CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-919

PHARMACOLOGY REVIEW(S)

Memorandum

RE: Pharmacology/Toxicology Review

NDA: 21-919
Sponsor: Amylin Pharmaceuticals, San Diego, CA
Drug: BYETTA (exenatide)

Background: Amylin has submitted an NDA for the use of BYETTA as monotherapy for the treatment of type 2 diabetes mellitus. BYETTA currently has marketing approval for use in patients already receiving metformin, a sulfonylurea, a thiazolidinedione, a sulfonylurea plus metformin, or a thiazolidinedione plus metformin.

Recommendation on approvability: Approval (AP)

Recommendation for nonclinical studies: Additional nonclinical studies were not required to support the change from combination therapy to monotherapy. The nonclinical studies previously reviewed under NDA 21-773 (BYETTA combination therapy) by Dr. John Colerangle are sufficient to support the safety of subcutaneous administration of exenatide to type 2 diabetic patients as monotherapy at the MRHD (10 µg BID). No additional nonclinical studies are required.

Recommendations on labeling: Because new nonclinical information was not submitted in NDA 21-919, the nonclinical-related text located in the "Use in Specific Populations" and "Carcinogenesis, Mutagenesis, Impairment of Fertility" sections of the package insert for NDA 21-919 will be the same as the text contained in the current BYETTA package insert approved under NDA 21-773.

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

/s/

Brian T Hummer
8/27/2008 04:32:47 PM
PHARMACOLOGIST

Karen Davis-Bruno
8/28/2008 01:56:30 PM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-919**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**6/30/04**
DRUG NAME:	(b) (4)
INDICATION:	**Type II Diabetes Mellitus**
SPONSOR:	**Amylin Pharmaceuticals Inc., San Diego, California**
DOCUMENTS REVIEWED:	**Electronic**
REVIEW DIVISION:	**Division of Metabolic and Endocrine Drug Products (HFD-510)**
PHARM/TOX REVIEWER:	**John Colerangle, DVM, Ph.D., DABT.**
PHARM/TOX SUPERVISOR:	**Karen Davis-Bruno, Ph.D.**
DIVISION DIRECTOR:	**David Orloff, MD.**
PROJECT MANAGER:	**Lina Aljuburi, PharmD.**

Date of review submission to Division File System (DFS): April 11, 2005.

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Approval (AP).

B. Recommendation for nonclinical studies: The non-clinical studies reviewed is sufficient to support the safety of subcutaneous administration of exenatide to Type II diabetic patients at the MRHD (10 µg BID). No further studies are required.

C. Recommendations on labeling

Carcinogenesis, Mutagenesis, Impairment of Fertility

In a 104-week carcinogenicity study in rats at doses of 18, 70, 250 µg/kg/day by bolus SC injection, an increased incidence of thyroid C-cell adenomas in females was observed at 250 µg/kg/day at systemic exposures 130 times the human exposure at 20 µg/day based on AUC.

In a 104 week carcinogenicity study in mice at doses of 18, 70, 250 µg/kg/day by bolus SC injection, no evidence of tumors was observed at doses up to 250 µg/kg/day at systemic exposures 12 to 95 times the human exposure at 20 µg/day based on AUC.

Exenatide was not mutagenic or clastogenic with or without metabolic activation in the Ames bacterial mutagenicity assay or chromosomal aberration assay in Chinese hamster lung cells. Exenatide was negative in the *in vivo* mouse micronucleus assay.

In mouse fertility studies with SC doses of 6, 68 and 760 µg/kg/day, males were treated for 4 weeks prior to and throughout mating and females were treated 2 weeks prior to mating throughout mating until gestation day 7. No adverse effect on fertility was observed at 760 µg/kg/day (systemic exposures up to 260 times human exposure at 20 µg/day based on AUC comparisons).

Pregnancy Category C

Exenatide has been shown to cause reduced fetal and neonatal growth and skeletal effects in mice at systemic exposures 3 times human exposure (following a 20 µg/day dose based on AUC comparisons) and in rabbits at systemic exposures 12 times human exposure (after a 20 µg/day dose based on AUC). There are no adequate and well controlled studies in pregnant women. Exenatide should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

In female mice given SC doses of 6, 68, 760 µg/kg/day beginning 2 weeks prior to mating throughout mating until gestation day 7 there were no adverse fetal developmental findings observed at 760 µg/kg/day (systemic exposures up to 260 times human exposure at 20 µg/day based on AUC comparisons). In pregnant mice given SC doses of 6, 68, 460, 760 µg/kg/day from gestation day 6 through 18 (organogenesis) cleft palate (some with holes) and irregular skeletal ossification of rib and skull bones were observed at 6 µg/kg/day (systemic exposures 3 times human exposure at 20 µg/day based on AUC comparisons).

In pregnant rabbits given SC doses of 0.2, 2, 22, 156, 260 µg/kg/day from gestation day 6 through 18 (organogenesis) irregular skeletal ossifications were observed at 2 µg/kg/day (systemic exposures 12 times human exposure at 20 µg/day based on AUC comparisons).

In pregnant mice given SC doses of 6, 68, 760 µg/kg/day from gestation day 6 through lactation day 20 (weaning) an increased number of neonates were found dead postpartum days 2-4 in dams given 6 µg/kg/day (systemic exposures 3 times human exposure at 20 µg/day based on AUC comparisons).

Nursing Mothers
It is not known whether exenatide is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for clinically significant adverse reactions in nursing infants from exenatide a decision should be made whether to discontinue producing milk for consumption or discontinue the drug, taking into account the importance of the drug to the lactating woman. Studies in lactating mice have demonstrated that exenatide is secreted into breast milk at levels up to 2.5% higher than obtained in the plasma following subcutaneous dosing.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Exenatide is a DPP IV (dipeptidylpeptidase IV) resistant synthetic peptide which extends its duration of action relative to mammalian GLP-1 (7-36) amide. Many, but not all activities of Exenatide appear to be mediated by binding and subsequent activation of the GLP-1 receptor. Exenatide acts through binding to the GLP-1 receptor to reduce plasma glucose and lower HbA_{1c}. Long term reductions of HbA_{1c} are seen in diabetic rats. Exenatide decreases fasting glucose concentrations in rat and mouse models of type 2 diabetes and in monkey. Glycemic control occurs in conjunction with the reduction in the rate of plasma glucose (from increased glucose–dependent insulin secretion, improved insulin sensitivity and increased pancreatic β-cell mass) via glucose-dependent reduction in gastric emptying, reduced food consumption, and suppression of inappropriately elevated glucagon. By limiting the rate at which nutrients enter the GI tract, exenatide attenuates postprandial glucose elevations. Chronic administration of exenatide to normal glycemic mice rats and monkeys at systemic exposure multiples of 400X, 100X and 450X respectively the clinical dose of 20 µg/day did not produce signs of hypoglycemia or related neurological signs or pathology. The effects of reduced food consumption and decreased body weight were noted in the 2-year rat (not mouse) carcinogenicity study where this contributed to the increased survival compared to the heavier control rats. Gravid mice and rabbits were particularly sensitive to the decreased food consumption and subsequent weight loss with exenatide in the reproductive toxicity evaluations.

Exenatide exposures increase with dose (linear kinetics except for pregnant rabbits) but do not show dose limiting accumulation. Metabolism occurs by proteolytic cleavage into progressively smaller peptides to amino acids predominately in the renal tubules. Elimination occurs by renal excretion. Less than 3% of the administered dose crosses the placenta in rats, mice, rabbits (0-0.025) and ex vivo human placenta (0.008-0.017). Exenatide is minimally secreted (2.5%) into milk from mice. Developmental effects observed include delayed fetal/neonatal growth, peri- and neonatal mortality in the absence of maternal toxicity and at higher doses adverse maternal food consumption and body weight. The pregnant rabbit is very sensitive to exenatide toxicity as a function of the greater than dose proportional exposure following repeated SC dosing. Water consumption is dramatically reduced in these animals and it is possible that decreased renal clearance of exenatide (and metabolism) may be contributors to this elevated exposure.

The drug substance and product were tested by BID SC injection in mice, rats and monkeys chronically. Injection site changes consisted of those changes expected from repeated SC injection (inflammation, hemorrhage, fibrosis, epithelial hyperplasia minimal to slight). To compare lots of exenatide drug substance with impurities across three different manufacturers; 28-day toxicity studies in mice were performed. Genetic toxicity studies (Ames, chromosomal aberration) with these lots were also unremarkable. Heat inactivated exenatide was tested in a 28-day mouse toxicity using representative batches from all three manufacturing sources to assess degradent toxicity. No difference in target organ toxicity was observed with the different batches. However some detectable anti-exenatide antibodies were observed.

Exenatide is a synthetic peptide of the lizard (Gila Monster) proexendin gene therefore its antigenicity in rodents and monkeys is not unexpected. Anti-exendin antibodies at titers ≥ 1:125 in monkey resulted in altered pharmacokinetics but were not considered neutralizing based on the observed pharmacologic activity (reduced body weight). The sponsor has suggested that the alteration in pharmacokinetics reflects decreased renal filtration due to antibody binding which results in decreased renal clearance the primary metabolic/excretory pathway of exenatide. Anti-exenatide antibodies were observed as early as one month dosing in monkey.

Toxicity studies using drug lots from different manufacturing sources (Star, Bachem, Mallincrodt) were tested in 28 day mouse studies with no difference in toxicologic profile. However 2/20 mice given the Star drug lot showed very low anti-exenatide antibodies (titer 1:25). Heat degraded exenatide from these three manufacturers also demonstrated no differences compared to the untreated exenatide. Only 1 mouse showed any positive anti-exenatide titers (1:5) albeit very low. Interestingly it appears that the Star manufactured drug substance lots are the only ones showing a positive antibody response and these are also the drug lots used for the subchronic and chronic toxicity studies some of which show positive antibody titers.

Species	Rat	Mouse	Monkey
Body Weight	↓	↑ F (3 Mo) - (6 Mo)	↓
Food consumption	↓	↑	↓
Parotid Salivary gland basophilia	+	+	Mononuclear infiltrate +
Pancreas islet cell hyperplasia	lymphocytic infiltration +	-	+
Anti-exenatide antibody Titer ≥ 1:25	- + (titer 1:5) 1-2/8 MD, HD	-	+
Injection site inflammation	+	+	+

The significance of the parotid salivary gland basophilia in rodents and mononuclear infiltration in monkey is unclear. Historically this is a tissue not routinely sampled in toxicology studies. Basophils are related to mast cells and have phagocytic activity.
Pancreatic islet cell hypercellularity is noted in monkeys. Exenatide including the natural form exendin-4 from lizard and GLP-1 and its analogues have been shown to increase β-cell mass in vitro and in vivo. However this does not appear to be a preneoplastic lesion based on the lack of tumorigenicity observed in the carcinogenicity evaluation.

Exenatide did not show a mutagenic or clastogenic potential with or without metabolic activation in *in vitro* Ames or chromosomal aberration assay in CHO cells or *in vivo* in the mouse micronucleus assay.

Lifetime carcinogenicity evaluations in rats and mice demonstrate increased thyroid C-cell adenomas in female rats at exposures 130X the clinical dose of 20 µg/day. Mice did not demonstrate a tumorigenic potential.

Exenatide treatment during organogenesis results in impaired fetal/neonatal growth and skeletal effects at exposures 3X MRHD.

B. Pharmacologic activity

(b) (4) (exenatide) is an incretin mimetic that mimics several glucoregulatory actions of the endogenous incretin, GLP-1 both in vitro and in vivo including decreased fasting and post-prandial glucose. The actions of exenatide are partially mediated through binding to the human pancreatic GLP-1 receptor, leading to the glucose-dependent enhancement of both synthesis and secretion of insulin from pancreatic beta cells via a cyclic AMP-dependent mechanism and β-cell proliferation. Actions of exenatide noted in vivo include sustained improvement in beta-cell function. Glucose control is also enhanced via suppression of inappropriately elevated glucagon secretion, slowing of gastric emptying, and reduction in food intake with accompanying weight loss. Decreased glycosylated HbA_{1c} is observed.

C. Nonclinical safety issues relevant to clinical use

Injection site inflammatory, hemorrhagic, fibrotic, exudative and degenerative changes were observed across species. Parotid gland basophilia of unclear relevance was observed in the rat (5X MRHD) and mouse (10X MRHD). In monkeys, there was a treatment-related increase in percentage of animals that tested positive for anti-exenatide antibodies suggesting that the drug may be antigenic to monkey. 5% of control animals tested positive for anti-AC2993 antibodies compared to 38%, 25% and 50% for the 0.6, 6.7 or 75 µg/kg BID groups respectively. The positive finding in some control animals (which may be due to contamination or background error) undermines the accuracy of this study. However, NOAEL for anti-exenatide antibodies is < 0.6 µg/kg BID (< 6X MRHD). Teratologic finding that occurred at maternal NOAEL (3 µg/kg/d BID = 3X MRHD) in mice during organogenesis were cleft palate with/without holes and delayed ossification of ribs and skull (interfrontal) bone. In pregnant rabbits irregular skeletal ossifications were also seen with treatment during organogenesis. In pregnant mice treated from organogenesis through weaning, neonatal death was observed post-partum days 2 to 4.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

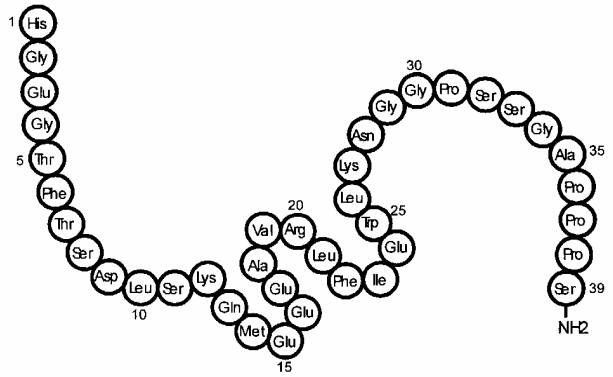
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-773 (IND 57,725).
Review number: 1.
Sequence number/date/type of submission: 000/July 2, 2004/ Commercial NDA.
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Amylin Pharmaceuticals, Inc; 9373 Towne Centre Drive; San Diego, CA 92121.
Manufacturer for drug substance: Bachem, 3132 Kashiwa St., Torrance, CA 90505; Mallinckrodt Inc., 675 McDonnel Boulevard, St. Louis, MO 63134; and Star Biochemical, 20910 Higgins Court, Torrance, CA 90501.

Reviewer name: John Colerangle, DVM, Ph.D., DABT.
Division name: Division of Metabolic and Endocrine Drug Products (DMEDP).
HFD#: 510
Review completion date: March 1, 2005.

Drug:

Trade name: (b) (4) Byetta (International trade name).
Generic name: Exenatide/Exendin-4.
Code name: AC2993, AC2993A, AC002993, LY2148568.
Chemical name: L-histidylglycyl-L-glutamylglycyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-aspartyl-L-luecyl-L-seryl-L-lysyl-L-glutaminyl-L-methionyl-L-glutamyl-L-glutamyl-L-glutamyl-L-alanyl-L-valyl-L-arginyl-L-leucyl-L-phenylalanyl-L-isoleucyl-L-glutamyl-L-tryptophanyl-L-leucyl-L-lysyl-L-asparaginylglycylglycyl-L-prolyl-L-seryl-L-serylglycyl-L-alanyl-L-prolyl-L-prolyl-L-prolyl-L-serinamide, hydrate (IUPAC)
CAS Registry Number: 141732-76-5
Molecular Formula/ Molecular Weight: $C_{184}H_{282}N_{50}O_{60}SCXH_2O$, where x is variable. MW for anhydrous: 4186.6
Structure:



Relevant INDs/NDAs/DMFs: IND 57,725, (b) (4), DMF 17227, DMF 17114.

Drug Class: Synthetic peptide, antihypoglycemic, incretin mimetic. Exenatide was originally isolated from the saliva of the Gila monster. The amino acid in the central region has 8 amino acids common to

GLP-1 (7-36) amide. These amoni acids lie in the same face of the α-helix suggesting a common active binding site. However, exendin-4 is not a GLP-1 analogue.
Indication: Type II diabetes.

Clinical formulation: There are two strengths (0.3 and 1.0 mg/ml, GR434R01 and GR344R01, respectively). Each is a 1 ml single dose, sterile formulation in 30mM acetate buffer pH 4.5 with mannitol added as an iso-osmolality modifier.

Route of administration: Subcutaneous Injection.

Disclaimer: Some tables, graphs and text were taken from sponsor's submission.
Studies reviewed within this submission:
Single Dose Toxicity Studies
An IV toxicity study in mouse.
A single and a rising dose toxicity study in rats by subcutaneous injection.
A rising dose subcutaneous toxicity study in monkey.

Repeat Dose Toxicity Studies
Rat
14 days IV toxicity study in rat.
28 days subcutaneous toxicity study in rat.
91 days subcutaneous toxicity study in rat.

Mouse
28 days subcutaneous (BID) toxicity study in mouse.
91 days subcutaneous (BID) toxicity study in mouse.
91 days subcutaneous (QD) toxicity study in mouse.
182 days subcutaneous (BID) toxicity study in mouse.

Monkey
5 days subcutaneous (QD) toxicity study in monkey.
28 days subcutaneous (QD) toxicity study in monkey.
91 days subcutaneous (BID) toxicity study in monkey.
273 days subcutaneous (BID) toxicity study in monkey.

Genetic Toxicology Studies
Salmonella-E. coli reverse mutation assay.
Salmonella-E. coli reverse mutation assay (Bachem).
Salmonella-E. coli reverse mutation assay (Mallinckrodt).
Chromosome aberration in Chinese Hamster Ovary cells.
Chromosome aberration in Chinese Hamster Ovary cells (Bachem).
Chromosome aberration in Chinese Hamster Ovary cells (Mallinckrodt).
In vivo mouse micronucleus assay.

Carcinogenicity Studies
104 weeks study in rats.
96 weeks (females) and 98 weeks (males) studies in mouse.

Reproductive Toxicology Studies
Fertility and general reproductive toxicology study in mouse.
Embryo-fetal development study in mouse.
Embryo-fetal development study in rabbit.
Comparative evaluation of the effects on normal development and growth of embryo and fetus in rabbits at doses that cause depression in food consumption and matched pair-fed animals.

The toxicokinetics of AC2993 and pharmacodynamics of plasma glucose in pregnant rabbits.
Perinatal and postnatal development study in mouse.

Special Toxicology Studies
Neutralizing anti-exenatide antibody production in NIH Swiss mice.
Effects of Anti-AC2993 antibodies on toxicokinetics, body weight changes and histological change in pancreas of Cynomolgus monkeys administered AC2993 BID by subcutaneous injection for 9 Months.
Antigenicity of exenatide in mice, rats and monkeys.
28-Day toxicity evaluation of heat-degraded AC2993 in CD-1 mice administered subcutaneously twice daily.

Studies not reviewed within this submission: The toxicokinetic studies conducted separately were reviewed with their respective toxicology studies.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Nonclinical pharmacology studies have shown that exenatide and GLP-1 bind to and stimulate GLP-1 receptors equipotently, as demonstrated by the production of cyclic adenosine monophosphate (cAMP) in human- and rat-based receptor systems. Receptor activation with exenatide occurred only while the ligand was present within the incubation media suggesting that the extended duration of pharmacologic action of exenatide in vivo compared to GLP-1 relates to an increased plasma half life. Distribution of binding sites within mouse tissue for exenatide is similar to that observed for GLP-1, with most notable binding in the lateral septum and basal forebrain, as well as within circumventricular organs including the *area postrema*. Significant binding was also observed in the pancreas and the outer cortex of the kidney. Within the pancreas, binding densities of exenatide were focally distributed within the islets of Langerhans, presenting a functional target for circulating concentrations of exenatide to act to effect the secretion of insulin.

Anti-diabetic actions thought to be mediated via GLP-1 receptor occur at 0.1-1 μg/kg exenatide in various species. These primary pharmacodynamic actions of exenatide in animals are listed as follows:

- Decreased fasting and postprandial glucose
- Decreased glycosylated hemoglobin (HbA_{1c})
- Stimulation of insulin secretion
- Suppression of glucagon secretion
- Increased insulin sensitivity
- Slowing of gastric emptying
- Neogenesis of pancreatic islets
- Suppression of food intake and weight loss

In vitro studies revealed that exenatide has a direct action on isolated pancreatic rat islets to stimulate release of insulin, and the results are consistent with published reports confirming a glucose-dependent insulinotropic action. Studies in the perfused rat pancreas showed that exenatide potentiates both first- and second-phase insulin secretion, further suggesting that the glucose dependence of the insulinotropic effect may reside, in part, at the level of the pancreas. A parallel study in this system showed that the exenatide-induced suppression of glucagon was still present when both insulin and somatostatin (endogenous suppressors of glucagon secretion) were removed from the system, suggesting an additional direct effect at the level of the α-cell.

In vivo, exenatide improves fasting and postprandial plasma glucose concentrations through multiple mechanisms of action. In nondiabetic animals, exenatide administration potently and dose dependently

reduced plasma glucose concentrations by up to 37% in mice and 35% in rabbits but not in rats. In contrast, a paradoxical increase in plasma glucose levels in response to exenatide in nondiabetic rats appears to be mediated by release of catecholamines. Exenatide did not increase either glucose or lactate in adrenalectomized rats or in rats treated with the β-adrenoceptor blocker propranolol. Using the *db/db* and *ob/ob* mouse models of type 2 diabetes, treatment with exenatide resulted in dose-dependent reductions in plasma glucose concentrations. The maximum plasma glucose reductions achieved were 37% and 30% in *db/db* and *ob/ob* mice, respectively. In diabetic (ZDF) rats, subchronic treatment with exenatide (5 to 8 weeks) prevented the onset of diabetes as reflected by lower HbA1c concentrations. While published in vitro studies examining direct tissue actions of exenatide to promote glucose uptake remain equivocal (studies in isolated muscle, fat and liver preparations), 6-week treatment with exenatide in diabetic ZDF rats and in obese, glucose-intolerant (*fa/fa*) rats resulted in a robust improvement in insulin sensitivity, partly independent of reduced body weight. The acute administration of exenatide to diabetic rhesus monkeys resulted in a dose-dependent reduction in plasma glucose of up to 41%. Several studies examining the glucose-lowering action of exenatide in diabetic animals suggest that this action is glucose-dependent in nature as evidenced by a more potent glucose-lowering action when glucose is high.

Exenatide may contribute to the maintenance of islet cell mass and function, through complementary actions to stimulate islet cell proliferation and neogenesis. In vitro studies report a direct action of exenatide to stimulate β-cell neogenesis from putative pancreatic endocrine "stem cells" in primary cultures of pancreatic precursor cells, primary islet cultures, and pancreatic cell lines. To further enhance β-cell mass, exenatide has been reported to suppress apoptosis of β-cells via a protein kinase A-mechanism, decreased activation of caspases, and increased expression of the anti-apoptotic gene Bcl-2. In nine animal studies reported so far, the improvement in glycemic control achieved with exenatide occurred in conjunction with enhanced islet function and increased islet mass. Other studies in animals suggest that additional metabolic conditions, such as hyperglycemia, may be required to promote islet neogenesis following activation of GLP-1 receptor signaling. Together, these findings indicate that exenatide acts to enhance and sustain the appropriate β-cell mass required to maintain normal glucose control (glucose-dependent stimulation of islet neogenesis) and support the concept that exenatide therapy may improve disease states characterized by β-cell deficiency. (It should be noted that no exenatide-related neoplastic lesions were found in pancreas of mice and rats in the 2-year carcinogenicity evaluation.)

Exenatide produced a potent action to slow gastric emptying in rats in a dose-related manner. Studies in rats have shown that there is a reversal of this action of exenatide during hypoglycemia, suggesting that this effect is glucose-dependent. Exenatide thereby regulates the inflow of nutrients into the circulation, contributing to reduced postprandial glucose concentrations.

In nondiabetic mice and rats, acute administration of exenatide dose dependently reduced food intake by up to 75%. A reduction in daily food intake and body weight was seen in both diabetic, fatty Zucker (ZDF) rats and in nondiabetic, obese *fa/fa* rats that received exenatide daily or BID for 6 weeks. These changes were associated with improvements in insulin sensitivity and with reductions in hyperinsulinemia and hyperlipidemia. In an environmental model of diet-induced obesity, expected increases in food intake, body weight, plasma glucose, insulin, and triglycerides were dose dependently decreased with exenatide infusion in high-fat fed mice. At the highest dose of exenatide tested, high-fat, diet-induced changes were completely reversed to (normal) levels observed in lean-fat fed control mice. This is consistent with chronic dosing of CD-1 mice where food consumption and body weights are generally unremarkable. Changes in body weight were due to decreased fat mass without effects on lean body mass. In diet-induced obese rats, exenatide dose dependently reduced body weight gain and caloric intake compared with high-fat fed controls. Plasma concentration-response relationships in rats yielded a plasma EC50 for body weight change after 28 days of treatment of 14.3 pM (59.9 pg/mL).

In conclusion, experimental studies exploring the pharmacology of exenatide support the concept that this incretin mimetic acts through multiple mechanisms to potently and immediately promote lowering of plasma glucose levels and to promote long-term actions to significantly lower HbA1c. Exenatide decreases fasting glucose levels in all animal models of type 2 diabetes assessed to date (rat, mouse, and monkey) and exhibits a durable effect to lower HbA1c levels in diabetic rats. Improvements in glycemic control are achieved via both modulation of the rate of glucose appearance in the circulation (slowing of gastric emptying rate, reduced food intake, suppression of glucagon secretion), and modulation of the rate of glucose clearance from the blood (glucose-dependent insulin secretion, improved insulin sensitivity, increased β-cell mass). Exenatide can thus be expected to have therapeutic value for the long-term treatment of patients with type 2 diabetes mellitus. The results from glucose-lowering studies in several animal species support an efficacious dosage range of 0.01 to 1 μg/kg BID.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Exenatide mimics several glucoregulatory actions of the endogenous incretin, GLP-1 both in vitro and in vivo. The actions of exenatide are partially mediated through binding to the human pancreatic GLP-1 receptor, leading to the glucose-dependent enhancement of both synthesis and secretion of insulin from pancreatic beta cells via a cyclic AMP-dependent mechanism. Actions of exenatide noted in vivo include sustained improvement in beta-cell function. Glucose control is also enhanced via suppression of inappropriately elevated glucagon secretion, slowing of gastric emptying, and reduction in food intake with accompanying weight loss.

Drug activity related to proposed indication: Exendin-4 is proposed to act through the GLP-1 receptor and to have many of the antidiabetic effects of GLP-1. In normal and diabetic rats and mice, tissue binding distribution is identical to GLP-1 (including brain lateral septum and basal forebrain, *area postrema* and *medulla oblongata*). Significant binding was observed in pancreas and outer cortex of the kidney. There was wide distribution throughout the pancreas, but focal distribution within the Islets of Langerhans. Potency for stimulating cAMP production in human and rat based receptor systems was similar for Exendin-4 and GLP-1. EC_{50} in RINm5f cells (rat insulinoma pancreatic cell line) was 0.31 nM for AC2993 and 0.23 nM for GLP-1. Duration of effect on cAMP production was similar (~ 30 min) for both compounds. AC2993 was ~ 1000 times more potent than GLP-1 in reducing plasma glucose concentration after a single SC dose to hyperglycemic *db/db* mice. Both compounds induced ~30 % maximum decrease in glucose in monkeys or mice but the ED_{50} for GLP-1 was approximately 10,000-fold greater than Exendin-4. The magnitude of glucose lowering effect was related to the pre-existing plasma glucose concentration. This effect has also been well characterized for GLP-1. It is suggested that the glucose lowering ability of GLP-1 is dependent on glucose concentrations in the plasma and thus hypoglycemic effects are not likely to occur. This is a potential advantage of use of GLP-1 or Exendin-4 in treatment of diabetes. Sponsor suggests that the mouse is a better model than rat because blood glucose response in the mouse was more similar to the human response than the response in the rat.

2.6.2.3 Secondary pharmacodynamics

The secondary pharmacological effects of exenatide was examined in studies on the cardiovascular (mouse, rat, monkey), renal (rat, mouse), gastrointestinal exocrine (rat), and endocrine (rat) systems. Exenatide has been shown to produce a dose-dependent increase in mean arterial blood pressure and heart rate in nondiabetic rats. This effect was partially blocked with concomitant antagonism of α-adrenoceptors using phentolamine. The increase in blood pressure diminished subsequent to repeated dosing, suggesting the effect in rats was not relevant to chronic administration of exenatide. There were no findings indicative of chronic hypertension in a 91-day toxicity study or in a 2-year carcinogenicity study in rats, with survival being higher among exenatide-treated groups. While transient increases in blood pressure were observed in rats, no hemodynamic effects were observed with GLP-1 or exenatide administration in mice, dogs, calves, or monkeys. Acute toxicological studies designed to elicit potential cardiodynamic effects of exenatide were performed in non-human primates, and no effect of exenatide

was observed on ECG, arterial pressure, or heart rate at doses up to 10,000 times those proposed for antidiabetic therapy. Also, no effects on cardiovascular performance including QTc interval were observed in a 9-month toxicity study in monkeys. When exenatide was administered acutely to nondiabetic human subjects or to subjects with type 2 diabetes over a 30-fold dose range (0.01 – 0.3 µg/kg), it did not significantly alter blood pressure. When dosed appropriately (5 or 10 µg BID), exenatide did not change blood pressure during chronic administration for up to 12 months in patients with type 2 diabetes. Overall, cardiodynamic effects reported for exenatide in rats are highly species specific. Furthermore, in these long-term controlled clinical studies, no prolongation in QTc interval, as determined from ECG over-reads, was observed. It appears that the cardiovascular effects reported for exenatide in rats are highly species specific.

Exenatide-related effects on the urinary/renal system were evaluated in anesthetized rats. Exenatide produced an acute, profound diuresis (rat and monkey) and exhibited a durable effect to lower HbA1c in diabetic rats. Improvements in glycemic control were achieved via modulation of both the rate of glucose appearance in the circulation (slowing of gastric emptying, reduced food intake, suppression of inappropriately elevated glucagon secretion) and modulation of the rate of glucose clearance (enhanced glucose-dependent insulin secretion, improved insulin sensitivity, increased β-cell mass). Exenatide has been shown to produce an acute, profound diuresis and natriuresis in rats following intravenous doses of ≥0.8 µg/kg. No net effect on potassium excretion was noted; however, calcium excretion was increased after single, IV dosing (≥0.21 µg/rat). Pressure diuresis is one of several mechanisms that might explain this. However studies using a pressure clamp to maintain high renal blood flow in rats indicate that this is not the only operative mechanism (i.e. urine and K^+ are maintained). Similar, albeit less potent, diuretic and natriuretic effects were observed following IV exenatide administration in anesthetized mice. No renal pharmacology studies were performed in monkeys. However, no renal toxicity (pathology, electrolytes) was observed in long-term toxicity studies in mice, rats, and monkeys. Increased water consumption is a consequence of the diuresis seen in rats and to a lesser extent in mice. Water consumption was significantly decreased in pregnant rabbits at ≥ 22 µg/kg contributing to maternal MTD.

Acute administration of exenatide did not affect serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (at approximately 60 µg/kg), or thyroid hormones T3 and T4 (at approximately 0.8 µg/kg) in nondiabetic rats. A recent external study in rats has shown that Subcutaneous (SC) injection of exenatide at 6-120 µg/kg significantly lowered plasma TSH levels for up to 12 hours following injection.

2.6.2.4 Safety Pharmacology

Organ System Evaluated	Species/ Strain	Method of Administration/ Vehicle/Formulation	Doses (µg/kg)	Number and Sex per Group	Noteworthy Findings	GLP	Study Number/ Location
Nervous	Mice/ICR	IV/ saline solution	0, 30, 300, 1500	8-10 M	≥300 µg/kg decreased grip strength, limb tone ≥30 µg/kg transient decreases in spontaneous motor activity	Non-GLP	REST98095, Section 4.2.1.3.1
Cardiovascular	Cynomolgus monkey/ *Macaca fascicularis*	SC/ saline solution	0, 30, 300, 1000	3 M,1 F used at each dose	≤1000 µg/kg no cardiovascular effects ≥30 µg/kg decreases in activity	GLP	REST98100R1, Section 4.2.1.3.2
Cardiovascular	Cynomolgus monkey/ *Macaca fascicularis*	SC BID/ AC-2993-F12 (vehicle)	0, 2.2, 18, 150 µg/kg/day for 273 days	6 M, 6 F	≤150 µg/kg/day no qualitative or quantitative electrocardiographic changes following 9 months dosing[a]	GLP	REST00120R1, Section 4.2.3.2.10

IV = intravenous SC = subcutaneous BID = Dose divided and administered twice daily M = Male F = Female.

[a] Qualitative and quantitative electrocardiographic data obtained as part of 273-day repeat-dose toxicity study. General toxicity data summarized in Section 2.6.7.7.9.

