$\bar{x}$	0.030	0.026	0.019	0.024	-0.004	0.056	0.023*	0.073	0.264	0.513
	S.D.	0.036	0.034	0.028	0.038	0.031	0.052	0.033	0.046	0.094	0.123
	N	15	15	15	15	15	15	15	15	15	15

* = p≤0.05

Food consumption: No difference in food consumption

Toxicokinetics: See the PK/TK section

For embryofetal development studies:

In-life observations:

- None of the does aborted.

Terminal and necropsy evaluations:

Dams:

- 14/18 (77.8%), 16/18 (88.9%), 14/18 (77.8%) and 15/18 (83.3%) animals in control, 0.03, 0.1 and 0.3 mg/kg/day were gravid, respectively. ICH guidelines require 16 to 20 gravid animals for evaluation. With only 14 to 16 animals evaluated per group, this study barely meets the minimum requirement. It is not clear why the sponsor didn't start the study with

a larger number of animals/dose since in an earlier study they had found significantly lower pregnancy rate in all groups.

Summary of Group Mean Maternal and Fetal Observations at Cesarean Section

Dose (mg/kg/day)	0		0.03		0.1		0.3	
	No. (S.D.)	%	No. (S.D.)	%	No. (S.D.)	%	No. (S.D.)	%
Rabbits on Study:	18	-	18	-	18	-	18	-
Rabbits that were gravid:	14	77.8	16	88.9	14	77.8	15	83.3
Rabbits that died:	0	0.0	0	0.0	0	0.0	0	0.0
Rabbits sacrificed early:	0	0.0	0	0.0	0	0.0	0	0.0
Non-gravid:	0	0.0	0	0.0	0	0.0	0	0.0
Gravid:	0	0.0	0	0.0	0	0.0	0	0.0
Rabbits that aborted:	0	0.0	0	0.0	0	0.0	0	0.0
Rabbits delivered early:	0	0.0	0	0.0	0	0.0	0	0.0
Rabbits examined at cesarean section:	18	100.0	18	100.0	18	100.0	18	100.0
Non-gravid:	4	22.2	2	11.1	4	22.2	3	16.7
Gravid:	14	77.8	16	88.9	14	77.8	15	83.3
Dams with resorption only:	0	0.0	0	0.0	0	0.0	1	6.7
Dams with non-viable fetuses only:	0	0.0	0	0.0	0	0.0	0	0.0
Does with viable fetuses:	14	100.0	16	100.0	14	100.0	14	93.3
Viable fetuses/doe:	6.6(2.34)	-	6.0(3.06)	-	5.2(2.67)	-	5.8(2.54)	-
Total implantations/doe	7.5(2.21)	-	6.5(3.64)	-	5.9(3.04)	-	6.6(2.35)	-
Post-implantation loss/doe	0.9(1.17)	-	0.5(0.82)	-	0.6(1.15)	-	0.8(0.94)	-
Corpora lutea/dam:	10.4(2.68)	-	10.2(2.74)	-	9.2(2.55)	-	9.6(2.35)	-
Pre-implantation Loss(%)[a]	26.9(17.63)	-	35.8(29.32)	-	37.8(27.57)	-	28.7(26.66)	-
Post-implantation Loss (%)[b]	12.3(16.91)	-	5.1(7.62)	-	8.5(7.59)	-	15.3(25.64)	-
Fetal sex distribution								
Male	49	52.7	51	53.1	40	54.8	48	55.2
Female	44	47.3	45	46.9	33	45.2	39	44.8
Mean fetal body weight (grams)								
Male	47.4(6.91)	-	48.2(5.54)	-	53.1(5.93)	-	49.0(3.71)	-
Female	45.9(6.83)	-	47.8(4.54)	-	49.8(5.00)	-	49.5(3.75)	-

[a] = Group Mean % Pre-implantation loss by Dam = $\frac{\text{(Corpora Lutea - Implantations)}}{\text{Corpora Lutea}} \times 100$

[b] = Group Mean % Post-implantation loss by Dam = $\frac{\text{(Implantations - Viable Fetuses)}}{\text{Implantations}} \times 100$

- = Not applicable

Summary of Incidence of Fetal Malformations

Dose (mg/kg/day)	0		0.03		0.1		0.3	
No. of litters examined:	14		16		14		15[a]	
No. of fetuses examined externally:	94		96		73		87	
No. of fetuses examined viscerally:	93[b]		96		73		87	
No. of fetuses examined skeletally:	93		96		73		87	
No. of fetal skulls examined skeletally:	50		53		41		47	
No. of fetal skulls examined sectionally:	43		43		32		40	
Total Malformations:	No.	%	No.	%	No.	%	No.	%
Fetuses with soft tissue malformations:	0	0.0	0	0.0	2	2.7	1	1.1
Fetuses with skeletal malformations:	0	0.0	1	1.0	1	1.4	1	1.1
Total fetuses with malformations:	0	0.0	1	1.0	3	4.1	2	2.3
Litters with soft tissue malformations:	0	0.0	0	0.0	2	14.3	1	6.7
Litters with skeletal malformations:	0	0.0	1	6.3	1	7.1	1	6.7
Total litters with malformations:	0	0.0	1	6.3	3	21.4	2	13.3

a = Includes one totally resorbed litter.
b = One dead fetus, not skeletally or viscerally evaluated.

Offspring:

- There were no statistical differences in the total number of implantation sites, total number of viable fetuses, non-viable fetuses, corpora lutea, early or late resorption, fetal sex distribution, fetal body weight or in the number and percentage of pre- and post-implantation losses.

- There were no fetal malformations in the control group.
- **Low dose** malformations: 1/96 fetuses (1 with fused sternabrae)
- **Mid dose** malformations: 3/73 fetuses (1 fetus with moderate hydrocephalus, 1 with fused sternabrae and 1 with truncus communis and intraventricular septal defect. each from different dam)
- **High dose** malformations: 2/87 fetuses (1 fetus with fused sternabrae, 1 fetus with truncus communis and intraventricular septal defect).
- **Visceral variation included hypoplastic gallbladders from one fetus in each 0, 0.03, 0.1 mg/kg/day group. This was also seen in 3 fetuses of from one doe in the 0.3 mg/kg/d (HD) animals**. The incidence of hypoplasia in historical controls is very low. Of the 10 labs examined, only 1/10 labs reported gallbladder hypoplasia (1/110 rabbits).
- Skeletal variations were seen in all groups (see table). Statistically significant decreases were observed in the number of fetuses exhibiting incomplete ossification of the 5th sternebrae in the 0.1 and 0.3 mg/kg/day and 13th full pair of ribs in the 0.03 and 0.1 dose groups compared to controls. The biological significance of these findings is not clear.

Type and Incidence of Skeletal Variations

Dose (mg/kg/day)	0				0.03				0.1				0.3			
Variations Observed	Fetuses		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses		Litters	
	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Skull Incomplete Ossification	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	4.2	2	14.
Sternebrae Bipartite 6th (Xiphoid Process)	0	0.0	0	0.0	1	1.1	1	7.1	0	0.0	0	0.0	0	0.0	0	0.
Sternebrae Incomplete Ossification																
2nd	0	0.0	0	0.0	1	1.0	1	6.3	0	0.0	0	0.0	0	0.0	0	0.
3rd	0	0.0	0	0.0	1	1.0	1	6.3	0	0.0	0	0.0	0	0.0	0	0.
4th	2	2.2	1	7.1	1	1.0	1	6.3	0	0.0	0	0.0	1	1.1	1	7.
5th	42	45.2	11	78.6	37	36.5	13	81.3	18*	24.6	10	71.4	17*	19.5	10	71.
6th (Xiphoid Process)	10	10.8	4	28.6	17	17.7	9	56.3	9	12.3	5	35.7	13	14.9	7	50.
Sternebrae Unossified 5th	7	7.5	3	21.4	6	6.3	6	37.5	9	12.3	5	35.7	4	4.5	2	14.
6th (Xiphoid) Process)	1	1.1	1	7.1	1	1.0	1	6.3	2	2.7	2	14.3	2	2.3	2	14.
Sternebrae Asymmetrical 2-4	0	0.0	0	0.0	0	0.0	0	0.0	1	1.4	1	7.1	0	0.0	0	0
Sternebrae Asymmetrical 2-5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.1	1	7
Ribs 13th Ossification Center Right	0	0.0	0	0.0	1	1.0	1	6.3	0	0.0	0	0.0	0	0.0	0	0
13th Float Right	4	4.3	3	21.4	4	4.2	4	25.0	9	12.3	4	28.6	2	2.3	2	14
13th Float Left	2	2.2	2	14.3	0	0.0	0	0.0	0	0.0	0	0.0	4	4.5	3	21
13th Float Pair	1	1.1	1	7.1	1	1.0	1	6.3	3	4.1	3	21.4	0	0.0	0	0
13th Full Right	1	1.1	1	7.1	4	4.2	4	25.0	1	1.4	1	7.1	2	2.3	2	14
13th Full Left	4	4.3	4	28.6	9	9.4	7	43.8	9	12.3	6	42.8	8	9.2	7	50
13th Full Pair	59	63.4	14	100.0	36*	37.5	13	81.3	31*	42.5	11	78.5	45	51.7	13	92.8
13th Rudimentary Right	2	2.2	2	14.3	6	6.3	5	31.3	4	5.5	3	21.4	8	9.2	7	50.0
13th Rudimentary Left	6	6.4	6	42.8	6	6.3	4	25.0	5	6.8	5	35.7	1	1.1	1	7.1
13th Rudimentary Pair	4	4.3	2	14.3	4	4.2	4	25.0	6	8.2	6	42.8	5	5.7	4	28.5

*-p≤0.05

Summary:
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. 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 or body weight gains except for periodic decrease in body weight gains (Days 22-24 MD and HD). In the earlier study sponsor used doses that were 10 fold higher than doses used in this study. The study was not valid since the pregnancy rate was too low and adequate numbers of pregnant doses were not available for assessment.
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. Preponderance of skeletal variations were observed in the sternebrae 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 5th sternebrae 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. The incidence of gallbladder hypoplasia was higher in HD group than control (3 vs. 1). Gallbladder hypoplasia is a very rare finding in new Zealand white rabbits. Out of 10 labs (n= 3968 rabbits), only 1/10 labs has reported this findings (1/110 rabbits). Thus greater incidence of gallbladder hypoplasia in HD rabbits is concerning.

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 sponsor should have repeated the study using the same doses used in study # REST98110 (0.3, 1 and 3 mg/kg/day). The developmental NOEL was 0.3 mg/kg/day (9 X human dose). Doses used in this study were too low. The highest dose should have had maternal toxicity. According to sponsor, there was a decrease in body weight loss on Days 22-24 of gestation in the 0.03, 0.1 and 0.3 mg/kg/day (57, 89 and 57% ↓, respectively). However, only the MD and HD dams had significantly lower weight gain. There was an increase in the incidence of gallbladder hypoplasia in HD group. The reported incidence of gallbladder hypoplasia in historical control data is very rare. However, since this finding was not observed in the rabbit study conducted with 10-fold higher dose of pramlintide, and was also observed in controls the relationship to study drug is unclear.

Study title: Study for effects on pre- and postnatal development including maternal function in rats

Key study findings: 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).