2.6.2.5 Pharmacodynamic drug interactions

Nonclinical studies to evaluate potential drug interactions have not been conducted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Type of Study[a]	Test System	Method of Administration	Testing Facility	Study Number	Location
Primary Pharmacodynamics					
Receptor Pharmacology	RINm5f cell membranes	-	Amylin Pharmaceuticals, Inc.	RESTP98011	4.2.1.1.1
Receptor Pharmacology	RINm5f cell membranes	-	Amylin Pharmaceuticals, Inc.	RESTP98012	4.2.1.1.2
Receptor Pharmacology	RINm5f cell membranes	-	Amylin Pharmaceuticals, Inc.	RESTP98013	4.2.1.1.3
Receptor Pharmacology	RINm5f cells	-	Amylin Pharmaceuticals, Inc.	RESTP98014	4.2.1.1.4
Autoradiography- Brain tissue	Rat; mouse	-	Amylin Pharmaceuticals, Inc.	RESTP98017	4.2.1.1.5
Effects on isolated pancreatic islets	Rat	-	Amylin Pharmaceuticals, Inc.	RESTP98008	4.2.1.1.6
Receptor Pharmacology	RINm5f cells	-	Amylin Pharmaceuticals, Inc.	RESTP98019	4.2.1.1.7
Receptor Pharmacology of putative metabolites	RINm5f cell membranes	-	Amylin Pharmaceuticals, Inc.	RESTT03366	4.2.1.1.31
Effects on adipocytes, soleus muscle, and hepatocytes	Rat	-	Amylin Pharmaceuticals, Inc.	RESTP98018	4.2.1.1.8
Effect on renal function, blood pressure, glucose, and lactate	Rat	IV	Amylin Pharmaceuticals, Inc.	RESTP98009	4.2.1.1.9
Effect of adrenalectomy on glucose/ lactate/blood pressure responses	Rat	IV	Amylin Pharmaceuticals, Inc.	RESTP99039	4.2.1.1.10
Effect of β-adrenergic receptor blockade on glucose and lactate responses	Rat	IV	Amylin Pharmaceuticals, Inc.	RESTT01024	4.2.1.1.11
Effect on plasma glucose	Mouse	IP	Amylin Pharmaceuticals, Inc.	RESTP99013	4.2.1.1.12
Effect on glycemic control	Mouse	SC, IV	Amylin Pharmaceuticals, Inc.	RESTP98107R1	4.2.1.2.3

Type of Study[a]	Test System	Method of Administration	Testing Facility	Study Number	Location
Effect on plasma glucose	Mouse	SC	Amylin Pharmaceuticals, Inc.	RESTT01219	4.2.2.2.1
Effect on plasma glucose	Rabbit	IV, SC	Amylin Pharmaceuticals, Inc.	RESTT01218R1	4.2.2.2.3
Effect on plasma glucose	Mouse	IP	Amylin Pharmaceuticals, Inc.	RESTP98003	4.2.1.1.13
Effect on insulin and glucagon	Mouse	IP	Amylin Pharmaceuticals, Inc.	RESTP99009	4.2.1.1.14
Effect on plasma glucose	Rat	IP	Amylin Pharmaceuticals, Inc.	RESTP98030	4.2.1.1.15
Effect on glycemic control	Rat	SC	Amylin Pharmaceuticals, Inc.	RESTT01185	4.2.1.1.16
Effect on glycemic control	Rat	SC	Amylin Pharmaceuticals, Inc.	RESTT03126	4.2.1.1.17
Effect on glycemic control	Rat	SC	Amylin Pharmaceuticals, Inc.	RESTT02015	4.2.1.1.18
Islet neogenesis - overview	In vitro; rat; mouse	multiple	Amylin Pharmaceuticals, Inc.	RESTT03088	4.2.1.1.19
Islet neogenesis	Rat	SC	Amylin Pharmaceuticals, Inc.	RESTT02273R1	4.2.1.1.20
Insulinotropic actions	Rat	IV	Amylin Pharmaceuticals, Inc.	RESTP99003	4.2.1.1.21
Glucagonostatic actions	Rat	IV	Amylin Pharmaceuticals, Inc.	RESTP98025	4.2.1.1.22
Effect on glucagon	Rat	IV	Amylin Pharmaceuticals, Inc.	RESTP99004	4.2.1.1.23
Effect on glucagon	Rat	IV	Amylin Pharmaceuticals, Inc.	RESTP99016	4.2.1.1.24
Effect on gastric emptying	Rat	SC	Amylin Pharmaceuticals, Inc.	RESTP98029	4.2.1.1.25
Effect on gastric emptying	Rat	IV	Amylin Pharmaceuticals, Inc.	RESTP99011R1	4.2.1.1.26
Effect on food intake	Rat; mouse	IP, ICV	Amylin Pharmaceuticals, Inc.	RESTP98004	4.2.1.1.27
Effect on food/water intake, body weight, and urine output	Rat	IP	Amylin Pharmaceuticals, Inc.	RESTP98005	4.2.1.1.28
Effect on body weight and glycemic control	Mouse	SC	Amylin Pharmaceuticals, Inc.	RESTT02197	4.2.1.1.29
Effect on food intake and body weight	Rat	SC	Amylin Pharmaceuticals, Inc.	RESTT02205	4.2.1.1.30

Type of Study[a]	Test System	Method of Administration	Testing Facility	Study Number	Location
Secondary Pharmacodynamics					
Effect on cardiovascular system and renal function	Rat	IV	Amylin Pharmaceuticals, Inc.	REST98009	4.2.1.1.9
Cardiac inotropic action	Rat	IV, IP	Amylin Pharmaceuticals, Inc.	REST98020	4.2.1.2.1
Effect on cardiovascular system	Rat	IV, IP	Amylin Pharmaceuticals, Inc.	REST98021	4.2.1.2.2
Effect on cardiovascular system	Rat	IV	Amylin Pharmaceuticals, Inc.	REST99039	4.2.1.1.10
Effect on cardiovascular system	Rat	IV	Amylin Pharmaceuticals, Inc.	REST01024	4.2.1.1.11
Effect on cardiovascular system and renal function	Mouse	SC, IV	Amylin Pharmaceuticals, Inc.	REST98107R1	4.2.1.2.3
Effect on cardiovascular system[b]	Monkey	SC	(b) (4)	REST98100R1	4.2.1.3.2
Effect on renal function	Rat	IV	Amylin Pharmaceuticals, Inc.	REST98010	4.2.1.2.4
Effect on renal function	Rat	IV	Amylin Pharmaceuticals, Inc.	REST98015	4.2.1.2.5
Effect on gastric acid secretion	Rat	SC	Amylin Pharmaceuticals, Inc.	REST98022	4.2.1.2.6
Effect on exocrine pancreatic secretion	Rat	SC	Amylin Pharmaceuticals, Inc.	REST98023	4.2.1.2.7
Effect on reproductive hormone secretion	Rat	SC	Amylin Pharmaceuticals, Inc.	REST00192	4.2.1.2.8
Effect on T3/T4 levels	Rat	SC	Amylin Pharmaceuticals, Inc.	REST04015	4.2.1.2.9
Effect of area postrema lesioning on food intake	Rat	SC	Amylin Pharmaceuticals, Inc.	REST01186	4.2.1.2.10
Effect of area postrema lesioning on gastric emptying actions	Rat	SC	Amylin Pharmaceuticals, Inc.	REST02204	4.2.1.2.11

[a] All studies are non-GLP unless noted.
[b] Report contains a GLP-compliance statement.

Type of Study[a]	Test System	Method of Administration	Testing Facility	Study Number	Location
Safety Pharmacology					
Effects on central nervous system	Mice	IV	(b) (4)	REST98095	4.2.1.3.1
Effects on cardiovascular system[b]	Monkey	SC		REST98100R1	4.2.1.3.2
Effects on cardiovascular system[b]	Monkey	SC		REST00120R1	4.2.3.2.10
Effects on toxicokinetics[b]	Monkey	SC		REST01187R1	4.2.3.2.10.2
Effects on toxicokinetics, food/water intake, and body weight[b]	Mouse	SC		REST02325R1	4.2.3.2.3
Effects on toxicokinetics, food/water intake, and body weight[b]	Rat	SC		REST02246R1	4.2.3.2.6
Effect on food/water intake[b]	Rabbit	SC		REST02022	4.2.3.5.4
Pharmacodynamic Drug Interactions	-	-	-	-	-
Other	-	-	-	-	-

[a] All studies are non-GLP unless noted.
[b] Report contains a GLP-compliance statement.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary (This was obtained from the sponsor's summary).

The pharmacokinetics of exenatide was assessed in rats following subcutaneous (SC), intravenous (IV), and intraperitoneal (IP) administration. The pharmacokinetic parameters of exenatide were also assessed in mice, rabbits, and monkeys following single or multiple, SC or IV injection. No consistent differences in the pharmacokinetic parameters for exenatide were observed between male and female mice, rats, and monkeys; therefore, the pharmacokinetic parameters in the pharmacokinetic and the non-clinical toxicokinetic studies were not separated by gender. In general, for SC administration, Cmax and AUC increased in proportion to dose for the mouse, rat, and rabbit (dose ≤20 µg/kg in rabbit) and the Tmax ranged from 0.25 to 1.75 h. The terminal t½ after SC injection was prolonged in the mouse, rat, and rabbit when compared to the terminal t½ after IV injection, suggesting that absorption of exenatide is the rate-limiting factor for determining terminal t½ after SC administration.

The potential for exenatide to cross the placental barrier was assessed in vivo in mice, rats, and rabbits and ex vivo using human placental tissue. The potential of exenatide to cross the placental barrier was low with a maximum fetal to maternal ratio of 0.025. Exenatide used during pregnancy is, therefore, expected to result in limited direct exposure to the fetus. In addition, exenatide was present in the milk of lactating

CD-1 mice at a level of approximately 2.5% of the plasma concentration after SC administration at 760 µg/kg/day; indicating limited excretion of exenatide into milk of lactating mice.

In anesthetized rats, no major in vivo metabolites of exenatide were observed in the plasma following doses up to 20 mg/animal. Additional studies were done to characterize exenatide clearance. The potential metabolic basis for exenatide's longer plasma half-life and pharmacodynamics versus that observed for GLP-1 (a related incretin peptide) was examined by in vitro comparison of exenatide and GLP-1 enzymatic degradation by purified dipeptidyl peptidase IV (DPP-IV). In these studies, exenatide was found to be resistant to proteolytic cleavage by DDP-IV. Since clearance of peptides by renal filtration and metabolism is known to occur, this route of elimination was investigated for exenatide. The dominant role of the kidneys in the clearance of exenatide was further assessed in a renal-ligation model in rats; AUC, Cmax, and t½ all significantly increased and clearance decreased. Analysis of post-administration urine samples from rats failed to reveal significant concentrations of intact exenatide suggesting that reabsorption and proteolytic degradation may occur in the renal tubule after filtration. Metabolism was further evaluated using in vitro assays of kidney membrane preparations from mouse, rat, rabbit, monkey, and human tissue sources, demonstrating the potential for metabolism of intact exenatide within renal tubules. Tubular re-absorption and degradation of exenatide would explain the relative absence of immunoreactive (full length) exenatide in the urine of rats. The potential role of the liver in metabolism and excretion was assessed in models of liver injury including thioacetamide- and D-galactosamine-induced liver injury models of cirrhosis and acute hepatitis, respectively, in rats. These studies demonstrated no significant difference in pharmacokinetic parameters in rat models with either acute or chronic liver injury versus controls. In summary, the pharmacokinetic data suggest that compromised liver function did not alter the clearance of exenatide and that exenatide is eliminated predominantly by the kidney.

2.6.4.2 Methods of Analysis

Analytical assays were developed and validated for the quantitation of exenatide in mouse, rat, rabbit, dog, monkey, and human plasma to support nonclinical and clinical pharmacokinetic studies. The exenatide immunoradiometric assay (IRMA) was used in initial nonclinical studies. The exenatide IRMA was replaced by the exenatide immunoenzymetric assay (IEMA) to increase assay sensitivity. The exenatide IEMA was used for the quantification of exenatide in most nonclinical and clinical studies. In addition to assays to quantify exenatide, an assay was developed and validated to detect the presence of anti-exenatide antibody in specimens from nonclinical and clinical studies. The resulting anti-exenatide enzyme-linked immunosorbent assay (ELISA) provided a means of screening plasma specimens for the presence of anti-exenatide antibodies. A modification of this method was used to evaluate cross-reactivity of anti-exenatide antibodies to endogenous peptides (GLP-1 and glucagon) that have some sequence homology to exenatide.

Detection of Exenatide Concentrations in Plasma by IRMA: The exenatide immunoradiometric assay (IRMA) was developed and validated for the quantitation of exenatide in rat, rabbit, dog, monkey, and human plasma to support initial nonclinical studies. The exenatide IRMA is a (b) (4) assay that uses two monoclonal antibodies. Sponsor stated that the capture antibody EXE4:2-8 is specific for exenatide as it recognizes a C-terminal epitope of exenatide and does not cross-react with GLP-1(7-36) or glucagon. The detecting antibody GLP1:3-3 recognizes the N-terminal epitope on exenatide, GLP-1 (7-36), and glucagons and is ^{125}I-labelled. The assay is specific for exenatide due to the selectivity of the EXE4:2-8 capture antibody. Since both antibodies need to bind to the peptide in order to generate an assay signal, cross-reactivity with other peptides or metabolites is minimized. The lower and upper limits of quantitation for the assay are 62.8 pg/mL and 2512.0 pg/mL exenatide, respectively. Assay accuracy and precision range from 95.5% to 114% and 3 to 17.3% respectively.

Detection of Exenatide Concentrations in Plasma by IEMA: The exenatide immunoenzymetric assay (IEMA), based upon the exenatide IRMA, was developed for increased assay sensitivity and to eliminate the use of radioactive materials required in the IRMA. The methods are very similar, differing primarily in the method of detection and the limits of quantitation. The exenatide IEMA has been used to evaluate specimens for nonclinical and clinical studies.

The exenatide IEMA is a (b) (4) assay using the same two monoclonal antibodies used in the exenatide IRMA but employs fluorescent detection in place of the previous radiometric (^{125}I) method. The capture antibody EXE4:2-8 is specific for exenatide as it recognizes a C-terminal epitope of exenatide and does not cross-react with GLP-1(7-36) or glucagons and is biotinylated. The detecting antibody GLP1:3-3 recognizes the N-terminal epitope on exenatide, GLP-1 (7-36), and glucagon. The assay is specific for exenatide due to the selectivity of the capture antibody. Since both antibodies need to recognize the peptide in order to generate a signal for this assay, cross-reactivity with other peptides or metabolites is minimized. The assay accuracy and precision range from 93.8-110.3% and 8.7-26.7% respectively.

Determination of Anti-Exenatide Antibody in Plasma Specimens by ELISA: An enzyme-linked immunosorbent assay (ELISA) capable of detecting antibodies with affinity for exenatide was developed, validated and used to detect anti-exenatide antibodies in plasma specimens from CD-1 mice, cynomolgus monkeys, or humans. Additionally, the assay was used to detect anti-exenatide antibodies in plasma specimens from rats as a research assay. The ELISA detection reagent detects IgG, IgA, and IgM responses specific to exenatide and therefore allowed detection of multiple immunoglobulin classes.

Sponsor stated that in the anti-exenatide ELISA, plasma specimens were assayed with and without excess soluble exenatide to correct for assay signal not associated with anti-exenatide, anti-GLP-1, or anti-glucagon antibodies (i.e., signal from nonspecific binding). Specimens were diluted 1:5 in two different dilution buffers: in sample diluent and the second in sample diluent containing an excess of exenatide (0.1 mg/ml). Additional serial dilutions were prepared, as needed (1:25, 1:125, 1:625, etc.), to determine a titer. Each diluted sample was added to a microtiter plate with exenatide non-covalently bound to the wells. The samples were incubated to allow adherence of anti-exenatide antibodies present in the plasma to the exenatide-coated wells. Specific antibody binding was ascertained by incubating goat anti-(species of interest) Ig conjugated to horseradish peroxidase followed by signal generation with the addition of the peroxidase substrate *o*-phenylenediamine. The color signal was detected at a wavelength of 490 nm. As a negative control, three different negative plasma samples are assayed in each assay. As positive controls, the reactivity of the goat anti-Ig-horseradish peroxidase conjugate and exenatide binding to the microtiter plate wells were assessed in each assay.

Assay specificity was demonstrated by inhibition of exenatide binding by soluble peptides. Exenatide levels present in the plasma specimen at less than 8 ng/ml do not affect the results. The LLQ = 1 µg/ml for mouse antibodies and 0.5 µg/ml for affinity purified human antibody pool. Insulin (0.1 mg/ml), with no homology to exenatide, did not inhibit exenatide binding to either of the monoclonal antibodies. Soluble GLP-1 and glucagon (0.1 mg/ml) both inhibited exenatide binding to GLP1:3-3, the binding epitope of which is shared by each of the peptides. In contrast, exenatide binding to EXE4:2-8, which is specific to exenatide, was not inhibited by either GLP-1 or glucagon. Therefore, this assay format may detect autoantibodies generated to plasma GLP-1 or glucagon.

Assay for Cross-reactivity of GLP-1 and Glucagon in Plasma Positive for Anti-exenatide Antibody (Cross-reactivity ELISA): The anti-exenatide ELISA established that some patients produce antibodies in response to exenatide treatment. Furthermore, using monoclonal antibodies, it was observed that this assay also detects antibodies that are cross-reactive to common epitope(s) between exenatide, GLP-1 and

glucagon. Therefore, the validated anti-exenatide ELISA was adapted and used to determine if antibody cross-reactivity to GLP-1 or glucagon was present in plasma positive for anti-exenatide antibody.

Sponsor stated that the cross-reactivity ELISA is a validated qualitative assay that compares inhibition of exenatide binding to antibodies in the presence of excess exenatide, GLP-1(7-36) or glucagon to a sample without competitive peptide added. The amount of competitive peptide added, 0.1 mg/ml, is at least 1000-fold excess over physiological levels of GLP-1 and glucagon, which range from 20 to 100 pg/ml. In addition, competitive peptide levels at both 0.1 and 0.5 mg/ml achieved near maximal inhibition. Results are reported using % Inhibition values for the competitive peptide (either GLP-1 or glucagon). Sponsor stated that this assay has the ability to determine cross-reactivity to GLP-1 and glucagon in specimens positive for anti-exenatide antibody at antibody concentrations above 0.5 µg/ml.

The assay is described as follows: Plasma specimens are diluted 1:5 in:

- sample diluent (buffer)
- sample diluent containing an excess of exenatide (0.1 mg/ml)
- sample diluent containing an excess of GLP-1(7-36) (0.1 mg/ml)
- sample diluent containing an excess of glucagon (0.1 mg/ml)

Each diluted sample was then added to the microtiter plate and incubated to allow unbound antibodies present in the plasma to bind with the exenatide coated wells. Exenatide-specific antibody binding was detected by incubating goat anti-human (or goat anti-mouse for the monoclonal controls) Ig conjugated to horseradish peroxidase. The amount of enzyme bound determined the color generation following addition of an *o*-phenylenediamine solution. The resulting signal was measured at 490 nm. The inhibition of ELISA signal when compared between the sample in buffer and the sample with competitive peptide was used to determine the cross-reactivity. In cases where the amount of anti-exenatide antibody in the plasma specimen is very low, the resulting low optical density (OD) values in the competitive peptide assay format may result in a false % Inhibition due to high variability at these low assay response levels. Therefore, results were evaluated in a (b) (4) process to determine if an anti-exenatide antibody positive sample is positive or negative for cross-reactivity.

Anti-exenatide monoclonal antibodies, one specific to exenatide (EXE4:2-8) and one capable of cross-reacting with exenatide, GLP-1 and glucagon (GLP1:3-3), were used to evaluate assay specificity and sensitivity during method validation. The monoclonal antibody GLP1:3-3 was demonstrated to be positive for cross-reactivity with GLP-1 and glucagon at an antibody concentration of 0.5 µg/ml. The monoclonal antibody EXE4:2-8 and purified, pooled human antiexenatide antibody-positive sample showed a positive response specifically to exenatide at antibody concentrations of 1 µg/ml and 10 µg/ml, respectively, and both concentrations were not cross-reactive to GLP-1 or glucagon.

2.6.4.3 Absorption
Pharmacokinetic Parameters in Sprague-Dawley Rats

Pharmacokinetic Parameters for Exenatide in Anesthetized Rats

Route	IV		SC		IP	
Dose[a]	C_0 (nM) (pg/mL)	AUC (nmol•h/L) (pg•h/mL)	C_{max} (nM) (pg/mL)	AUC (nmol•h/L) (pg•h/mL)	C_{max} (nM) (pg/mL)	AUC (nmol•h/L) (pg•h/mL)
0.5 nmol (2 µg)	2.9 (12,139)	0.69 (2888)	0.6 (2512)	1.16 (4856)	0.26 (1088)	0.63 (2637)
5 nmol (21 µg)	70 (293,020)	18 (75,348)	4.1 (17,163)	13 (54,418)	3.9 (16,325)	13.6 (56,930)
50 nmol (210 µg)	645 (2,699,970)	172 (719,992)	28 (117,208)	112 (468,832)	35 (147,766)	128 (535,808)

[a] Dose reported as per animal. Dose/kg would be approximately 6, 60, and 600 µg exenatide/kg.

Single-dose Pharmacokinetic Parameters for Exenatide Determined After IV and SC Administration in Rats

		Route of Administration	
Parameter	Dose/rat	IV (Bolus)	SC
Bioavailability	5 nmol (21 µg) 50 nmol (210 µg)	- -	75± 3 % 65 ± 9 %
T_{max}	0.5 nmol (2 µg) 5 nmol (21 µg) 50 nmol (210 µg)	- - -	0.5 h 0.5 h 0.5 h
C_{max}, SC C_o, IV	0.5 nmol (2 µg) 5 nmol (21 µg) 50 nmol (210 µg)	2.9 ± 0.4 nM (12139 pg/mL) 70 ± 3 nM (293,020 pg/mL) 645 ± 12 nM (2,699,970 pg/mL)	0.6 ± 0.1 nM (2512 pg/mL) 4.1 ± 1.5 nM (17,163 pg/mL) 28 ± 4 nM (117,208 pg/mL)
AUC_{0-6h}	0.5 nmol (2 µg) 5 nmol (21 µg) 50 nmol (210 µg)	0.69 ± 0.08 nmol•h/L (2888 pg•h/mL) 18 ± 0.9 nmol•h/L (75,348 pg•h/mL) 172 ± 5 nmol•h/L (719,992 pg•h/mL)	1.16 ± 0.11 nmol•h/L (4856 pg•h/mL) 13 ± 0.1 nmol•h/L (54,418 pg•h/mL) 112 ± 18 nmol•h/L (468,832 pg•h/mL)
AUC/Dose[a]	0.5 nmol (2 µg) 5 nmol (21 µg) 50 nmol (210 µg)	- - -	809 907 781
Clearance	0.5 nmol (2 µg) 5 nmol (21 µg) 50 nmol (210 µg)	8.3 ± 0.7 mL/min[b] 4.8 ± 0.4 mL/min[b] 3.7 ± 0.5 mL/min[b]	- - -

[a] Dose approximately equivalent to 6, 60, and 600 µg/kg, respectively.
[b] Values obtained from continuous IV infusion.

Exenatide shows rate limiting absorption SC vs. IV.

Protein Binding and Distribution in Blood Cells: Binding of exenatide to human erythrocytes was evaluated at concentrations of 0.0, 0.25, and 2.5 µg/ml using 3.2 ng/ml (^{125}I)Y^{39}-exenatide as a tracer. Approximately 82% of exenatide is associated with the plasma fraction and 18% associated with erythrocytes. Binding of exenatide to serum albumin and other plasma components was not determined.

Summary of Pharmacokinetic Parameters in SD Rats, CD-1 Mice and New Zealand White Rabbits

	Dose	Rat		Mouse		Rabbit	
Route of Administration	(µg/kg)[a]	IV	SC	IV	SC	IV	SC
Bioavailability		-	65-75%	-	-	-	-
T_{max} (h)		-	0.5	-	0.25-0.5	-	0.4-1.75
C_{max} (pg/mL)	2	-	2512	-	-	-	1766
	3.6		-		3468		-
	20		17,163		31,072		13,415
	200		117,208		318,507		340,808
AUC (pg•h/mL)	2	2888	4856	-	-	-	4767
	3.6	-	-		2687		-
	20	75,348	54,418		21,939		55,420
	200	719,992	468,832		228,931		1,331,694
AUC/dose	2	-	809	-	-	-	2383
	3.6		-		746		-
	20		907		1097		2771
	200		781		1145		6658
Terminal $t_{1/2}$		18-41 min	90-216 min	10.1 min[b]	-	43 min[b]	-

[a] Rats were dosed at 2, 21, and 210 µg exenatide/animal, which was approximately equivalent to 6, 60, and 600 µg/kg, respectively.
[b] Calculated from the 20µg/kg exenatide dose.
- Not measured or not applicable.

2.6.4.4 Distribution

Tissue Distribution: Sponsor stated that Exenatide (a 39-amino acid peptide) like other peptides is generally metabolized throughout the body into peptide fragments or individual amino acids. These peptides and amino acids may be redistributed into the general circulation for utilization into newly created peptides and proteins or as an energy resource in the liver. Therefore, evaluation of exenatide whole body distribution was not technically feasible by existing methods. Exenatide binding to human RBCs (0, 0.25, 2.5 µg/ml) resulted in 82% association with plasma proteins and 18% with RBCs.

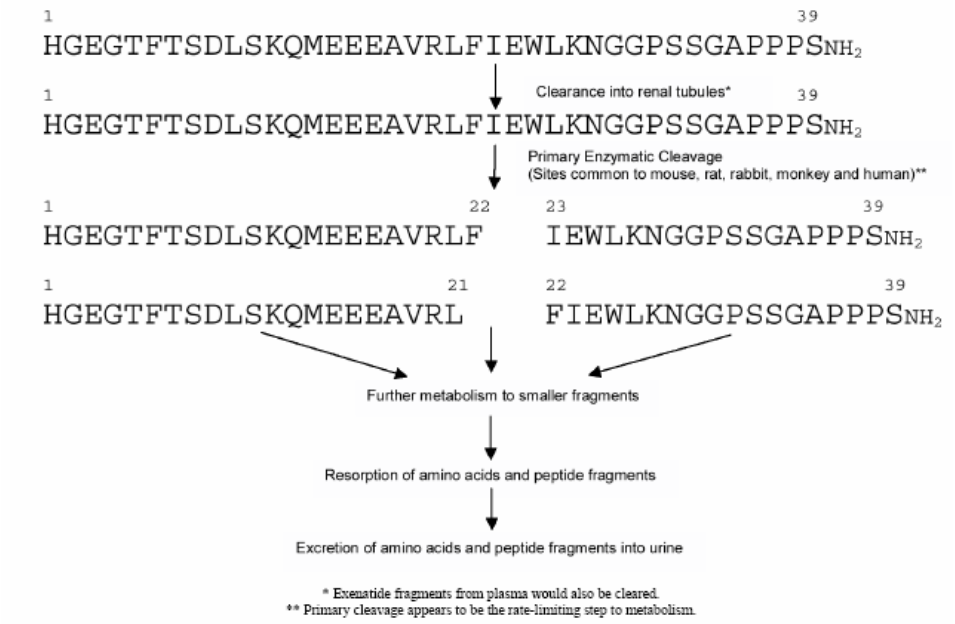
2.6.4.5 Metabolism

Studies have been performed to investigate exenatide degradation in vivo. In general, no significant fragments of exenatide were found in plasma following IV or SC injection in rats. Based upon the rat data, characterization of exenatide metabolites from monkey and human plasma or urine were not performed because at the lower exenatide concentrations resulting from the lower doses used in these species, any metabolite would be expected to be below the limit of detection of the available analytical techniques. Because of this lack of identifiable metabolites in the rat, additional studies were done in vivo and in vitro to characterize how exenatide degradation could occur.

Characterization of In Vivo Exenatide Metabolites Following Intravenous or Subcutaneous Administration Into Anesthetized Rats: Following the start of a 10 min IV infusion of 10 mg exenatide or a SC injection of 20 mg exenatide, plasma was withdrawn at -15, 10, 15, 20, 30, and 60 min. The plasma samples were analyzed by RP-HPLC with UV detection to identify possible exenatide metabolites. The major exenatide-related component for both types of administration was exenatide itself. Sponsor stated that there were several, very minor, peaks in the chromatograms of plasma samples from IV-infused rats that were not present in the control sample: these peaks could not be identified as metabolites of exenatide. Due to low concentrations, there was insufficient material to identify possible

metabolites from the plasma of SC-treated rats. In conclusion exenatide metabolites could not be identified in these high dose studies.

Possible Metabolic Pathways

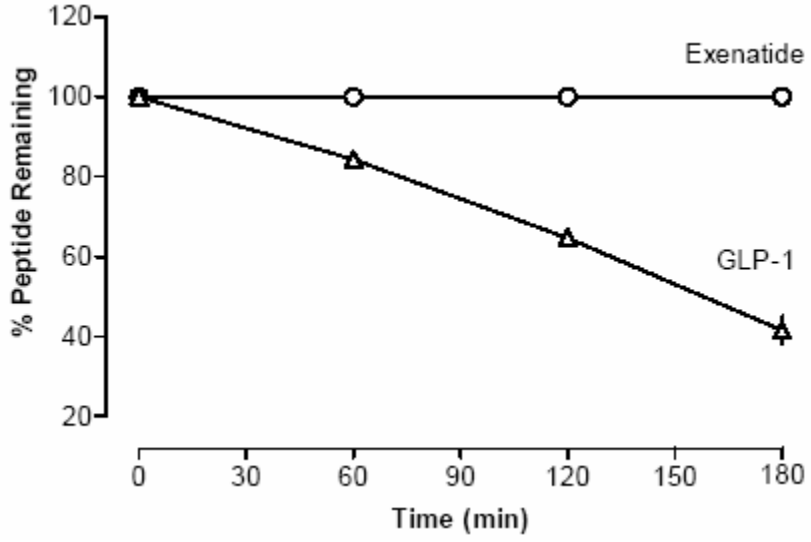


In Vitro Metabolism

Dipeptidyl Peptidase-IV (DPP-IV)

The potential metabolic basis for the longer plasma half-life and pharmacodynamics of exenatide versus that observed for GLP-1 (a related incretin peptide) was examined by in vitro comparison of exenatide and GLP-1 metabolism by purified DPP-IV. In vitro studies demonstrated that exenatide is a poor substrate for human DPP-IV, relative to GLP-1 (Figure below). Therefore, this peptidase is unlikely to be involved in exenatide metabolism in vivo.

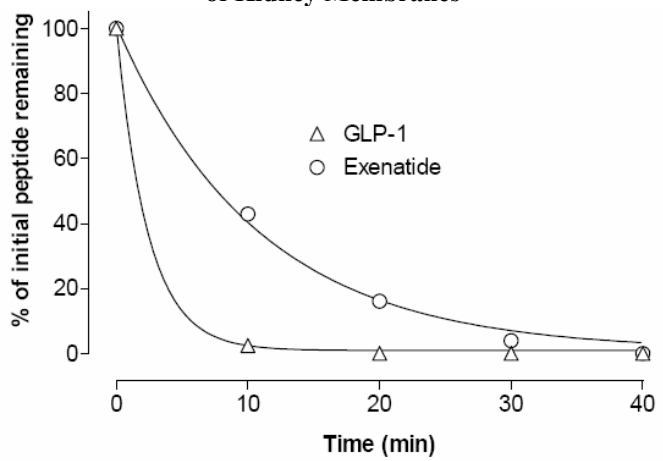
GLP-1 But Not Exenatide Is Degraded in the Presence of DPP-IV In Vitro



Kidney Membrane

Since the kidney was the major route of elimination for exenatide and there were not significant levels of full-length peptide present in rat urine, studies were done to investigate the potential of kidney membrane preparations to degrade exenatide in vitro. In the presence of rat kidney membranes, exenatide degradation was 4- to 5-fold slower compared to GLP-1. However, both peptides were completely degraded after 40 min in this model system (Figure below).

In Vitro Degradation of Exenatide Is 4- to 5-fold Slower Compared to Degradation of GLP-1 in the Presence of Kidney Membranes



Membrane preparations from mouse, rabbit, monkey, and human kidneys were also shown to degrade exenatide. A preliminary assessment of the in vitro degradation products of exenatide was made using kidney membrane preparations of mouse, rat, rabbit, monkey, and human. The data in below show the primary in vitro degradation products detected by species. Two primary cleavage sites were common to all of the species tested. These cleavage sites were between amino acids 21 and 22 (leucine and phenylalanine, respectively) or between amino acids 22 and 23 (phenylalanine and isoleucine, respectively). It should be noted that in all cases, except the cleavage after the thirty-fourth amino acid in the mouse kidney membrane samples, the degradation products from both sides of the primary cleavage sites were detected. Additional degradation products were identified that appeared to be formed after the primary cleavage. A majority of these secondary products were detected in the mouse and rat kidney membrane samples with relatively few seen in the rabbit, monkey, or human samples.

Primary In Vitro Exenatide Degradation Products Identified From Exenatide Digested With Kidney Membrane Preparations

In vitro Primary Metabolite	Mouse	Rat	Rabbit	Monkey	Human
Exenatide (1-21)	+	+	+	+	+
Exenatide (22-39)	+	+	+	+	+
Exenatide (1-22)	+	+	+	+	+
Exenatide (23-39)	+	+	+	+	+
Exenatide (1-15)	+	+	-	-	-
Exenatide (16-39)	+	+	-	-	-
Exenatide (1-14)	-	+	-	-	-
Exenatide (15-39)	-	+	-	-	-
Exenatide (1-34)	+	-	-	-	-

+ = identified as present in digests - = not identified.

These data demonstrate the potential for exenatide to be degraded within the kidney. The relative absence of immunoreactive, full-length exenatide in the urine of rats may be explained by renal degradation subsequent to glomerular filtration. In vivo, three of the four degradation products common to all species tested [exenatide (1-21), exenatide (1-22), exenatide (23-39)] were the only circulating metabolites identified and then only when renal-ligated rats were dosed with 10 and 20 mg exenatide/rat, IV and SC, respectively. This data suggest similar degradation of exenatide may occur in locations outside the kidney. In contrast, no circulating metabolites could be identified in plasma from anesthetized, non-ligated rats dosed at 10 and 20 mg/rat, IV and SC, respectively.

2.6.4.6 Excretion

Nonclinical studies were performed to assess the clearance of exenatide in vivo and in vitro. The role of the liver in exenatide degradation and clearance was evaluated and found not to contribute significantly, if at all, to the clearance of exenatide. Described below are results of nonclinical pharmacokinetic and drug metabolism studies that indicate that the kidney may be a site for the proteolytic degradation of exenatide and the major contributor to exenatide clearance. Sponsor stated that the relative absence of immunoreactive exenatide in the urine, however, suggests that proteolytic degradation may occur in the renal tubule after filtration. Analysis of plasma following IV and SC administration of exenatide to rats did not reveal major degradation products, further supporting the role of the kidneys as the major source of clearance for exenatide.

In Vivo Studies

Liver

Determination of Plasma Pharmacokinetics of a Single IV Dose of Exenatide in a D-Galactosamine-Induced Rat Liver Injury Model: The pharmacokinetic profile of a single IV bolus dose of exenatide was determined in rats with D-galactosamine-induced acute liver injury (model of acute hepatitis) and compared to the pharmacokinetic profile obtained in control rats. Acute liver injury was confirmed by an increase of plasma ALT and AST activities. Twenty-four hours after injection of either D-galactosamine or saline, rats were administered a single bolus 210-µg dose of exenatide IV and the pharmacokinetics of exenatide were determined over the following 6-h period. Pharmacokinetic parameters were than calculated using noncompartmental analysis of the plasma exenatide concentration-time data. Mean terminal t½, CL, and AUC values of exenatide in rats administered D-galactosamine were not significantly different (p >0.05) from those in control rats. The results indicate that the pharmacokinetics of exenatide are not altered in this model of acute hepatitis, suggesting that liver metabolism does not contribute to exenatide excretion.

Summary of PK Parameters of a Single, IV 210-µg Dose of Exenatide in Control and D-Galactosamine-treated Male Sprague-Dawley Rats

Group	$t_{½}$ (min)	CL (mL/min)	AUC_{0-360} (µg•min/mL)
Control (n=4)	29.68 ± 3.75	0.74 ± 0.99	379.61 ± 238.20
Treated[a] (n=4) (Mean ± Std.Dev.)	34.42 ± 3.94	0.80 ± 1.01	277.58 ± 156.05

[a] There were no statistically significant (i.e., p > 0.05) differences between treated and control groups.

Determination of Plasma PK of a Single, IV Dose of Exenatide in a Thioacetamide-Induced Rat Liver Injury Model: To determine if chronic liver injury altered the pharmacokinetics of exenatide,

cirrhosis was induced in male Sprague-Dawley rats by IP administration of 200 mg/kg thioacetamide for 12 weeks (a model of cirrhosis). After 12 weeks of treatment, liver injury in the thioacetamide-treated group was indicated by increased mean plasma AST and ALT activities, and decreased mean plasma BUN concentration, relative to the control group. Cirrhosis was confirmed histopathologically. Sponsor stated that microscopic changes in kidneys were not remarkable. Approximately 72 h following the last injection of thioacetamide or saline, exenatide was administered IV in a single bolus 210-µg dose and serial plasma exenatide concentrations determined for 360 min post-dosing. Plasma exenatide concentration versus time data was evaluated by noncompartmental analysis. Mean terminal t½, CL and AUC values of exenatide in the thioacetamide-treated group were not significantly different from those in the control group. The results indicate that the pharmacokinetics of exenatide is not significantly altered in this model of cirrhosis, suggesting that the liver does not contribute to exenatide excretion.

PK Parameters of a Single, 210-µg IV Dose of Exenatide in Control and Thioacetamide-treated Male Sprague-Dawley Rats

Group	$t_{½}$ (min)	CL (mL/min)	AUC_{0-360} (µg·min/mL)
Control	33.05 ± 3.29	0.62 ± 0.44	497.51 ± 284.24
Treated[a] (Mean ± S.D.)	28.64 ± 5.45	0.56 ± 0.42	545.40 ± 335.01

[a] There were no statistically significant (i.e., p > 0.05) differences between treated and control groups.

Kidney

To assess the involvement of the kidneys in clearance of exenatide, experiments were carried out in renal-ligated animals. It was determined that there was a significant decrease in clearance of exenatide and an increased t½ in renal-ligated animals versus control animals, suggesting that the kidneys have an important role in the clearance of exenatide. Three metabolites [exenatide (1-20),
exenatide (1-22), and exenatide (23-29)] were present at very low levels in renal-ligated rats that could not be identified in control animals suggesting that exenatide and/or exenatide metabolites were cleared from the system by the kidney before they could accumulate. A study using intact rats to evaluate the amount of exenatide excreted into the urine, found that there was negligible urinary excretion of immunoreactive exenatide.

Effects of Functional Nephrectomy on Clearance of Exenatide in Rats: Functional nephrectomy was achieved by acute ligation of the renal arteries and veins in anesthetized SD rats. Exenatide was infused intravenously for 150 min at 5 nmol/h (21 µg/ml/h). Exenatide plasma concentration during and after infusion was measured with an exenatide immunoradiologic assay (IRMA). Exenatide plasma concentration approached steady-state in 30 min at a concentration of 19.0 ± 4.5 nM (79,534 ± 18,837 pg/ml) in control rats but was 83.4 ± 39.0 nM (349,112 ± 163,254 pg/ml) in renal-ligated rats after 150 min (steady-state was not reached). Clearance of exenatide in control rats was 4.3 ml/min and was decreased to 0.86 ml/min in renal-ligated rats indicating that the kidney is responsible for a majority of exenatide elimination in rats. The terminal t½ in control rats was 66.9 min but increased to 326 min (~5-fold) in renal-ligated rats. These data indicate that exenatide is cleared from plasma predominantly at the kidney.

Characterization of In Vivo Exenatide Metabolites Following IV or SC Administration Into Anesthetized Renal-Ligated Rats: Previously described studies using renal-ligated rats demonstrated that the kidney is the major site of clearance for exenatide. This study along with studies using high doses of exenatide in the rat did not result in detectable levels of metabolites, suggesting that exenatide is

cleared from plasma predominantly by filtration. To investigate exenatide metabolite formation in vivo, without contribution from the kidney and compare the resulting metabolite profile between two different routes of administration (IV and SC), male SD rats were renal-ligated, dosed with exenatide (10 mg/animal IV, 20 mg/animal SC), and blood samples were collected and analyzed. The resulting metabolite profile was compared between the two different routes of administration, IV and SC. Plasma samples at each of the time points collected were evaluated for the presence of exenatide and potential exenatide metabolites using LC/MS/MS. Three in vivo metabolites were identified in this study: exenatide (1-22), exenatide (23-39) and exenatide (1-20). Concentration of the metabolites could not be determined because they were present at trace levels (estimated at < 3% of exenatide levels by comparing peak areas of exenatide to metabolite in the same sample). In a renal-ligated rat that had received exenatide by IV, exenatide (1-22) and exenatide (23-39) were identified. From a renal-ligated rat that had received exenatide by SC dose, exenatide (1-20) and exenatide (23-39) were identified. These metabolites were not identified in rats that received the sham operation suggesting that the exenatide and/or exenatide metabolites were cleared by the kidney or by further metabolism before they could accumulate to levels that could be detected using the LC/MS/MS technique. The lower limit of detection of the method was 50 ng/ml exenatide and the level of exenatide in all specimens was at least 10,000 ng/ml. Two of the metabolites identified in this study, exenatide (1-22) and exenatide (23-39) were tested for agonist and antagonist activity using an in vitro assay and neither was active.

In Vivo Metabolism

Species:	**Rat**		**Renal-ligated Rat**	
Gender (M/F)	M	M	M	M
Vehicle/Formulation	saline	saline	saline	saline
Route of Administration	IV	SC	IV	SC
Dose (per animal)	10 mg	20 mg	10 mg	20 mg
Assay	HPLC	HPLC	LC/MS/MS	LC/MS/MS
Parent Concentration	7-134 µg/mL	4-12 µg/mL[a]	410-4740 µg/mL[b]	72-115 µg/mL[b]
Metabolite Concentration	ND[c]	ND		
Exenatide (1-20)	ND	ND	ND	trace
Exenatide (1-22)	ND	ND	trace	ND
Exenatide (23-39)	ND	ND	trace	trace
Study Number	REST98163		REST03235	
Location	4.2.2.4.2		4.2.2.4.5	

[a] exenatide concentration determined by exenatide IRMA.
[b] exenatide concentration determined by exenatide IEMA.
[c] ND = not detected.

Measurement of Exenatide Excretion in Rat Urine: Exenatide was infused IV at a rate of 260 µg/h for 2 h into male SD rats. Urine and plasma were collected and assayed for exenatide content by IRMA, which is specific for full-length exenatide and is known not to recognize exenatide fragments. Urine exenatide concentration reached a maximum of 1.47 ± 1.03 ng/ml after 60 min of infusion. Plasma exenatide concentration at 90 min was 14.5 ± 1.0 µg/ml (represents approximate Css). The exenatide plasma to urine ratio was 11,364 to 1 at 90 min. The total exenatide each animal received was 520 µg over 2 h. The estimated total maximum amount of exenatide excreted in urine over 2 h was 35.3 ng. Therefore, only ~ 0.007% of total intact exenatide was excreted into the urine over this time period at high doses that may have exceeded the clearance capacity for pharmacological doses. These findings support the concept that exenatide is metabolized following renal filtration so that exenatide does not appear in the urine in significant amounts.

Exenatide Excretion in Rat Urine

Species:	Rat		
Gender (M/F) / Number of animals	M/3-5		
Feeding condition	Fed		
Vehicle/Formulation	saline		
Method of Administration	IV infusion over 2 h		
Dose (mg/kg)	260 µg/h		
Assay	Exenatide IRMA		
Excretion route	Urine[a]	Plasma	Total
Time			
1 h	1469 ± 1028 pg/mL	--	
1.5 h	1277 ± 676 pg/mL	14.5 ± 1.0 µg/mL	
0-2 h	35.3 ng		520 µg
Urine/plasma ratio	0.007%		

[a] The 0-2 h total excreted intact exenatide amount was estimated from the measured concentration and an average urine flow rate of 200 µL/min.

2.6.4.7 Pharmacokinetic drug interactions

Non-clinical drug interaction studies were not performed.

2.6.4.8 Other Pharmacokinetic Studies – Pregnancy-related PK.

Measurement of Exenatide Excretion in CD-1 Mouse Milk: Exenatide was measured in milk from CD-1 mice on Day 14 of lactation. Female mice, 3/group, were dosed by repeat-dose SC injection at 0, 3, 34, or 380 µg/kg BID (0, 6, 68, or 760 µg/kg/day). Milk was collected approximately 1 h post first dose on Day 14 of lactation; a blood sample was collected after the milk collection. Specimens were assayed by exenatide IEMA. The presence of exenatide in the milk of animals that did not receive exenatide during dosing suggests that contamination occurred during the collection of the samples or that there is some nonspecific background in the assay. However, the data do suggest that low concentrations of exenatide are present in milk at 380 µg/kg/dose exenatide. The concentration of exenatide in milk is 2.5% of the plasma concentration at the highest dose administered [% exenatide in milk = 100* (5654 pg/ml - 717 pg/ml)/194,993 pg/ml].

Exenatide Concentration in Plasma and Milk of CD-1 Mice

Dose (µg/kg/dose)	Plasma (pg/mL)	Milk (pg/mL)
0	<LowStd	717[a]
3	553	951
34	7342	1151[a]
380	194993	5654

[a] n = 2, for other groups n = 3

In Vivo Evaluation of Exenatide Transport Across the Placental Barrier: The potential of exenatide to cross the placental barrier in mice, rats, and rabbits was evaluated. Pregnant female Sprague-Dawley rats were dosed with a single, SC injection at 21 or 210 µg exenatide. Blood, amniotic fluid, and fetal blood were collected when maternal exenatide plasma concentrations were expected to be maximal based on previous studies (t = 30 min). The samples were assayed for exenatide concentration by exenatide IRMA. Exenatide was not detectable in the amniotic fluid or fetal blood (assay LLOQ = 63 pg/ml). Therefore, detectable levels of exenatide did not cross the placenta of pregnant rats despite high maternal plasma concentrations [232.16 nM (971, 822 pg/ml) for the 210 µg dose].

The plasma concentrations of exenatide in maternal and fetal plasma samples were evaluated in pregnant female CD-1 mice and NZ White rabbits after repeat-dose SC BID administration of exenatide at doses ranging from 6 to 760 µg/kg/day and 2 to 260 µg/kg/day respectively. The samples were assayed for exenatide concentration by the exenatide immunoenzymatic assay (IEMA, assay LLOQ = 2.5 pg/ml). Samples were collected approximately 1.5 h after dosing to allow time for the distribution of maternal exenatide into the fetus if such transfer occurred. The data demonstrate that the potential of exenatide to cross the placental barrier is very low in the mouse and rabbit (i.e., mean ratios for fetal plasma exenatide concentrations to maternal plasma exenatide concentrations ranged from 0.008 to 0.025 in the mouse and 0 to 0.008 in the rabbit).

Ex Vivo Evaluation of Exenatide Human Placental Transfer: The potential of exenatide to cross the human placental barrier was evaluated using an ex vivo human placental perfusion system. The integrity of each placenta was demonstrated using radioactive antipyrine before being used in the experiment to evaluate placental transfer. Placentas were then perfused with perfusate (maternal side) containing a control peptide known not to cross the placental barrier (insulin) plus either 300 or 3000 pg/ml exenatide. These concentrations were chosen because they represent 1 and 10 times the Cmax expected from the highest clinical dose of exenatide (10 µg SC BID). Samples of maternal and fetal perfusate were collected over 120 min and assayed to determine the concentrations of exenatide and insulin. The data with the ex vivo human placental perfusion system (i.e., mean ratios for fetal side plasma exenatide concentrations to maternal side plasma exenatide concentrations) ranged from 0.008 to 0.017, consistent with ratios found in the whole animal experiments in rats, mice, and rabbits described above. The data show that the potential of exenatide to cross the placental barrier is low. Thus exenatide used during pregnancy should result in minimal direct exposure of the peptide to the fetus. Sponsor stated that under some circumstances antibodies were formed (antigenicity) which appeared to slow the clearance of exenatide further supporting that the kidney is the primary pathway for clearance of exenatide.

2.6.4.9 Discussion and Conclusions

Pharmacokinetic parameters for exenatide were determined in mouse, rat, rabbit, and monkey. The pharmacokinetics of exenatide in males and females were not different and therefore, the phamacokinetic parameters in the toxicology studies were calculated as combined data. In general, for the subcutaneous route of administration, exenatide Cmax or AUC increased in proportion to dose and the Tmax ranged from 0.25 to 1.75 h. The terminal t½ after SC injection was prolonged in the mouse, rat and rabbit when compared to the terminal t½ after IV injection, suggesting that absorption of exenatide is the rate-limiting factor in determining terminal t½ after SC administration. Exenatide clearance was studied in models of liver and kidney impairment in rats. These studies showed that there was no significant difference in pharmacokinetic parameters in rat models of either acute or chronic liver injury versus controls. However, AUC, Cmax and terminal t½ all significantly increased and clearance decreased in rats with renal ligation. These data suggest that exenatide is cleared predominantly by the kidney. There were no detectable exenatide metabolites identified in studies performed in intact rats at very high doses of exenatide. The relative absence of immunoreactive (full-length) exenatide in the urine of rats, suggests that proteolytic degradation likely occurs in the renal tubule after filtration. Studies performed using membrane preparations from rat, mouse, rabbit, monkey, and human kidneys support this hypothesis.

Studies done in rats, mice, rabbits, and humans to evaluate the potential for exenatide to cross the placental barrier show that the maximum fetal to maternal ratio is low (0.025). These data suggest that maternal exenatide exposure during pregnancy would result in minimal direct exposure to the fetus. Exenatide was present in the milk of lactating CD-1 mice at a level of approximately 2.5% of the plasma concentration after SC administration of 380 µg/kg BID.

2.6.4.10 Tables and figures to include comparative TK summary

2.6.4.11 Pharmacokinetics: Absorption After a Single Dose - SC

Species:	Mouse	Rat	Rabbit	Monkey*	Human
Gender (M/F)/Number of animals	M/36,F/36 (4/time point)	M/4-6	F/4	M/6, F/6	M/22, F/6
Feeding condition	Fed	Fasted	Fed	Fed	Fed
Vehicle/Formulation	PBO-F10/AC2993-F1	Saline	PBO-F10/(AC2993-F1, AC2993-F2)	PBO-F12/ AC2993-F7	AC2993-F8
Method of Administration	SC	SC	SC	SC BID	SC
Dose (µg/kg)	3.6, 20, 200	6, 60, 600	2, 20, 200	1.1, 9, 75	10 µg/subject
Sample Type	Plasma	Plasma	Plasma	Plasma	Plasma
Assay	Exenatide IRMA	Exenatide IRMA	Exenatide IRMA	Exenatide IEMA	Exenatide IEMA
PK parameters:					
T_{max} (h)	0.5, 0.25, 0.25	0.5	0.4, 1.75, 1.31	0.5, 0.62, 0.67	2.5
C_{max} (pg/mL)	3468, 31072, 318507	2512, 17163, 117208	1766, 13415, 340808	3140, 32002, 211634	251
AUC (pg•h/mL)	2687, 21939, 228930	4856, 54418, 468832	4767, 55420, 1331694	5121, 61019, 500354	1199
(time for calculation - h)	(0-2)(0-2)(0-4)	(0-6)	(0-8)	(0-12)	(0-∞)
$t_{1/2}$ (min)	--	90-216	--	--	160
Bioavailability	--	65-75%	--	--	--
Study Number	REST01219	REST98144R1	REST01218R1	REST01187R1	2993-118
Location	4.2.2.2.1	4.2.2.2.2	4.2.2.2.3	4.2.3.2.10.2	5.3.1.1.1

Single dose pharmacokinetic parameters were also calculated during the toxicokinetic studies (see Section 2.6.7.2 and Section 2.6.7.3).
* Data taken from day one of a 273-day toxicity study; contains GLP-compliance statement.
M = male, F = female

2.6.4.12 Pharmacokinetics: Absorption After a Single Dose – IV

Species:	Mouse	Rat	Rabbit	Human
Gender (M/F)/Number of animals	M/36, F/36 (4/timepoint)	M/4-5	F/3	M/22, F/6
Feeding condition	Fed	Fasted	Fed	Fed
Vehicle/Formulation	PBO-F10/AC2993-F1	Saline	PBO-F10/AC2993-F1	AC2993-F8
Method of Administration	IV	IV	IV	IV
Dose (µg/kg)	20	6, 60, 600	20	1 µg/subject
Sample Type	Plasma	Plasma	Plasma	Plasma
Assay	Exenatide IRMA	Exenatide IRMA	Exenatide IRMA	Exenatide IEMA
PK parameters:				
AUC (pg•h/mL) (time for calculation – h)	–	2888, 75348, 719992 (0-6)	--	--
$t_{1/2}$ (min)	10	18-41	43	--
Clearance (mL/min)	--	3.7-8.3	--	11.68 L/h
Study Number	REST01219	REST98144R1	REST01218R1	2993-118
Location	4.2.2.2.1	4.2.2.2.2	4.2.2.2.3	5.3.1.1.1

2.6.4.13 Pharmacokinetics: Study in Pregnant or Nursing Animals

Placental transfer				
Species:	**Mouse**	**Rat**	**Rabbit**	**Human**
Gestation day/Number of animals:	18/5	17-21/ 4-5	24/5	Birth[d]/ 3
Vehicle/Formulation:	PBO-F11/AC2993-F4	Saline	PBO-F11/AC2993-F4	AC2993-F1
Method of Administration:	SC (BID)	SC	SC (BID)	Perfusion, ex vivo
Dose (µg/kg/dose):	3, 34, 230, 380[a]	21, 210 (µg per animal)	1, 11, 78, 130[b]	300, 3000 pg/mL
Assay:	exenatide IEMA	exenatide IRMA	exenatide IEMA	exenatide IEMA
Time (h)	1.5	0.5	1.5	2
Concentration				
Maternal Plasma (pg/mL)	655, 20870, 194087, 11126136	971822	27, 6690, 368211, 431670	241, 1890
Fetal Plasma (pg/mL)	18, 162, 3987, 7584	ND[c]	<LowStd, 62, 467, 806	<LowStd, 24
Amniotic Fluid (pg/mL)	--	ND[c]	--	--
Fetal/Maternal plasma ratio	0.018, 0.008, 0.025, 0.015	--	0, 0.008, 0.002, 0.003	0.012, 0.008-0.017
Study Number:	REST01004	REST99014R1	REST01007	REST00123R2
Location:	4.2.2.3.2	4.2.2.3.3	4.2.2.3.4	4.2.2.3.5

[a] Multidose study - dosed BID for days of gestation 6 through 18.
[b] Multidose study - dosed BID for days of gestation 6 through 24.
[c] ND = not detectable.
[d] Study was done ex vivo with human placentas from normal term or cesarean section deliveries.

2.6.4.14 Pharmacokinetics: Study in Pregnant or Nursing Animals (continued)

Excretion into milk	
Species:	**CD-1 Mouse**
Lactating date/Number of animals:	day14 / 3 per group
Vehicle/Formulation:	PBO-F11/AC2993-F4
Method of Administration:	SC BID
Dose (µg/kg/dose):	0, 3, 34, 380
Assay:	exenatide IEMA
Time (h)	1
Concentration (pg/mL):[a]	
Milk:	4937
Plasma:	194993
Milk/plasma:	2.5%
Study Number:	REST04054
Location:	4.2.2.5.6

[a] Values determined at the highest dose of 380 µg/kg/dose.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

In Report REST03391R1 the sponsor explains their calculations across species. AUC values for the cynomolgus monkey are derived from the 273-day chronic SC toxicity study with BID exenatide administration. The sponsor calculated that for each µg/kg of SC administered exenatide an AUC value of 6279 pg.h/ml resulted. Likewise, AUC values for CD-1 mice, taken from the 182-day chronic toxicity study with SC administered exenatide BID results in 1096 pg.h/ml for each µg/kg administered. AUC for pregnant New Zealand White rabbits was complicated by the fact that consumption of water decreased with dose and the magnitude of the decrease changes with the number of doses. This was relevant because exenatide is metabolized and cleared through the kidney. Thus for pregnant rabbits the PK was non-linear and the sponsor chose to use mean AUC as a more conservative estimation of exposure multiples in the rabbit rather than a linear calculated value. Rats were dosed by a single SC administration not BID. Exposures were estimated from a 90-day toxicity study to be 818 pg/h/ml µg/kg administered. The table on the next page summarizes the sponsor's calculated exposure multiples.

Exposure Multiples From SC Administration Relative to Systemic Exposure in Humans at the Highest Clinical dose of 10 µg BID

Dose (µg/kg/d)	AUC Value[a] (pg.h/ml)	AUC Value in Humans[b]	Exposure Multiples Based on Relative Systemic Exposure[c]
Mice (Once daily)			
18	13,662	2,076	7
70	53,130	2,076	26
250	189,750	2,076	91
Mice (BID)			
6	6,576	2,076	3
18	19,728	2,076	10
68	74,528	2,076	36
116	127,136	2,076	61
460	504,160	2,076	243
760	832,960	2,076	401
Rat (Once daily)			
18	14,724	2,076	7
70	57,260	2,076	28
250	204,500	2,076	99
Pregnant Rabbit (BID)			
0.2	456	2,076	0.2
2	24,328	2,076	12
22	429,766	2,076	207
156	2,973,334	2,076	1,432
260	7,221,500	2,076	3,479
Monkey (BID)			
1.2	7,534	2,076	4
2.2	13,814	2,076	7
13.4	84,138	2,076	41
18	113,022	2,076	54
150	941,850	2,076	454

[a]Values except for rabbit were derived from equations obtained from analysis of TK data, rabbit values are mean AUC values. See REST03391R1, Section 4.2.3.7.7.1 and REST03392, Section 4.2.3.7.7.2 for explanation.
[b]Total daily exposure calculated from data obtained in Clinical study 2993-118 for 10 µg SC doses using the [mean $AUC_{(t=0-600min)}$ x 2] to obtain total daily exposure.
[c]Species AUC÷Human AUC.

2.6.5.6 Pharmacokinetics: Plasma Pharmacokinetics in Rat Liver Injury Models

Study type:	**Plasma pharmacokinetics in rat liver injury models**			
Species:	**Rat**			
Mode of liver injury:	D-galactosamine-Induced		Thioacetamide-Induced	
Type of liver injury	acute	control	chronic	control
Gender (M/F) / Number of animals	M/4	M/4	M/4	M/6
Feeding condition	Fasted		Fasted	
Vehicle/Formulation	Saline		Saline	
Method of Administration	IV		IV	
Dose (µg/animal)	210		210	
Sample Type	Plasma		Plasma	
Assay	Exenatide IEMA		Exenatide IEMA	
PK parameters[a]				
AUC (µg•min/mL)	277.58 ± 156.05	379.61 ± 238.20	545.40 ± 335.01	497.51 ± 284.24
(time for calculation – min)	(0-360)	(0-360)	(0-360)	(0-360)
$t_{1/2}$ (min)	34.42 ± 3.94	29.68 ± 3.75	28.64 ± 5.45	33.05 ± 3.29
Clearance (mL/min)	0.80 ± 1.01	0.74 ± 0.99	0.56±0.42	0.62 ± 0.44
Study Number	REST02101		REST02139	
Location	4.2.2.5.2		4.2.2.5.1	

[a] There were no statistically significant (i.e. p > 0.05) differences between treated and control groups.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: Single and repeat dose toxicity studies were conducted in the mouse, rat and monkey. Exenatide caused no mortality and minimal toxic responses following single, IV dose in mice at doses up to 1500 µg/kg, as a SC dose in rats up to 10,000 µg/kg, and as a SC dose in monkeys up to 5000 µg/kg.

Following subacute exposure (28 days) to exenatide, mean body weight of rats and monkeys (but not mice) was decreased due to decreased food consumption. Weight of the thymus was decreased in monkeys dosed 1000 µg/kg/d (3592X MRHD, AUC) which correlated with the lymphoid depletion observed microscopically. Increased incidence of basophilic foci in the parotid salivary gland (minimal severity) was observed in mice dosed 760 µg/kg/d (520X MRHD, AUC). Very low titers of anti-exenatide antibody were evident in 2/20 (titers ≤ 1:25) mice treated with exenatide manufactured by Star. Anti-exenatide antibody was not evident in mice treated with exenatide manufactured by Bachem or Mallinckrodt. In monkeys, very low titers (<1:5) of anti-exenatide antibody were observed at doses ≥ 10 µg/kg/d (19X MRHD, AUC). Anti-exenatide antibody detection was not performed in the rat study. While NOAEL could not be established in the mouse, NOAELs for the rat and monkey toxicity is 100 µg/kg/d (58X MRHD, AUC) and µg/kg/d (273X MRHD, AUC) respectively.

Subchronic exposure (91 days) of mice to exenatide at doses of 3, 34 and 380 µg/kg BID was well tolerated. A high incidence of basophilic foci in the parotid salivary gland (minimal to mild severity) was observed in mice at doses ≥ 6 µg/kg/d (3X MRHD, AUC). A high incidence of mandibular lymph node hemorrhage was observed in mice dosed 760 µg/kg/d (520X MRHD, AUC). In another subchronic (91 days) toxicity study, mice dosed with exenatide at 18, 70 and 250 µg/kg QD followed by a 30-day recovery period showed reversible increases in incidence of basophilic foci in the parotid salivary gland at doses ≥ 18 µg/kg/d (12X MRHD, AUC). The physiologic significance of this lesion remains unclear, but the lack of neoplastic sequelae of the lesion indicate that the basophilic foci of the parotid salivary gland in mice may not be an adverse effect. Subchronic exposure (91 days) of rats and monkeys to exenatide caused decreased body weight gain which correlated with decreased food consumption. Reversible decreases in body weight gain were observed in rats at doses ≥ 18 µg/kg/d (5X MRHD, AUC). Decreased body weight gain was observed in monkeys at doses ≥ 0.6 µg/kg BID (3X MRHD, AUC). Reversibility was not assessed in monkeys. A low incidence and minimal severity of basophilic foci (reversible) was observed in female rats at 18 µg/kg/d (5X MRHD, AUC) and 250 µg/kg/d (129X MRHD, AUC). At the end of the recovery period, relatively high incidences of vacuolar change (adrenal gland), lymphocyte infiltration (pancreas), and a low incidence of basophilic foci (parotid salivary gland) with minimal severity were noted in 250 µg/kg/d (129X MRHD, AUC) male rats. NOAEL could not be established in the rat study since microscopic evaluation was performed on only a few selected organs/tissues. In the monkey, the target organs toxicity of minimal to mild severity were observed in the lung (inflammation, hemorrhage, syncytial giant cells), endometrium (hemorrhage), pancreas (hypercellular islet) and stomach (focal inflammation) at doses ≥ 6.7 µg/kg BID (65X MRHD, AUC). NOAEL for the monkey study was 0.6 µg/kg BID (3X MRHD, AUC). The potential of exenatide to elicit an immune response in rats was low. In monkeys, 5% of control animals tested positive for anti-AC2993 antibodies compared to 38%, 25% and 50% for the 0.6, 6.7 or 75 µg/kg BID groups respectively. There seems to be a treatment-related increase in percentage of animals that tested positive suggesting that the drug may be antigenic to monkey. However, the positive finding in some control animals (which may be due to contamination or background error) undermines the accuracy of this study. Moreover, with the exception of one 75 µg/kg BID (1004X MRHD, AUC) animal that had an antibody titer of 125, the rest of the treated animals had antibody titer of 25 regardless of treatment group. Systemic exposure increased with dose in the monkey suggesting that the anti-exenatide antibody formed is not neutralizing.

Chronic toxicity studies were conducted in mice (182 days) and monkeys (273 days). Mortality was observed in mice at all dose levels including control but not in monkeys. The incidence of death was not dose-dependent in the mouse. While there was no treatment-related effects on body weight/body weight gain in the mouse, body weight gain decreased dose-dependently in treated monkeys. The target organs of toxicity in the mouse include the eye (retinal atrophy, corneal mineralization, cataract), testis (degeneration of seminiferous tubules), parotid salivary gland (basophilia), bone marrow (hyperplasia) and injection sites (inflammation, hemorrhage, fibrosis, epithelial hyperplasia). Except for the basophilia in the parotid salivary gland observed at all doses, most of the remaining toxicities were limited to the HD of 380 µg/kg BID (260X MRHD, AUC) group. Anti-exenatide antibody reactivity was not different between control and exenatide-treated mice. NOAEL could not be established because of the ophthalmology findings, tissue reaction at the injection sites and the parotid gland basophilia observed at all doses. In monkeys, the target organs of toxicity include the brain (mononuclear cell infiltration, hemorrhage), thyroid (follicular distension, epithelial degeneration - males), adrenal gland (mineralization - males, nodular hypertrophy – females), kidney (tubular dilatation - males), heart (mononuclear cell infiltration - males), skeletal muscle (lymphoid cell infiltrate - males), pancreas (vacuolation, fibrosis, mononuclear cell infiltrate, hypercellular islet – males and females), sciatic nerve (fibrosis - males), uterus (protein deposits - females), stomach (lymphoid hyperplasia, lymphoplasmacytic infiltrate), colon (cystic dilatation), cecum (pigmented macrophages), jejunum (cytoplasmic vacuolation), rectum (inflammation)- all the GI lesions were observed in females except for the pigmented macrophages observed in a HD males; injection sites (epidermal hyperplasia – males). Most of the toxic effects occurred in the 9 µg/kg BID (1360X MRHD, AUC) and 75 µg/kg BID (994X MRHD, AUC) groups. NOAEL was 1.1 µg/kg BID (8X MRHD, AUC) based on histopathology. One of 12 (8%) control monkeys, 9/12 monkeys (75%) each receiving 1.1 µg/kg/BID and 9.0 µg/kg/BID and 8/12 monkeys (67%) receiving 75 µg/kg/BID were found positive for anti-exenatide antibody. Titers of 1:125 or greater were obtained in 5/12 (42%) monkeys receiving 1.1 µg/kg/BID, 4/12 (33%) monkeys receiving 9.0 µg/kg/BID and 2/12 (17%) monkeys receiving 75 µg/kg/BID. Increases in Cmax and AUC generally correlated with anti-exenatide antibody titers ≥1:125 suggesting that anti-exenatide antibody was not neutralizing. Moreover, exenatide-related effects on body weight were not correlated with anti-exenatide antibody.