Study no.: REST98129R1
Volume #, and page #: 1.67, 1-364
Conducting laboratory and location: ℃ ℶ

Date of study initiation: Jan 24, 1995 through June 29, 1995
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 97-806CB non-radiolabeled (— purity)
Formulation/vehicle: AC-0137 and vehicle (control) were in clear liquid form provided to the testing facility by the sponsor. The dosing volume was 1.2 ml/kg.
Methods:

Species/strain: male and female SD rats — CD®BR VAF, C
Doses employed: **0, 0.2, 0.5, 1.2 mg/kg/day once a day**
Route of administration: SC
Study design: Total of 48 female rats/group were treated. Twenty four/group were treated from gestation Day 6 through gestation Day 19. Twenty four/group were treated from gestation Day 6 through lactation Day 21 to raise F1 generation. The F1 generations were mated and fetuses were examined again. Two females were mated with 1 male rat.

Study for Effects on Pre- and Postnatal Development Including Maternal Function in Rats [REST98129R1]

Study Design

Pramlintide (mg/kg/day)	Dose Volume (mL/kg)	Route	F_0 Females		F_1 Generation		F_1 Females
			Sacrificed		Approximate Number Mated		Sacrificed
			Day 20 of Gestation	Day 21 of Lactation	Males	Females	Day 20 of Gestation
0.0	1.2	SC	24	24	24	24	24
0.2	1.2	SC	24	24	24	24	24
0.5	1.2	SC	24	24	24	24	24
1.2	1.2	SC	24	24	24	24	24
Lot # 94-0806CB							

Number/sex/group: 24 male and 48 female rats/dose
Parameters and endpoints evaluated: drug effects on gonadal function and weight, estrous cycle, mating behavior, conception rate, the early and late stages of gestation, parturition and lactation.

Results:

***For embryofetal development studies*:**

F0 Generation, Gestation Day 20:

Mortality: No mortality during gestation period or on day of sacrifice (day 20)
Clinical signs:

- No clinical signs were noted in control or LD females.
- In MD and HD dose, vasodilation of the pinna was seen in all animals. Some of the animals showed signs of abnormal gait, tremor, decreased motor activity and cold to the touch near to the time of delivery. One dam had a mass (1x 0.5x 0.5 cm) in the right inguinal region noted prior to cesarean section. HD group had also vasodilation of the limbs.

Body weight: There were no overall statistically significant changes in body weight or body weight gains. However, on body weight gain of the 1.2 mg/kg/day groups appeared to be less than control.
Food consumption: There were significant decreases in food intake of dams of MD (-4%) and HD (-10%) group on gestation Days 17-20.
Toxicokinetics: No TK data

Terminal and necroscopic evaluations of dams/fetuses terminated on gestation Day 20 (D20G):

Dams:

- None of the animals aborted or delivered early.
- Gravidity: 192 females were mated. 40/48, (83.3%), 42/48 (87.5%), 42/48 (87.5%) and 45/48 (93.8%) of the control, LD, MD and HD females were pregnant, respectively. There were no significant differences in gravidity between the treated and control animals.
- There were no significant differences in the number if viable fetuses, nonviable fetuses, early resorption, late resorption, total number of implantation, corpora lutea, fetal sex distribution, mean fetal body weight, pre- and post-implantation losses

Offspring ():

There was no incidence of fetal malformation or developmental variation from dams **sacrificed on gestation day 20.**

- Two fetuses in control group had umbilical herniation
- One fetus in LD group exhibited transposition of the trachea and esophagus as well as major heart and vessel malformation. A second fetus exhibited situs invertus. Both were considered incidental.
- Placenta in one HD group fetus was pale while another in HD group had umbilical herniation.

Study for Effects on Pre- and Postnatal Development Including Maternal Function in Rats [REST98129R1]

Maternal (F_0) Observations at Cesarean Section

Pramlintide (mg/kg/day)	0.0		0.2		0.5		1.2	
	No.	%	No.	%	No.	%	No.	%
Cesarean Section	24	100.0	24	100.0	24	100.0	24	100.0
Non-gravid	6	25.0	2	8.3	2	8.3	2	8.3
Gravid	18	75.0	22	91.7	22	91.7	22	91.7
Dams with resorptions only	0	0.0	0	0.0	0	0.0	0	0.0
Number of early resorptions	24	—	30	—	18	—	18	—
Number of late resorptions	0	—	1	—	0	—	0	—
Dams with viable fetuses	18	100.0	22	100.0	22	100.0	22	100.0
Viable fetuses/dam	14.5	—	13.0	—	12.9	—	13.6	—
Total implantation/dam	15.8	—	14.4	—	13.7	—	14.4	—
Post-implantation loss/dam	1.3	—	1.4	—	0.8	—	0.8	—
Corpora Lutea/dam	17.9	—	16.8	—	17.5	—	17.0	—
Pre-implantation Loss (%)[a]	11.0	—	13.9	—	21.2	—	13.8	—
Post-implantation Loss (%)[b]	8.4	—	9.5	—	6.0	—	5.8	—

Study for Effects on Pre- and Postnatal Development Including Maternal Function in Rats [REST98129R1]

Incidence of Fetal (F_1) Soft Tissue and Skeletal Malformations

Pramlintide (mg/kg/day)	0.0		0.2		0.5		1.2	
No. of litters examined	18		22		22		22	
No. of fetuses examined:								
externally	261		285		284		299	
viscerally	134		151		147		156	
skeletally	127		134		137		143	
Total Malformations	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Fetuses with:								
soft tissue malformations	2	1.5	2	1.3	0	0.0	1	0.6
skeletal malformations	0	0.0	0	0.0	0	0.0	0	0.0
Total fetuses with malformations	2	0.8	2	0.7	0	0.0	1	0.3
Litters with:								
soft tissue malformations	1	5.6	2	9.1	0	0.0	1	4.5
skeletal malformations	0	0.0	0	0.0	0	0.0	0	0.0
Total litters with malformations	1	5.6	2	9.1	0	0.0	1	4.5

F0 Generation, Lactation Day 21:

Clinical signs:

- No clinical signs were noted in control.
- In LD group, red vaginal discharge from two dams (due to delivery)
- Mid dose dams all exhibited vasodilation of pinna during lactation period. Similar signs were also seen in HD group plus soft stool and vasodilation of limbs

Body weight: There were no overall statistically significant changes in maternal body weight. However, on Day 8-21 lactation, the mean body weight of HD dams was lower (~5%, p>NS) than controls.

A statistically significant decrease in body weight gains in HD pups on Days 7-8 (-54%) and 9-10 (-82%) was noted. The overall body weight gains (lactation Day 0-21) in the HD pups was 22% less than control (p=NS).

Food consumption: There were significant decreases in food intake of dams of HD (-12.5%).

Terminal and necroscopic evaluations of dams/fetuses terminated after lactation:

Dams:

- None of the dams exhibited dystocia or agalactia
- There were no significant differences in the number of litters delivered, weaned or stillborns.
- Although not significant, the total number of neonates delivered in the HD group was less than control.

Study for Effects on Pre- and Postnatal Development Including Maternal Function in Rats [REST98129R1]

Summary Data for Dams (F_0) and Neonates (F_1) at Delivery

Pramlintide (mg/kg/day)	0.0		0.2		0.5		1.2	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Rats								
Designated for delivery	24	—	24	—	24	—	24	—
Gravid	22	(91.7)	20	(83.3)	23	(95.8)	20	(83.3)
Non-gravid	2	(8.3)	4	(16.7)	1	(4.2)	4	(0.0)
Dams								
Delivering	22	(100.0)	20	(100.0)	23	(100.0)	20	(100.0)
Gravid/did not deliver	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
With stillborn	2	(9.1)	6	(30.0)	3	(13.0)	3	(15.0)
With agalactia/dystocia	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Length of gestation (days)	21.8	—	22.2*	—	22.2*	—	22.1	—
Number of litters delivered	22	(100.0)	20	(100.0)	23	(100.0)	20	(100.0)
Neonates per litter at delivery	13.4		13.3		12.9		13.0	

* $p \leq 0.05$; — = Not Applicable

Offspring (F1)

- There were no significant differences in the number, viability and sex distribution or body weight of the neonates at birth.
- No test article related toxicity in neonates was observed.
- There were significant decreases in body weight of MD (11%) and HD (18%) neonates compared to controls.
- There were no skeletal malformations at any dose.
- There were no significant differences in surface righting, negative geotaxis, air drop, galton whistle, pinna detachment, incisor eruption, fur growth, eye opening, pupil constriction, vaginal opening, preputial separation, rotorod performance, spontaneous locomotor activity, m-maze performance and, passive avoidance performance.
- A significant increase (control 7.4 s vs. HD 9.1s) in grasping and holding in HD was noted. The biological significance of this finding is not clear.

- No signs of toxicity in post-weaning F1 animals.
- **A significant decrease in body weight of male and female pups of all dose groups on Day 14 and 21 (-15%)** was noted suggesting that milk from the treated rats still affecting the growth of the pups. There were no significant differences in body weight among groups on Day 106).

Study for Effects on Pre- and Postnatal Development Including Maternal Function in Rats [REST98129R1]

Number, Viability and Sex of Neonates (F_1)

Pramlintide (mg/kg/day)	0.0		0.2		0.5		1.2	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Total neonates delivered	295		265		296		259	
Total viable	293	(99.3)	245	(92.8)	283	(95.6)	251	(96.9)
Total stillborn	2	(0.7)	14	(5.3)	11	(3.7)	3	(1.2)
Total dead day 0/born alive	0		5	(1.9)	2	(0.7)	5	(1.9)
Total males delivered	160	(54.2)	135	(51.1)	134	(45.3)	132	(50.9)
Total females delivered	135	(45.8)	129	(48.9)	162	(54.7)	127	(49.1)
Neonate survival (%)								
Days 0-4, prior to standardization		(96.2)		(96.3)		(97.2)		(95.2)
Neonate survival (%)								
Days 4-21, after standardization		(97.7)		(98.0)		(100.0)		(98.0)

Study for Effects on Pre- and Postnatal Development Including Maternal Function in Rats [REST98129R1]

Group Mean Body Weights (grams) of Neonates (F_1) During Lactation

Pramlintide (mg/kg/day)	0.0		0.2		0.5		1.2	
	Male	Female	Male	Female	Male	Female	Male	Female
Body weight								
Day 0	6.1	5.7	6.2	6.0*	6.1	5.9*	6.1	5.8
Day 4[a]	9.4	8.8	9.1	8.9	9.3	9.0	9.0*	8.6
Day 7	15.6	14.8	14.6	14.3	14.6*	14.2*	14.1*	13.7*
Day 14	33.1	31.9	30.2*	29.9*	29.7*	28.7*	28.0*	27.1*
Day 21	53.7	51.7	50.6*	49.1*	49.3*	47.7*	45.5*	44.1*

*$p \leq 0.05$; [a] prior to standardization

For postnatal development studies:

In-life observations:

Dams (F1):

- There were no signs of drug related toxicity in any F1 dam during gestation.
- None of the dams died during gestation.
- 83 F1 dams were mated and 71 were gravid. 19/21 (90.5%), 17/18(94.4%), 19/22(86.4%) and 16/18(88%) of control, 0.2, 0.5 and 1.2 mg/kg/d were gravid, respectively. **There were no significant differences among groups.**
- None of the dams (F1) aborted or delivered early.
- There were no significant differences in body weight of F1 dams during gestation.