Genetic toxicology: Genotoxicity was assessed in three bacterial reverse mutagenesis (Ames assay) studies, one for each of the manufacturers of exenatide, including Star, Bachem and Mallinckrodt. Cytogenetic assays of mutagenicity (clastogenicity) were performed in vitro, for each of the manufacturers of exenatide. Genotoxicity was further examined in vivo by the mouse micronucleus assay with exenatide manufactured by Star. Exenatide tested negative under the conditions of the battery of genotoxicity studies conducted.

Carcinogenicity: The carcinogenic potential of exenatide was investigated in rodent bioassays. Once daily subcutaneous administration of exenatide at doses of 18, 70 and 250 µg/kg/d to mice for 96 weeks (females) and 98 weeks (males) did not demonstrate any carcinogenic findings. Once daily subcutaneous administration of exenatide at doses of 18, 70 and 250 µg/kg/d to rats for 104 weeks was associated with increased incidence of thyroid C-cell adenoma in all drug treated females relative to controls. The incidence in HD females is 23% relative to controls (8% and 5% for control groups 1 and 2 respectively) and is greater than the sponsor's historical control mean (5%) and range (0-10%).

Reproductive toxicology: The potential of exenatide to cause reproductive or developmental toxicity was evaluated in mice and rabbits. Exenatide does not produce hypoglycemia in normal animals based on its mechanism of action and study data. Therefore maternal hypoglycemia does not occur to confound the reproductive toxicology evaluations. In fertility and general reproductive toxicity studies, male and

female mice were dosed at 3, 34 and 380 µg /kg BID (3X, 50X and 520X MRHD, AUC). There were no treatment-related effects on mating and fertility in both sexes or estrous cycling in treated females. There was a dose-dependent decrease (not SS) in number of motile sperm by 7%, 8% and 20% at 3, 34 and 380 µg/kg BID respectively. There were dose-dependent decreases (not SS) in number of corpora lutea, implantations and viable embryos in treated females relative to control. Post-implantation loss was increased by 2 to 3-fold (not dose-related) in treated mice, but the differences were not statistically significant relative to control. NOAEL for mating and fertility is 380 µg/kg BID (520X MRHD, AUC).

In a mouse teratology study, exenatide doses of 3, 34, 230 and 380 µg/kg BID, SC (3X, 50X, 243X and 520X MRHD, AUC) were evaluated. In addition, extra pregnant mice were exposed to the same doses of exenatide and used to assess the extent of placental transfer. Food consumption was decreased in all treated dams relative to control. Abortions, 1/25 each, were observed in dams at dosed 34 (50X MRHD) and 380 µg/kg BID (520X MRHD) while premature delivery was observed in 1/25 dams each at doses ≥ 34 µg/kg BID (50X MRHD). Two and five fetuses from the control and treated groups respectively had multiple findings (cleft palate with/without hole, interfrontal ossification site, cervical ribs and wavy ribs). The only teratologic finding that occurred at maternal NOAEL (3 µg/kg/d BID = 3X MRHD) is the non-dose-related increased incidences of cleft palate (litter), delayed ossification sites in rib pairs (fetal) and increased interfrontal (skull) ossification (litter, fetal). All other findings (decreases in implantations, litter sizes, live fetuses and fetal weights, wavy ribs, delayed ossification of the thoracic and lumbar vertebrae) occurred at doses ≥ 34 µg/kg BID (≥ 50X MRHD). The TK data showed that the potential of exenatide to cross the placental barrier is very low in mice. Maternal NOAEL is 3 µg/kg BID (3X MRHD) based on the abortions observed. Developmental NOAEL is also 3µg/kg BID (3X MRHD) based on dose-related lower body weights in fetuses at higher doses, cleft palate and wavy ribs. Since the potential of exenatide to cross the placental barrier is very low, the fetal findings observed may be a consequence of the dose-related decreased nutritional state of the dams during gestation or maternal toxicity. Sponsor stated that dams with compromised nutritional state during organogenesis, produced fetuses with decreased body weights and delays in normal fetal maturation (e.g. wavy ribs).

In a rabbit teratology study, pregnant female rabbits were dosed at 0.1, 11, 78, or 130 µg/kg BID, SC resulting in total daily doses of 0.2 (0.2X), 22 (207X), 156 (1432X) and 260 µg/kg/day (3479X MRHD). A satellite group of 25 female rabbits were exposed to the same doses of exenatide to assess the extent of placental transfer which was 0, 0.009, 0.001, and 0.002 fetal: maternal plasma concentrations respectively. Mortality was observed in 1/20 does each at 0.2 µg/kg/day (0.2X MRHD) and 22 µg/kg/day (207X MRHD). Abortion and premature delivery occurred in 1/20 does each at 22 µg/kg/day (207X MRHD) and 156 µg/kg/day (1432X MRHD) dose groups respectively. These events were considered unrelated to the test article because they were not dose-dependent, the death of one doe appeared to be related to an injury, and the abortion and delivery of a single doe in a study is within the historical control incidence for the testing facility. Dose-dependent decreases in body weight gain which correlated with decreased food consumption were observed in treated does relative to control.

The only treatment-related fetal effect observed at the maternal NOAEL (0.2 µg/kg/day = 0.2X MRHD) is an increased incidence (3.1%) of dead or resorbed conceptuses/litter relative to control (0%). The incidence of this finding is less than the historic control mean of 3.7% and falls within the range (0-22%). All other treatment-related fetal effects (higher incidence of dead or resorbed conceptuses/litter, resorptions, umbilical hernia, small gall bladder, angulated hyoid, delayed ossifications, fused ribs and fused sternal centra) occurred at doses ≥ 22 µg/kg/day (207X MRHD). Maternal NOAEL = 0.2 µg/kg/day (0.2X MRHD) based on dose-related decrease in weight gain during the dosage period. The developmental NOAEL is also 0.2 µg/kg/day (0.2X MRHD) based on the developmental toxicity (higher incidence of dead or resorbed conceptuses/litter, resorptions, umbilical hernia, small gall bladder, angulated hyoid, delayed ossifications, fused ribs and fused sternal centra) observed at doses ≥ 22

μg/kg/day (207X MRHD). The potential of exenatide to cross the rabbit placental barrier is very low. Therefore the fetal findings observed may be a consequence of the reduced nutritional state of the dams during gestation or maternal toxicity. Sponsor stated that dams with compromised nutritional state during organogenesis produced fetuses with decreased body weights and delays in normal fetal maturation (e.g., resorptions, umbilical hernia and delays in ossifications).

Another rabbit teratology study was performed to better define the NOAEL with regard to fetal effects and to clarify the role of exenatide-related decreases in food consumption and body weight on developmental effects. In this study, pregnant rabbits were administered 1, 11 and 130 μg/kg BID SC exenatide resulting in total daily doses of 2 (12X MRHD), 22 (9207X MRHD), and 260 μg/kg/day (3479X MRHD). Three additional groups were pair-fed (fed the same average daily amount of food) to match the three respective exenatide-dosed groups. Rabbits that were administered exenatide exhibited profound, dose-related decreases in food and water consumption and loss in body weight. Clinical indicators of starvation (β-hydroxybuterate and K) and body weight loss were more pronounced in the exenatide-treated groups than in the pair-fed groups. Based on the severity of the body weight loss and anorexia, the MTD in pregnant rabbits was exceeded at doses ≥22 μg/kg/day exenatide. As in the previous rabbit study, developmental toxicity occurred only at doses ≥22 μg/kg/day exenatide, doses that exceeded the MTD in pregnant rabbits. None of the fetuses from pair-fed dams and from the dams administered 2 μg/kg/day exenatide had umbilical hernias. Skeletal variations were present in similar incidences in both exenatide and pair-fed groups, suggesting these effects were a consequence of compromised maternal condition. Thus, exenatide is not a developmental toxicant in rabbits; the NOEL for developmental toxicity was 2 μg/kg/day exenatide (12X MRHD).Rabbit TK indicates greater exposure than mice, rat or monkey based on dose. Decreased water consumption coincides with the unusually high exenatide exposures. The sponsor suggests that since this drug is cleared by the kidney, impaired clearance may explain the increased sensitivity of the pregnant rabbit to exenatide toxicity.

In a developmental and perinatal/postnatal reproduction toxicity study, pregnant mice were administered exenatide at doses of 3, 34 and 380 μg/kg BID SC resulting in total daily doses of 6 (3X), 68 (50X) and 760 μg/kg/d (520X MRHD). One of 25 (F0) female mice died at all dose levels. The HD (520X MRHD) female died while delivering a litter. The death at the HD might be drug-related because it occurred in the HD group and the other mice in this dose group had increased incidences of stillbirths and pup deaths on LD1 (Lactation Day 1). Although the cause of death could not be determined, sponsor indicated that the deaths in the 6 (3X MRHD) and 68 μg/kg/day (50X MRHD) dose groups were not considered drug-related because the incidences were not dose-dependent. There were no treatment-related effects on corpora lutea, implantations, litter sizes and resorptions in cesarean-sectioned F1 females. No treatment-related effects on preputial separation or day of vaginal patency in the F1 generation mice, learning or memory, mating or fertility, cesarean-sectioning parameters or the incidence of fetal alterations in F2 generation mice were observed. The maternal (F0) NOAEL < 6 μg/kg/d (<3X MRHD) due to mortality at doses ≥ 6 μg/kg/d, decreased body weight gain and food consumption at doses ≥ 68 μg/kg/d (50X MRHD). NOAEL for fetal viability and growth is 6 μg/kg/d (3X MRHD) because doses ≥ 68 μg/kg/d (≥ 50X MRHD) caused reduced pup body weights preweaning, increased incidence of still births, decreased number if live births, and the 760 μg/kg/day increased perinatal mortality and reduced body weight gains postweaning.

Special toxicology: Anti-exenatide antibody production in NIH Swiss mice was investigated to determine if the anti-exenatide antibody is neutralizing by observing its effect on the glucose lowering activity of exenatide. The mice treated with exenatide showed a consistent drop in plasma glucose levels an hour after IP administration regardless of the duration of treatment with exenatide, GLP-1, or vehicle. Sponsor stated that no measurable anti-exenatide antibody titers were established with the treatment of exenatide for up to 8 weeks.

Monkeys dosed for > 90 days at ≥ 18 µg/kg/d had greater than dose proportional exposure which correlated with anti-exenatide antibody titers ≥ 1:125. 25% of the monkeys at 9 months were positive. This affected kidney clearance. A study to determine the effects of anti-exenatide antibody on toxicokinetics, body weight changes and histological change in pancreas of Cynomolgus monkeys administered exenatide BID by subcutaneous injection for 9 months, showed that there were no effects of antibody formation on decreased body weight gain and increased pancreas islet cellularity in the treated groups. Except for one, monkeys with antibody titer >125 exhibited a larger plasma exenatide AUC value at sample days 90, 180 and 273 relative to the AUC value on day 1. Based on this evaluation, an antibody titer >125 caused a change in plasma pharmacokinetics, probably by slowing renal clearance due to increased plasma protein binding. Anti-exenatide antibodies were not neutralizing with regard to the biological responses evaluated in this study.

Exenatide was weakly antigenic or non-antigenic in rodents but antigenic in monkeys. Anti-exenatide antibodies were noted following 1 month of treatment, and were present following 9 months of treatment, resulting in 8 months of exposure to anti-exenatide antibody in monkeys. The formation of anti-exenatide antibody in monkeys was not dose-dependent. The presence of anti-exenatide antibody at titers ≥1:125 resulted in altered pharmacokinetics in monkeys but was not neutralizing. Sponsor stated that there were no apparent adverse effects of anti-exenatide antibody formation in monkeys such as injection sites reactions, anaphylaxis, delayed-type hypersensitivity, autoimmune (dermal reactions, arthritis, anemia or aplasias, mucocutaneous reactions) or antibody-antigen-complex-related pathology (arthritis, nephropathies).

2.6.6.2 Single-dose toxicity

REPORT #	ROUTE	SPECIES	DOSE (µg/kg)	KEY STUDY FINDINGS
REST98095	IV injection	Mouse	0, 30, 300, 1500	• No mortality or signs of serious toxicity at any dose. • Doses ≥300 µg/kg decreased grip strength and limb tone. • Doses ≥30 µg/kg transiently decreased spontaneous motor activity. • No gross changes at necropsy.
REST98098	SC injection	Rat	Rising-dose 100, 300, 1000, 3000, 10,000, 30,000 Single-dose 30, 300, 3000	• No mortality or signs of serious toxicity at any dose. • Doses ≥10,000 µg/kg caused hunched posture, fur staining, & piloerection. • No mortality or signs of serious toxicity at any dose. • Decreased body weight at HD relative to LD. • No gross changes at necropsy.
REST98099R1	SC injection	Monkey	Rising-dose 100, 300, 1000, 3000, 5000	• No mortality or signs of serious toxicity at any dose. • Doses ≥5000 µg/kg caused decreased food consumption (qualitative estimate).

Exenatide caused no mortality and minimal toxic responses following single, IV dose in mice at doses up to 1500 µg/kg, as a SC dose in rats up to 30,000 µg/kg, and as a SC dose in monkeys up to 5000 µg/kg. Therefore, the median lethal dose values for exenatide in mice, rats, and monkeys were >1500 µg/kg, >30,000 µg/kg, and >5000 µg/kg, respectively.

2.6.6.3 Repeat-dose toxicity

REPORT #	ROUTE	SPECIES	DURATION	DOSE	KEY STUDY FINDINGS
REST98099 2.6.6.3.1	SC Daily	Monkey	5 Days	5000 µg/kg/d	• No deaths or signs of toxicity. • Decreased food consumption (qualitative estimate) and feces. • Decrease in body weight (9%). • No hematology or clinical chemistry changes. • NOAEL < 5000 µg/kg/d.
REST98097	IV Daily	Rat	14 Days	0, 10, 100, 1000 µg/kg/d	• Doses ≥ 100 µg/kg/d caused transient hypoactivity, decreased food consumption (Day 8) in males. • 1000 µg/kg/d decreased body weight in males (10%)

2.6.6.3.2					and increased (33%) adrenal weight in females. • NOAEL = 100 µg/kg/d.
REST02075 **2.6.6.3.3**	SC BID	Mouse	28 Days	0, 380 (total daily dose = 760) µg/kg/d	• No deaths. Mice in the HD group showed unkempt appearance. • Increased incidence of basophilic foci in the parotid salivary gland occurred in the HD group compared to zero in control. Severity is minimal. • Anti-exenatide antibody positive titer was observed in 2/20 males (≤1:25) given 760 µg/kg/d (manufactured by Star). None of the mice given the batches manufactured by Bachem and Mallinckrodt developed anti-exenatide antibodies.
REST98082 **2.6.6.3.4**	SC Daily	Rat	28 Days	0, 10, 100, 1000 µg/kg/d	• No exenatide-related mortality was observed. • An increased incidence in hypoactivity was observed among males and females treated at ≥10 µg/kg/day. • A dose- related increase in salivation was noted after dosing in males and females treated at ≥10 µg/kg/day. • Mean body weights decreased dose-dependently being significant in HD males (11.3%). Body weight gain decrements of 10-24% (M) and 35-45% (F) were observed at doses ≥100 µg/kg/day. • Mean food consumption decreased by 15% and 22% in HD males and females respectively. • There was a dose-related increase in relative adrenal weights in treated females at ≥10 µg/kg/day exenatide, with increases of 14.8%, 16.1%, and 24% at 10, 100, and 1000 µg/kg/day, respectively. These organ weight changes had no correlative microscopic changes. • NOAEL = 100 µg/kg/day (51-58X MRHD, AUC) based on decreased body weight gain at the HD.
REST98079 **2.6.6.3.5**	SC Daily	Monkey	28 Days	0, 10, 100, 1000 µg/kg/d	• No treatment-related mortality. • Increased incidence in mucous membrane pallor was noted in treated groups relative to control. • Mean body weight was decreased by 18% (M) and 24% (F) in the HD group, being significant in HD males. This correlates with the decreased food consumption of 68% (M) and 47% (F) in the HD group. • Small thymus was observed among 1/3 males and 1/3 females in the HD group. They were accompanied by decreased spleen weights in HD males (43.6%) and decreased thymus weights in HD males (48.3%) and females (58.0%). • The decreased thymus weight correlated with microscopic findings of lymphoid depletion in the thymuses from HD males (1/3) and females (3/3). • Sponsor attributed the changes in spleen and thymus to the physiologic stress of weight loss and inappetence at the HD. • 2/6 LD and HD monkeys were anti-exenatide antibody positive. Low titers (<1:5) of anti-exenatide antibody were noted at ≥10 µg/kg/day. • NOAEL = 100 µg/kg/day (237X MRHD, AUC) based on the decreased body weight and histopathology at the HD.

Human AUC data derived from data in Clinical Study 2993-118 for 10-µg SC BID doses as [mean $AUC_{(0-t)}$ x 2 = 2076 pg.h/ml] to obtain total daily exposure.

MOUSE

2.6.6.3.6

Study title: **A 91 Days Toxicity Study of AC2993 Administered BID by Subcutaneous Injection to Mice.**

Key study findings:

- Mortality occurred at all dose levels including control. Hence, the test article could not be implicated.
- Mean body weight of females was increased relative to those of controls at week 13.
- Triglyceride level was significantly decreased in MD (females) and in HD males and females.
- Albumin and globulin were slightly but significantly increased in males at LD and MD respectively.
- Calcium was slightly but significantly increased in LD females.
- A high incidence of basophilic foci in the parotid salivary gland with minimal to mild severity was noted in all treated mice. A high incidence of sciatic nerve degeneration and inflammation was noted at the injection sites of HD males relative to control. A high incidence of mandibular lymph node hemorrhage of minimal to mild severity was noted in the HD group relative to controls.
- The target organs of toxicity include the parotid salivary gland (basophilic foci) and the mandibular lymph node (hemorrhage).
- NOAEL = 380 µg/kg/BID (260X MRHD, AUC).

Study no.: REST99051
Volume # and page #: N/A.
Conducting laboratory and location: (b) (4)
Date of study initiation: February 1, 2000.
GLP compliance: Yes (USA).
QA report: yes (X) no ( )
Drug, lot #, and % purity: Lot # 99-1001TP, 97% pure.
Methods

Doses: Animals were dosed subcutaneously BID at 3, 34 and 380 µg/kg giving total daily doses of 6, 68 and 760 µg/kg/d.
Species/strain: Mouse/CD-1.
Number/sex/group or time point (main study): 20/sex/group (control); 21/sex/group (LD, MD & HD).
Route, formulation, volume, and infusion rate: Subcutaneous injection. 4.9 ml/kg (control); 0.6 ml/kg (LD); 1.8 ml/kg (MD); 4.9 ml/kg (HD).
Satellite groups used for toxicokinetics or recovery: 54/sex/group.
Age: 7-8 weeks at study initiation.
Weight (non-rodents only): N/A.
Study design:

Group No.	Number of (M/F)	Test Article	Dose Level (µg/kg BID)	Total Daily Dose (µg/kg)	Dose Vol. (ml/kg)	Dose Conc. (µg/µl)	Number (M/F) Sacrificed on Days 91-92
1	20/20[1]	0	0	0	4.9	0	20/20
2	21/21[1]	AC2993	3	6	0.6	0.005	21/21
3	21/21[1]	AC2993	34	68	1.8	0.019	21/21
4	21/21[1]	AC2993	380	760	4.9	0.078	21/21
5	54/54[2]	AC2993	3	6	0.6	0.005	None
6	54/54[2]	AC2993	34	68	1.8	0.019	None
7	54/54[2]	AC2993	380	760	4.9	0.078	None

[1] Animals for toxicokinetics and toxicity evaluations (Groups 1-4)
[2] Animals for toxicokinetics evaluations only (Groups 5-7)

Observation times and results

Mortality: Daily.
Based on the lack of significant tissue findings and the clustered occurrence of deaths during study Days 78-80, the deaths were considered unrelated to the test article. Sponsor stated that the deaths were most likely caused by handling during dosing and/or scheduled bleeding.

Dose (µg/kg)	0 (n = 20)	3 (n = 21)	34 (n = 21)	380 (n = 21)
Sex	**M**	**M**	**M**	**M**
# DEAD	105 AC Day 79 119 FD Day 61 120 FD Day 78	202 FD Day 80 204 ACC Day 80 207 HS Day 67 214 ACC Day 78 217 ACC Day 78	301 FD Day 29 306 FD Day 79 319 ACC Day 79 320 ACC Day 78	
Total Deaths	**3**	**5**	**4**	**0**
Dose (µg/kg)	**0 (n = 20)**	**3 (n = 21)**	**34 (n = 21)**	**380 (n = 21)**
SEX	**F**	**F**	**F**	**F**
# DEAD		1219 ACC Day 79		1408 ACC Day 29 1410 HS Day 80 1414 HS Day 52 1415 FD Day 77
Total Deaths	**0**	**1**	**0**	**4**

ACC = Accidental death; FD = Found dead; HS = Humane sacrifice

The cause of death for most animals was undetermined. Control male no. 119 had moderate edema and marked hemorrhage in the lungs. Mild hemorrhage was present in the lungs of 380 mg/kg female no. 1408. Marked hemorrhage was present in one kidney of humanely sacrificed 380 mg/kg female no. 1414. Sponsor stated that the lymphoid necrosis observed in control male no. 120 was probably due to autolysis as several other tissues in this animal were autolytic. The lack of significant findings in other tissues from other early decedent animals, the clustered occurrence of deaths during study days 78-80, and the lack of a dose response for males suggest that the deaths were most likely caused by handling during dosing and not test article related.

ANIMALS SACRIFICED IN THE TOXICOKINETIC GROUP DUE TO POOR HEALTH

Dose (µg/kg)	0 (n = 20/sex/group)		3 (n = 54/sex/group)		34 (n = 54/sex/group)		380 (n = 54/sex/group)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
SACRIFICED	**0**	**0**	**0**	**1**	**2**	**2**	**1**	**0**

Clinical signs: Twice daily.

No treatment-related clinical signs were noted. All animals showed varying degrees of staining, scabbing, thin hair/alopecia and occasional rough coat and erythema in the vicinity of the injection sites. Sponsor stated these findings tended to be more pronounced in the control and HD animals due to increased injection volume and not the test article.

Body weights: Measured prior to dosing, weekly during dosing and at necropsy.

Body weight (g) changes in males were not significantly different from that of control. Body weight changes in females are indicated below.

Body Weights:

Dose (µg/kg)	Control (n = 20/sex/group)	3 (n = 21/sex/group)	34 (n = 21/sex/group)	380 (n = 21/sex/group)
Sex	**F**	**F**	**F**	**F**
Pre-test	23.0 ± 1.2	23.1 ± 1.3	23.3 ± 1.6	23.0 ± 1.3
Week 13	30.2 ± 1.9	31.7* ± 2.1	32.6* ± 2.1	32.6* ± 1.9
Gain	7.2	8.6 (19%↑)	9.3 (29%↑)	9.6 (33%↑)

*** = p <0.05 (statistically significant from control)**

Food consumption: Weekly.

No treatment-related effects on food consumption.

Ophthalmoscopy: Conducted on main study animals prior to dosing and during the last week of dosing.

Dose (µg/kg)	Control (n = 20/sex/group)		3 (n = 21/sex/group)		34 (n = 21/sex/group)		380 (n = 21/sex/group)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Unilateral Central corneal opacity	1	1	0	0	2	0	0	0
Unilateral Cortical cataract	0	0	0	0	0	0	1	0

EKG: Not conducted.

Hematology: Blood samples were collected from main study animals (groups 1-4) prior to the morning dose on days 78 and 79 for routine hematology evaluation.

No treatment-related effects.

Clinical chemistry: Blood samples were collected from main study animals (groups 1-4) prior to the morning dose on days 78 and 79 for routine hematology evaluation.

Dose (µg/kg)	Control	3	34	380
Sex	**M**	**M**	**M**	**M**
Alb (g/dl)	3.4	3.8** (12%↑)	3.5	3.4
Glob (g.dl)	2.4	2.5	2.9** (21%↑)	2.4
Trig (mg/dl)	186	143	137	111**
Sex	F	F	F	F
Ca (mg/dl)	10.1	10.8** (7%↑)	10.3	10.2
Trig (mg/dl)	161	118	97** (40%↓)	91** (44%↓)

**** = p <0.05** ↑ ↓

Urinalysis: Urine samples were obtained by cystocentesis at necropsy.

No treatment-related effects.

Gross pathology: Tissues/organs isolated for gross pathology examinations are indicated in the histopathology table.

Except for red discoloration at injection sites, there were no other treatment-related gross findings.

Dose (µg/kg)	Control (n = 20/sex/group)		3 (n = 21/sex/group)		34 (n = 21/sex/group)		380 (n = 21/sex/group)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Injection site discoloration and scabs	9	6	1	0	1	5	7	7

Organ weights: Organs weighed are indicated in the histopathology table.

No treatment-related effects.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Histopathology findings related to dosing trauma included epithelial hyperplasia/acanthosis, hemorrhage, inflammation, ulceration and fibrosis/fibroplasia at injection sites. These findings as well as the nerve degeneration at some injection sites were due to direct needle trauma and/or extension of inflammation/fibrosis from repeated injections. The pancreas, adrenal glands, kidneys and parotid salivary glands from all dose groups were processed for examination. For the remaining tissues, only those from control and HD groups were processed for examination.

Dose (µg/kg) BID	**0**		**3**		**34**		**380**	
n	**20**		**21**		**21**		**21**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Parotid salivary gland Basophilic foci	0	1(1)	10(1)	12(1)	19 16(1) 3(2)	20 18(1) 2(2)	17 15(1) 2(2)	18 16(1) 2(2)
Pancreas Enlarged Islets	1(1)	2 1(2) 1(3)	1(1)	3(2)	2(2)	2(2)	2(2)	3(2)
Sciatic nerve Degeneration at injection site	0	2 1(1) 1(2)	-	-	-	-	4 3(2) 1(3)	0
Inflammation	0	1(1)	-	-	-	-	4 1(1) 3(2)	1(1)
Mandibular lymph node Hemorrhage	1(2)	1(1)	-	-	-	-	2 1(1) 1(2)	5 1(1) 4(2)
Skin at injection site Inflammation	4 1(1) 3(2)	7 6(1) 1(2)	-	-	-	-	1(2)	6 1(1) 4(2) 1(3)
Fibrosis/fibroplasia	2 1(2) 1(4)	13 2(1) 8(2) 3(3)	-	-	-	-	0	15 3(1) 9(2) 3(3)
Epithelial hyperplasia/acanthosis	3 1(1) 2(2)	1(1)	-	-	-	-	0	0

1 = minimal; 2 = mild; 3 = moderate; marked

Toxicokinetics: Blood samples for TK were collected on Days 1, 30, 60 and 91 at 0.5, 1, 2, 4, 6 and 12 hr post dose.

Daily Dose µg/kg/day	Dose µg/kg	Sex	C_{max} (pg/mL)				AUC [b] (pg·h/mL)			
			Day 1	Day 30	Day 60	Day 91	Day 1	Day 30	Day 60	Day 91
6	3	M	14,420	4426	4946	4627	7582	3183	3595	3426
		F	3961	4873	3556	3687	2294	3261	2543	3250
		M/F	**8145**	**4694**	**4251**	**4157**	**4443**	**3245**	**3095**	**3485**
68	34	M	31,823	28,403	25,574	61,355	24,300	21,456	33,387	56,699
		F	37,756	25,841	29,043	65,440	23,849	23,406	23,609	45,974
		M/F	**34,789**	**27,122**	**27,308**	**63,398**	**24,075**	**22,799**	**28,498**	**51,389**
760	380	M	996,992	541,875	693,429	565,345	737,219	464,407	608,018	633,253
		F	428,782	498,430	555,323	546,782	376,807	404,318	562,673	476,576
		M/F	**712,887**	**520,153**	**624,376**	**556,063**	**557,010**	**434,363**	**585,345**	**539,949**

Total daily $AUC_{(0\text{-}10hr)}$ for the MRHD (10 µg BID = 20 µg/day) = 2076 pg.h/ml

Antibody sample: Blood was collected from the 3 animals/sex/group (LD, MD & HD) not used for toxicokinetic sampling and from all control group animals sacrificed on Day 92 (no sooner than 24 hours after the morning dose on Day 91). In addition, blood samples were obtained pre-study from 5 males and 5 females not randomized into the study. ELISA was used to determine anti-exenatide antibody.

Anti-Exenatide Antibody Titer

Dose (µg/kg) BID	0		3		34		380	
Sex	M	F	M	F	M	F	M	F
Anti-Exenatide Antibody Positive titer/Total assayed	0/17	0/20	0/2	0/2	0/1	0/3	0/3	0/1

2.6.6.3.7

Study title: Subcutaneous TK Study of AC2993 in CD-1 Mice with Selective Measurements of Biological Response following 91 Days Exposure

This study was conducted to evaluate the systemic exposure to AC2993 in the strain of mice and at the dose levels used in the carcinogenicity study; to define the food consumption, water consumption, and body weight gain; and to examine the parotid salivary gland for histopathological alterations. This study uses single SC bolus dosing as does the carcinogenicity evaluation.

Key study findings:

- Reversible mean body weight increases were noted in MD (8%) and HD (6%) females relative to control.
- Basophilic foci were noted in the parotid salivary glands from all treated animals. Severity was minimal to moderate in MD males and HD females. This effect was almost completely reversed.
- Water consumption decreased in MD females (33%) during week 1. By week 13, water consumption was increased in MD (30%) and HD (18%) females. This effect was not reversed in HD females.
- Cmax and AUC both increased with increasing dose.
- NOAEL could not be established since only the parotid salivary gland was examined. Other tissues were not examined.

Study no.: REST02325
Volume # and page #: N/A.
Conducting laboratory and location: (b) (4) (b) (4).
Date of study initiation: January 3, 2003.
GLP compliance: Yes.
QA report: yes (X) no ( )
Drug, lot #, and % purity: Lot # 02-0106TP, 95.8% pure; Lot # 01-0102TP 95.1% pure.
Methods

Doses: 18, 70 and 250 µg/kg once daily by bolus subcutaneous injection.
Species/strain: Mouse/CD-1.
Number/sex/group or time point (main study): 20/sex/group (control & HD); 10/sex/group (LD & MD).
Route, formulation, volume, and infusion rate: Subcutaneous injection; 947 µl/kg (LD), 1400 µl/kg (MD), 3205 µl/kg (HD), 3205 µl/kg (control).
Satellite groups used for toxicokinetics or recovery: 20/sex/group for TK.
Age: 7 weeks at study initiation.
Weight (non-rodents only): N/A.

Study design:

Group Assignments			
Group Number	Dose Level (µg/kg)	Number of Animals[a,b] Male (animal numbers)	Female (animal numbers)
Main Study:			
1	0	20 (1001 – 1020)	20 (1181 – 1200)
2	18	10 (1021 – 1030)	10 (1201 – 1210)
3	70	10 (1031 – 1040)	10 (1211 – 1220)
4	250	20 (1041 – 1060)	20 (1221 – 1240)
Toxicokinetic:			
5 (Day 91)	18	20 (1061 – 1080)	20 (1241 – 1260)
6 (Day 91)	70	20 (1081 – 1100)	20 (1261 – 1280)
7 (Day 91)	250	20 (1101 – 1120)	20 (1281 – 1300)
8 (Day 1)	18	20 (1121 – 1140)	20 (1301 – 1320)
9 (Day 1)	70	20 (1141 – 1160)	20 (1321 – 1340)
10 (Day 1)	250	20 (1161 – 1180)	20 (1341 – 1360)

[a]Ten animals/sex/main study group at 0 and 250 µg/kg were randomly selected and maintained for a 30-day recovery following the end of the treatment period.
[b]An extra two animals/sex were assigned to each toxicokinetic group to be utilized as replacements, which was not necessary. Because animals were not utilized as replacements, they were used for additional samples at the 6-hour postdose interval.