Offspring:

- There were no significant differences 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 percent of the pre- and post-implantation losses.
- All F2 fetuses were viable except for two in MD group. One of the fetuses exhibited craniorachischisis, exencephaly and bilateral anophthalmia. The other displayed

exencephaly, cleft lip, cleft palate, protruding tongue, anophthalmic left eye and hydramnios.

Summary:
Female — 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 numbers 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. The decrease in fetal weight suggests negative effects of Symlin delivered via milk or negative effect of Symlin on lactation. 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 differences 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). The HD (1.2 mg/kg/day reduced body weight of dams (F0) in the lactation (allowed to deliver) by 23%.

Summary of Reprotoxicity Studies:
In the rat fertility study, male (13/dose) and female (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. 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. Sperm counts and sperm motility were not examined. 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 was noted (12%). Eight of the females in 3 mg/kg/d died at the time of parturition. The fetuses from 7/8 of these 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. A biologically significant decrease in the number of litters delivered in the 3 mg/kg/d dams was observed. 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 associated with dystocia, 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. The number of fetuses examined was not adequate to make a valid evaluation of skeletal malformation in this study.

In rat teratology study, female 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**. 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.

In the first rabbit teratology, 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. 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. Due to abnormally low pregnancy rate, this study was considered invalid due to inadequate numbers for evaluation and a second study was performed with nearly 10 fold lower doses of pramlintide.

In this second embryo-fetal development study, female (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.
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 sternebrae 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 5th sternebrae in the mid and high dose and 13 full pair of ribs in the low and mid dose groups were noted but were within historical control range. Visceral variations were primarily confined to the gall bladder and kidneys. The greater incidence of gallbladder hypoplasia in the HD group (3 vs. 1 control) is a concern since the incidence was greater than historical control data (1/10 labs reported 1/110 rabbits), but the relationship to drug treatment is unclear since the finding was not reproduced in the rabbit teratology study evaluating 10-fold higher doses.

In the pre- and postnatal development study, female – 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 pups 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 was 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. The decrease in weight may have been due to negative effect of Symlin in the milk or negative effect of Symlin on lactation. 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 differences 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.

Labeling recommendations:

Sponsor's recommendation:

Our recommendation:

Carcinogenesis, Mutagenesis, impairment of fertility:

Carcinogenicity:

[ ]

Mutagenesis: SYMLIN was not mutagenic in the Ames test and did not increase chromosomal aberration in the human lymphocyte assay. Symlin was not clastogenic in the in vivo mouse micronucleus test or in the chromosomal aberration assay utilizing Chinese hamster ovary cells

Impairment of Fertility: Administration of 0.3, 1 or 3 mg/kg/day of — (8, — and 82 times [ ] had no significant effects on fertility in males or female rats. The highest dose of 3 mg/kg/day resulted in dystocia in 8/12 dams secondary to significant decreases in serum calcium levels.

Pregnancy: Teratogenic effects:

[ ]

Toxicology conclusions:

Toxicity of pramlintide was evaluated in mice (SC), and in rats and dogs by both SC and IV. Since acute studies were not reviewed fully, only a brief description of acute studies will be presented here. The 26 WK rat, dog, and 52 WK dog toxicology studies were reviewed in full and detailed review of these studies can be found in the main review text.

Acute Toxicity Studies:
After a single subcutaneous dose of 500 mg/kg (rat) or 2.0 mg/kg (dog) or an intravenous dose of 10.0 mg/kg (rat and dog), no drug-related mortality occurred. At subcutaneous doses of 250 mg/kg or higher in rats' abnormal gait and stance were observed. These signs occurred shortly after dosing and were transient, disappeared within 30 minutes to 4 hours after dosing. Clinical signs in dogs treated with 1 mg/kg or higher (SC) included diarrhea. When given intravenously, diarrhea, decreased activity and emesis occurred at doses of 0.3, 1.0 and 10.0 mg/kg or greater, respectively.

Repeat Dose Toxicity Studies
In a 28 day study, pramlintide doses up to 2 mg/kg/day (SC) was well-tolerated by mice. Small but significant changes in some erythrocyte parameters were noted. Following subcutaneous injection, higher erythrocyte counts and hemoglobin and hematocrit values, as compared to control, occurred in the 2.0 mg/kg-dosed groups. Serum glucose (0.3 mg/kg) and lactate (2.0 mg/kg) values were lower and phosphorus values (2.0 mg/kg) were higher than the concurrent control values. In both studies injection site irritation, observed in all groups including the vehicle controls, was characterized by hemorrhage and cellulitis or epidermatitis.
In the 28-day study, pramlintide was administered BID at total daily doses of 0.2, 0.5 and 1.2 mg/kg to rats. Vasodilation of the extremities was observed at ≥0.2 mg/kg. Serum glucose (≥0.2 mg/kg), total protein (≥0.5 mg/kg) and albumin (≥1.2 mg/kg) values were lower compared to control. Heart weights were lower than control values in the 1.2 mg/kg-dosed males; however, there were no changes in the heart histology.
A bridging 28-day subcutaneous study in rats compared the toxicologic effects of pramlintide lots manufactured by three different bulk drug manufacturers [ ] Plasma concentrations were similar for the three products. Irritation induced at the injection site was similar across the three products. There were no signs of systemic toxicity.
In dogs administered pramlintide subcutaneously at doses up to 2.0 mg/kg for 14 days, irritation at the injection site was considered to be treatment-related. Diarrhea occurred intermittently in females at 0.3 mg/kg and in males and females at 1.0 and 2.0 mg/kg. Dogs administered 0.3 mg/kg pramlintide by intravenous injection for 14 days experienced emesis, quivering and decreased activity. Diarrhea occurred at all doses with a dose-related increase in frequency.
In the 28-day study, the principal findings were postdose vasodilation (≥0.2 mg/kg), and lower body weight gains (≥0.5 mg/kg), shorter prothrombin times (≥0.5 mg/kg), lower serum phosphorus and mean urine specific gravity levels (≥0.2 mg/kg) as compared to the controls. The injection sites were discolored in treated groups as well as the vehicle control.

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 [ ] CD®BR rats (30 /sex/group). Approximately, 10 rats/sex/group were examined at 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 weeks than at 13 weeks suggesting greater inflammation with repeated SC administration.

26-WK dog study (REST98123R1) evaluated 0 (control), 0.2, 0.5 and 1.2 (HD) mg/kg/day pramlintide SC. In this study, the dose was divided into two daily injections 12 hrs apart. The volume of injections for control and HD were 0.6 ml/kg/day. There were no mortalities. Pramlintide treatment appeared to increase the incidence of vomiting which may have contributed to decrease in body weight of dogs. HD males had significantly lower body weight at Week 13 (-20%) and Week 26 (29%). The effect was not apparent in females at any dose levels. There were no significant pramlintide related effects on ophthalmology, ECG, blood pressure or clinical pathology evaluations. There were no changes in organ weights. Only notable finding was the inflammatory lesions at the injection sites. Since the severity of the lesions increased with injection volume (control and HD group) and increases in study duration (13 WK vs. 26 WK) and resolved during the recovery, the injection site findings were not attributed to the drug substance. There was no evidence of any systemic toxicity at doses up to 1.2 mg/kg/day (84X human dose based on AUC). The minor elevations in adjusted liver and kidney weights were not accompanied by any histological lesions and pramlintide was considered not to produce any organ toxicity.

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 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.

Genotoxicity:
A standard battery of tests was 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 mutagenic, with and without metabolic activation in two bacterial mutagenicity tests. The bacterial mutagenicity of drug from the 3 bulk drug suppliers [redacted] were also tested. Pramlintide produced by three different suppliers was negative in the bacterial mutagenicity assay. The chromosomal aberration and clastogenicity of

pramlintide was tested in human lymphocytes and mouse micronucleus assay, respectively. Under the assay conditions, pramlintide was negative for both chromosomal aberration and clastogenicity. The potential ability of pramlintide to induce forward gene mutation was tested in genetically engineered AS52/XPRT mammalian cell lines from Chinese hamster ovary cells. Pramlintide did not appear to induce forward gene mutation in this mammalian cell line.

Reprotoxicity:
The potential for pramlintide to affect the reproductive performance or fertility was evaluated in the adult rat. The teratogenic potential of the compound was assessed in both the rat and the rabbit by administering the compound to pregnant females of both species during the period of organogenesis and then evaluating the fetuses immediately prior to parturition. Pramlintide was also evaluated in the rat to determine if it had the potential to affect labor and parturition or the potential to affect the physical or neurological development or reproductive performance of the F1 generation or the fetuses produced by the F1 generation. For summary of findings, see pp 113-115)

Carcinogenicity:
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 those 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.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Fred Alavi
9/5/01 12:43:12 PM
PHARMACOLOGIST
Full review of the Symlin NDA 21-332 [pramlintide acetate].
Please, sign off on the Symlin review

Jeri El Hage
9/5/01 02:00:06 PM
PHARMACOLOGIST

PHARMACOLOGY AND TOXICOLOGY SUMMARY

Drug: pramlintide, Symlin ®
Indication: Adjunctive therapy to insulin to improve glycemic control in type 1 and 2 diabetics
Doses: 30 [redacted] 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 ß-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 pentogastrin-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 [redacted]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 [ ]
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 [ ]
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 [ ]
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 [ ]
Female — 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 [redacted]

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 sternebrae 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 5th sternebrae 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: [ ]
Specific tests and findings are described:

1. Ames tests conducted on product from 3 different manufacturers [ ] of pramlintide were negative.
2. In vitro chromosomal aberration test using human lymphocytes: Since pramlintide was cytotoxic at concentrations higher than 1670µg/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): — 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/ foliculitis, 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.

Incidence of selected injection site findings by grade*

Sex	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	Control2
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 %	100 %
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.

Incidence of rats with fasciitis/fibrosis at injection site

Sex	Male					Female				
Group	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 ($p<0.001$).

Incidence of tumors in male and female rats

Sex	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 1	28/50	28/49	33/50	36/50	25/50	43/50	37/50	38/50	40/50	34/50

1 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 [redacted]:CD®(SD)BR rats is about 47% (range 1 to 70%) in males and 70% (range 26 to 92%) in females [redacted] 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.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Fred Alavi
6/12/01 04:56:53 PM
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
PHARMACOLOGIST

45 Day Meeting Checklist

NDA 21-332, Symlin

NONCLINICAL PHARMACOLOGY/TOXICOLOGY

ITEM	YES	NO	COMMENT
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	X		
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	X		adequate
3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	X		
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA (genotox, reprotox, adequate duration of chronic tox, carcinogenicity)			Have electronic files of the carcinogenicity studies been submitted for statistical review? Yes Ames test in E.coli, Salmonella Chrome Abs using human lymphocyte AS52/XPRT mammalian cell forward gene mutation assay Micronucleus test mouse bone marrow Acute tox studies in dogs and rats. 14 Day SC, IV rat and dog studies 28 Day SC mouse, rat and dog studies 26 WK SC rat and dog studies 52 WK SC dog study 104 WK SC Carci study in mouse, rats Special tox studies in rabbits Segment I, II, III Repro in rats Segment II Repro in rabbits

ITEM	YES	NO	COMMENT
5) Were the studies adequately designed (ie., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?	X		
6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (ie., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	X		
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	X		
8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m2 or comparative serum/plasma AUC levels?	X		

ITEM	YES	NO	COMMENT
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	X		
10) Reasons for refusal to file:			

Reviewing Pharmacologist

Supervisory Pharmacologist

Carcinogenicity of SYMLIN was tested once daily for 104 weeks in mice (0.2, 0.5 and 1.2 mg/kg) and rats (0.04 0.2 and 0.5 mg/kg).
ANimal to human exposure based on AUC data. The highest dose of Symlin is given to type II patients at maximum of 120 µg TID and type I diabetic patients at — µg QID by SC injections.