Observation times and results

Mortality: Twice daily.

One control male (1/20) was found dead on Day 74 and one HD male (1/20) was found dead on Day 7. While the demise of these animals could not be determined, sponsor stated that they were not considered treatment-related.

Clinical signs: Daily.

Except for scabbed areas at some of the injection sites, there were no other treatment-related clinical findings.

Body weights: (g) - Prior to dosing and weekly thereafter.

Dose (µg/kg/d)	**0**		**18**		**70**		**250**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Week -1	27.6	23.2	27.6	23.2	27.6	23.2	27.6	23.2
Week 13	37.6	30.5	37.5	32.4	37.0	33.0*(8%↑)	37.5	32.4*(6%↑)
Recovery week 17	31.0	32.2					39.2	34.0

*** p < 0.05**

Food consumption: Weekly.
No treatment-related effects.

Water consumption: Weekly.

Dose (µg/kg/d)	**0**		**18**		**70**		**250**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Week 1	8.4	6.3	6.2	6.2	6.3	4.2*(33%↓)	6.2	5.2
Week 13	7.9	8.3	8.8	8.7	8.0	10.8**(30%↑)	9.4	9.8(18%↑)
Recovery week 17	7.8	8.2					8.5	9.6*(17%↑)

*** p < 0.05; ** p< 0.01**

Ophthalmoscopy: Not conducted.

EKG: Not conducted.

Hematology: Not conducted.
Clinical chemistry: Not conducted.

Urinalysis: Not conducted.

Gross pathology: Tissues/organs collected are indicated in the histopathology table.

No treatment-related macroscopic lesions were noted were noted at terminal and recovery sacrifice.

Organ weights: Not evaluated.

Histopathology: Adequate Battery: yes ( ), no (X)—explain
Peer review: yes (X), no ( )

This is a TK study. Therefore only representative samples of the parotid salivary gland (target organ) were processed from all main study animals for examination.

Dose (µg/kg/d)	**0**		**18**		**70**		**250**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Parotid salivary gland Lymphocyte infiltration	0	0	1(1)	0	1(1)	0	0	0
Basophilic foci	0	0	7 5(1) 2(2)	9 7(1) 2(2)	8 5(1) 2(2) 1(3)	10 5(1) 5(2)	6 2(1) 4(2)	9 4(1) 4(2) 1(3)
RECOVERY	**M**	**F**					**M**	**F**
N	**10**	**10**					**10**	**10**
Lymphocyte infiltration	1(1)	0					1(1)	1(1)

1 = minimal; 2 = mild; 3 = moderate

Toxicokinetics: Blood samples were collected from TK animals on Days 1 and 91 at 0.5, 1, 2, 3, 4 and 6 hours post dose.

TK Data

Dose µg/kg/day	Sex	C_{max} (pg/mL)		AUC_{0-6h} (pg·h/mL)	
		Day 1	Day 91	Day 1	Day 91
18	M/F	9286	29,363	10,113	25,425
70	M/F	37,293	63,700	32,508	58,403
250	M/F	92,253	168,867	123,241	197,295

Total daily $AUC_{(0-10hr)}$ for the MRHD (10 µg BID = 20 µg/day) = 2076 pg.h/ml

2.6.6.3.8

Study title: **A 182-Day Toxicity Study of AC2993 Administered BID by Subcutaneous Injection to CD-1 Mice**

Key study findings:

- 2/20 control males, 5/25 LD males, 7/25 MD males, 2/25 HD males, 5/20 control females, 4/25 LD females, and 3/25 HD females died or were sacrificed in a moribund condition during the study. Deaths associated with the bleeding procedure (cardiac puncture) occurred in 1/20 control males, 4/25 LD males, 3/25 MD males, 1/20 control females, 2/25 LD females, and 3/25 HD females. 1/20 control males died as a result of a probable urinary tract obstruction; 1/25 LD males was sacrificed moribund due to an accidental spinal cord injury; 1/25 MD males died as a result of either a urinary tract inflammation, an inflammation and hemorrhage from skin lesions, or a hemorrhage of the thoracic cavity; and 1/25 HD males died due to ulcerative skin lesions. Sponsor stated that the cause

of death for the remaining animals was undetermined but not considered related to treatment with AC 2993.

- Ophthalmology results showed that 1/25 males each in the LD and MD groups had phthisis bulbi and retinal atrophy respectively. 2/23 HD males had retinal atrophy and corneal edema while another HD male had keratitis. 1/25 MD female had phthisis bulbi. Retinal atrophy and cataract were reported separately in 2/25 HD females.
- MCV was slightly but significantly increased in HD males relative to control. Leukocytes and eosinophils were also slightly but significantly increased in HD females relative to control.
- Weight of the epididymis was slightly but significantly decreased in HD males relative to control. Heart weight was slightly but significantly decreased in HD males and females with no correlative histopathology. Aspermia was noted in 1/23 HD males along with degeneration of seminiferous tubule. Weights of the pituitary and thyroid/parathyroid were significantly decreased in HD males with no correlative histopathology. Relative weight of the lung was slightly but significantly decreased in HD females. Absolute weight of the liver and thyroid/parathyroid were slightly but significantly increased in HD females with no correlative histopathology.
- The target organs of toxicity include the eye (retinal atrophy, corneal edema, corneal mineralization, keratitis, phthisis bulbi, cataract), injection site (inflammation, hemorrhage, fibrosis, epithelial hyperplasia), testis (degeneration of seminiferous tubules), parotid salivary gland (basophilia), bone marrow hyperplasia (females).
- NOAEL could not be established based on the basophilia observed in the parotid salivary gland and ophthalmology findings at all doses. Pthisis bulbi (shrinkage and wasting of the eyeball) was observed at the LD (1/25 males). However, it is not clear if pthisis bulbi precedes development of retinal atrophy or if they are different pathological entities.

Study no.: REST00119
Volume # and page #: N/A.
Conducting laboratory and location: (b) (4) (b) (4).
Date of study initiation: 10/25/00.
GLP compliance: Yes (USA, UK & Japan)
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 00-0606TP, purity 92.9%.

Methods

Doses: Animals were dosed with 9, 58 and 380 µg/kg BID for 182 days (26 weeks or 6.5 months) to give total doses of 18, 116 and 760 µg/kg/d.

Species/strain: Mouse/CD-1.
Number/sex/group or time point (main study): 20/sex/group – control and 25/sex/group for all other study groups.
Route, formulation, volume, and infusion rate: Subcutaneous injection, 900, 3050 and 4875 µl/kg/dose.
Satellite groups used for toxicokinetics or recovery: 10/sex/group for TK.
Age: 7 weeks at study initiation.
Weight (non-rodents only): N/A.

Observation times and results

Mortality: Daily.

Dose (µg/kg) BID	Animals found dead or sacrificed in extremis		Died post bleeding	
	M	F	M	F
0	2/20	4/20		1/20
9	4/25	2/25	1/25	2/25
58	6/25		1/25	
380	2/25	1/25		2/25

Empty cells indicate zero incidence.

Causes of Death:

Dose (µg/kg) BID	Deaths associated with bleeding procedure		Other causes of death determined by the sponsor
	M	F	
0	1/20	1/20	1/20 control males died as a result of a probable urinary tract obstruction (bilateral dilatation of pelvis, hydronephrosis-bilateral, lymphocytic infiltration-unilateral).
9	4/25	2/25	1/25 males was sacrificed moribund due to an accidental spinal cord injury.
58	3/25		1/25 males died as a result of either a urinary tract inflammation, an inflammation and hemorrhage from skin lesions, or a hemorrhage of the thoracic cavity.
380		3/25	1/25 males at 380 µg/kg/dose died due to ulcerative skin lesions

Empty cells indicate zero incidence.

SUMMARY OF DEATHS

Dose (µg/kg) BID	Total Deaths	Deaths associated with bleeding procedure	Other causes of death determined by the sponsor	Undetermined causes of death
0	7/40	3/40	1/40 - probable urinary tract obstruction (bilateral dilatation of pelvis, hydronephrosis-bilateral, lymphocyt -ic infiltration-unilateral).	3/40
9	9/50	6/50	1/50 - spinal cord injury	2/50
58	7/50	3/50	1/50 - urinary tract inflammation, an inflammation and hemorrhage from skin lesions, or a hemorrhage of the thoracic cavity.	3/50
380	5/50	3/50	1/50 - ulcerative skin lesions.	1/50

Sponsor stated that the cause of death of the remaining animals was undetermined but not considered related to treatment with AC 2993.

Clinical signs: Daily.

No clinical signs associated with AC2993 treatment were observed. Findings of scabbed areas and/or abrasions were noted throughout the study in both control and treated groups of both sexes, generally at a higher incidence during the latter half of the study. These findings were considered secondary to the physical trauma associated with local injection site inflammation.

Body weights: Weekly.

No treatment-related effects on body weight.

Food consumption: Weekly.

No treatment-related effects on food consumption.

Ophthalmoscopy: Conducted at months 3 and 6.

Males

Dose (µg/kg) BID	0	9	58	380
Pretest	0/20	0/25	0/25	0/25
Month 3	0/20	0/25	1/23 (RA)*	2/23 (RA)*
Month 6	0/20	1/25 (PB)	1/23 (RA)*	2/23 (RA)*+CE; 1/23 (K)

PB = Phthisis bulbi; RA = retinal atrophy; CE = corneal edema; K = keratitis; * same animal

Females

Dose (µg/kg) BID	0	9	58	380
Pretest	0/20	0/25	0/25	0/25
Month 3	1/20 (K)	0/25	0/25	1/25(RA*);1/25(C**)
Month 6	0/17	0/24	1/25 (PB)	1/25(RA*);1/25(C**)

PB = Phthisis bulbi; RA = retinal atrophy; CE = corneal edema; K = keratitis; * or ** same animal; C = cataract

EKG: Not conducted

No data.

Hematology: Blood samples for hematology evaluation were collected pretest and at months 3 and 6 (prior to the AM dose). The animals had free access to drinking water and food prior to blood collection.

Dose (µg/kg) BID	0	9	58	380
MALES				
MCV (fl)	55.6	56.2	54.9	57.9*
FEMALES				
Leukocytes (x K/mm^3)	3.74	4.62	4.93	5.7**
Eosinophils (x 10^3/ul)	0.103	0.106	0.123	0.178*

*** p < 0.05; ** p < 0.01**

Clinical chemistry: Blood samples for clinical chemistry evaluation were collected pretest and at months 3 and 6 (prior to the AM dose). The animals had free access to drinking water and food prior to blood collection.

Males - Unremarkable

Dose (µg/kg) BID	0	9	58	380
FEMALES				
BUN (mg/dl)	27.1	34.8*	33.1	36.9**
Albumin/globulin ratio	1.00	1.14*	1.06	1.10

*** p < 0.05; ** p < 0.01**

Urinalysis: Urine samples were collected at necropsy by cystocentesis and pooled by group and sex.

Unremarkable.

Gross pathology: Organs/tissues isolated for gross pathology examination is indicated in the list of addendum. Bone marrow smears were collected at scheduled sacrifice.

There were no treatment-related macroscopic findings. Red discoloration noted at injection sites was considered a result of the injection and not related to treatment with AC2993.

Organ weights: Organs weighed are indicated in the list of addendum.

Dose (µg/kg) BID	0	9	58	380
MALES				
Epididymis (g)	0.14	0.12	0.12	0.11*
Epididymis/b. wt.% x10	3.69	3.31	3.27	2.99*
Heart (g)	0.23	0.21	0.22	0.21*
Heart/b. wt.% x10	6.10	5.66	5.99	5.44*
Pituitary (mg)	1.4	2.0	2.0	4.0**
Pituitary/b. wt.% x10	4.47	6.22	6.35	11.06**
Thyroid/parathyroid (mg)	7.0	7.0	7.0	9.0*
FEMALES				
Heart/b. wt.% x10	6.18	5.62*	5.65	5.36**
Liver (g)	1.72	1.87	1.86	1.94**
Lung/b. wt.% x10	8.26	7.67	7.78	7.40**
Thyroid/parathyroid (mg)	7.0	8.0	8.0	9.0**

*** p < 0.05; ** p < 0.01**

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Males

Dose (µg/kg) BID	0	9	58	380
Epididymis Aspermia				1/23(2)
Eye Cornea, mineralization				1/23(1)
Retinal atrophy			1/23(1)	2/23(1)
Injection sites Hemorrhage	2/20(1)	1/25(1)	1/25(1)	5/25 4/25(1) 1/25(2)
Inflammation	3/20(1)	2/25(1)	3/25(1)	6/25 5/25(1) 1/25(2)
Fibrosis	14/20 12/20(1) 2/20(2)	8/25(1)	13/20 12/20(1) 1/20(2)	15/20 13/20(1) 2/20(2)
Hyperplasia, epithelial	1/20(1)	5/25(1)	1/25(1)	5/25 4/25(1) 1/25(2)
Liver Inflammation	2/20(1)		1/25(1)	4/25(1)
Salivary gland, Parotid Basophilic	0/17	22/22 13/23(1) 7/23(2) 2/23(3)	16/20 16/20(1)	19/24 12/24(1) 6/24(2) 1/24(3)
Skin, subcutis Inflammation			1/5(1)	5/13(1)
Fibrosis	3/5(1)	2/2(1)	5/5 3/5(1) 2/5(2)	13/13 8/13(1) 5/13(2)
Testis Degeneration, seminiferous tubule	1/20(1)			3/25(1)

1 = trace; 2 = mild; 3 = moderate; 4 = severe; empty cells = zero incidence

Females

Dose (µg/kg) BID	0	9	58	380
Bone marrow, femur Hyperplasia				1/25(1)
Bone marrow, sternum				

Hyperplasia				1/25(1)
Eye Retinal atrophy				1/25(1)
Cataract				1/25(2)
Mineralization, cornea				1/25(1)
Injection sites Hemorrhage	3/20(1)	1/25(1)	1/25(1)	5/25 3/25(1) 2/25(2)
Fibrosis	7/20(1)	3/25(1)	15/25(1)	10/25 9/25(1) 1/25(2)
Hyperplasia, epithelial	2/20(1)		3/25(1)	4/25(1)
Inflammation	13/20 12/20(1) 1/20(2)	17/25 15/25(1) 2/25(2)	18/25(1)	14/25(1)
Lymph node, inguinal Lymphoid hyperplasia				1/25(2)
Histiocytosis				1/25(2)
Sciatic nerve Fibrosis				1/25(1)
Degeneration				1/25(2)
Salivary gland, Parotid Altered foci, basophilic		21/24 16/24(1) 5/24(2)	19/19 12/19(1) 6/19(2) 1/19(3)	24/25 10/25(1) 12/25(2) 2/25(3)

1 = trace; 2 = mild; 3 = moderate; 4 = severe; empty cells = zero incidence

Toxicokinetics: Blood samples were collected pretest and at 0.5, 1, 2, 3, 4, and 6 hours post-AM dose on Days 1 and Day 91. On Day 182, blood samples were collected at the same time points. The animals were not fasted prior to blood collection.

Daily Dose µg/kg/day	Dose µg/kg	Sex	C_{max} (pg/mL)			AUC_{0-6h} (pg h/mL)		
			Day 1	Day 91	Day 182	Day 1	Day 91	Day 182
18	9	M/F	10,450	8726	12,848	7745	8527	10,562
116	58	M/F	71,018	70,649	59,769	52,186	61,311	54,789
760	380	M/F	560,693	605,329	684,224	429,798	543,957	538,670

Total daily $AUC_{(0-10hr)}$ for the MRHD (10 µg BID = 20 µg/day) = 2076 pg.h/ml

Plasma Collection for Antibody Analysis: Blood samples for the determination of anti-AC2933 antibodies were collected at pretest and at necropsy from all surviving main study animals. The samples were collected approximately 24 hours after the final dose in each case. Anti-exenatide antibody detection was carried out using ELISA.

Dose (µg/kg) BID	**0**		**9**		**58**		**380**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Positive Titer 1:5/Total	0/18 (0%)	2/15 (13.3%)	0/9 (0%)	0/9 (0%)	0/8 (0%)	2/12 (16.7%)	0/8 (0%)	0/9 (0%)

RAT

2.6.6.3.9

Study title: **Subcutaneous TK Study Of AC2993 In SD Rats With Selective Measurements Of Biological Response With A 91-Days Exposure**

This study was conducted to evaluate the systemic exposure to AC2993 in the strain of rats and at the dose levels used in the carcinogenicity study; to define the food consumption, water consumption, and body weight gain; and to examine the parotid salivary gland and pancreas for histopathological alterations. The study utilized bolus SC dosing to mimic the dosing in the carcinogenicity study not the actual BID dosing.

Key study findings:

- One HD male was found dead on Day 35. Cause of death could not be determined.
- Body weight gain was decreased by 29-34% in treated males and by 23-25% (not dose-related) in treated females. At the end of the recovery period, mean body weight was lower in HD males relative to control. However, body weight gain in the HD group was greater than in controls.
- Food consumption was decreased by 11% and 24% in HD males and females respectively, with partial recovery.
- Reversible increase in water consumption was noted in all treated rats relative to controls.
- Reversible increases (not dose-related) in adrenal and liver weights were observed in treated rats. Weight of the thyroid/parathyroid gland was decreased in LD and MD males but increased in HD males. Relative weight of the thyroid/parathyroid gland was increased in MD females. Reversible decrease in prostate weight was noted in MD and HD males. Partial reversible increase in relative weight of the testis was increased in HD. Ovarian weight was decreased in HD females at the end of the recovery period. The increased relative organ weights may be due to the decreased body weight gains observed since there were no histopathology correlates.
- A low incidence and minimal severity of basophilic foci (reversible) was observed in LD and HD females. At the end of the recovery period, relatively high incidences of vacuolar change (adrenal gland), lymphocyte infiltration (pancreas) and a low incidence of basophilic foci (parotid salivary gland) with minimal severities were noted in HD males.
- Seven specimens tested positive for anti-exenatide antibodies (One animal in the control group had a titer of 1:125. One LD male and female had a titer ≥ 1:25. All other titer-positive animals had a titer of 1:5 (1 MD male, 2 HD males and 1 HD female). Thus the potential of AC2993 to elicit an immune response in rats over a 3-month period is low.
- Red discoloration at injection sites was observed in both control and treated rats with no dose-related effect.
- Both Cmax and AUC increased with increasing dose.
- NOAEL could not be established since only selected organs were examined macroscopically.

Study no.: REST 02246

Volume # and page #: N/A.

Conducting laboratory and location: (b) (4) (b) (4)

Date of study initiation: December 9, 2002.

GLP compliance: Yes.

QA report: yes (X) no ( )

Drug, lot #, and % purity: Lot # 00-0606TP, 93% pure; Lot # 01-0102TP, 95% pure.

Methods

Doses: 18, 70, 250 µg/kg/d – animals were dosed once daily for 91 days by bolus subcutaneous injection.

Species/strain: Rat/SD.

Number/sex/group or time point (main study): 20/sex/group (control and HD); 10/sex/group (LD and MD).
Route, formulation, volume, and infusion rate: Subcutaneous injection; 360 µl/kg (LD), 700 µl/kg (MD), 833 µl/kg (HD).
Satellite groups used for toxicokinetics or recovery: 24/sex/group on Day 1 and 16/sex/group on Day 91 for TK.
Age: 9 weeks old at study initiation.
Weight (non-rodents only): N/A.
Study Design:

Group Assignments			
Group Number	Dose Level (µg/kg)	Number of Animals[a]	
		Male (animal numbers)	Female (animal numbers)
Main Study:			
1	0	20 (1001 – 1020)	20 (1181 – 1200)
2	18	10 (1021 – 1030)	10 (1201 – 1210)
3	70	10 (1031 – 1040)	10 (1211 – 1220)
4	250	20 (1041 – 1060)	20 (1221 – 1240)
Toxicokinetic:			
5 (Day 91)	18	16 (1061 – 1076)	16 (1241 – 1256)
6 (Day 91)	70	16 (1077 – 1092)	16 (1257 – 1272)
7 (Day 91)	250	16 (1093 – 1108)	16 (1273 – 1288)
8 (Day 1)	18	24 (1109 – 1132)	24 (1289 – 1312)
9 (Day 1)	70	24 (1133 – 1156)	24 (1313 – 1336)
10 (Day1)	250	24 (1157 – 1180)	24 (1337 – 1360)

[a]Ten animals/sex/main study group at 0 and 250 µg/kg were randomly selected and maintained for a 30-day recovery following the end of the treatment period.

Observation times and results

Mortality: Daily.

One out of 20 HD males was found dead on Day 35. Cause of death could not be determined.

Clinical signs: Daily.

Treatment-related salivation was observed in all treated animals during the treatment phase of the study. While this effect is not dose related, it was observed more frequently in more HD animals than in LD and MD animals. During the recovery phase, only a single incidence of salivation was observed in HD males.

Body weights: Measured prior to treatment and weekly thereafter.

Body weight (g)

Dose (µg/kg/d)	0		18		70		250	
Sex	M	F	M	F	M	F	M	F
Week 1	325	228	330	226	320	229	320	219
Week 13	528	307	475**	287	465**	288	455*	280**
Wt. gain	203	79	145	61	145	59	135	61
Wt. gain decrement (%)	-	-	29	23	29	25	34	23
Recovery week 14	517	300					462**	283
Recovery week 17	553	313					510**	299
Wt. gain	36	13					48	16
Wt. gain decrement (%)	-	-					-	-

* $p < 0.05$; ** $p < 0.01$

Food consumption: Weekly.

Food consumption (g/day)

Dose (µg/kg/d)	0		18		70		250	
Sex	M	F	M	F	M	F	M	F
Week 1	23	17	22	16*(6%↓)	20*(13%↓)	15**(12%↓)	19**(17%↓)	13**(24%↓)
Week 13	27	20	26	19	25	18	24**(11%↓)	16**(20%↓)
Recovery week 14	26	18					28	19
Recovery week 17	27	20					26	18

* p < 0.05; ** p < 0.01

Water consumption: Weekly.

Water consumption (g/day)

Dose (µg/kg/d)	0		18		70		250	
Sex	M	F	M	F	M	F	M	F
Week 1	30	26	41**(37%↑)	48**(85%↑)	41**(37%↑)	44**(69%↑)	41**(37%↑)	44**(69%↑)
Week 13	37	31	42	49**(58%↑)	47**(27%↑)	54**(74%↑)	50**(35%↑)	44**(42%↑)
Recovery week 14	37	38					45	35
Recovery week 17	36	34					38	35

* p < 0.05; ** p < 0.01

Ophthalmoscopy: Not conducted.
No data.

EKG: Not conducted.
No data.

Hematology: Not conducted.
No data.

Clinical chemistry: Not conducted.
No data.

Urinalysis: Not conducted.
No data.

Gross pathology: Tissues/organs collected from all main study animals are indicated in the histopathology table.

Dose (µg/kg/d)	0		18		70		250	
Sex	M	F	M	F	M	F	M	F
N	10	10	10	10	10	10	10	10
Injection site, L. flank Red discoloration	0	4	2	0	2	0	1	3
Injection site, L. shoulder Red discoloration	1	0	0	0	1	2	1	2
Injection site, R. flank Red discoloration	2	3	1	2	3	3	2	2
Injection site, R. shoulder Red discoloration	3	2	0	1	1	0	2	3
Kidney Dilatation, pelvic	0	0	0	0	0	0	1	0
Urinary bladder Calculus/calculi	0	0	0	0	0	0	2	0
Recovery	M	F					M	F
n	10	10					10	10
Kidney Dilatation, pelvic	2	0					0	0

Organ weights: Organs weighed are indicated in the histopathology table.

Dose (µg/kg/d)	0		18		70		250	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Adrenal (mg)	68	69	76	84	87**(30%↑)	82**(19%↑)	82**21(%↑)	82**(19%↑)
Adrenal/BW % x 10^3	13	23	16*(23%↑)	30*(30%↑)	19*(46%↑)	31*(35%↑)	18*(39%↑)	30*(30%↑)
Thyroid/Pthy (mg)	30	21	22*(27%↓)	20	25*(16%↓)	23	25*(19%↑)	20
Thyroid/Pthy/BW % x 10^3	6	7	5*(17%↓)	7	5	9*(29↑%)	6	7
Liver/BW %	3.4	-	3.6	-	3.7*(9%↑)	-	3.8**(12%↑)	-
Prostate (g)	0.8	-	0.8	-	0.6*(25%↓)	-	0.6*(25%↓)	-
Testis/BW % x 10	6.9	-	7.3	-	7.6	-	8.1*(17%↑)	-
RECOVERY								
N	**10**	**10**					**10**	**10**
Epididymis/BW % x 10	2.8	-					2.9*(3%↑)	-
Liver (g)	19	-					17*(11%↓)	-
Testis/BW % x 10	5.8	-					6.7*(16%↑)	-
Ovary (mg)	-	130					-	98*(25%↓)

* $p < 0.05$; ** $p < 0.01$

Histopathology: Adequate Battery: yes ( ), no (X)—explain
Peer review: yes (X), no ( )

This is a TK study. Therefore only representative samples of the adrenal glands, pancreas, parotid salivary gland (target organ) and thyroid glands were processed from all main study animals for examination.

Histopathology Data

Dose (µg/kg/d)	0		18		70		250	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Adrenal gland, cortex Vacuolar change	2 1(1) 1(2)	0	3(1)	0	2(1)	0	3(1)	0
Parotid salivary gland Basophilic foci	0	0	0	1(1)	0	0	0	1(1)
RECOVERY								
N	**10**	**10**					**10**	**10**
Adrenal gland, cortex Vacuolar change	3(1)	0					4(1)	0
Pancreas Lymphocyte infiltraton	5(1)	0					7(1)	0
Parotid salivary gland Basophilic foci	0	0					1(1)	0

1 = minimal; 2 = mild

Plasma Anti-AC2993 antibodies: Blood samples were collected from all main study animals prior to scheduled necropsy at 24 hr following the last dose. Anti-exenatide antibody was detected by ELISA.

Anti-Exenatide Antibody

Dose (µg/kg/d)	0		18		70		250	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Titer 1:5/Total	0/10	0/14	0/8	0/10	0/6	1/9	2/8	1/9
Titer 1:25/Total	0/10	0/14	1/8	0/10	0/6	0/9	0/8	0/9
Titer 1:125/Total	1/10	0/14	0/8	1/10	0/6	0/9	0/8	0/9

Toxicokinetics: Blood samples were collected on Days 1 and 91 at 0.5, 1, 2, 3, 4, 6, 9 and 12 hr post dose from the TK animals.

Dose µg/kg/day	Sex	C_{max} (pg/mL)		AUC_{0-6h} (pg·h/mL)	
		Day 1	Day 91	Day 1	Day 91
18	M	12,407	10,505	19,866	10,693
	F	14,480	8310	20,474	9663
	M/F	**13,444**	**9408**	**20,188**	**10,178**
70	M	21,910	30,370	40,753	50,783
	F	45,480	38,798	51,560	46,348
	M/F	**31,338**	**34,584**	**45,619**	**48,554**
250	M	129,933	126,600	195,309	266,284
	F	187,000	204,225	202,166	269,458
	M/F	**158,467**	**162,863**	**201,764**	**268,094**

Total daily $AUC_{(0-10hr)}$ for the MRHD (10 µg BID = 20 µg/day) = 2076 pg.h/ml

MONKEY

2.6.6.3.10

Study title: **A 91 Days Toxicity Study of AC2993 Administered BID by Subcutaneous Injection to Cynomolgus Monkeys**

Key study findings:

- Inappetence was the only treatment-related clinical sign that occurred during the first two weeks of dosing in HD males and during the first week in HD females. Food consumption was statistically significantly decreased only in HD males by 51% and 23% during weeks 1 and 2 respectively.
- Though not statistically significant, body weight of HD males was relatively lower throughout the dosing period compared to those of control.
- There was a slight but significant decrease in hemoglobin concentration in HD males relative to control.
- BUN was significantly increased by 33% and 29% in males dosed with 0.6 and 6.7 µg/kg BID but not in the HD group. While this may be suggestive of nephropathy, there is no correlative histopathology.
- There seems to be a dose-related decrease in absolute weights of the spleen (males only) and thymus (males and females) but the decrements are not significant. Absolute weights of the heart and kidney were also decreased in males but not in a dose-related manner.
- Small thymuses were observed in 1/4 males and 1/4 females at 6.7 µg/kg and in 1/4 males and 3/4 females at 75 µg/kg. This explains the decrease in absolute weight observed. There is no correlative histopathology.
- A focal inflammation was observed in the stomach of 1/4 HD males and a focal congestion in 1/4 MD females. Severity was minimal.
- Minimal inflammation of the lung was observed in 1/4 LD males and 1/4 HD females. Syncytial giant cells were observed in the lungs of 1/4 HD female. Focal and multifocal lung hemorrhages were observed in 1/4 HD males and 1/4 MD females respectively.
- Multifocal endometrial hemorrhage of minimal to mild severity was observed in 1/4 females each at the MD and HD.
- Pigmented histiocytes were observed in the mesenteric lymph nodes all treated and some control animals. While incidence of occurrence appears to be dose related, severity is not.
- Injection sites were observed with chronic inflammation and fibrosis or fibroplasia.

- There seems to be a treatment-related increase in percentage of animals that tested positive for anti-AC2993 antibodies suggesting that the drug may be antigenic.
- The target organs of toxicity appear to be the lung, uterus and stomach.
- NOAEL = 0.6 µg/kg BID (3X MRHD, AUC) based on the stomach, lung and endometrial findings at the MD and HD.

Study no.: REST99050
Conducting laboratory and location: (b) (4)., (b) (4)
Date of study initiation: January/February 2000.
GLP compliance: Yes (USA and Japan).
QA report: yes (X) no ( )
Drug, lot #, and % purity: Lot # 99-1002TP, 85% pure.
Formulation/vehicle: AC2993 stock solutions (0.3 mg/ml) were diluted with placebo (control article) to provide concentrations of 50, 100 and 300 µg/ml.

Methods

Doses: 0.6, 6.7 and 75 µg/kg BID (total daily doses of 1.2, 13.4 and 150 µg/kg).
Species/strain: Monkey/Cynomolgus
#/sex/group or time point (main study): 4/sex/group.
Satellite groups used for toxicokinetics or recovery: All main study animals were used for TK.
Age: 3 to 4 years.
Weight: 2.5 to 4.4 kg (M); 2.3 to 2.8 kg (F).
Route, form, volume, and infusion rate: Subcutaneous, solution, volume = 12, 67 and 250 µl/kg for LD, MD and HD respectively.

Observation times and results

Mortality: Daily.
None.
Clinical signs: Daily.

The only treatment-related clinical sign was inappetence that was observed mostly in the HD group.

AC2993 Dose Level (µg/kg BID)	Total Number of Days of Inappetence Observed/Total Number of Days Possible	
	Male	Female
0	2/368	11/368
0.6	2/368	7/368
6.7	6/368	13/368
75	17/368	21/368

Body weights: Weekly.

Week 13 Mean Body Wt. (kg)

DOSE (µg/kg) BID	0		0.6		6.7		75	
Sex	M	F	M	F	M	F	M	F
Day -1	3.5	2.6	3.0	2.5	3.2	2.5	3.0	2.6
Week 13 wt.	4.0	2.8	3.2	2.6	3.4	2.6	3.0	2.4
Wt. Gain	0.5	0.2	0.2	0.1	0.2	0.1	0.0	- 0.2
Wt. gain decrement	-	-	0.3	0.1	0.3	0.1	0.5	0.4
% Decrement	-	-	60	50	60	50	100	200

Food consumption: Daily estimation.

Food Consumption Values (Average Daily Number of Biscuits)

DOSE (µg/kg) BID	0		0.6		6.7		75	
Sex	M	F	M	F	M	F	M	F
Day -1	7.8	5.1	8.0	6.3	8.4	5.0	8.2	6.1
Week 13 wt.	10.5	6.9	11.1	8.3	9.7	7.3	8.3	7.4
Gain	2.7	1.8	3.1	2.0	1.3	2.3	0.1	1.3
Decrement	-	-	-	-	1.4	0.5	1.7	0.5
% Decrement	-	-	-	-	52	28	94	28

Ophthalmoscopy: Conducted on all animals pre-dosing and during the last week of dosing.

No treatment-related ophthalmic findings.

EKG: Conducted on all animals pre-dosing and during the last week of dosing. Sponsor did not state when EKG was conducted with respect to dosing.

No treatment-related ECG findings.

Hematology: Blood samples were collected 1 week before dosing, prior to dosing on Day 1 and prior to the first dose on one day in weeks 4, 8 and 13 for routine hematology evaluation.