Symlin Daily Doses	Mouse AUC 0-120 m ng.min/ml	Rat AUC0-120m	Human AUC 0-300 m	Mouse to human Exposure	Rat to human exposure
0.04 mg/kg		267			2.5
0.2 mg/kg	3340	923		32	8.87
0.5 mg/kg	7012	2566		67	24.6
1.2 mg/kg	16499			159	
Highest dose in human 360 µg			104		

The formulation that will be marketed contains pramlintide as the acetate salt, metacresol as preservative, acetic acid sodium acetate, mannitol and water for injection.

/s/

Fred Alavi
1/25/01 10:52:58 AM
PHARMACOLOGIST
The 45-day checklist for NDA 21-332, Symlin was check and found fileab
le by the Pharm/Tox reviewer.
Jeri, I revised the 45-Day checklist and added the NDA and drug name t
o the list

Jeri El Hage
1/25/01 11:03:13 AM
PHARMACOLOGIST

Statistical Review and Evaluation

Preclinical Animal Carcinogenicity Review

NDA #	21-332
Drug Name	Symlin ™ Injection (pramlintide acetate)
Applicant	Amylin Pharmaceuticals 9373 Towne Centre Drive, San Diego, CA 92121 (619) 552-2200
Documents Reviewed	EDR NDA Pharmacology / Toxicology Section 5.5.4.1 and 5.8.3.1.3.1 (mouse) Section 5.5.4.2 and 5.8.3.1.3.2 (rat)
Indication	[redacted]
Submission Date	Original IND: June, 1992 NDA: December 07, 2000
Final Review Date	June 22, 2001
Statistical Reviewer	Lillian Patrician, MS, MBA

I. Background

The sponsor reports that SYMLIN ™ (pramlintide acetate) is an amylinomimetic agent, the first of a new class of therapeutic compounds, which under pharmacologic experimental investigations indicate that it should be effective in diabetes mellitus as an adjunct to insulin treatment. The proposed indication is [ ] Pramlintide is a synthetic analogue of human amylin, a pancreatic ß -cell hormone that is secreted along with insulin, and has actions that complement those of insulin in maintaining metabolic control.

The carcinogenic potential of pramlintide was evaluated in 2-year studies in the mouse and the rat with daily subcutaneous administration, which was chosen because it was a possible human therapeutic route. The sponsor's analysis did not determine carcinogenicity at any dose in either sex of either species.

II. AC-0137 104-week Subcutaneous Oncogenicity Study in the Mouse

(Section 5.5.4.1 and 5.8.3.1.3.1, pharmtox/tox/98108.pdf)

The sponsor's study report REST98108R1 is dated May, 2000 with final report in July, 1999 and amendment in February, 2000. The protocol is dated January, 1995 and the experiment was conducted [ ] Treatment began January, 1995 with the last necropsy competed January, 1997.

This study included male and female — CD-1 (ICR) BR [ ] mice. Animals were not more than 8 weeks of age at start of dosing. At randomization, all animals were within 20% of the mean body weight for their sex. Male mice ranged in weight between 26 and 38 grams. Females were 20 to 33 grams.

The study began with more male and female animals than what was planned for randomization into the experiment. This included an additional 10 mice of each sex randomly selected for health check purposes, then killed within hours of arrival, and examined macroscopically. The remaining animals were then acclimated for 3 weeks before being randomly assigned to dose groups of Control 1, Control 2, 0.2, 0.5, and 1.2 mg/kg pramlintide administered subcutaneously each day for 104 weeks (51 per sex and dose group). They were caged in groups of 3 by sex. Dosing volumes were 2 and 5 mL/kg/day for the 0.2 and 0.5 mg/kg/day groups, respectively, and 12 mL/kg/day for the 1.2 mg/kg/day and vehicle control groups.

Each mouse was weighed before treatment on the first day of dosing, weekly for the first 16 weeks, once every 4 weeks thereafter, and before necropsy. Food consumption per cage of mice was determined weekly for the first 16 weeks and then 1 out of every 4 weeks thereafter.

On a daily basis, detailed observations of clinical signs were done for evidence of reaction to treatment and for ill health. Throughout the study, animals were examined twice daily to identify dead or moribund animals. At weekly intervals, all animals were given detailed physical examination and were palpated to aid the detection of superficial swellings and tissue masses. Any debilitated animals judged to be *in extremis* were sacrificed, examined macroscopically, and sampled for designated tissues. Interim sacrifices were also performed on humane grounds to prevent unnecessary or prolonged suffering. Sacrifices included complete necropsy, which involved dissection of organs and histopathological examination of 55 separate tissue samples. Tissues at injection sites were examined from all mice.

Microscopic examinations of tissue were performed for all animals of 2 Control groups and the high dose group (1.2 mg/kg/day) following the scheduled treatment period, and for all animals killed or dying during the study. Microscopic examination was also performed if macroscopic examination reports revealed tissues with significant abnormalities (gross lesions). Because of mortality in the male mice, all surviving male mice were sacrificed at Week 96-97 while females were terminated in Week 104-105.

Sponsor's Analysis - Mouse: All treatment comparisons were for treated groups against combined control groups. The sponsor reported finding no statistical relationship between pramlintide dose and survival and no adverse treatment-related effect on causes of morbidity and mortality. There was no evidence that pramlintide was tumorigenic in mice when administered subcutaneously.

> There were no statistically significant effects on body weight, food consumption, red cell count or white blood cell count. Sores were observed at the dose sites in all groups with the incidence related to the volume of the dose rather than to pramlintide. There was an increased incidence of early demise due to subcutaneous masses as a function of dose volume, particularly in males.
>
> The only neoplastic finding related to the dosing procedure was injection site sarcomas. These first appeared during the second year of study. The incidence, especially in males, was related to the dose volume and not to pramlintide. In males, the incidences in the groups administered 0.2 and 0.5 mg/kg were statistically lower than the control groups and the incidence in the 1.2 mg/mL group was not statistically different from controls.

A total of 163 male and 174 female mice died or were killed in extremis during the study. The sponsor reports that within each sex, survival was comparable across all control and treated groups. The sponsor reports survival at Week 96 time of terminal sacrifice for males as 29%, 27%, 45%, 43%, and 37% for Control 1, Control 2, low, medium, and high doses, respectively. That for females at Week 104 was 29%, 29%, 29%, 37%, and 30% for Control 1, Control 2, low, medium, and high doses, respectively. These percentages vary slightly from those of the FDA

Statistical Reviewer *(see page 5)* who performed computerized calculations based on number live at end-of-treatment. In both cases, there was no evidence of mortality showing a relationship to treatment.

Major causes of death are summarized in the sponsor's Table 221 as follows.

Sponsor's AC-0137: Pramlintide 104-week Subcutaneous Oncogenicity Study in the Mouse
Causes of Death

Pramlintide (mg/kg)

	Males [n=162/255]					Females [n=174/255]				
Mouse	**0.0 C1**	**0.0 C2**	**0.2 L**	**0.5 M**	**1.2 H**	**0.0 C1**	**0.0 C2**	**0.2 L**	**0.5 M**	**1.2 H**
# Examined	36	37	28	29	32	36	34	36	32	36
Injection site sarcoma	12	11	1	4	12	2	2	1	0	0
Amyloidosis	4	5	7	5	4	3	1	5	0	4
Urogenital tract lesion	3	4	10	5	4	1	3	3	1	0
Hemolymp. Tumor	1	2	2	3	1	4	8	2	4	5
Neurological lesion *	0	1	0	1	3	4	2	3	7	10

*** No clinical signs, no gross observations, and no microscopic findings recorded in the individual animal data that support this description (most deaths appeared due to mechanical trauma to the spinal cord).**

The sponsor reports that the most frequent clinical observation was sores on the backs of both the control and treated mice, primarily at the injection site. The higher dosing volume (12 mL/kg) of vehicle administered per dose to mice in the control and high dose groups was determined to be associated with the higher mean incidence of sores at the injection sites for these groups than for the low and medium dose groups.

Although mean bodyweight gains were higher during the first 28 weeks of study, bodyweight changes and food consumption appeared to be unaffected by treatment in all dose groups and sexes. There were no treatment related clinical signs observed.

> Body weights and gains and food consumption intervals were analyzed using analysis of variance. Pair wise comparisons with combined controls used the Dunnett's test. A regression test was performed to determine linear relationship between increasing dose and response. Survival probability functions were estimated by the Kaplan-Meier technique. Survival curves were compared by the log-rank procedure to the start of the terminal kill phases (Week 105 for females and Week 97 for males).

FDA Statistical Reviewer's Analysis - Mouse: All analyses used the Carcinogenicity Evaluation System established for the Office of Biostatistics in CDER at FDA, and followed the statistical methodology defined for that system. The survival data analysis used the methods described in the papers of Cox (Regression Models and Life Tables, Journal of the Royal Statistical Society, B, 34, 187-220, 1972), and of Gehan (A Generalized Wilcoxon Test for Comparing Arbitrarily Singly Censored Samples, Biometrika, 52, 203-223, 1965). The analysis also applied the death-rate method described in the paper of Peto et al. ('Guidelines for Simple, Sensitive Significance Tests for Carcinogenic Effects in Long Term Animal Experiments' in Long Term and Short Term Screening Assays for Carcinogens: A Critical Appraisal, International Agency for Research on Cancer Monographs, Annex to Supplement 2, World Health Organization, 311-426, 1980). Tumor data analysis used the Peto methods and the method of exact permutation trend test.

Reviewer's Summary of Data
Pramlintide 104-week Subcutaneous Oncogenicity Study in the Mouse

	Males [n=162 / 255]				Females [n=173 / 255]			
104-week Mouse Study	0.0 C1+2	0.2 L	0.5 M	1.2 H	0.0 C1+2	0.2 L	0.5 M	1.2 H
# Animals in study	102	51	51	51	102	51	51	51
# Live end-of-treatment - males (week 95) - females (week 103)	29	23	22	19	32	15	19	16
Survival rates end-of-treatment - males (week 95) - females (week 103)	28.4	45.1	43.1	37.3	31.4	29.4	37.3	31.4
Mortality during treatment	73	28	29	32	70	36	32	35
Mortality rate during treatment	71.6	54.9	56.9	62.7	69.6	70.6	62.7	68.6
Survival (weeks 0-52)	93	46	45	45	90	48	42	46
Survival rate (weeks 0-52)	91.2	90.2	88.2	88.2	88.2	94.1	82.4	90.2
Survival rate (weeks 0-91)	38.2	45.1	47.1	47.1	57.8	54.9	47.1	52.9
Mortality (weeks 0-52)	9	5	6	6	12	3	9	5
Mortality (weeks 53-78)	24	7	8	14	17	8	14	11
Mortality (weeks 79-91)	30	16	13	7	14	12	4	8
Mortality (end-of-treatment) - males (weeks 92-95) - females (weeks 92-103)	10	0	2	5	27	13	5	11
Mortality Terminal Sacrifice - males (weeks 96-97) - females (weeks 104-105)	29	23	22	19	32	15	19	16

1. Survival Analysis - Mouse: The National Cancer Institute developed a computer program to perform trend tests and homogeneity tests as described in 'Trend and Homogeneity Analysis of Proportions and Life Table Data' by Thomas, Breslow, and Gart. The program was incorporated into the Carcinogenicity Evaluation System established for the Office of Biostatistics in CDER at FDA. Actual times and numbers of animals that die at the individual times (if there are two or more that die at a time point) are entered into the Thomas-Breslow-Gart NCI program. The NCI program performs both trend and homogeneity tests.