DOSE (µg/kg) BID	0		0.6		6.7		75	
Sex	M	F	M	F	M	F	M	F
HGB (g/dl)	14.1	12.3	13.9	13.1	12.6	13.2	12.7*(10%↓)	12.3

***p < 0.05**

Clinical chemistry: Blood samples were collected 1 week before dosing, prior to dosing on Day 1 and prior to the first dose on one day in weeks 4, 8 and 13 for routine clinical chemistry evaluation.

DOSE (µg/kg) BID	0		0.6		6.7		75	
Sex	M	F	M	F	M	F	M	F
BUN(mg/dl)	21±3	21±4	28±3*(33%↑)	28±5	27±3*(29%↑)	26±3	26±2	25±4

***p < 0.05**

Urinalysis: Urine samples were obtained by cystocentesis at necropsy.

No treatment-related effects.

Gross pathology: Organs/tissues isolated for gross pathology examination is indicated in the histopathology table.

Empty cells indicate zero incidence

DOSE (µg/kg) BID	0		0.6		6.7		75	
Sex	M	F	M	F	M	F	M	F
n	4	4	4	4	4	4	4	4
Thymus - Small					1	1	1	3
Stomach - lesion					1			
Stomach - focal lesion						1	1	
Cecum - pink focus			1					
Auxillary lymph node – dark discoloration					1			
Inguinal lymph node – dark discoloration					1			
Thyroid – small							1	
Ovaries – unilateral, enlarged						1		
Tail lesion							1	1
Injection site lesion		2						

Organ weights: Organs weighed are indicated in the histopathology table.

Week 13 Data

DOSE (µg/kg) BID	0		0.6		6.7		75	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Thymus (g)	4.8	1.8	4.8	1.5	3.3	1.3	1.9	1.0
Thymus wt. relative to body wt.	1.1	0.7	1.5	0.6	1.1	0.5	0.7	0.4
% Decrease in absolute wt.	-	-	-	17	31	28	60	44

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Tissues from animals in all dose groups were processed for microscopic examination.
Empty cells indicate zero incidence

Dose (µg/kg/BID)	0		0.6		6.7		75	
Organ/Tissue Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Uterus Endometrial hemorrhage, multifocal						1/4(2)		1/4(1)
Lung Syncytial giant cells, focal								1/4(1)
Lung Subacute inflammation, interstitial			1/4(1)					1/4(1)
Lung Hemorrhage, focal & multifocal						1/4(1)	1/4(1)	
Stomach Acute inflammation, focal								1/4(1)
Stomach Congestion, focal							1/4(1)	
Mesenteric lymph node Pigmented histiocytes, multifocal	2/4(2)	3/4(2)	3/4 1/4(1) 2/4(2)	3/4(2)	4/4 2/4(2) 2/4(3)	4/4 1/4(1) 2/4(2) 1/4(3)	4/4 1/4(1) 2/4(2) 1/4(3)	4/4 1/4(1) 3/4(2)
Injection site (SC) Chronic/chronic active inflammation	3/4 2/4(1) 1/4(2)	2/4 1/4(1) 1/4(2)	2/4(1)	1/4(1)	2/4(1)		3/4 1/4(1) 2/4(2)	4/4 1/4(1) 3/4(2)
Injection site (SC) Fibrosis/fibroplasia	3/4 1/4(2) 2/4(4)	4/4 2/4(2) 2/4(4)	3/4 1/4(2) 2/4(4)	3/4 3/4(2)	3/4 2/4(2) 1/4(4)	1/4 1/4(4)	4/4 2/4(2) 2/4(4)	4/4 1/4(1) 3/4(4)

1 = minimal; 2 = mild; 3 = moderate; 4 = severe

Toxicokinetics: Blood samples were collected prior to the first dose and at 0.5, 1, 2, 4, 6, 9 and 12 hr following the first dose on Day 1 and on one day during weeks 4, 8 and 13.

Daily Dose µg/kg/day	Dose µg/kg	Sex	C_{max} (pg/mL)				$AUC_{0-12\,h}$ (pg·h/mL)			
			Day 1	Day 30	Day 60	Day 90	Day 1	Day 30	Day 60	Day 90
1.2	0.6	M	2214	2189	2698	2398	3209	3353	6572	6646
		F	2865	1919	4788	3156	4289	3551	9527	6123
		M/F	**2540**	**2054**	**3743**	**2831**	**3749**	**3452**	**8050**	**6347**
13.4	6.7	M	16,844	19,016	14,113	41,252	36,299	46,778	54,494	159,783
		F	16,756	14,192	12,887	33,873	40,024	40,816	35,996	111,770
		M/F	**16,800**	**16,604**	**13,500**	**37,537**	**38,162**	**43,797**	**45,245**	**135,776**
150	75	M	311,261	278,143	288,818	423,846	654,064	740,226	1,499,184	1,858,685
		F	235,039	479,886	323,365	759,854	555,463	1,394,959	1,481,474	2,309,482
		M/F	**273,150**	**379,015**	**306,092**	**591,850**	**604,763**	**1,067,592**	**1,490,329**	**2,084,084**

Total daily $AUC_{(0-10hr)}$ for the MRHD (10 µg BID = 20 µg/day) = 2076 pg.h/ml

Antibody Evaluation: Plasma anti-AC2993 antibodies were measured using an ELISA assay. The results of the anti-AC2993 antibody values in the study specimens are presented below.

Summary of all anti-AC2993 Positive Animals

Dose Group (µg/kg/day)	0	1.2	13.4	150
Total Animals	44	8	8	8
Positive Animals	2	3	2	4
Percent Positive	5	38	25	50

All specimens with calculated SD scores below 3.00 were reported as negative and all above 3.00 were reported as the positive. The positive specimens were further diluted in the two sample diluents and assayed to calculate the titer point, highest dilution giving a positive SD score.

Anti-AC2993 Positive specimens

Animal Number	Dose Group (µg/kg/day)	SDscore	Titer Point
2803	0	27.51	1:5
2815	0	5.76	1:5
1203	1.2	4.49	1:5
1204	1.2	100.9	1:25
2203	1.2	23.37	1:25
1303	13.4	37.63	1:25
1304	13.4	18.75	1:25
1401	150	69.56	1:125
1402	150	20.35	1:25
1404	150	35.22	1:25
2401	150	27.35	1:25

5% of control animals tested positive for anti-AC2993 antibodies compared to 38, 25 and 50% for the 0.6, 6.7 or 75 µg/kg BID groups. There seems to be a treatment-related increase in percentage of animals that tested positive suggesting that the drug may be antigenic to monkey. However, the positive finding in some control animals (which may be due to contamination or background error) undermines the accuracy of this study. Moreover, with the exception of one HD animal that had an antibody titer of 125, the rest of the treated animals had antibody titer of 25 regardless of treatment group. Systemic exposure increased with dose in the monkey suggesting that the anti-exenatide antibody formed is not neutralizing. Sponsor stated that the efficacy of the drug was not inhibited in a 28-day clinical trial suggesting that the anti-exenatide antibody is not neutralizing.

2.6.6.3.11

Study title: A **273 Days Toxicity Study of AC2993 Administered BID By Subcutaneous Injection to Cynomolgus Monkeys**

Key study findings:

- RBC, hemoglobin and hematocrit were decreased in HD animals relative to control. MCV and MCH were decreased only in MD animals. Reticulocyte count was also decreased in MD and HD animals.
- Relative weights of the thyroid/parathyroid and brain were increased in all treated groups but achieved statistical significance in the HD group. It is not clear if the mononuclear cell infiltration (brain) and follicular distension (thyroid) observed in some HD animals could explain the increase in relative weights.
- Positive serum titers of anti-KLH antibodies were found in 5 out of 6 (83 %), 6 out of 6 (100%), 5 out of 6 (83%) and 6 out of 6 (100%) animals immunized with KLH antigen from Groups 1-4 respectively. This demonstrated that the animals had ample humoral integrity.
- The target organs of toxicity include the brain (mononuclear cell infiltration, hemorrhage), thyroid (follicular distension, epithelial degeneration), adrenal gland (mineralization, nodular hypertrophy), kidney (tubular dilatation), heart (mononuclear cell infiltration), skeletal muscle (lymphoid cell

infiltrate), pancreas (vacuolation, fibrosis, mononuclear cell infiltrate, hypercellular), sciatic nerve (fibrosis), uterus (protein deposits), stomach (lymphoid hyperplasia, lymphoplasmacytic infiltrate), colon (cystic dilatation), cecum (pigmented macrophages), jejunum (cytoplasmic vacuolation), injection sites (epidermal hyperplasia) and rectum (inflammation).

- NOAEL 2.2 µg/kg BID (4X MRHD, AUC) based on histopathology.

Study no: REST00120
Volume # and page #: N/A.
Conducting laboratory and location: (b) (4), (b) (4)
Date of study initiation: November 8, 2000.
GLP compliance: Yes (USA, UK, Japan).
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 00-0606TP, 93% pure.

Methods

Doses: Animals were dosed twice daily with AC2993 1.1, 9 and 75 µg/kg via subcutaneous injection for 273 consecutive days. The drug was administered at 6 pre-designated sites on the back of each animal. Injection sites were rotated in a systematic manner.
Species/strain: Monkey/ Cynomolgus.
Number/sex/group or time point (main study): 6/sex/group.
Route, formulation, volume, and infusion rate: Subcutaneous; 29, 90 and 250 µl/kg for the LD, MD and HD respectively.
Satellite groups used for toxicokinetics or recovery: Same animals were used for TK studies.
Age: 2.8-7.3 years.
Weight: 2.0-4.6 kg (M); 1.7-3.5 kg (F).

Observation times and results

Mortality: Daily.

None.

Clinical signs: Daily.

Low food consumption and watery stool was observed throughout all dose groups including controls. Emesis and dehydration were also observed. Sponsor stated that the incidence of these findings in laboratory-housed animals is typically high when there are repeated stressful study procedures and/or GI parasitic infections. Microscopic evaluation of portions of the GI tract from all animals (including controls) confirmed the presence of GI parasites and mononuclear cell infiltration that was likely the source of, or contributed to the overall incidence of watery stool.

Body weights: Weekly.

Group Mean Body Weight (Combined sex)

Dose (µg/kg/d)	0	2.2	18	150
Day -1	2.6	2.6	2.7	2.5
Day 273	3.0	2.9	2.9	2.5
Body wt. gain (%)	13	10 (-3)	7 (-6)	0 (-13)

Food consumption: Daily qualitative assessment.

No data. Food consumption was stated to be generally low in all dose groups including controls.

Ophthalmoscopy: All animals were examined prior to dosing and at 3, 6 and 9 months following initiation of dosing.

There were no treatment-related changes. 1/6 HD males (FN15736M) had hyper reflective streaks with detached retina dorsal to the macula. Sponsor stated that the hyper-reflectivity was likely related to the apparent detached retina. In the absence of ocular histological changes, this was not considered test article-related.

EKG: ECG's were recorded all animals prior to dosing and at 3, 6 and 9 months following initiation of dosing.

There were no test article-related ECG changes.

Hematology: Blood samples for hematology were collected from all animals prestudy and prior to a.m. dosing at 3, 6 and 9 months following initiation of dosing.

Month 9 Data

Dose (µg/kg/d)	0	2.2	18	150
RBC (10^6/µl)	6.87	6.53	6.60	6.32*
Hemoglobin (g/dl)	12.4	12.0	12.5	11.6*
Hematocrit (%)	36.7	35.5	36.6	34.2*
MCV (fl)	53.6	54.4	55.5*	54.4
MCH (pg)	18.0	18.4	19.0*	18.5
Reticulocyte (10^5/µl)	0.59	0.47	0.41*	0.42*

*** p<0.05**

Clinical chemistry: Blood samples for clinical chemistry were collected from all animals prestudy and prior to a.m. dosing at 3, 6 and 9 months following initiation of dosing.

No treatment-related changes in serum chemistry.

Urinalysis: samples were obtained by cystocentesis during necropsy.

No treatment-related changes in urinalysis parameters measured.

Gross pathology: Organs/tissues isolated for gross pathology examination is indicated in the list of addendum.

Empty cells indicate zero incidence

Dose (µg/kg/d)	0		2.2		18		150	
Sex	M	F	M	F	M	F	M	F
Thymus - Decreased size	1/6(2)	1/6(1)	2/6 1/6(2)* 1/6(4)	2/6(1)	1/6(4)		3/6 1/6(1) 1/6(3) 1/6(4)	1/6(1)
Testis (L) - Decreased size			1/6(4)*					

1 = minimal; 2 = mild; 3 = moderate; 4 = marked; * = same animal

Organ weights: Organs weighed are indicated in the list of addendum.

Relative wt. (g/kg body wt.); Absolute wt. (g)

Dose (µg/kg/d)	0	2.2	18	150
Thyroid/parathyroid (g/kg)	0.119 ± 0.032	0.155 ± 0.050	0.152 ± 0.047	0.154 ± 0.044*
Brain (g/kg)	20.67 ± 3.66	21.83 ± 3.40	22.33 ± 4.97	24.64 ± 4.79*

*** p<0.05**

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Tissues from animals in all dose groups were processed for microscopic examination.
Empty cells indicate zero incidence

Dose (µg/kg/d)	0		2.2		18		150	
Sex	M	F	M	F	M	F	M	F
Brain - meninges Mononuclear cell infiltration					2/6(2)	1/6(2)	1/6(2)	
Brain - perivascular Mononuclear cell infiltration							1/6(1)	
Brain - submeningeal Hemorrhage								1/6(2)
Thyroid Follicular distension			1/6(1)				1/6(2)	
Degeneration, follicular epithelium					1/6(2)		1/6(1)	
Adrenal gland Mineralization			1/6(1)		1/6(1)		1/6(1)	
Nodular hypertrophy, cortex								1/6(1)
Kidney Dilatation, tubular lumen	1/6(2)		1/6(2)		3/6(2)		2/6(2)	
Heart – epicardium Mononuclear cell infiltrate							1/6(1)	
Myocardium Mononuclear cell infiltrate					1/6(2)		1/6(2)	
Skeletal muscle Lymphoid cell infiltrate							1/6(2)	
Pancreas Vacuolation, cytoplasm	1/6(1)				3/6 2/6(1) 1/6(2)		4/6 2/6(1) 2/6(2)	3/6 1/6(1) 2/6(2)
Fibrosis - interstitial							1/6(2)	
Hypercellular - islet							2/6(2)	3/6 1/6(1) 2/6(2)
Mononuclear cell infiltrate					1/6(1)		1/6(1)	
Injection sites - epidermis Hyperplasia			2/6 1/6(1) 1/6(2)		1/6(2)		5/6(2)	
Sciatic nerve Fibrosis							1/6(2)	
Uterus – myometrium Protein deposits						1/6(2)		1/6(2)
Stomach - mucosa Lymphoplasmacytic infiltrate		1/6(1)				2/6 1/6(1) 1/6(2)		3/6 1/6(1) 2/6(2)
Lymphoid hyperplasia						1/6(2)		2/6 1/6(1) 1/6(2)
Colon – mucosal crypt								

Cystic dilatation								1/6(1)
Cecum – submucosa Pigmented macrophages							1/6(1)	
Inflammation						1/6(2)		1/6(2)
Jejunum – mucosa Vacuolation, cytoplasmic								1/6(3)
Rectum – mucosa Inflammation						1/6(2)		1/6(2)

1 = minimal; 2 = mild; 3 = moderate; 4 = marked; * = same animal

Additional Histopathology Evaluation of the Pancreas:

In the chronic toxicity study done with cynomolgus monkeys, four groups of 6 males and 6 females each were administered 0 (vehicle control) 1.1, 9 or 75 µg/kg BID (0, 2.2, 18 or 150 µg/kg/day, respectively) for 273 days. Five of 12 HD animals exhibited pancreatic islet cell hypercellularity which was not observed in control, LD or MD animals. In contrast, no such changes were reported in a subchronic (91-day) toxicity study that was done with subcutaneously administered AC2993 in cynomolgus monkeys. In the 91-day study, four groups of 4 males and 4 females each were administered 0 (vehicle control) 0.6, 6.7 or 75 µg/kg BID (1.2, 6.7 or 150 µg/kg/day, respectively) for 91 days. Sponsor stated that since the reported pancreatic changes are subtle, it is possible that the pathologist for the subchronic study missed them. Thus, it was decided to have the pathologist for the chronic study peer review the pancreas tissues from the subchronic study. Because the contract laboratory that did the subchronic study ((b) (4) (b) (4) is no longer in business, Amylin Pharmaceuticals, Inc. elected to appoint Richard Hiles, PhD of Amylin Pharmaceuticals, Inc. as the Study Director for this peer review. (b) (4) (b) (4) was the reviewing pathologist.

Following the peer pathology review of cynomolgus monkeys pancreas tissues collected from the 91-day toxicity study of subcutaneously administered AC2993, minimal to mild hypercellularity of the pancreatic islets of Langerhans was identified in 1/8, 1/8 and 3/8 animals from the control, mid-dose (13.4 µg/kg/day) and high-dose (150 µg/kg/day) groups. The character of the islet hypercellularity was similar to that present in 5/12 high-dose monkeys administered subcutaneous AC2993 for 9 months in a chronic toxicity study done at (b) (4). Although a single control female in the subchronic (91-day) study had hypercellularity of the pancreatic islets, the overall slight increase in the incidence of this lesion in HD animals treated for 91 days, and the apparent persistence and similar incidence of this finding in animals administered 150 µg/kg/day for 9 months suggests an association of AC2993 administration to this lesion. A special stain [Gomori Aldehyde Fuchsin (GAF)] was used to identify ß-cells in the islets of Langerhans; there were no apparent differences in the density or distribution of GAF-positive cells in the pancreas in animals with islet hypercellularity.

Summary of Pathology Findings on Increased Islet Cellularity in Cynomolgus Monkeys Exposed to AC2993 for 91-days

Dose (µg/kg BID)	**0**		**0.6**		**6.7**		**75**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Pancreas - Hypercellularity	**0/4**	**1/4(2)**	**0/4**	**0/4**	**0/4**	**1/4(2)**	**1/4(1)**	**2/4(2)**

1 = minimal; 2 = mild

Gomori's Aldehyde Fuchsin (GAF) Positive Islet Cells in Cynomolgus Monkeys Exposed to AC2993 for 91-days

Group 1 (0 µg/kg/day)		Group 2 (1.2 µg/kg/day)		Group 3 (13.4 µg/kg/day)		Group 4 (150 µg/kg/day)	
Animal No. & Sex	Score1	Animal No.	Score	Animal No.	Score	Animal No.	Score
1101M	2	1201M	1	1301M	2	1401M	3
1102M	1	1202M	2	1302M	1	1402M	2
1103M	2	1203M	3	1303M	3	1403M	3
1104M	1	1204M	1	1304M	3	1404M	1
2101F	2	2201F	3	2301F	2	2401F	2
2102F	2	2202F	3	2302F	3	2402F	3
2103F	3	2203F	3	2303F	2	2403F	2
2104F	3	2204F	3	2304F	3	2404F	2
Mean±SD	2.0±0.8		2.4±0.9		2.4±0.7		2.3±0.7

1 = 30-50% of total islets; 2 = 50-80% of total islets; 3 = 80-100% of total islets

Toxicokinetics: Blood samples were collected prior to a.m. dosing and at 0.5, 1, 2, 4, 6, 9 and 12 hours post a.m. (and prior to p.m.) dosing on days 1, 90, 180 and 273.

Daily Dose µg/kg/day	Dose µg/kg	Sex	C_{max} (pg/mL)				$AUC_{0-12\,h}$ (pg·h/mL)			
			Day 1	Day 90	Day 180	Day 273	Day 1	Day 90	Day 180	Day 273
2.2	1.1	M/F	3140	3858	4505	3681	5121	8429	14,279	8317
18	9	M/F	32,002	49,941	136,995	149,605	61,019	290,411	788,730	1,411,201
150	75	M/F	211,634	221,080	199,961	197,043	500,354	736,288	777,046	1,031,391

Total daily $AUC_{(0-10hr)}$ for the MRHD (10 µg BID = 20 µg/day) = 2076 pg.h/ml

Serum Anti-KLH antibodies: 100µg KLH (Keyhole limpet hemocyanin – a sensitizing agent) in a 1:1 emulsion of sterile saline, USP and Incomplete Freund's Adjuvant was administered by intradermal injection to 3 animals/sex/group during weeks 34 or 35 following the a.m. dose (prior to the p.m. dose and before animals received their morning food ration). Blood samples for serum analysis of anti-KLH antibodies were obtained prior to dosing with KLH as well as at approximately 7 day intervals following dosing throughout the remainder of the study.

Pre-KLH and Day 274 Summary of Anti-KLH Positive Animals With Titer at or Above 1:125

Dose Group	1		2		3		4	
Dose	0 µg/kg BID		1.1 µg/kg BID		9 µg/kg BID		75 µg/kg BID	
Day	PRE	274	PRE	274	PRE	274	PRE	274
Total Animals	6	6	6	6	6	6	6	6
Positive Animals ≥ 1:125	0	5	0	6	0	5	0	6
% Positive Animals	0%	83%	0%	100%	0%	83%	0%	100%
Lowest Titer	0	25	0	125	0	25	0	125
Highest Titer	25	625	25	625	25	625	25	3125

Positive serum titers of anti-KLH antibodies were found in 5 out of 6 (83 %), 6 out of 6 (100%), 5 out of 6 (83%) and 6 out of 6 (100%) animals immunized with KLH antigen from Groups 1-4 respectively. This demonstrated that the animals had ample humoral integrity.

Anti-Exenatide Antibodies: Plasma anti-AC2993 antibodies were measured using an ELISA assay. Specimens were collected from all animals at pre-drug dose and at day 275. All specimens calculated SDscores below 3.00 were reported as negative and all above values were reported as the calculated value. The results of the anti-AC2993 antibody values in the study specimens are presented below.

Dose Group (Dose)	Placebo		1.1 µg/kg/BID		9.0 µg/kg/BID		75 µg/kg/BID	
	Pre-Drug	Day 275	Pre-Drug	Day 275	Pre-Drug	Day 275	Pre-Drug	Day 275
Total Animals	12	12	12	12	12	12	12	12
Positive Animals	1	0	3	9	1	9	1	8
Percent Positive	8	0	25	75	8	75	8	67

The positive specimens were further diluted in the two sample diluents and assayed to calculate the titer point, highest dilution giving a positive SDscore.

Anti-AC2993 Positive Specimens

Animal Number	Dose Group (µg/kg/BID)	SDscore		Titer Point	
		Pre-dose	Day 275	Pre-dose	Day 275
FN15706M	Placebo	3.8	Negative	Negative	ND
FN14937F	1.1	Negative	147.2	ND	1:125
FN15707M	1.1	Negative	3.0	ND	1:5
FN15711F	1.1	Negative	191.0	ND	1:625
FN15728F	1.1	Negative	114.2	ND	1:125
FN15729F	1.1	44.8	41.2	1:25	1:25
FN15734M	1.1	Negative	48.8	ND	1:125
FN15737M	1.1	43.8	142.8	1:25	1:125
FN15742F	1.1	4.2	3.0	1:5	1:5
FN15746M	1.1	Negative	22.6	ND	1:25
F4288CQF	9.0	Negative	7.0	ND	1:5
FN15708M	9.0	Negative	50.3	ND	1:25
FN15709M	9.0	17.8	16.1	1:5	1:25
FN15713F	9.0	Negative	73.5	ND	1:125
FN15722M	9.0	Negative	80.1	ND	1:125
FN15726F	9.0	Negative	6.7	ND	1:5
FN15733M	9.0	Negative	29.3	ND	1:25
FN15740M	9.0	Negative	66.1	ND	1:125
FN15741M	9.0	Negative	170.7	ND	1:125
FN14007F	75	Negative	8.2	ND	1:5
FN14015F	75	Negative	28.0	ND	1:25
FN15702M	75	Negative	50.3	ND	1:125
FN15705M	75	Negative	28.0	ND	1:25
FN15714M	75	Negative	42.5	ND	1:125
FN15717F	75	7.6	6.7	<1:25	1:5
FN15718F	75	Negative	34.2	ND	1:25
FN15744M	75	Negative	3.9	ND	1:5

One of 12 animals (8%) not receiving AC2993 was found positive, 9 of 12 animals (75%) receiving 1.1 µg/kg/BID were found positive, 9 of 12 animals (75%) receiving 9.0 µg/kg/BID were found positive and 8 of 12 animals (67%) receiving 75 µg/kg/BID were found positive. Titers of 1:125 or greater were obtained in 5 out of 12 (42%) in animals receiving 1.1 µg/kg/BID, 4 out of 12 (33%) in animals receiving 9.0 µg/kg/BID and 2 out of 12 (17%) in animals receiving 75 µg/kg/BID. These results suggest that anti-exenatide antibody titers ≥1:125 were not neutralizing since increases in Cmax and AUC generally correlated with anti-exenatide antibody titers ≥1:125. Moreover, exenatide-related effects on body weight were not correlated with anti-exenatide antibody.

Histopathology Inventory for NDA # 21-773

Study	00119	00119	00120	00120	00120	00120
Species	91-D Mouse	91-D Mouse	182-D Mouse	91-D Monkey	273-D Monkey	91-D Rat
Adrenals	X*	X*	X*	X*	X*	X*
Aorta	X	X	X	X	X	X
Bone Marrow smear	X	X	X	X	X	X
Bone (femur)	X	X	X	X	X	X
Brain	X	X	X*	X*	X*	X*
Cecum	X	X	X	X	X	X
Cervix	X*	X*	X	X	X	X
Colon	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X
Epididymis	X*	X*	X*	X*	X*	X*
Esophagus	X	X	X	X	X	X
Eye	X	X	X	X	X	X
Fallopian tube	X	X	X	X	X	X
Gall bladder	X	X	X	X	X	X
Gross lesions	X	X	X	X	X	X
Harderian gland	X	X	X	X	X	X
Heart	X*	X*	X*	X*	X*	X*
Ileum	X	X	X	X	X	X
Injection site	X	X	X	X	X	X
Jejunum	X	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*	X*
Lachrymal gland	X	X	X	X	X	X
Larynx						
Liver	X*	X*	X*	X*	X*	X*
Lungs	X*	X*	X*	X*	X*	X*
Lymph nodes, cervical						
Lymph nodes, mandibular	X	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X	X
Mammary Gland	X	X	X	X	X	X
Nasal cavity						
Optic nerves	X	X	X	X	X	X
Ovaries	X*	X*	X*	X*	X*	X*
Pancreas	X	X	X	X	X	X
Parathyroid	X*	X*	X*	X*	X*	X*
Peripheral nerve						
Pharynx						
Pituitary	X*	X*	X*	X*	X*	X*
Prostate	X	X	X	X	X	X
Rectum	X	X	X	X	X	X
Salivary gland	X	X	X	X	X	X
Sciatic nerve	X	X	X	X	X	X
Seminal vesicles	X	X	X	X	X	X
Skeletal muscle	X	X	X	X	X	X
Skin	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*	X*
Sternum	X	X	X	X	X	X
Stomach	X	X	X	X	X	X
Testes			X*	X*	X*	X*
Thymus	X*	X*	X*	X*	X*	X*
Thyroid	X*	X*	X*	X*	X*	X*
Tongue	X	X	X	X	X	X
Trachea	X	X	X	X	X	X
Urinary bladder	X	X	X	X	X	X
Uterus	X*	X*	X*	X*	X*	X*
Vagina	X	X	X	X	X	X
Zymbal gland						

X, histopathology performed
*, organ weight obtained

6.6.6.4 Genetic toxicology

6.6.6.4.1 Study Title: Mutagenicity test with AC2993 in the Salmonella-Escherichia coli/Mammalian microsome reverse mutation assay with a confirmatory assay.

Key Study findings:

- AC2993 did not increase the number of revertants/plate in any of the tester strains with or without S9 mix.
- AC2993 was not mutagenic under the conditions of this study.

Study No: REST98093

Volume # and Page #: N/A.
Conducting Laboratory and location: (b) (4)
Date of Study Initiation: January 1, 1998.
GLP Compliance: Yes (U.S.A., U.K.)
QA- Reports Yes (x) No ( ):
Drug Lot # and % purity: Lot # 97-1210RP. Purity was not provided.

Methods:

Strains/Species/Cell line: S. typhimurium: TA98, TA100, TA1535, TA1537; E. coli-WP2uvrA.

Doses used in definitive study: 33.3, 100, 333, 1,000, 3,330 and 5,000 µg/plate.

Basis of dose selection: Based on cytotoxicity (growth inhibition).

Negative Controls: Deionized water.

Positive Controls: Dose unit µg/plate.

	TA98	TA100	TA1535	TA1537	WP2uvrA
- S9	2-NF 1.0	NaN_3 2.0	NaN_3 2.0	ICR-191 2.0	4-NNO 1.0
+ S9	B(a)P 2.5	2-AA 2.5	2-AA 2.5	2-AA 2.5	2-AA 25.0

2-NF =2=nitrofluorene; B(a)P = benzo(a)pyrene; 2-AA = 2-aminoanthracene; NaN3 = sodium azide; 4-NNO = 4-nitroquinoline-N-oxide.

Incubation and sampling times: The test article, tester strains and S9 mix (where appropriate) were combined in molten agar which was overlaid onto a minimal agar plate. The plates were incubated at 37 ± 2°C for 54 ± 4 hr after which revertant colonies were counted.

Results

Study Validity: Three replicates per dose were evaluated. The number of revertant colonies/plate for the vehicle controls and plates containing test article were counted manually. The number of revertant colonies/plate for positive controls were counted by automated colony counter except for the positive controls for tester strain TA98 in the absence of S9 mix which were counted manually. The highest dose used was 5,000 µg/plate. Positive controls showed appropriate increases in colony numbers. The numbers of revertant colonies for the negative and positive controls were within the limits of the historical data of the testing facility. The test article is considered to be positive, if it produced at least a 2-fold increase in mean number of revertants per plate in at least one of the tester strains and a dose-related increase in number of revertants per plate relative to control. The study appears to be adequately performed.

Study Outcome: In both the initial and confirmatory mutagenicity assays, no positive increases in the number of revertants per plate were observed in any of the tester strains either in the presence or absence of S9 mix. AC2993 was not mutagenic under the conditions of this study.

Initial Mutagenicity Assay (Mean Revertants/Plate ± SD)

Metabolic Activation	Test/Control Article	Dose Level (µg/plate)	Assay 1 Mean Revertant Colony Counts Per Plate (±SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without S9	Vehicle Control	0	15 (2)	84 (4)	10 (2)	7 (1)	17 (2)
	Exenatide	33.3	14 (4)	89 (4)	8 (4)	6 (3)	10 (3)
		100	14 (8)	89 (8)	8 (2)	6 (2)	13 (5)
		333	14 (2)	85 (12)	14 (3)	9 (7)	19 (4)
		1000	14 (8)	78 (11)	12 (6)	6 (1)	14 (5)
		3330	16 (2)	91 (11)	7 (3)	7 (2)	18 (6)
		5000	14 (2)	92 (9)	9 (4)	5 (2)	19 (5)
	Positive Control		2NF 169 (28)	NAz 604 (60)	NAz 540 (44)	ICR 1004 (51)	4NQO 449 (63)
With S9	Vehicle Control	0	23 (3)	90 (16)	11 (2)	8 (4)	16 (5)
	Exenatide	33.3	26 (14)	97 (2)	12 (6)	9 (3)	12 (6)
		100	31 (9)	91 (0)	9 (2)	10 (2)	14 (2)
		333	24 (8)	102 (8)	9 (2)	7 (4)	16 (3)
		1000	29 (8)	94 (3)	14 (2)	10 (2)	16 (5)
		3330	32 (8)	104 (9)	7 (3)	12 (1)	15 (2)
		5000	38 (4)	104 (7)	13 (2)	10 (2)	15 (3)
	Positive Control		BaP 356 (82)	2AA2 969 (49)	2AA2 145 (8)	2AA2 169 (17)	2AA25 386 (26)

N/A = not applicable SD = standard deviation
ICR = ICR-191 2.0 µg/plate BaP = benzo(a)pyrene 2.5 µg/plate 2NF = 2-nitrofluorene 1.0 µg/plate
2AA2 = 2-aminoanthracene 2.5 µg/plate 2AA25 = 2-aminoanthracene 25 µg/plate NAz = sodium azide 2.0 µg/plate 4NQO = 4-nitroquinoline-*N*-oxide 1.0 µg/plate

Confirmatory Assay (Mean Revertants/Plate ± SD)

Metabolic Activation	Test/Control Article	Dose Level (µg/plate)	Assay 2 Mean Revertant Colony Counts Per Plate (± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without S9	Vehicle Control	0	15 (3)	96 (4)	10 (0)	8 (5)	11 (2)
	Exenatide	33.3	19 (4)	101 (16)	9 (2)	7 (2)	13 (1)
		100	16 (3)	94 (16)	9 (2)	6 (1)	15 (3)
		333	26 (7)	93 (11)	10 (4)	5 (1)	12 (2)
		1000	21 (8)	98 (7)	9 (1)	7 (1)	15 (9)
		3330	23 (3)	92 (13)	14 (6)	7 (3)	14 (4)
		5000	21 (1)	83 (18)	10 (1)	7 (2)	13 (4)
	Positive Control		2NF 107 (6)	NAz 717 (36)	NAz 649 (20)	ICR 507 (91)	4NQO 210 (74)
With S9	Vehicle Control	0	23 (8)	90 (6)	12 (5)	10 (1)	12 (1)
	Exenatide	33.3	25 (4)	100 (11)	10 (1)	10 (2)	12 (3)
		100	24 (1)	96 (19)	15 (6)	5 (2)	12 (2)
		333	31 (8)	88 (7)	12 (2)	12 (5)	16 (4)
		1000	32 (4)	101 (20)	9 (5)	12 (1)	13 (4)
		3330	29 (4)	105 (5)	10 (1)	8 (4)	12 (6)
		5000	33 (4)	107 (17)	10 (5)	9 (1)	14 (3)
	Positive Control		BaP 392 (17)	2AA2 680 (148)	2AA2 137 (16)	2AA2 163 (18)	2AA25 355 (18)

N/A = not applicable SD = standard deviation
ICR = ICR-191 2.0 µg/plate BaP = benzo(a)pyrene 2.5 µg/plate 2NF = 2-nitrofluorene 1.0 µg/plate
2AA2 = 2-aminoanthracene 2.5 µg/plate 2AA25 = 2-aminoanthracene 25 µg/plate NAz = sodium azide 2.0 µg/plate 4NQO = 4-nitroquinoline-*N*-oxide 1.0 µg/plate

6.6.6.4.2 Study Title: Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with AC2993

Study performed to compare the genotoxicity of exenatide from new manufacturer (Bachem)

Key Study findings:

- AC2993 did not increase the number of revertants/plate in any of the tester strains with or without S9 mix.
- AC2993 was not mutagenic under the conditions of this study.

Study No: REST02099

Volume # and Page #: N/A.
Conducting Laboratory and location: (b) (4)
Date of Study Initiation: June 7, 2002.
GLP Compliance: Yes (U.S.A., U.K.)
QA- Reports Yes (x) No ( ):
Drug Lot # and % purity: Lot # 01-0108AP. 91.5% pure.

Methods:

Strains/Species/Cell line: S. typhimurium: TA98, TA100, TA1535, TA1537; E. coli-WP2uvrA.

Doses used in definitive study: 33.3, 100, 333, 1,000, 3,330 and 5,000 µg/plate.

Basis of dose selection: Based on cytotoxicity (growth inhibition).

Negative Controls: Deionized water.

Positive Controls: Dose unit µg/plate.

	TA98	TA100	TA1535	TA1537	WP2uvrA
- S9	2-NF 1.0	NaN_3 2.0	NaN_3 2.0	ICR-191 2.0	4-NNO 1.0
+ S9	B(a)P 2.5	2-AA 2.5	2-AA 2.5	2-AA 2.5	2-AA 25.0

2-NF =2=nitrofluorene; B(a)P = benzo(a)pyrene; 2-AA = 2-aminoanthracene; NaN3 = sodium azide; 4-NNO = 4-nitroquinoline-N-oxide.

Incubation and sampling times: The test article, tester strains and S9 mix (where appropriate) were combined in molten agar which was overlaid onto a minimal agar plate. The plates were incubated at 37 ± 2°C for 54 ± 4 hr after which revertant colonies were counted.

Results

Study Validity: Three replicates per dose were evaluated. The number of revertant colonies/plate was counted by automated colony counter. The highest dose used was 5,000 µg/plate. Positive controls showed appropriate increases in colony numbers. The numbers of revertant colonies for the negative and positive controls were within the limits of the historical data of the testing facility. The test article is considered to be positive, if it produced at least a 2-fold increase in mean number of revertants per plate in at least one of the tester strains and a dose-related increase in number of revertants per plate relative to control. The study appears to be adequately performed.

Study Outcome: In both the initial and confirmatory mutagenicity assays, no positive increases in the number of revertants per plate were observed in any of the tester strains either in the presence or absence of S9 mix. AC2993 was not mutagenic under the conditions of this study.

Initial Mutagenicity Assay (Mean Revertants/Plate ± SD)

Metabolic Activation	Test/Control Article	Dose Level (µg/plate)	Assay 1 Mean Revertant Colony Counts Per Plate (± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without S9	Vehicle Control	0	12 (7)	77 (10)	12 (4)	6 (4)	15 (4)
	Exenatide	33.3	12 (6)	92 (13)	8 (1)	5 (3)	16 (5)
		100	15 (4)	88 (13)	10 (5)	2 (1)	14 (3)
		333	14 (4)	89 (17)	12 (5)	8 (3)	17 (4)
		1000	11 (3)	93 (10)	9 (3)	5 (4)	10 (4)
		3330	10 (4)	89 (15)	9 (3)	4 (2)	13 (2)
		5000	12 (7)	100 (6)	9 (3)	10 (2)	14 (5)
	Positive Control		2NF 162 (7)	NAz 1069 (33)	NAz 735 (14)	ICR 520 (21)	4NQO 294 (45)
With S9	Vehicle Control	0	19 (4)	82 (10)	16 (1)	6 (4)	12 (4)
	Exenatide	33.3	24 (5)	93 (13)	6 (1)	8 (6)	18 (2)
		100	24 (5)	89 (14)	10 (4)	9 (1)	14 (3)
		333	25 (9)	100 (8)	9 (4)	10 (1)	19 (4)
		1000	17 (3)	82 (5)	11 (2)	7 (4)	13 (5)
		3330	22 (3)	110 (18)	10 (4)	9 (3)	14 (4)
		5000	31 (7)	139 (9)	9 (3)	7 (2)	11 (1)
	Positive Control		BaP 379 (21)	2AA2 1014 (109)	2AA2 79 (8)	2AA2 77 (20)	2AA25 350 (12)

SD = standard deviation
ICR = ICR-191 2.0 µg/plate BaP = benzo(a)pyrene 2.5 µg/plate 2NF = 2-nitrofluorene 1.0 µg/plate
2AA2 = 2-aminoanthracene 2.5 µg/plate 2AA25 = 2-aminoanthracene 25 µg/plate NAz = sodium azide 2.0 µg/plate 4NQO = 4-nitroquinoline-*N*-oxide 1.0 µg/plate

Confirmatory Mutagenicity Assay (Mean Revertants/Plate ± SD)

Metabolic Activation	Test/Control Article	Dose Level (µg/plate)	Assay 2 Mean Revertant Colony Counts (± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without S9	Vehicle Control	0	12 (3)	75 (14)	11 (6)	7 (1)	15 (6)
	Exenatide	33.3	9 (1)	74 (10)	15 (5)	9 (2)	16 (6)
		100	13 (1)	80 (6)	13 (3)	5 (2)	15 (3)
		333	15 (1)	79 (12)	11 (3)	7 (1)	19 (3)
		1000	16 (5)	79 (11)	16 (6)	9 (2)	19 (6)
		3330	13 (3)	75 (8)	10 (5)	4 (3)	15 (4)
		5000	18 (4)	82 (9)	11 (4)	7 (4)	14 (3)
	Positive Control		2NF 141 (19)	NAz 969 (44)	NAz 655 (98)	ICR 1783 (160)	4NQO 205 (24)
With S9	Vehicle Control	0	19 (8)	77 (8)	12 (3)	11 (6)	8 (7)
	Exenatide	33.3	33 (6)	82 (9)	13 (1)	13 (3)	8 (5)
		100	32 (3)	83 (12)	10 (5)	7 (3)	9 (5)
		333	27 (2)	85 (5)	9 (3)	9 (1)	8 (3)
		1000	27 (4)	80 (10)	15 (5)	10 (9)	9 (5)
		3330	30 (1)	94 (9)	8 (2)	10 (4)	6 (1)
		5000	40 (2)	89 (9)	11 (6)	8 (3)	9 (4)
	Positive Control		BaP 328 (18)	2AA2 588 (23)	2AA2 112 (6)	2AA2 116 (17)	2AA25 985 (47)

SD = standard deviation
ICR = ICR-191 2.0 µg/plate BaP = benzo(a)pyrene 2.5 µg/plate 2NF = 2-nitrofluorene 1.0 µg/plate
2AA2 = 2-aminoanthracene 2.5 µg/plate 2AA25 = 2-aminoanthracene 25 µg/plate NAz = sodium azide 2.0 µg/plate 4NQO = 4-nitroquinoline-*N*-oxide 1.0 µg/plate

6.6.6.4.3 Study Title: Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with AC2993

Study was performed to compare the genotoxicity of exenatide from new manufacturer (Mallinckrodt)

Key Study findings:

- AC2993 did not increase the number of revertants/plate in any of the tester strains with or without S9 mix.
- AC2993 was not mutagenic under the conditions of this study.

Study No: REST02098

Volume # and Page #: N/A.
Conducting Laboratory and location: (b) (4)
Date of Study Initiation: June 7, 2002.

GLP Compliance: Yes (U.S.A., U.K.)
QA- Reports Yes (x) No ( ):
Drug Lot # and % purity: Lot # 01-0802BP. 95.8% pure.

Methods:

Strains/Species/Cell line: S. typhimurium: TA98, TA100, TA1535, TA1537; E. coli-WP2uvrA.

Doses used in definitive study: 33.3, 100, 333, 1,000, 3,330 and 5,000 µg/plate.

Basis of dose selection: Based on cytotoxicity (growth inhibition).

Negative Controls: Deionized water.

Positive Controls: Dose unit µg/plate.

	TA98	TA100	TA1535	TA1537	WP2uvrA
- S9	2-NF 1.0	NaN_3 2.0	NaN_3 2.0	ICR-191 2.0	4-NNO 1.0
+ S9	B(a)P 2.5	2-AA 2.5	2-AA 2.5	2-AA 2.5	2-AA 25.0

2-NF =2=nitrofluorene; B(a)P = benzo(a)pyrene; 2-AA = 2-aminoanthracene; NaN3 = sodium azide; 4-NNO = 4-nitroquinoline-N-oxide.

Incubation and sampling times: The test article, tester strains and S9 mix (where appropriate) were combined in molten agar which was overlaid onto a minimal agar plate. The plates were incubated at 37 ± 2°C for 54 ± 4 hr after which revertant colonies were counted.

Results

Study Validity: Three replicates per dose were evaluated. The number of revertant colonies/plate was counted by automated colony counter. The highest dose used was 5,000 µg/plate. Positive controls showed appropriate increases in colony numbers. The numbers of revertant colonies for the negative and positive controls were within the limits of the historical data of the testing facility. The test article is considered to be positive, if it produced at least a 2-fold increase in mean number of revertants per plate in at least one of the tester strains and a dose-related increase in number of revertants per plate relative to control. The study appears to be adequately performed.

Study Outcome: In both the initial and confirmatory mutagenicity assays, no positive increases in the number of revertants per plate were observed in any of the tester strains either in the presence or absence of S9 mix. AC2993 was not mutagenic under the conditions of this study.

Initial Mutagenicity Assay (Mean Revertants/Plate ± SD)

Metabolic Activation	Test/Control Article	Dose Level (µg/plate)	Assay 1 Mean Revertant Colony Counts Per Plate (± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without S9	Vehicle Control	0	18 (9)	71 (10)	11 (5)	8 (1)	11 (5)
	Exenatide	33.3	14 (2)	77 (12)	15 (4)	6 (6)	10 (4)
		100	20 (11)	90 (10)	14 (5)	7 (5)	16 (6)
		333	16 (6)	75 (17)	11 (3)	10 (6)	16 (3)
		1000	15 (3)	85 (11)	15 (7)	8 (2)	20 (2)
		3330	11 (4)	79 (6)	11 (6)	11 (4)	18 (10)
		5000	12 (3)	87 (18)	17 (2)	6 (2)	17 (3)
	Positive Control		2NF 208 (7)	NAz 1068 (108)	NAz 707 (34)	ICR 736 (193)	4NQO 246 (23)
With S9	Vehicle Control	0	25 (3)	87 (9)	13 (8)	8 (2)	21 (1)
	Exenatide	33.3	32 (8)	90 (4)	12 (6)	12 (3)	18 (2)
		100	28 (6)	96 (19)	13 (6)	12 (2)	22 (6)
		333	27 (6)	85 (18)	12 (4)	15 (7)	17 (7)
		1000	29 (1)	91 (3)	16 (2)	11 (5)	19 (2)
		3330	27 (5)	95 (4)	14 (5)	13 (3)	19 (1)
		5000	35 (13)	91 (12)	17 (3)	14 (3)	18 (1)
	Positive Control		BaP 352 (10)	2AA2 690 (198)	2AA2 127 (17)	2AA2 88 (3)	2AA25 619 (47)

SD = standard deviation
ICR = ICR-191 2.0 µg/plate BaP = benzo(a)pyrene 2.5 µg/plate 2NF = 2-nitrofluorene 1.0 µg/plate
2AA2 = 2-aminoanthracene 2.5 µg/plate 2AA25 = 2-aminoanthracene 25 µg/plate NAz = sodium azide 2.0 µg/plate 4NQO = 4-nitroquinoline-*N*-oxide 1.0 µg/plate

Confirmatory Mutagenicity Assay (Mean Revertants/Plate ± SD)

Metabolic Activation	Test/Control Article	Dose Level (µg/plate)	Assay 2 Mean Revertant Colony Counts Per Plate (± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without S9	Vehicle Control	0	15 (3)	66 (6)	14 (6)	6 (0)	20 (3)
	Exenatide	33.3	14 (4)	63 (4)	12 (3)	8 (4)	21 (4)
		100	13 (3)	69 (1)	15 (4)	8 (3)	16 (3)
		333	13 (1)	63 (9)	13 (3)	8 (3)	16 (8)
		1000	17 (3)	66 (9)	10 (1)	7 (4)	18 (7)
		3330	10 (5)	64 (12)	14 (5)	10 (8)	17 (2)
		5000	15 (5)	78 (14)	14 (6)	5 (3)	15 (2)
	Positive Control		2NF 199 (13)	NAz 844 (70)	NAz 627 (34)	ICR 1668 (66)	4NQO 121 (42)
With S9	Vehicle Control	0	23 (2)	64 (11)	14 (2)	8 (3)	17 (9)
	Exenatide	33.3	27 (8)	64 (7)	7 (3)	8 (3)	21 (6)
		100	22 (3)	59 (15)	13 (3)	9 (2)	16 (8)
		333	27 (7)	49 (3)	14 (3)	5 (2)	17 (4)
		1000	34 (10)	66 (5)	11 (3)	8 (3)	13 (3)
		3330	27 (7)	75 (5)	10 (1)	11 (4)	17 (2)
		5000	35 (6)	85 (8)	15 (1)	11 (5)	14 (4)
	Positive Control		BaP 263 (11)	2AA2 219 (12)	2AA2 103 (9)	2AA2 62 (8)	2AA25 561 (17)

SD = standard deviation
ICR = ICR-191 2.0 µg/plate BaP = benzo(a)pyrene 2.5 µg/plate 2NF = 2-nitrofluorene 1.0 µg/plate
2AA2 = 2-aminoanthracene 2.5 µg/plate 2AA25 = 2-aminoanthracene 25 µg/plate NAz = sodium azide 2.0 µg/plate 4NQO = 4-nitroquinoline-*N*-oxide 1.0 µg/plate

6.6.6.4.4 Study Title: Mutagenicity test on AC2993 measuring chromosomal aberration in Chinese hamster ovary (CHO) cells.

Key findings:

Confirmatory Chromosomal Aberrations Assay without Metabolic Activation

- No visual signs of toxicity were observed in any of the test cultures.
- No reductions were observed in the mitotic indices of the cultures analyzed relative controls.
- No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplicafion were observed at the concentrations analyzed.

Confirmatory Chromosomal Aberrations Assay with Metabolic Activation

- No visual signs of toxicity were observed in any of the test cultures.
- Reductions of 6%, 35%, and 21% were observed in the mitotic indices of the cultures treated with 625, 1250, and 5000 µg/ml relative to control cultures.

- No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.
- Overall, AC2993 tested negative for inducing chromosome aberrations in CHO cells with and without metabolic activation under the conditions of the study.

Study No: REST98094.

Volume # and Page #: N/A.
Conducting Laboratory: (b) (4)
Date of Study Initiation: January 27, 1998.
GLP Compliance: Yes (U.S.A.)
QA- Reports Yes (x) No ( ):
Drug Lot Number and % purity: Lot # 97-1210RP. Purity was not provided.

Methods

Strains/Species/Cell line: Chinese hamster ovary cell line.

Doses used in definitive study: 625, 1250, 2500, 5000 µg/ml.

Basis of dose selection: The highest dose was selected as the dose/concentration that inhibited cell growth (cytotoxicity) by 50%.

Negative Controls: Deionized water.

Positive Controls: Mitomycin C (- S9); Cyclophosphamide (+ S9).

Incubation and sampling times:
Aberration assay without metabolic activation: Cultures were initiated by seeding approximately 1.2 x 10^6 cells per 75 cm^2 flask into 10 ml of complete McCoy's 5a medium. One day after culture initiation, the cells were incubated at 37°C with the test article at predetermined concentrations for approximately 3.0 hours for the initial assay or 17.8 hours in the confirmatory assay. The cultures were then washed with buffered saline. In the initial assay, the cells were refed with complete McCoy's 5a medium and incubated for the rest of the culture period up to the time of harvest with 0.1 µg/ml (b) (4)® present during the last 2.0 hours of incubation. In the confirmatory assay, cells were refed with complete McCoy's 5a medium with 0.1 µg/ml (b) (4)® and harvested 2.0 hours later.

Aberrations Assay with Metabolic Activation: Cultures were initiated as previously described. One day after culture initiation, the cultures were incubated at 37°C for approximately three hours in the presence of the test article and the S9 reaction mixture in McCoy's 5a medium without FBS. After the three-hour exposure period the cells were washed twice with buffered saline and the cells were refed with complete McCoy's 5a medium. The cells were incubated for the rest of the culture period up to the time of harvest with 0.1 µg/ml (b) (4)® present during the last 2.0 hours of incubation. The cultures were then harvested.

RESULTS:
Study Validity: Two replicate cultures per dose were evaluated. The method of counting was not disclosed. The negative (untreated) and vehicle control cultures contain less than ~5% cells with aberration. The positive control cultures contain significantly higher ($p<0.01$) number of cells with aberrations than the vehicle controls. The assay tested the highest applicable dose (10 mM or 5 mg/ml).

The test article is considered positive if a statistically significant increase in the number of cells with chromosomal aberrations is observed at one or more concentrations. The chromosomal aberrations should show a dose response-response relationship. Overall, the study was valid and adequately performed.

Study Outcome:

Chromosomal Aberrations Assay without Metabolic Activation

Initial assay: No visual signs of toxicity were observed in any of the test cultures. No reductions were observed in the mitotic indices of the cultures analyzed as compared with the solvent control cultures. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.

Confirmatory Assay: No visual signs of toxicity were observed in any of the test cultures. No reductions were observed in the mitotic indices of the cultures analyzed as compared with the solvent control cultures. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplicafion were observed at the concentrations analyzed.

Chromosomal Aberrations Assay with Metabolic Activation

Initial assay: No visual signs of toxicity were observed in any of the test cultures, except for floating debris in the cultures treated with 5000 µg/ml. Reductions of 57%, 5%, and 41% were observed in the mitotic indices of the cultures treated with 625, 1250, and 2500 µg/ml as compared with the solvent control cultures. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.

Confirmatory Assay: No visual signs of toxicity were observed in any of the test cultures. Reductions of 6%, 35%, and 21% were observed in the mitotic indices of the cultures treated with 625, 1250, and 5000 µg/ml as compared with the solvent control cultures. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.

Conclusion: AC2993 tested negative for inducing chromosome aberrations in CHO cells with and without metabolic activation under the conditions of the study.

Initial Chromosome Aberration Assay

Metabolic Activation	Test/Control Article	Dose Concentration (µg/mL)	Assay 1 (Total 200 Cells Counted)				
			Mitotic Index (%)	Endoreduplicated Cells (%)	Polyploid Cells (%)	Cells With Chromosomal Aberrations (%)	Cells With > 1 Chromosomal Aberrations (%)
Without S9	Negative (media)	0	8.2	0.0	0.5	1.0	0.5
	Vehicle Control	0	3.4	0.0	0.0	1.5	0.5
	Exenatide	625	6.7	0.0	1.0	1.5	0.0
		1250	6.6	0.0	0.5	1.5	0.5
		2500	3.6	0.0	1.5	2.0	0.5
		5000	11.5	0.0	0.0	0.5	0.0
	MMC (50 cells)	1.50	3.0	0.0	3.0	50.0*	32.0*
With S9	Negative (media)	0	9.0	2.5	0.5	2.5	0.5
	Vehicle Control	0	10.6	2.5	0.5	2.0	0.0
	Exenatide	625	4.6	5.5	0.5	4.5	0.5
		1250	10.1	1.0	0.0	0.5	0.0
		2500	6.3	0.5	1.0	2.5	0.0
		5000	13.9	4.5	0.5	0.5	0.0
	CP (50 cells)	5.00	8.9	0.0	1.5	62.0*	32.0*

[a] Times listed are approximate N/A = not applicable *Fisher's Exact Test p ≤ 0.01 MMC = mitomycin C CP = cyclophosphamide h = hour

Confirmatory Chromosome Aberration Assay

Metabolic Activation	Test/Control Article	Dose Concentration (µg/mL)	Assay 2 (Total 200 Cells Counted)				
			Mitotic Index (%)	Endoreduplicated Cells (%)	Polyploid Cells (%)	Cells With Chromosomal Aberrations (%)	Cells With > 1 Chromosomal Aberrations (%)
Without S9	Negative (media)	0	6.6	0.0	1.5	1.0	0.0
	Vehicle Control	0	6.2	0.0	0.5	1.0	0.0
	Exenatide	625	7.8	0.0	1.0	1.5	0.0
		1250	8.1	0.0	1.0	0.5	0.0
		2500	8.7	0.0	0.0	1.5	0.0
		5000	7.6	0.0	1.0	0.0	0.0
	MMC (50 cells)	0.100	5.5	0.0	1.5	15.0*	2.5
With S9	Negative (media)	0	10.5	0.5	1.0	0.0	0.0
	Vehicle Control	0	11.3	0.5	1.0	1.5	0.0
	Exenatide	625	10.6	0.0	3.0	2.5	0.0
		1250	7.3	2.5	2.5	1.0	0.0
		2500	11.3	0.0	2.0	1.0	0.0
		5000	8.9	0.0	0.5	1.0	0.0
	CP (50 cells)	5.00	4.5	0.0	4.5	34.0*	10.0*

[a] Times listed are approximate N/A = not applicable *Fisher's Exact Test p ≤ 0.01 MMC = mitomycin C CP = cyclophosphamide h = hour

6.6.6.4.5 Study Title: Mutagenicity test on AC2993 measuring chromosomal aberration in Chinese hamster ovary (CHO) cells.

Study was performed to compare the genotoxicity of exenatide from new manufacturer (Bachem)

Key findings:

Confirmatory Chromosomal Aberrations Assay without Metabolic Activation

- No visual signs of toxicity were observed in any of the test cultures.
- Reductions of 0% and 7% were observed in the mitotic indices of cultures treated with 2500 and 5000 µg/ml, respectively, relative to solvent control cultures.
- No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplicafion were observed at the concentrations analyzed.

Chromosomal Aberrations Assay with Metabolic Activation

- No visual signs of toxicity were observed in any of the test cultures.
- Reductions of 25, 24%, 19% and 22% were observed in the mitotic indices of cultures treated with 625, 1250, 2500 and 5000 µg/ml, respectively, relative to solvent control cultures.
- No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.

- AC2993 tested negative for inducing chromosome aberrations in CHO cells with and without metabolic activation under the conditions of the study.

Study No: REST02305.

Volume # and Page #: N/A.
Conducting Laboratory: (b) (4)
Date of Study Initiation: January 27, 1998.
GLP Compliance: Yes (U.S.A.)
QA- Reports Yes (x) No ( ):
Drug Lot Number and % purity: Lot # 97-1210RP, 93.8% pure.

Methods

Strains/Species/Cell line: Chinese hamster ovary cell line.

Doses used in definitive study: 625, 1250, 2500, 5000 µg/ml.

Basis of dose selection: The highest dose was selected as the dose/concentration that inhibited cell growth (cytotoxicity) by 50%.

Negative Controls: Deionized water.

Positive Controls: Mitomycin C (- S9); Cyclophosphamide (+ S9).

Incubation and sampling times:
Aberration assay without metabolic activation: Cultures were initiated by seeding approximately 1.2 x 10^6 cells per 75 cm^2 flask into 10 ml of complete McCoy's 5a medium. One day after culture initiation, the cells were incubated at 37°C with the test article at predetermined concentrations for approximately 3.0 hours for the initial assay or 17.8 hours in the confirmatory assay. The cultures were then washed with buffered saline. In the initial assay, the cells were refed with complete McCoy's 5a medium and incubated for the rest of the culture period up to the time of harvest with 0.1 µg/ml (b) (4) present during the last 2.0 hours of incubation. In the confirmatory assay, cells were refed with complete McCoy's 5a medium with 0.1 µg/ml (b) (4) and harvested 2.0 hours later.

Aberrations Assay With Metabolic Activation: Cultures were initiated as previously described. One day after culture initiation, the cultures were incubated at 37°C for approximately three hours in the presence of the test article and the S9 reaction mixture in McCoy's 5a medium without FBS. After the three-hour exposure period the cells were washed twice with buffered saline and the cells were refed with complete McCoy's 5a medium. The cells were incubated for the rest of the culture period up to the time of harvest with 0.1 µg/ml (b) (4) present during the last 2.0 hours of incubation. The cultures were then harvested.

RESULTS:
Study Validity: Two replicate cultures per dose were evaluated. The method of counting was not disclosed. The negative (untreated) and vehicle control cultures contain less than ~5% cells with aberration. The positive control cultures contain significantly higher ($p<0.01$) number of cells with aberrations than the vehicle controls. The assay tested the highest applicable dose (10 mM or 5 mg/ml). The test article is considered positive if a statistically significant increase in the number of cells with chromosomal aberrations is observed at one or more concentrations. The chromosomal aberrations should show a dose response-response relationship. Overall, the study was valid and adequately performed.

Study Outcome:

Chromosomal Aberrations Assay without Metabolic Activation

Initial assay: Chromosomal aberrations were analyzed from the cultures treated with 625, 1250, 2500, and 5000 µg/ml. No visual signs of toxicity were observed in any of the test cultures. No reductions were observed in the mitotic indices of the cultures analyzed as compared with the solvent control cultures. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed. Based on the results from the initial trial, the confirmatory non-activation aberrations assay was conducted testing concentrations of 313, 625, 1250, 2500, and 5000

µg/ml. Treatment periods were for 20 and 3 hours without and with metabolic activation, respectively, and the cultures were harvested 20 hours from the initiation of treatment.

Confirmatory Assay: Chromosomal aberrations were analyzed from the cultures treated with 625, 1250, 2500, and 5000 µg/ml. No visual signs of toxicity were observed in any of the test cultures. Reductions of 0% and 7% were observed in the mitotic indices of the cultures treated with 2500 and 5000 µg/ml, respectively, relative to solvent control cultures. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplicafion were observed at the concentrations analyzed.

Chromosomal Aberrations Assay with Metabolic Activation

Initial assay: No visual signs of toxicity were observed in any of the test cultures. Reductions of 0% and 12% were observed in the mitotic indices of the cultures treated with 2500 and 5000 µg/ml, respectively, relative to solvent control cultures. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.

Based on the results from the initial trial, the confirmatory aberrations assay with metabolic activation was conducted testing concentrations of 313, 625, 1250, 2500, and 5000 µg/ml. Treatment period was for 3 hours and cultures were harvested 20 hours from the initiation of treatment.

Confirmatory Assay: No visual signs of toxicity were observed in any of the test cultures. Reductions of 25, 24%, 19% and 22% were observed in the mitotic indices of the cultures treated with 625, 1250, 2500 and 5000 µg/ml, respectively, relative to the solvent control cultures. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.

Conclusion: AC2993 was considered negative for inducing chromosome aberrations in CHO cells with and without metabolic activation.

Initial Chromosome Aberration Assay

Metabolic Activation	Test/Control Article	Dose Concentration (µg/mL)	Assay 1 (Total 200 Cells Counted)				
			Mitotic Index (%)	Endoreduplicated Cells (%)	Polyploid Cells (%)	Cells With Chromosomal Aberrations (%)	Cells With Gaps or Chromosomal Aberrations (%)
Without S9	Negative (media)	0	13.7	0.0	0.0	0.0	1.0
	Vehicle Control	0	14.2	0.0	0.0	0.0	0.5
	Exenatide	625	-	0.0	0.0	0.5	0.5
		1250	-	0.5	0.0	0.5	0.5
		2500	-	0.0	0.0	0.0	0.0
		5000	19.3	0.0	0.0	0.5	1.0
	MMC (100 cells)	0.75	-	0.0	0.0	47.0*	48.0*
With S9	Negative (media)	0	11.5	1.0	0.0	1.0	1.0
	Vehicle Control	0	14.5	0.5	0.0	0.0	0.0
	Exenatide	625	-	0.0	0.5	0.5	1.0
		1250	-	1.0	0.0	0.5	0.5
		2500	15.9	1.0	0.0	0.0	0.0
		5000	12.7	0.0	0.0	1.0	1.0
	CP (100 cells)	7.50	-	0.0	2.0	50.0*	53.0*

[a] Times listed are approximate N/A = not applicable *Fisher's Exact Test $p \leq 0.01$ MMC = mitomycin C CP = cyclophosphamide
- = not tested

Confirmatory Chromosome Aberration Assay

Metabolic Activation	Test/Control Article	Dose Concentration (µg/mL)	Assay 2 (Total 200 Cells Counted)				
			Mitotic Index (%)	Endoreduplicated Cells (%)	Polyploid Cells (%)	Cells With Chromosomal Aberrations (%)	Cells With Gaps or Chromosomal Aberrations (%)
Without S9	Negative (media)	0	7.1	0.0	0.0	2.0	3.0
	Vehicle Control	0	14.0	0.0	0.0	0.0	2.0
	Exenatide	625	-	0.0	0.0	1.0	2.5
		1250	-	0.0	0.5	1.5	3.5
		2500	14.8	0.0	0.0	0.0	0.5
		5000	13.7	0.0	0.0	0.5	3.0
	MMC (100 cells)	0.200	-	0.0	0.0	71.0*	77.0*
With S9	Negative (media)	0	12.7	0.0	0.0	1.0	6.5
	Vehicle Control	0	12.3	0.0	0.0	3.0	5.5
	Exenatide	625	9.2	0.0	0.0	0.5	3.0
		1250	9.4	0.5	0.0	0.5	3.0
		2500	10.0	0.0	0.0	0.5	2.0
		5000	9.6	1.0	0.0	1.0	2.0
	CP (100 cells)	7.50	-	0.5	0.0	57.0*	61.0*

* Times listed are approximate N/A = not applicable *Fisher's Exact Test p ≤ 0.01 MMC = mitomycin C CP = cyclophosphamide
- = not tested

6.6.6.4.4 Study Title: Mutagenicity test on AC2993 measuring chromosomal aberration in Chinese hamster ovary (CHO) cells.

Study was performed to compare the genotoxicity of exenatide from new manufacturer (Mallinckrodt)

Key findings:

Confirmatory Chromosomal Aberrations Assay without and without Metabolic Activation

- No visual signs of toxicity were observed in any of the test cultures.
- No reductions were observed in the mitotic indices of the cultures analyzed as compared with the solvent control cultures.
- No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.
- AC2993 tested negative for chromosomal aberrations under the conditions of the study.

Study No: REST02304.

Volume # and Page #: N/A.
Conducting Laboratory: (b) (4)
Date of Study Initiation: January 27, 1998.
GLP Compliance: Yes (U.S.A.)
QA- Reports Yes (x) No ( ):
Drug Lot Number and % purity: Lot # 97-1210RP. 96.5% pure.

Methods

Strains/Species/Cell line: Chinese hamster ovary cell line.

Doses used in definitive study: 625, 1250, 2500, 5000 µg/ml.

Basis of dose selection: The highest dose was selected as the dose/concentration that inhibited cell growth (cytotoxicity) by 50%.

Negative Controls: Deionized water.

Positive Controls: Mitomycin C (- S9); Cyclophosphamide (+ S9).

Incubation and sampling times:
Aberration assay without metabolic activation: Cultures were initiated by seeding approximately 1.2 x 10^6 cells per 75 cm^2 flask into 10 ml of complete McCoy's 5a medium. One day after culture initiation, the cells were incubated at 37°C with the test article at predetermined concentrations for approximately 3.0 hours for the initial assay or 17.8 hours in the confirmatory assay. The cultures were then washed with buffered saline. In the initial assay, the cells were refed with complete McCoy's 5a medium and incubated for the rest of the culture period up to the time of harvest with 0.1 µg/ml (b) (4)® present during the last 2.0 hours of incubation. In the confirmatory assay, cells were refed with complete McCoy's 5a medium with 0.1 µg/ml (b) (4)® and harvested 2.0 hours later.