Intercurrent mortality rates for both male and female mice *(See Attachment # 2 on page 16)* were tested for linear trend according to time intervals 0-52; 53-78; 79-91; and 92-95 (males); and 92-103 (females). The results of the test for males and females show insufficient evidence to declare a significant trend in the intercurrent mortality rate (p-value of 0.6749 for males and 0.8052 for females) at the 0.05 level.

The Cox and Kruskal-Wallis tests (also known as generalized Wilcoxon test) were used to test for homogeneity of survival distributions of all dose groups, including Control (separately for males and females). There was insufficient evidence to declare statistically significant differences in survival distribution for males with a Cox test p-value of 0.2933 at the 0.05 level. There was also insufficient evidence to declare statistically significant differences for females, for which the Cox test for life table data shows a p-value of 0.9958.

The Kruskal-Wallis (generalized Wilcoxon test) gives more weight to early differences in death rates between groups than the Cox analysis. These also resulted in insufficient evidence to declare statistically significant differences in survival distributions for both male and female mice, with p-values of 0.4594 and 0.7168 for males and females, respectively.

Plots of Kaplan-Meier estimates illustrate survival distributions of the Control and treated groups for male and female mice *(Attachments 3M and 3F on pages 17-18).*

2. Tumor Data Analysis - Mouse: To test the positive linear trend in tumor rates, the analysis of the FDA/CDER/OB Carcinogenicity Evaluation System uses an extension of the Fisher exact test referred to as the method of exact permutation trend test. The analysis uses 3 definitions for a tumor's relation to cause of death. Using Peto et al. (1980), the 'tumor-caused death' followed Peto's 'death-rate method', whereas the second and third definitions of 'non-tumor-caused death' and 'unknown' followed the 'prevalence method'. This FDA Statistical Reviewer's analysis used time intervals of 0-52; 53-78; 79-91; 92-95 weeks; and terminal sacrifice (96-97) for males, and 0-52; 53-78; 79-91; 92-103 weeks; and terminal sacrifice (104-105) for females.

Not all animals in each dose group were microscopically examined. Only those animals in the Control and high dose group were sacrificed following end of treatment period, with the exception of tissues at injection sites which were examined for all animals. Microscopic

examinations of tissue were also performed for animals killed or dying during the study, and if macroscopic examination reports revealed tissues with significant abnormalities.

Because of this imbalance in the number of animals examined per dose group, and consequently, the number of tumors detected and reported, the FDA Reviewer's tumor data analysis includes a time-adjusted exact permutation dose-trend test only for tumor types at injection sites. Then a pairwise comparison between pooled controls and high dose group per sex was performed.

To adjust for multiplicity, this FDA Statistical Reviewer followed a standard decision rule in regard to the effect of multiple testings on inflating the overall false positive rate. A positive linear trend is considered not to occur by chance of variation alone if the p-value is less than 0.005 for common tumors, and less than 0.025 for rare tumors. A positive pairwise comparison is considered not to occur by chance of variation alone if the p-value is less than 0.01 for common tumors, and less than 0.05 for rare tumors with spontaneous tumor rate of 1% or less.

Using this adjustment, the analysis determined exact p-values when time-intervals for both of the following situations of lethality did not overlap: (1) tumors either fatal or non-fatal to all animals, and (2) tumors fatal only to some but not to all animals. In cases when the total number of tumor-bearing animals across all treatment groups is small, p-values from the asymptotic test will generally underestimate the true p-value and will be unstable, and instead the exact test should be used. The exact test is done using the Permutation test with general scores, which are the actual dose values.

There were no statistically significant linear trends for any injection site tumor types in male and female mice (exact p-values for males, 0.239 and for females, 0.952). Using a pairwise comparison between incidence rates of tumors in pooled controls and the 1.2 mg/kg/day high dose group, no statistically significant differences were found (exact p-values for males ranged from 0.310 to 0.996, and for females from 0.293 to 0.996).

3. Validity of Experiment - Mouse: The validity of the experiment depends on sufficient numbers of animals being exposed to drug/chemical over an adequate time period so as to be at risk of forming late-developing tumors. Some experts in the field have suggested that between weeks 80-90, a 50 percent survival rate of the 50 initial animals in each treatment and sex can be considered as a sufficient number at adequate exposure [proposed by Haseman and modified by the Division of Biometrics, CDER/FDA]. If the number of animals in each treatment group and sex group is less than or greater than 50, the percentage can vary. However, there should be 20 to 30 animals remaining live during these weeks. Additionally, Chu, Ceuto, and Ward ['Factors in the Evaluation of 200 National Cancer Institute Carcinogen Bioassays', Journal of Toxicology and Environmental Health, 8, 1981, pp. 251-280] propose that for studies in which there is no evidence of carcinogenic effect of the chemical/drug, animals in the high dose group should have greater than 50 percent survival at one year (52 weeks) into study.

The survival rates of male mice at weeks 80-91 were 38.2% to 47.1% and for females, 47.1% to 57.8%; the number remaining live during these weeks was 23 to 39 for males and 24 to 59 for females; and at one year (52 weeks) into study were 88.2% to 91.2% for males and 82.4% to 94.1% for females. Therefore, the study meets the above criteria for sufficient numbers of animals exposed over an adequate time period.

The validity of the experiment also depends on the administration of a large enough drug dose so as to present a tumor challenge to the animals. The same paper by Chu, Ceuto, and Ward identifies dose adequacy according to:

(1) 'A dose is considered adequate if there is a detectable loss in weight gain of up to 10% in a dosed group relative to the control';
(2) 'The administered dose is also considered a Maximum Tolerated Dose (MTD) if dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical';
(3) 'In addition, doses are considered adequate if the dosed animals show a slightly increased mortality compared to the controls'.

The sponsor reported that although mean bodyweight gains were higher during the first 28 weeks of study, bodyweight changes and food consumption appeared to be unaffected by treatment in all dose groups and sexes. Therefore, the first criteria was not met. Also, because there were no treatment related clinical signs observed, the MTD does not appear to have been met since the were no clinical signs or severe histopathologic toxic effects attributed to the chemical. Furthermore, the third criteria was not met since dosed male animals had lower mortality (54.9% to 62.7%) compared to the pooled controls (71.6%).

III. AC-0137 104-week Subcutaneous Oncogenicity Study in the Rat

(Section 5.5.4.2 and 5.8.3.1.3.2, pharmtox/tox/98109/98109.pdf)

The sponsor's study report REST98109 is dated May 30, 2000. The protocol is dated October, 1994 and the experiment was conducted [redacted] Treatment began November, 1994. Last necropsy was completed November, 1996.

This study included male and female Sprague-Dawley [redacted] rats. Animals were not more than 8 weeks of age at start of dosing. Male rats ranged in weight between 195 and 283 grams. Females were 148 to 254 grams.

The study began with more male and female animals than what was planned for randomization into the experiment. This included an additional 10 rats of each sex randomly selected for health check purposes, then killed within hours of arrival, and examined macroscopically. The remaining animals were then randomly assigned to dose groups of Control 1, Control 2, 0.04,

0.2, and 0.5 mg/kg pramlintide administered subcutaneously each day for 104 weeks (50 per sex and dose group). They were caged in groups of 5 by sex.

After a 3-week period of acclimating to the laboratory environment, dosing began at volumes of 0.1 mg/mL and 0.4 mL/kg/day for the 0.04 mg/kg/day low dose group; 0.2 mg/mL and 1 mL/kg/day for the 0.2 mg/kg/day mid dose group; 0.2 mg/mL and 2.5 mL/kg/day for 0.5 mg/kg/day high dose group; and 2.5 mL/kg for placebo.

Each rat was weighed before treatment on the first day of dosing, weekly for the first 16 weeks, once every 4 weeks thereafter, and before necropsy. Food consumption per cage of rats was calculated as grams per animal per week. This was determined weekly for the first 16 weeks and then 1 out of every 4 weeks thereafter.

On a daily basis, detailed observations of clinical signs were done for evidence of reaction to treatment and for ill health. Throughout the study, animals were examined twice daily to identify dead or moribund animals. At weekly intervals, all animals were given detailed physical examination and were palpated to aid the detection of superficial swellings and tissue masses.

Any debilitated animals judged to be *in extremis* were sacrificed, examined macroscopically, and sampled for designated tissues. Interim sacrifices were also performed on humane grounds to prevent cannibalism, unnecessary or prolonged suffering, or autolysis. Sacrifices included complete necropsy, which involved dissection of organs and histopathological examination of separate tissue samples. Injection sites were examined from all rats.

Microscopic examinations of tissue were performed for all animals of 2 Control groups and the high dose group (1.2 mg/kg/day) following the scheduled treatment period, and for all animals killed or dying during the study. Microscopic examination was also performed if macroscopic examination reports revealed tissues with significant abnormalities (gross lesions). The sponsor reports that injection sites, skin/appendage histiocytic sarcoma, and pituitary gland tumors were also examined microscopically for animals in all treatment groups.

Surviving male rats were sacrificed at the scheduled time of Weeks 104-105. Due to high number of deaths in this sex group, female rats were terminated early at Weeks 101-102.

Sponsor's Analysis - Rat: All treatment comparisons were for treated groups against combined control groups. The sponsor reported finding no statistically significant relationship between pramlintide dose and survival and no adverse treatment-related effect on causes of morbidity and mortality. There was no evidence that pramlintide was tumorigenic in rats when administered subcutaneously.

> There were no changes in total or differential white cell count. The proportion of high-dose male decedents with pituitary tumors (a common rat lesion) was statistically elevated ($p < 0.001$). Transient red extremities were noted during the first few weeks of study. There were no statistical differences in either sex in body weight and food consumption. Fascitis/fibrosis were noted in all groups at the injection sites, increasing with dose volume and not with pramlintide dose. In the control groups of both sexes, the sites of vehicle injection were characterized by fascitis/fibrosis and edema, with hemorrhage, myopathy, dermatitis and dermal fibrosis. The lesions suggest that the vehicle, in and of itself, was an irritant to the subcutaneous tissue in the area of the injection site. There was a high incidence (>80%) of proliferative pituitary lesions (focal hyperplasia/adenoma/carcinoma) in all test and control groups, but with no pattern related to pramlintide dose. Such lesions are common in rats.
>
> The weight of biological evidence (tumor size, earliest occurrence, earliest occurrence contributing to demise, incidence relative to historical controls) supported the conclusion that pramlintide did not contribute to the incidence of proliferative pituitary lesions.

A total of 165 male and 163 female rats died or were killed in extremis during the study. The sponsor reports that within each sex, survival was comparable across all control and treated groups. Survival at the time of terminal sacrifice for males was 40%, 34%, 32%, 40%, and 24% for Control 1, Control 2, low, medium, and high doses, respectively. That for females was 38%, 36%, 30%, 36%, and 34% for Control 1, Control 2, low, medium, and high doses, respectively. These percentages vary slightly from those of the FDA Statistical Reviewer *(see page 11)* who performed computerized calculations based on number live at end-of-treatment. In both cases, there was no evidence of mortality showing a relationship to treatment.

The sponsor reports no observed treatment related clinical signs, other than the incidence of microscopic non-neoplastic findings at the site of injection. Because the findings were less severe in low and medium dose groups, it was deemed related to volume of test drug formulation. Bodyweight changes and food consumption appeared to be unaffected by treatment in all dose groups and sexes.

> 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.
>
> Group mean body weight gains for male and female animals in the high dose group were slightly lower than those of the control animals over the Week 28 to Week 52 period. These continued to be slightly reduced in male animals until the end of study. The group mean body weight gains of high dose females appeared to recover during the second half of study. There was no consistent pattern of variation in body weight data to indicate an effect of treatment in the lower dose groups.

All treatment comparisons were for treated groups against combined control groups. The sponsor reported finding no statistical relationship between pramlintide dose and survival and no adverse treatment-related effect on causes of morbidity and mortality. There was no evidence that pramlintide was tumorigenic in rats when administered subcutaneously.

FDA Statistical Reviewer's Analysis - Rat: The survival data analysis used the methods referenced in this Reviewer's Analysis for the mouse carcinogenicity study *(See page 04)*.

Reviewer's Summary of Data
Pramlintide 104-week Subcutaneous Oncogenicity Study in the Rat

	Males [n=165 / 250]				Females [n=162 / 250]			
104-week Rat Study	0.0 C1+2	0.04 L	0.2 M	0.5 H	0.0 C1+2	0.04 L	0.2 M	0.5 H
# Animals in study	100	50	50	50	100	50	50	50
# Live end-of-treatment - males (week 104) - females (week 100)	37	16	20	12	37	15	18	17
Survival rates end-of-treatment - males (week 104) - females (week 100)	38.2	33.3	45.1	25.5	39.2	31.4	37.3	35.3
Mortality during treatment	63	34	30	38	63	35	32	33
Mortality rate during treatment	61.8	66.7	54.9	74.5	60.8	68.6	62.7	64.7
Survival (weeks 0-52)	93	46	45	45	100	49	50	50
Survival rate (weeks 0-52)	92.2	96.1	98.0	96.1	98.0	96.1	98.0	98.0
Survival rate (weeks 0-91)	54.9	66.7	60.8	47.1	50.0	43.1	47.1	52.9
Mortality (weeks 0-52)	8	2	1	2	2	2	1	1
Mortality (weeks 53-78)	16	8	6	13	24	14	14	7
Mortality (weeks 79-91)	22	7	13	12	25	13	12	16
Mortality (end-of-treatment) - males (weeks 92-103) - females (weeks 92-100)	63	34	28	38	11	6	5	9
Mortality Terminal Sacrifice - males (weeks 104-106) - females (weeks 92-100)	37	16	22	12	38	15	18	17

1. Survival Analysis - Rat: Intercurrent mortality rates for both male and female rats *(See Attachment 5 on page 20)* were tested for linear trend using time intervals 0-52, 53-78, 79-91, and 92-103, 104-105 for male rats and 92-100, 101-102 for female rats. The results show insufficient evidence to declare a significant linear trend in the intercurrent mortality rates (p-values of 0.1233 for males and 0.8105 for females) at the 0.05 level.

The Cox and Kruskal-Wallis tests (generalized Wilcoxon test) were used to test for homogeneity of survival distributions of all dose group (separately for males and females). There was insufficient evidence to declare statistically significant differences in survival distribution for males with a Cox test p-value of 0.1217 at the 0.05 level. There was also insufficient evidence to declare statistically significant differences for females, for which the Cox test for life table data shows a p-value of 0.7690. The Kruskal-Wallis test also resulted in insufficient evidence to declare statistically significant differences in survival distributions for both male and female rats, with p-values of 0.0921 and 0.6472 for males and females, respectively.

Plots of Kaplan-Meier estimates illustrate survival distributions of the Control and treated groups for male and female rats *(Attachments 3M and 3F on pages 21-22)*.

2. Tumor Data Analysis - Rat: Time intervals for testing the positive linear trend in tumor rates were set at weeks 0-52, 53-78, 79-91, 92-103, and terminal sacrifice (weeks 104-105) for males. They were weeks 0-52, 53-78, 79-91, 92-100, and weeks 101-102 for females.

Only those animals in the Control and high dose group were microscopically examined following end of treatment. Additionally, all injection site tissues, skin/appendage histiocytic sarcoma, and pituitary gland tumors from animals in all treatment groups were examined microscopically. Tissues for animals killed or dying during the study were also examined. If macroscopic examination reports revealed tissues with significant abnormalities, these, too, were examined.

Because of this imbalance in the number of animals examined per dose group, and consequently, the number of tumors detected and reported, the FDA Statistical Reviewer's tumor data analysis includes a time-adjusted exact permutation dose-trend test only for tumor types at injection sites, skin/appendage histiocytic sarcoma, and pituitary gland tumors. Then a pairwise comparison between pooled controls and high dose group per sex was performed. Multiplicity adjustments followed the standard decision rule described in the analysis of the mouse study *(see page 07)*.

In male rats, there was a statistically significant linear trend for pituitary B-adenoma tumor types (exact p-value < 0.001 with a spontaneous tumor rate of 53% among the pooled controls for this common tumor in rats). While the incidence of skin and subcutis M-sarcoma at injection sites was notable, there was no statistically significant trend for this tumor type (exact p-value = 0.021 tested at the 0.005 significance level with a spontaneous tumor rate of 7% among the controls). The incidence of skin and subcutis B-fibrolipoma at injection sites had a marginally significant

dose-tumor positive linear trend (exact p-value of 0.034 tested at the 0.025 significance level with a spontaneous tumor rate of less than 1% in the controls).

Pairwise comparisons of incidence rates of tumors between the combined controls and the 0.5 mg/kg/day high dose group were then performed. There was a statistically significant difference found in male rats for pituitary B-adenoma (exact p-value 0.002 tested at the 0.01 significance level for pairwise comparisons with a spontaneous tumor rate of 53% among controls). No statistically significant differences were found in female rats.

Male Rats # fatal tumors (%)	Controls Pooled	Low Dose	Medium Dose	High Dose
Pituitary B-adenoma	53 (53%)	28 (56%)	33 (66%)	36 (72%)
Skin & Subcutis M-sarcoma - Injection Site	7 (7%)	1 (2%)	0 (0%)	7 (14%)
Skin & Subcutis B-fibrolipoma - Injection Site	0 (0%)	0 (0%)	0 (0%)	2 (4%)

3. Validity of Experiment - Rat: The validity of the experiment depends on sufficient numbers of animals being exposed to drug/chemical over an adequate time period so as to be at risk of forming late-developing tumors, as is explained in the Reviewer's Analysis of the mouse carcinogenicity study *(Refer to Validity of Experiment, pages 07-08).*

The survival rates of high dose rats at weeks 80-91 were 47.1% for males and 52.9% for females (the very same rates as for mice); the number of animals remaining live during these weeks was 24 to 56 for males and 22 to 51 for females; and at one year (52 weeks) into study were 92.2% to 98.0% for males and 96.1% to 98.0% for females. Therefore, the study meets the criteria for sufficient numbers of animals exposed over an adequate time period.

The sponsor reported that bodyweight changes and food consumption appeared to be unaffected by treatment in all dose groups and sexes. Therefore, the first criteria for validity of experiment was not met. Also, because there were no treatment related clinical signs observed (other than the incidence of microscopic non-neoplastic findings at the site of injection), the MTD may not have been met. There were relatively few clinical signs and the only histopathologic toxic effects attributed to the chemical was tumor incidence in male rats for pituitary B-adenoma, a common tumor type in rats with spontaneous tumor rate of 53% among the control groups.

Sufficient numbers of animals were exposed over an adequate time period, and the criteria for mortality did meet the validity of experiment. Dosed male animals had generally higher mortality (54.9% to 74.5%) compared to the pooled controls (61.8%), and dosed female rats also had higher mortality (62.7% to 68.6%) compared to controls (60.8%).

IV. Reviewer s Conclusion

Tumor Data Analysis: Even though the experimental validity of the mouse and rat studies was not confirmed, this examination of tumor data was consistent with the sponsor's results. Given the sample size of 50 animals per sex and dose group, the only statistically significant linear trend in tumor incidence was pituitary B-adenoma in male rats. Only this tumor type in male rats showed sufficient evidence to declare a statistically significant difference in tumor incidence between Control and high dose group. No other tumor type was found to be statistically significant for either species and sex.

Survival Analysis: Mice: Results on intercurrent mortality rates for both male and female mice show insufficient evidence to declare a significant trend in the intercurrent mortality rates (p-value of 0.6749 for males and 0.8052 for females) at the 0.05 level. There was insufficient evidence to declare statistically significant differences in survival distribution (Cox test p-value of 0.2933 for males and 0.9958 for females, and Kruskal-Wallis generalized Wilcoxon test p-value of 0.4594 for males and 0.7168 for females) at the 0.05 level.

Rats: Results on intercurrent mortality rates for both male and female rats show insufficient evidence to declare a significant linear trend in the intercurrent mortality rates (p-values of 0.1233 for males and 0.8105 for females) at the 0.05 level. There was insufficient evidence to declare statistically significant differences in survival distribution (Cox test p-value of 0.1217 for males and 0.7690 for females, and Kruskal-Wallis generalized Wilcoxon test p-value of 0.0921 for males and 0.6472 for females) at the 0.05 level.

Lillian Patrician, MS, MBA
Mathematical Statistician

Concur: J. Todd Sahlroot, Ph.D.
Statistician Team Leader

Karl Lin, Ph.D.
Expert Mathematical Statistician
(Applications in Pharmacology/Toxicology)

cc: Original NDA 21-332
HFD-510/Division Files
HFD-510/RMisbin/FAlavi/JRhee
HFD-715/SENevius/JTSahlroot/LPatrician/KLin
Chron.

This review has twenty-two [22] pages including six [06] attachments.

Attachment # 1

AC-0137 104-week Subcutaneous Oncogenicity Study in the Mouse

Study Design

Male Mouse

	Controls Pooled	0.2 mg/g/day	0.5 mg/g/day	1.2 mg/g/day	Total
	# dead	# dead	# dead	# dead	# dead
Time (weeks)					
0-52	9	5	6	6	26
53-78	24	7	8	14	53
79-91	30	16	13	7	66
92-95	10	0	2	5	17
96-97	29	23	22	19	93
Total	102	51	51	51	255

Female Mouse

	Controls Pooled	0.2 mg/g/day	0.5 mg/g/day	1.2 mg/g/day	Total
	# dead	# dead	# dead	# dead	# dead
Time (weeks)					
0-52	12	3	9	5	29
53-78	17	8	14	11	50
79-91	14	12	4	8	38
92-103	27	13	5	11	56
104-105	32	15	19	16	82
Total	102	51	51	51	255

Attachment # 2

AC-0137 104-week Subcutaneous Oncogenicity Study in the Mouse

Intercurrent Mortality Rates

Male Mouse

	Controls Pooled			0.2 mg/g/day			0.5 mg/g/day			1.2 mg/g/day		
	# Dead	# at risk	Cum % dead	# Dead	# at risk	Cum % dead	# Dead	# at risk	Cum % dead	# Dead	# at risk	Cum % dead
Time (weeks)												
0-52	9	102	8.8	5	51	9.8	6	51	11.8	6	51	11.8
53-78	24	93	32.4	7	46	23.5	8	45	27.5	14	45	39.2
79-91	30	69	61.8	16	39	54.9	13	37	52.9	7	31	52.9
92-95	10	39	71.6	0	23	54.9	2	24	56.9	5	24	62.7
96-97 Terminal Sacrifice	29	102	28.4	23	51	45.1	22	51	43.1	19	51	37.3

Female Mouse

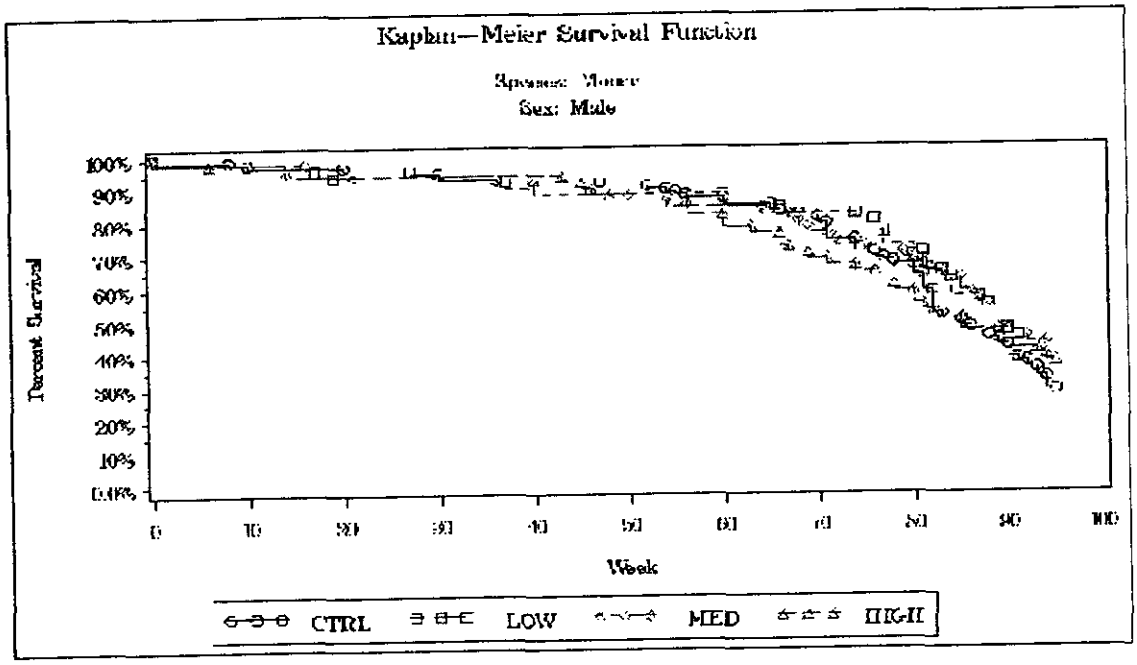
	Controls Pooled			0.2 mg/g/day			0.5 mg/g/day			1.2 mg/g/day		
	# Dead	# at risk	Cum % dead	# Dead	# at risk	Cum % dead	# Dead	# at risk	Cum % dead	# Dead	# at risk	Cum % dead
Time (weeks)												
0-52	12	102	11.8	3	51	5.9	9	51	17.6	5	51	9.8
53-78	17	90	28.4	8	48	21.6	14	42	45.1	11	46	31.4
79-91	14	73	42.2	12	40	45.1	4	28	52.9	8	35	47.1
92-103	27	59	68.6	13	28	70.6	5	24	62.7	11	27	68.6
104-105 Terminal Sacrifice	32	102	31.4	15	51	29.4	19	51	37.3	16	51	31.4

Attachment # 3M

AC-0137 104-week Subcutaneous Oncogenicity Study in the Mouse

Kaplan-Meier Survival Function

Male Mouse

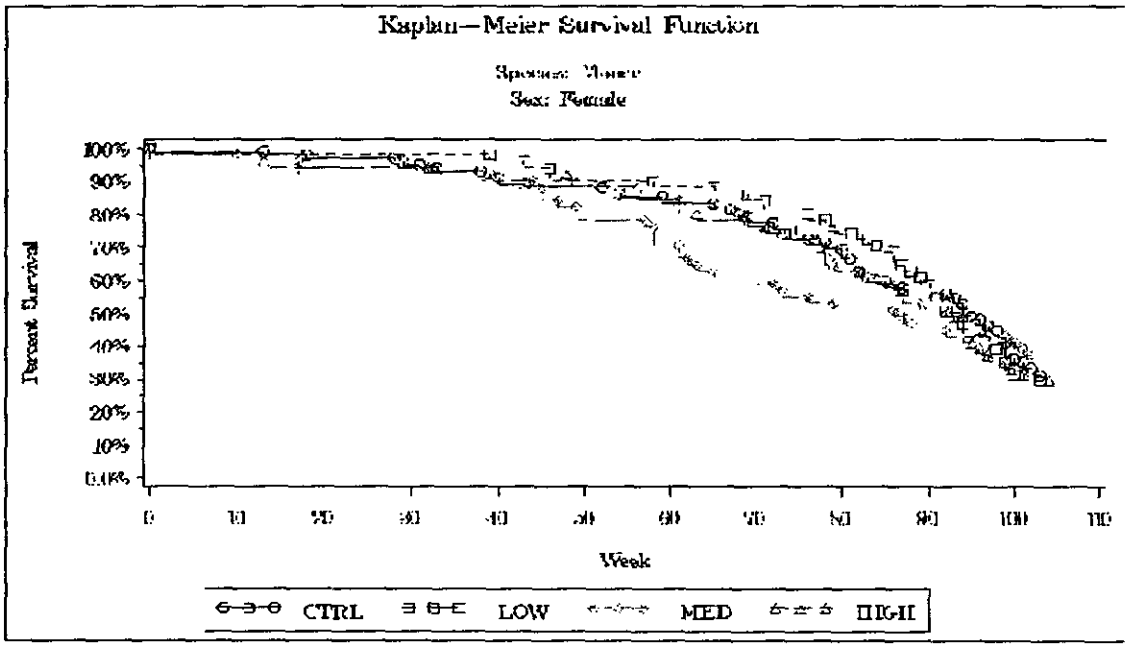


Attachment # 3F

AC-0137 104-week Subcutaneous Oncogenicity Study in the Mouse

Kaplan-Meier Survival Function

Female Mouse



Attachment # 4

AC-0137 104-week Subcutaneous Oncogenicity Study in the Rat

Study Design

Male Rat

	Controls Pooled	0.04 mg/g/day	0.2 mg/g/day	0.5 mg/g/day	Total
	# dead	# dead	# dead	# dead	# dead
Time (weeks)					
0-52	8	2	1	2	13
53-78	16	8	6	13	43
79-91	22	7	13	12	54
92-103	17	17	8	11	53
104-105	37	16	22	12	87
Total	100	50	50	50	250

Female Rat

	Controls Pooled	0.2 mg/g/day	0.5 mg/g/day	1.2 mg/g/day	Total
	# dead	# dead	# dead	# dead	# dead
Time (weeks)					
0-52	2	2	1	1	6
53-78	24	14	14	7	59
79-91	25	13	12	16	66
92-100	11	6	5	9	31
101-102	38	15	18	17	88
Total	100	50	50	50	250

Attachment # 5

AC-0137 104-week Subcutaneous Oncogenicity Study in the Rat

Intercurrent Mortality Rates

Male Rat

	Controls Pooled			0.04 mg/g/day			0.2 mg/g/day			0.5 mg/g/day		
	# Dead	# at risk	Cum % dead	# Dead	# at risk	Cum % dead	# Dead	# at risk	Cum % dead	# Dead	# at risk	Cum % dead
Time (weeks)												
0-52	8	100	8.0	2	50	4.0	1	50	2.0	2	50	4.0
53-78	16	92	24.0	8	48	20.0	6	49	14.0	13	48	30.0
79-91	22	76	46.0	7	40	34.0	13	43	40.0	12	35	54.0
92-103	17	54	63.0	17	33	68.0	8	30	56.0	11	23	76.0
104-105 Terminal Sacrifice	37	100	37.0	16	50	32.0	22	50	44.0	12	50	24.0

Female Rat

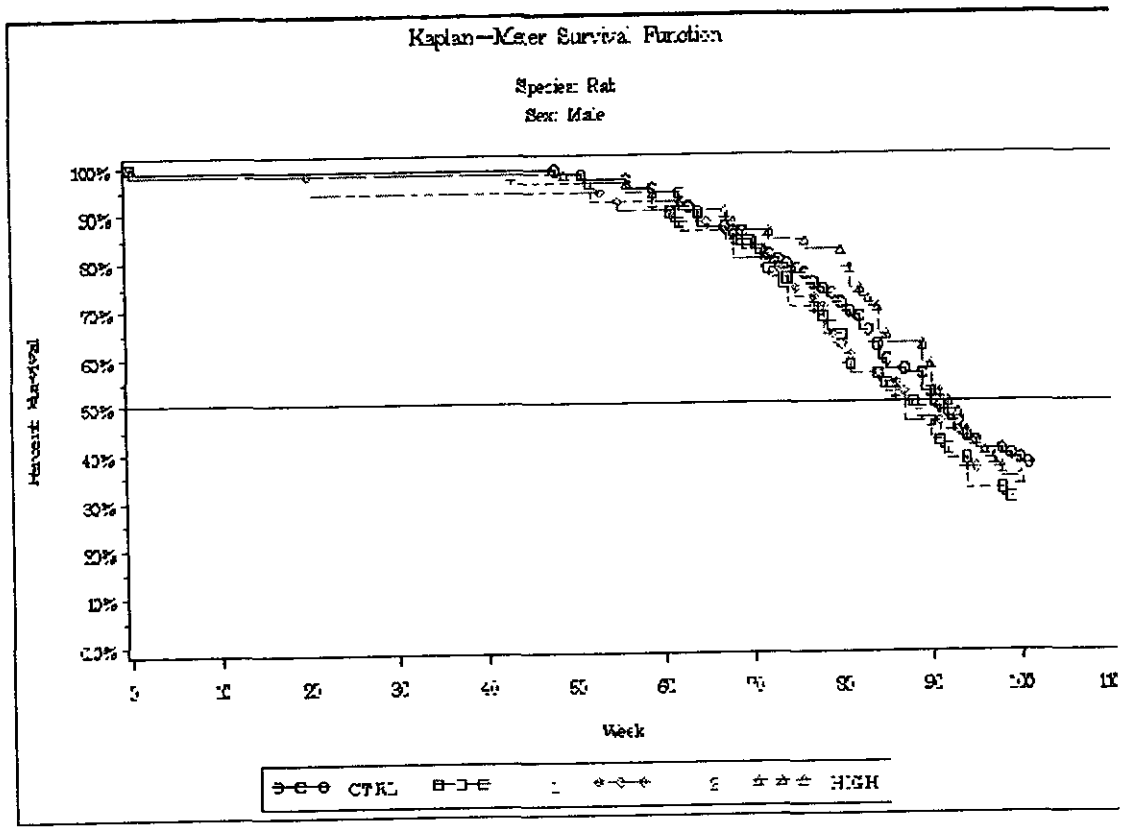
	Controls Pooled			0.04 mg/g/day			0.2 mg/g/day			0.5 mg/g/day		
	# Dead	# at risk	Cum % dead	# Dead	# at risk	Cum % dead	# Dead	# at risk	Cum % dead	# Dead	# at risk	Cum % dead
Time (weeks)												
0-52	2	100	2.0	2	50	4.0	1	50	2.0	1	50	2.0
53-78	24	98	26.0	14	48	32.0	14	49	30.0	7	49	16.0
79-91	25	74	51.0	13	34	58.0	12	35	54.0	16	42	48.0
92-100	11	49	62.0	6	21	70.0	5	23	64.0	9	26	66.0
101-102 Terminal Sacrifice	38	100	38.0	15	50	30.0	18	50	36.0	17	50	34.0

Attachment # 6M

AC-0137 104-week Subcutaneous Oncogenicity Study in the Rat

Kaplan-Meier Survival Function

Male Rat

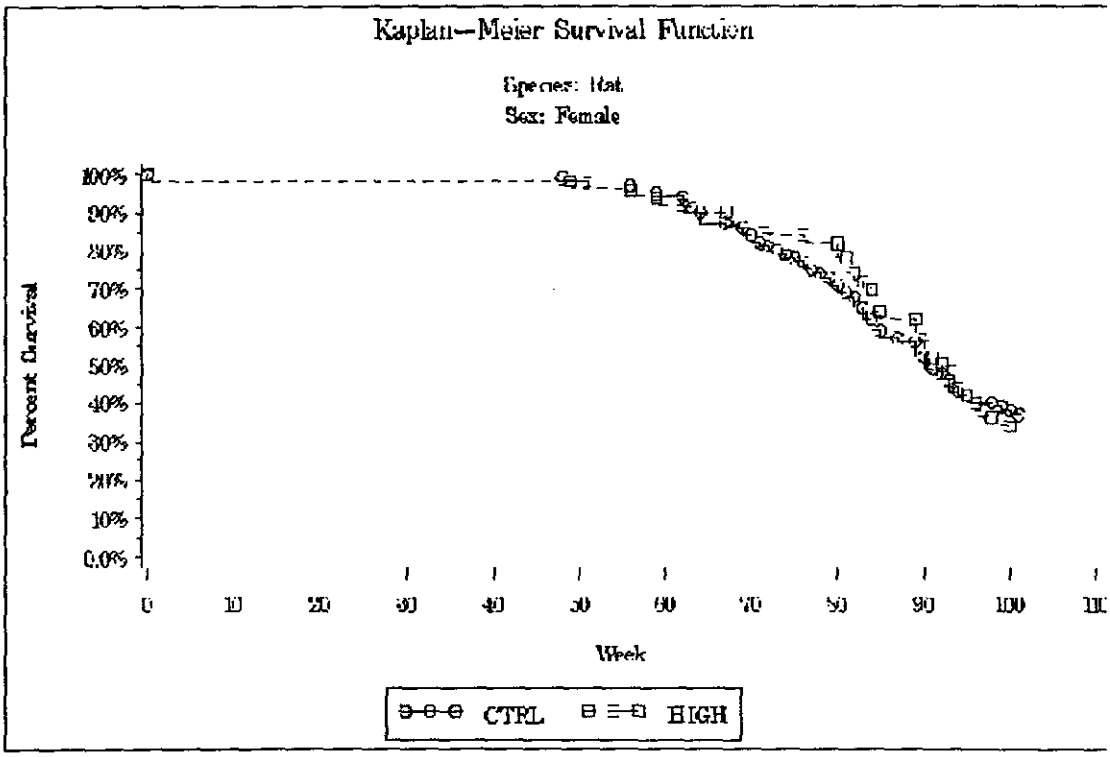


Attachment # 6F

AC-0137 104-week Subcutaneous Oncogenicity Study in the Rat

Kaplan-Meier Survival Function

Female Rat



This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Lillian Patrician
7/3/01 02:03:42 PM
BIOMETRICS
Paper print of this review has been signed by L Patrician, JT Sahlroot , & K Lin
Todd: You, Karl, and I have physically signed the paper printout of this final review version. The .pdf is now entered into DFS waiting for your electronic signature. Thanks in advance. Lillian

Todd Sahlroot
7/6/01 10:14:25 AM
BIOMETRICS

Karl Lin
7/9/01 09:26:49 AM
BIOMETRICS

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

REQUEST FOR CONSULTATION

TO (Division/Office): Todd Sahlroot, Ph.D., HFD-713			FROM: Julie Rhee, HFD-510	
DATE January 3, 2001	IND NO.	NDA NO. 21-332	TYPE OF DOCUMENT New NDA	DATE OF DOCUMENT December 8, 2000
NAME OF DRUG Symlin™ (pramlintide acetate)	PRIORITY CONSIDERATION		CLASSIFICATION OF DRUG	DESIRED COMPLETION DATE June 29, 2001

NAME OF FIRM: Amylin Pharmaceutical

REASON FOR REQUEST

I. GENERAL

- ☐ NEW PROTOCOL
- ☐ PROGRESS REPORT
- ☐ NEW CORRESPONDENCE
- ☐ DRUG ADVERTISING
- ☐ ADVERSE REACTION REPORT
- ☐ MANUFACTURING CHANGE/ADDITION
- ☐ MEETING PLANNED BY
- ☐ PRE--NDA MEETING
- ☐ END OF PHASE II MEETING
- ☐ RESUBMISSION
- ☐ SAFETY/EFFICACY
- ☐ PAPER NDA
- ☐ CONTROL SUPPLEMENT
- ☐ RESPONSE TO DEFICIENCY LETTER
- ☐ FINAL PRINTED LABELING
- ☐ LABELING REVISION
- ☐ ORIGINAL NEW CORRESPONDENCE
- ☐ FORMULATIVE REVIEW
- ☐ OTHER *(SPECIFY BELOW)*:

II. BIOMETRICS

STATISTICAL EVALUATION BRANCH	STATISTICAL APPLICATION BRANCH
☐ TYPE A OR B NDA REVIEW ☐ END OF PHASE II MEETING ☐ CONTROLLED STUDIES ☐ PROTOCOL REVIEW ☐ OTHER (SPECIFY BELOW):	☐ CHEMISTRY REVIEW ☐ PHARMACOLOGY ☐ BIOPHARMACEUTICS ☐ OTHER (SPECIFY BELOW):

III. BIOPHARMACEUTICS

- ☐ DISSOLUTION
- ☐ BIOAVAILABILTY STUDIES
- ☐ PHASE IV STUDIES
- ☐ DEFICIENCY LETTER RESPONSE
- ☐ PROTOCOL-BIOPHARMACEUTICS
- ☐ IN-VIVO WAIVER REQUEST

IV. DRUG EXPERIENCE

- ☐ PHASE IV SURVEILLANCE/EPIDEMIOLOGY PROTOCOL
- ☐ DRUG USE e.g. POPULATION EXPOSURE, ASSOCIATED DIAGNOSES
- ☐ CASE REPORTS OF SPECIFIC REACTIONS (List below)
- ☐ COMPARATIVE RISK ASSESSMENT ON GENERIC DRUG GROUP
- ☐ REVIEW OF MARKETING EXPERIENCE, DRUG USE AND SAFETY
- ☐ SUMMARY OF ADVERSE EXPERIENCE
- ☐ POISON RISK ANALYSIS

V. SCIENTIFIC INVESTIGATIONS

☐ CLINICAL	☐ PRECLINICAL

COMMENTS/SPECIAL INSTRUCTIONS:

Todd,

This consult request is for a statistical review of Symlin's carcinogenicity data. The data has been electronically submitted and is available thru network. The network path is \\CDSESUB1\N21332\N_000\2000-12-07\Pharmtox\datasets\98108 & 98109.

Within 98108 and 98109, there are 4 files each: the data definition table (define.pdf) and 3 SAS data files (food.xpt, tumor.xpt & weights.xpt). Thank you.

SIGNATURE OF REQUESTER	METHOD OF DELIVERY (Check one) ☐ MAIL ☐ HAND
SIGNATURE OF RECEIVER	SIGNATURE OF DELIVERER

/s/

Julie Rhee
1/3/01 11:43:00 AM

Executive CAC
Date of Meeting: Oct 30, 2001

Committee: Joseph DeGeorge, Ph.D., HFD-24, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Karen Davis-Bruno, Ph.D. HFD-510, Alternate Member
Jeri ElHage, HFD-510, Team leader
Fred Alavi, HFD-510, Presenting Reviewer

Author of Draft: Fred K. Alavi, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 21-332
Drug Name: Symlin
Sponsor: Amylin Pharmaceuticals, Inc.

Background:
The sponsor had submitted a two year bioassay for mouse (0, 0, 0.2, 0.5 and 1.2 mg/kg/day) and rat (0, 0, 0.04, 0.2 and 0.5 mg/kg/day) as part of NDA application.

The mouse carcinogenicity dose selection was based on AUC exposures. With the maximum dose of Symlin in type 1 and type 2 diabetic patients being 90 µg QID and 120 µg TID, the high dose in mouse provided AUC ratios 159 and 275 fold greater than the maximum human dose in type 1 and type 2 diabetic patients, respectively. Majority of the injection site showed evidence of chronic inflammation manifested by dermatitis, panniculitis /myopathy and fibrosis. The most notable tumor finding was increased incidence of sarcomas at the injection sites particularly in control and high dose males. There were no drug-related neoplastic findings.

The sponsor also based the rat carcinogenicity dose selection on AUC exposure ratios. The high dose in rat provided AUC exposures 20 and 34 fold greater than the maximum human dose in type 1 (90 µg QID) and type 2 (120 µg TID) diabetic patients, respectively. Since AUC ratios did not exceed 25-30 fold in type 1 diabetics use of AUC ratios for dose selection is not appropriate for the rat. However, the decrement in body weight at the end of the 2-year rat bioassay was 15% and 17% in the HD males and females relative to controls. The decreases in body weight suggest that 0.5 mg/kg/day is the maximum tolerated dose in rats. The histological findings at the injection sites were similar to mouse. The most notable neoplastic findings were injection site sarcomas in control and high dose rats and increased incidence of pituitary tumors in males. In female rats, there was an apparent dose related increase in the incidence of uterine polyps 1/50 (Cont1), 4/50 (Cont2), 1/50 (low dose), 4/50 (mid dose) and 8/50 (high dose). The polyps as percentage of affected animals were 2.5% for combined controls, 2%, 8% and 16% for LD, MD and HD rats, respectively. The incidence of benign uterine polyps from 5 to 10 contemporary studies in control Sprague-Dawley rats was requested from the sponsor. The median incidence of benign uterine polyp from 7 contemporary studies from [redacted] between 1993 and 1997 was 5, suggesting that incidence of uterine polyp in symlin treated rats

was within the historical control range. The incidence of pituitary tumors in males was also dose-dependent and statistically significant ($p<0.001$) in the high dose males; however, the incidence was within the historical range.

Executive CAC Recommendations and Conclusions:

Mouse Dose Selection:

- The Committee agreed that the mouse study was adequate and provided no evidence of carcinogenic potential.

Rat Dose Selection:

- The Committee agreed that the rat study was adequate based on the use of maximum tolerated dose established by decrements in body weight rather than AUC ratios.
- The Committee agreed that the rat study provided no evidence of carcinogenic potential.

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Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\
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/s/

Joseph DeGeorge
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