Aberrations Assay With Metabolic Activation: Cultures were initiated as previously described. One day after culture initiation, the cultures were incubated at 37°C for approximately three hours in the presence of the test article and the S9 reaction mixture in McCoy's 5a medium without FBS. After the three-hour exposure period the cells were washed twice with buffered saline and the cells were refed with complete McCoy's 5a medium. The cells were incubated for the rest of the culture period up to the time of harvest with 0.1 µg/ml (b) (4)® present during the last 2.0 hours of incubation. The cultures were then harvested.

RESULTS:
Study Validity: Two replicate cultures per dose were evaluated. The method of counting was not disclosed. The negative (untreated) and vehicle control cultures contain less than ~5% cells with aberration. The positive control cultures contain significantly higher ($p<0.01$) number of cells with aberrations than the vehicle controls. The assay tested the highest applicable dose (10 mM or 5 mg/ml). The test article is considered positive if a statistically significant increase in the number of cells with chromosomal aberrations is observed at one or more concentrations. The chromosomal aberrations should show a dose response-response relationship. Overall, the study was valid and adequately performed.

Study Outcome:

Chromosomal Aberrations Assay without Metabolic Activation
Initial assay: No visual signs of toxicity were observed in any of the test cultures. No reductions were observed in the mitotic indices of the cultures analyzed as compared with the solvent control cultures. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.

Based on the results from the initial trial, the confirmatory non-activation aberrations assay was conducted testing concentrations of 313, 625, 1250, 2500, and 5000 µg/ml. Treatment period was for 20 and 3 hours without and with metabolic activation, r5espectively, and cultures were harvested 20 hours from the initiation of treatment.

Confirmatory Assay: No visual signs of toxicity were observed in any of the test cultures. No reductions were observed in the mitotic indices of the cultures analyzed as compared with the solvent control cultures. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.

Chromosomal Aberrations Assay with Metabolic Activation

Initial assay: No visual signs of toxicity were observed in any of the test cultures. Reductions of 0%, and 10% were observed in the mitotic indices of the cultures treated with 2500 and 5000 µg/ml relative to the solvent control cultures. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.

Based on the results from the initial trial, the confirmatory aberrations assay with metabolic activation was conducted testing concentrations of 313, 625, 1250, 2500, and 5000 µg/ml. Treatment period was for 20 and 3 hours without and with metabolic activation, r5espectively, and cultures were harvested 20 hours from the initiation of treatment.

Confirmatory Assay: No visual signs of toxicity were observed in any of the test cultures. No reductions were observed in the mitotic indices of the treated cultures relative the solvent control cultures. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.

Conclusion: AC2993 was considered negative for inducing chromosome aberrations in CHO cells with and without metabolic activation.

Initial Chromosome Aberration Assay

Metabolic Activation	Test/Control Article	Dose Concentration (µg/mL)	Assay 1 (Total 200 Cells Counted)				
			Mitotic Index (%)	Endoreduplicated Cells (%)	Polyploid Cells (%)	Cells With Chromosomal Aberrations (%)	Cells With Gaps or Chromosomal Aberrations (%)
Without S9	Negative (media)	0	13.7	0.0	0.0	0.0	1.0
	Vehicle Control	0	14.2	0.0	0.0	0.0	0.5
	Exenatide	625	-	0.0	0.0	0.0	0.0
		1250	-	0.0	0.0	0.0	0.5
		2500	16.4	0.0	0.0	0.5	2.0
		5000	15.4	0.0	0.0	0.5	2.5
	MMC (100 cells)	0.75	-	0.0	0.0	47.0*	48.0*
With S9	Negative (media)	0	11.5	1.0	0.0	1.0	1.0
	Vehicle Control	0	14.5	0.5	0.0	0.0	0.0
	Exenatide	625	-	0.0	0.0	0.0	1.0
		1250	-	0.5	0.0	1.0	3.0
		2500	16.2	0.0	0.0	0.0	1.0
		5000	13.0	1.0	0.0	0.0	1.0
	CP (100 cells)	7.50	-	0.0	2.0	50.0*	53.0*

‡ Times listed are approximate N/A = not applicable *Fisher's Exact Test p ≤ 0.01 MMC = mitomycin C CP = cyclophosphamide
- = not tested

Confirmatory Chromosome Aberration Assay

Metabolic Activation	Test/Control Article	Dose Concentration (µg/mL)	Assay 2 (Total 200 Cells Counted)				
			Mitotic Index (%)	Endoreduplicated Cells (%)	Polyploid Cells (%)	Cells With Chromosomal Aberrations (%)	Cells With Gaps or Chromosomal Aberrations (%)
Without S9	Negative (media)	0	7.1	0.0	0.0	2.0	3.0
	Vehicle Control	0	14.0	0.0	0.0	0.0	2.0
	Exenatide	625	-	0.0	0.0	0.0	2.0
		1250	-	0.0	0.0	0.0	1.0
		2500	-	0.0	0.0	1.0	2.5
		5000	14.1	0.0	0.0	0.0	1.5
	MMC (100 cells)	0.200	-	0.0	0.0	71.0*	77.0*
With S9	Negative (media)	0	12.7	0.0	0.0	1.0	6.5
	Vehicle Control	0	12.3	0.0	0.0	3.0	5.5
	Exenatide	625	-	0.0	0.0	0.5	2.5
		1250	-	0.0	0.5	0.0	2.0
		2500	-	0.0	0.0	0.0	2.0
		5000	13.5	0.0	0.0	0.5	2.0
	CP (100 cells)	7.50	-	0.5	0.0	57.0*	61.0*

* Times listed are approximate N/A = not applicable *Fisher's Exact Test p ≤ 0.01 MMC = mitomycin C CP = cyclophosphamide
- = not tested

6.6.6.4.5 Study Title: In vivo mouse micronucleus assay with AC2993

Key findings:

- No significant increase in micronucleated PCEs was observed at any doses tested.
- AC2993 tested negative in the bone marrow micronucleus assay under the condition of this assay.

Study No: REST00078.

Volume # and Page #: N/A.
Conducting Laboratory: (b) (4)
Date of Study Initiation/completion: March 31, 2000/April 9, 2000.
GLP Compliance: Yes (U.S.A.).
QA- Reports Yes(x) No ( ):
Drug, Lot #, and % purity: 99-1002TP.

Methods:
Strains/Species/Cell line: Mouse/Crl:CD-1®(ICR)BR.
Doses used in definitive study: 34, 380 and 2000 µg/kg.
Basis of dose selection: The highest dose selected was 2000 µg/kg. Justification for dose selection was not provided.
Negative Controls: Formulation of vehicle was identical to that of test article but does not contain the active ingredient (AC2993).
Positive Controls: Cyclophosphamide.
Incubation and sampling times (Exposure Conditions): 1 day of dosing. Sampling at 24 and 48 hrs after dosing. The animals used (males) in this study were dosed subcutaneously with 34, 380 and 2000 µg/kg of AC2993. The positive control substance was administered by oral gavage. At 24 hrs post dose, 6 mice/group were sacrificed and bone marrow extracted for slide preparation. At 48 hrs post-dose, another 6 mice/group were selected from the HD and control groups, sacrificed and bone marrow extracted for slide preparation.

STUDY DESIGN:

Dosing Scheme for the Micronucleus Assay with AC2993

Target Treatment (μg/kg)	Dose Volume (mL/kg)	Route of Administration	Males/ Harvest Timepoint 24-Hour	48-Hour
34	0.6	subcutaneous	6	-
380	4.9	subcutaneous	6	-
2000	6.7	subcutaneous	6	6
Placebo for AC2993	6.7	subcutaneous	6	6
Positive Control, Cyclophosphamide, 80	10	oral gavage	6	-

RESULTS:

Study Validity: 2000 PCEs/animal were analyzed to determine the micronucleus frequency (expressed as percent micronucleated cells). Counting of PCEs from NCEs was done by microscopic evaluation of bone marrow smear slides using color differentiation. The study was valid because the mean incidence of micronucleated polychromatic erythrocytes (MNPCEs) did not exceed 5/1000 PCEs (0.5%) in the negative (vehicle) control. The mean incidence of MNPCEs in the positive control group were statistically significantly increased relative to the negative control. The test agent is said to be positive if a statistically significant increase in MNCPEs is observed for at least one dose level relative to control with significant dose-related response. Overall, the study was valid and adequately performed.

Study Outcome: The test article, AC2993, induced no signs of clinical toxicity in the treated animals and was not cytotoxic to the bone marrow (i.e., no statistically-significant decrease in the PCE:NCE ratio). A statistically significant increase in micronucleated PCEs was not observed at any dose level or harvest timepoint. The test article was evaluated as negative in the bone marrow micronucleus assay under the condition of this assay.

Test Article	Dose (μg/kg)	No./Sex of Animals	Harvest Time (h)	% Micronucleated PCEs (± SE)	Ratio PCE/NCE (± SE)
Vehicle	0	6 M	24	0.09 (0.03)	0.57 (0.04)
Vehicle	0	6 M	48	0.03 (0.02)	0.53 (0.03)
Exenatide	34	6 M	24	0.06 (0.02)	0.88 (0.05)
Exenatide	380	6 M	24	0.03 (0.01)	0.66 (0.03)
Exenatide	2000	6 M	24	0.03 (0.02)	0.82 (0.07)
Exenatide	2000	6 M	48	0.04 (0.02)	0.45 (0.06)
CP	80,000	6 M	24	1.60 (0.31)**	0.71 (0.07)

h = hour SC = subcutaneous PCE = polychromatic erythrocyte NCE = normochromatic erythrocytes CP = cyclophosphamide M = males
ANOVA and Dunnett's t-Test *= p < 0.05 **= p < 0.01 SE = standard error

2.6.6.5 Carcinogenicity

2.6.6.5.1 Study title: Carcinogenicity Study of AC2993 Administered Subcutaneously in Rats

Key study findings:

- No exenatide-related adverse effect was observed on survival rate. Survival rate was greater in the treated groups relative to controls.
- Body weight gain was slightly decreased (not SS) in the HD group. This correlated with the overall decreased food consumption.
- Injection site discoloration, thickening, and scab were observed in all treated animals including controls. Subcutaneous masses were observed in all treated groups including controls. Increased incidence of tan focus/foci of the lung were observed in treated groups relative to controls.

- Low titers of anti-exenatide antibody were detected at Week 36 as follows: 5/78, 2/40, 3/40 and 3/40 at 0 (both groups), 18, 70 and 250 µg/kg/d respectively. Exenatide was not antigenic in rats under the conditions of the study.
- None of the tumors observed was statistically significant, or dose-related.
- The incidence of thyroid C-cell adenoma was increased in all drug treated females relative to controls. The incidence in HD females is 23% relative to controls (8% and 5% for control groups 1 and 2 respectively) and is greater than the sponsor's historical control mean (5%) and range (0-10%). The thyroid C-cell adenomas may have been drug related.

Adequacy of the carcinogenicity study and appropriateness of the test model:
Sprague-Dawley rats were dosed once daily by subcutaneous administration of AC2993 at doses of 18, 70 and 250 µg/kg/d for 104 weeks. The test model (SD rat) is appropriate because the rat is a universal model routinely used for evaluating the toxicity and carcinogenicity of various classes of chemicals and for which there is a large historical database. The study was adequate because the study duration met the regulatory required duration for carcinogenicity studies (104 weeks); the doses evaluated provided adequate exposure multiples of 5X (LD), 23X (MD) and 130X (HD) the MRHD of 10 µg/day based on AUC; cumulative survival was greater in the treated groups relative to control; and mean body weight loss in the treated groups was slight (12-18%) over the 2 year period.

Evaluation of tumor findings:
- The incidence of thyroid C-cell adenoma was increased in all drug treated females (9/65-LD; 7/65-MD; 15/65-HD) relative to controls (5/65-Control 1 and 3/65-Control 2). The incidence in HD females is 23% relative to controls (8% and 5% for control groups 1 and 2 respectively) and is greater than the sponsor's historical control mean (5%) and range (0-10%).
- The thyroid C-cell adenomas may have been drug related.

Study no.: REST01052.
Volume # and page #: N/A.
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: April 27, 2001.
GLP compliance: Yes.
QA report: yes (X) no ( )
Drug, lot #, and % purity: Lot #01-0102TP, 95% pure; Lot # 00-0606TP, 93% pure.
CAC concurrence: Executive CAC did not concur with the doses selected for the rat. It was recommended that in order to receive concurrence on dose selection based on 25X AUC, the sponsor would need to provide appropriate exposure data rather than extrapolation on the basis of a single dose. Moreover, there may be a problem of excessive toxicity based on body weight changes.

The sponsor has submitted clinical PK data following a multiple dose study with 10 µg BID. Based on this data, the doses selected by the sponsor resulted in 12X, 28X and 95X the MRHD (10 µg BID = 2076 pg.h/ml, AUC) based on AUC. Excessive toxicity based on body weight changes was not observed throughout the carcinogenicity studies.

Methods

Doses: 0, 0, 18, 70, 250 µg/kg/d.
Basis of dose selection: AUC.
Species/strain: Rat/SD.
Number/sex/group (main study): 65/sex.group.

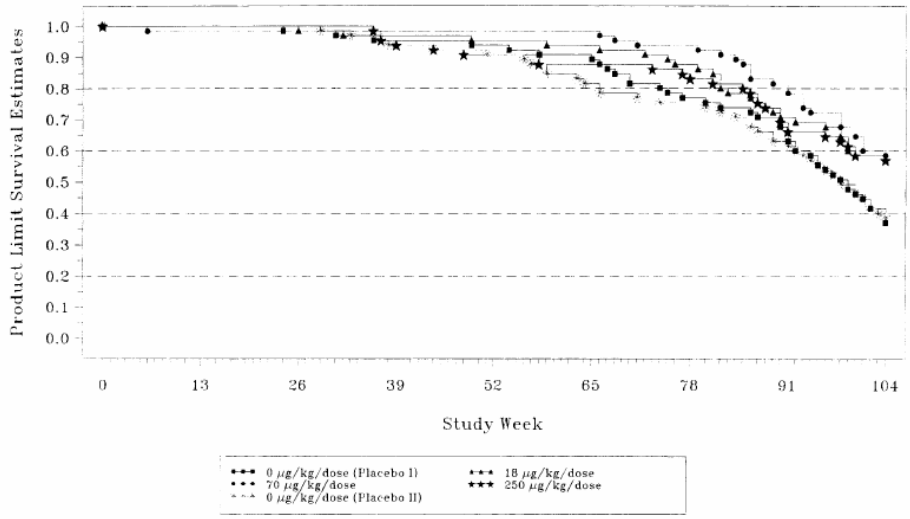
Route, formulation, volume: Subcutaneous administration; 360, 700, 833 µl/kg for the LD, MD and HD respectively. The control groups received 833 µl/kg each.
Frequency of dosing: Once daily.
Satellite groups used for toxicokinetics or special groups: Surviving main study animals were used for TK.
Age: 7 to 8 weeks old at study initiation.
Animal housing: Animals were individually housed in suspended, stainless steel, wire-mesh type cages in an environmentally-controlled room.
Restriction paradigm for dietary restriction studies: N/A.
Drug stability/homogeneity: Sponsor stated the test article was stable and homogeneous at the concentrations evaluated in the carcinogenicity study.
Dual controls employed: Yes.
Interim sacrifices: N/A.
Deviations from original study protocol: None

Observation times
Mortality: Daily.
Clinical signs: Daily.
Body weights: Measured predose, weekly during the first 16 weeks, and every 4 weeks thereafter.
Food consumption: Measured predose, weekly during the first 16 weeks, and every 4 weeks thereafter.
Histopathology: Peer review: yes (X), no ( )
Toxicokinetics: Approximately one week prior to study termination (~ 30 minutes postdose), blood samples were collected for determination of the plasma concentrations of the test article from the first five surviving rats/sex/control group, and from the first ten surviving rats/sex/treatment group.
Anti-Exenatide antibody: Specimens from each rat were collected before dose administration during Week 36 of the study. Collection before dose administration ensured that the plasma concentration of AC2993 would be below a concentration that would interfere with the antibody assay and collection of blood from each animal ensured that all animals in the study received approximately equal stress from the procedure. Antibody detection was by means of ELISA.

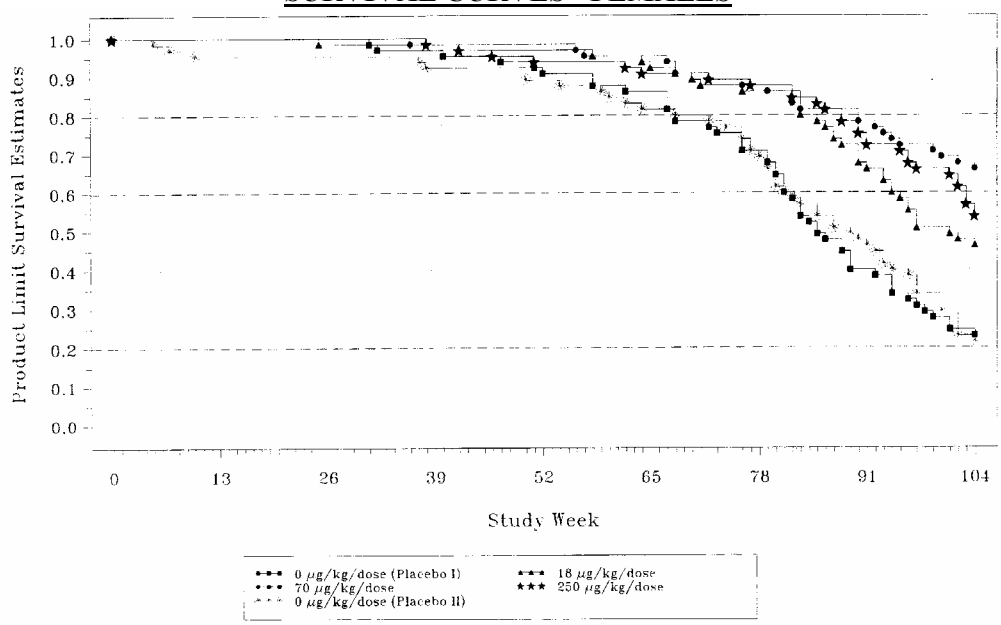
Results
Mortality:

Dose (µg/kg/d)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
# of Animals Start of treatment	65	65	65	65	65	65	65	65	65	65
Died/sacrificed moribund	48	52	44	49	40	45	43	50	45	49
Scheduled sacrifice	17	13	21	16	25	20	22	15	20	16
Cummulative Survival (%)	32	20	32	26	40	31	37	26	31	26

SURVIVAL CURVES – MALES



SURVIVAL CURVES - FEMALES



Clinical signs:
Treatment-related salivation was observed in all AC2993-treated groups in both sexes. Salivation was observed in almost all treated rats at least once during the study, with the time of onset being dose related, as increased salivation was generally noted during Weeks 3 to 4 at 250 µg/kg/d, Weeks 6 to 8 at 70 µg/kg/dose, and Weeks 16 to 18 at 18 µg/kg/dose and continued for the remainder of the study.

Body weights: (g) Mean body weight data.

Dose (µg/kg/d)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
Week 1	30.40	25.61	30.71	25.78	30.93	25.86	30.92	26.21	30.25	25.05*
Week 104	42.20	36.02	41.68	37.22	41.28	36.26	41.98	35.44	42.30	37.11
Body wt. gain	11.80	10.40	10.97	11.40	10.35	10.40	11.06	9.23	12.05	12.06

* $p < 0.05$

Food consumption: (g/day)

Dose (µg/kg/d)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
Week 1	7.09	6.40	7.45	7.98*	7.66*	7.70**	6.48**	6.29	6.55	7.27*
Week 104	6.02	5.94	6.20	6.14	6.39	6.11	6.16	6.13	5.73	5.83
Decrease	1.07	0.46	1.25	1.84	1.27	1.59	0.32	0.16	0.82	1.44

* p< 0.05; ** p< 0.01

Gross pathology: There were no treatment-related gross findings except for the gross findings noted at the injection sites. These findings were attributed to the trauma caused by repeated injection.

Summary of Macroscopic Observations - Males

Dose (µg/kg/d)	Severity	0_1		0_2		18		70		250	
Sex		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
# of Animals Examined		41	24	42	23	28	37	28	37	29	36
Injection site, Left flank		3	1	0	0	1	0	0	3	1	3
Discoloration, red	Minimal	1									
	Mild	1							3	1	3
	Moderate	1	1			1					
Thickened	Mild	0	0	0	0	0	0	1	0	0	0
Injection site, Left shoulder		3	4	1	0	1	3	1	3	0	4
Discoloration, red	Mild	3	4	1		1	2	1	3		4
	Moderate						1				
Mass	Present	0	0			2	0	0	0	0	0
Injection site, Right flank		0	1	0	0	1	3	1	5	2	1
Discoloration, red	Mild		1			1	3	1	4	2	1
	Moderate								1		
Discoloration, black	Moderate	0	0	0	0	0	0	0	0	1	0
Discoloration, gray	Mild	0	0			0	0	0	0	1	0
Injection site, Right shoulder		2	3	1	2	1	4	1	2	0	4
Discoloration, red	Mild	1	2	1	2	1	4		1		4
	Moderate	1	1					1	1		

DOS – Died or euthanized on study; SNC = Scheduled necropsy

Summary of Macroscopic Observations - Females

Dose (µg/kg/d)	Severity	0_1		0_2		18		70		250	
Sex		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
# of Animals Examined		51	14	51	14	35	30	22	43	30	35
Injection site, Left flank		7	0								
Discoloration, red	Minimal	1									
	Mild	6		4	0	3	3	1	5	1	0
Scab	Mild	1	0	0	0	1	0	0	0	0	1
Injection site, Left shoulder		3	1	3	1	8	1	4	4	1	2
Discoloration, red	Minimal	1		1		1					
	Mild	2	1	2	1	7	1	4	3	1	2
	Moderate								1		
Thickened	Mild	1	0								
	Moderate			0	0	1	0	0	0	0	0
Injection site, Right flank		6	1	6	1	3	4	1	3	2	5
Discoloration, red	Minimal							1		1	
	Mild	6	1	6		3	4		2	1	3
	Moderate				1				1		2
Thickened	Moderate			0	0	1	0	0	0	0	0
Injection site, Right shoulder		4	1	1	0	1	2	0	4	2	4
Discoloration, red	Minimal	2		1							1
	Mild	2	1			1	2		4	2	3
Skin, subcutis											
Mass	Present	51	14	50	9	16	12	8	17	11	20
Foot/feet											
Ulcer, plantar	Mild	6	4	0	0	0	0	0	0	0	0
Lung		1	0	0	1	3	4	0	6	2	3

Focus/foci, tan	Minimal	1									
	Mild				1	2	4		4	1	2
	Moderate								2	1	1
	Severe					1					

DOS – Died or euthanized on study; SNC = Scheduled necropsy

Histopathology:

Non-neoplastic:

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
Non-Neoplastic Findings:										
Parotid salivary gland										
Hypertrophy, basophilic, focal										
minimal	1	4	14	14	14	9	14	17	3	2
mild	0	0	9	14	15	18	10	11	0	0
moderate	0	0	6	4	2	8	5	12	0	0
severe	0	0	0	0	1	0	3	2	0	0
total	1	4	29	32	32	35	32	42	3	2

N/A = not assayed or measured
- = No noteworthy findings or findings not different from controls
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).

Neoplastic:

Summary of Neoplastic Lesions

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
Clinical Observations:	-	-	-	-	-	-	-	-	-	-
Hematology:	-	-	-	-	-	-	-	-	-	-
Number of Animals with Neoplastic Lesions										
Adipose Tissues										
Lipoma, bn, 1°	0	0	0	0	0	0	1	0	0	0
Hibernoma, bn, 1°	0	0	0	1	0	0	0	0	0	0
Hibernoma, mal, 1°	0	0	0	1	0	0	0	0	0	0
Adrenals glands										
Adenoma, cortical, bn, 1°	0	4	1	1	1	4	4	3	2	1
Carcinoma, cortical, mal, 1°	0	2	3	0	0	0	0	0	0	0
Pheochromocytoma, bn, 1°	3	3	1	3	4	3	6	2	12	2
Pheochromocytoma, mal, 1°	0	0	1	0	0	0	0	1	0	0
Brain										
Astrocytoma, mal, 1°	1	0	2	0	0	0	1	0	1	0
Hemangiosarcoma, mal, 1°	1	0	0	0	0	0	0	0	0	0
Granular cell tumor, mal, 1°	0	0	0	0	0	0	0	1	0	0
Meningioma, bn, 1°	0	1	2	0	0	0	0	0	0	0
Oligodendroglioma, bn, 1°	0	0	0	1	0	0	0	0	0	0
Papilloma, choroid plexus,bn,1°	0	0	0	0	0	0	0	1	0	0
Reticulosis, mal, 1°	0	1	0	0	0	0	0	0	0	0
Cavity, abdominal or thoracic										
Rhabdomyosarcoma, mal, 1°	0	0	0	0	1	0	0	0	0	0
Sarcoma, undiff, mal, 1°	1	0	0	0	0	0	0	0	0	0
Hibernoma, mal	0	0	0	0	0	0	1	0	0	0
Neuroendocrine tumor, mal, 1°	0	0	0	1	0	0	0	0	0	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cell or cellular B/A = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

Summary of Neoplastic Lesions Contd.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
Injection site, left flank	0	0	0	0	0	0	0	0	0	0
Injection site, left shoulder										
Sarcoma, undiff, mal, 1°	0	0	1	0	0	0	0	0	0	0
Injection site, right flank										
Fibrosarcoma, mal, 1°	0	0	0	0	0	0	0	0	1	0
Injection site, right shoulder										
Trichoepithelioma, bn, 1°	0	0	0	0	1	0	0	0	0	0
Kidneys										
Carcinoma, squamous cell, mal, 1°	0	0	1	0	0	0	0	0	0	0
Adenoma, tubular cell, bn, 1°	0	0	0	0	0	1	0	0	0	0
Carcinoma, tubular cell, mal, 1°	0	0	0	0	0	0	0	0	0	1
Lipoma, bn, 1°	0	0	0	0	0	0	0	0	0	1
Nephroblastoma, bn, 1°	0	1	0	0	0	0	0	0	0	0
Large intestine, cecum										
Fibroma, bn, 1°	0	0	0	0	0	0	0	0	0	1
Liver										
Adenoma, hepatocell, bn, 1°	0	2	0	1	1	1	2	1	2	2
Carcinoma, hepatocell, mal, 1°	0	1	0	0	1	0	1	1	0	0
Lymph nodes, all										
Hemangioma, bn 1°	0	0	0	0	1	0	0	0	0	0
Mammary glands										
Adenocarcinoma, mal, 1°	0	27	0	8	0	7	0	11	0	24
Adenoma, bn, 1°	0	4	0	3	0	1	0	1	0	1
Fibroadenoma, bn, 1°	0	26	0	12	0	17	2	13	0	21
Mediastinum										
Sarcoma, undiff, mal, 1°	0	0	0	0	0	0	0	0	1	0
Fibrosarcoma, mal, 1°	0	1	0	0	0	0	0	0	0	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular B/A = bronchiolar/alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

Summary of Neoplastic Lesions Contd.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
Multicentric neoplasm										
Lymphoma, mal, mc	2	1	0	1	0	1	2	0	1	0
Mast cell tumor, mal, mc	0	0	0	0	0	0	0	0	0	1
Sarcoma, histiocytic, mal, mc	2	2	1	4	0	0	0	0	1	0
Ovary	NA		NA		NA		NA		NA	
Adenoma, tubulostromal, bn, 1°		0		0		1		0		0
Carcinoma, tubulostromal, mal, 1°		0		0		0		1		0
Sex-cord/stromal tumor, bn, 1°		1		1		1		0		1
Sex-cord/stromal tumor, mal, 1°		0		0		0		0		1
Pancreas										
Adenoma, acinar cell, bn, 1°	0	0	0	0	0	1	0	0	0	0
Adenoma, islet cell, bn, 1°	3	1	3	1	4	2	5	2	2	2
Carcinoma, acinar cell, mal, 1°	0	0	0	0	0	0	0	1	0	0
Carcinoma, islet cell, mal, 1°	1	1	0	0	0	0	0	0	0	3
Parathyroid glands										
Adenoma, bn, 1°	1	0	2	0	0	0	1	0	4	0
Pituitary gland										
Adenoma, pars distalis, bn, 1°	36	55	31	47	26	56	29	48	29	49
Adenoma, pars intermedia, bn, 1°	0	0	1	0	0	0	0	0	0	0
Carcinoma, pars distalis, mal, 1°	0	0	0	0	0	0	0	1	0	0
Primary site unknown										
Adenocarcinoma, mal, 1°	0	0	0	0	0	0	0	0	1	0
Carcinoma, squamous cell, mal, 1°	0	0	0	0	0	0	0	1	0	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular B/A = bronchiolar/alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

Summary of Neoplastic Lesions Contd.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
Skin, all										
Adenoma, basal cell, bn, 1°	0	0	0	0	0	0	0	0	0	1
Carcinoma, squamous cell, mal, 1°	0	0	1	1	0	1	0	1	1	0
Papilloma, squamous, bn, 1°	0	0	0	1	1	0	0	0	1	0
Fibroma, bn, 1°	3	0	0	0	3	0	3	0	2	0
Fibrosarcoma, mal, 1°	0	2	0	1	1	1	0	0	1	3
Hemangiosarcoma, mal, 1°	1	0	0	1	0	0	0	0	1	0
Keratoacanthoma, bn, 1°	0	0	0	1	0	0	0	0	0	0
Lipoma, bn, 1°	1	0	0	0	0	0	0	0	1	0
Sarcoma, undiff, mal, 1°	0	0	1	0	0	0	0	0	1	0
Sarcoma, histiocytic, mal, 1°	0	1	0	3	0	0	0	0	0	0
Small intestine, all										
Adenocarcinoma, mal, 1°	0	0	0	0	0	0	0	0	1	0
Leiomyoma, bn, 1°	0	0	0	0	0	1	0	0	0	0
Stomach										
Carcinoma, squamous, mal, 1°	0	0	1	0	0	0	0	0	0	0
Testes		NA		NA		NA		NA		NA
Adenoma, interstitial cell, bn, 1°	3		5		4		2		1	
Mesothelioma, mal, 1°	0		0		1		0		0	
Thymus gland										
Thymoma, bn, 1°	0	0	0	2	0	0	0	0	0	1
Thyroid gland										
Adenoma, c-cell, bn, 1°	8	5	10	9	15	7	10	15	10	3
Adenoma, follicular cell, bn, 1°	0	0	0	0	1	0	0	2	0	1
Carcinoma, c-cell, mal, 1°	0	0	0	0	0	0	0	0	1	0
Carcinoma, follicular cell, mal 1°	0	0	0	0	0	1	0	0	1	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test;
Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

Summary of Neoplastic Lesions Contd.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
Tongue										
Carcinoma, squamous cell, mal, 1°	0	0	1	0	0	0	0	0	0	0
Urinary bladder										
Papilloma, transitional cell, be, 1°	0	0	1	0	0	0	0	0	0	0
Uterus with cervix	NA		NA		NA		NA		NA	
Granular cell tumor, bn, 1°		1		1		0		0		0
Leiomyoma, bn, 1°		0		0		1		1		0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test;
Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

Toxicokinetics: Based on 91-day Rat TK from bolus SC administration.

Dose (µg/kg/d)	0 (Control 1)	18	70	250	0 (Control 2)
AUC_{0-6h} (ph.h/ml)	M + F	M + F	M + F	M + F	M + F
Day 1	N/A	20,188	45,619	201,764	N/A
Day 91	N/A	10,178	48,554	268,094	N/A
C_{30min} (pg/ml)	31	13,413	47,179	208,635	14

Total daily $AUC_{(0-10hr)}$ for the MRHD (10 µg BID = 20 µg/day) = 2076 pg.h/ml

Anti-exenatide antibody data: Summary of ELISA positive Results

Dose Group	1	2	3	4	5	Total
Total Number	38	40	40	40	40	198
Number of Positives	2	2	3	3	3	13
Percent Positive	5.3	5.0	7.5	7.5	7.5	6.6

Based on the ELISA results, AC2993 has very low antigenic potential in rats.

2.6.6.5.2 Study title: Carcinogenicity Study of AC2993 Administered Subcutaneously in Mice

Key study findings:

- There were no exenatide-related effects on survival. Cummulative survival is greater in treated versus control groups. Clinical findings, or macroscopic pathology were unremarkable in any of the treated groups relative to controls.
- Body weight gain decreased by 23-27% in males and by 15-24% in females relative to controls. The decreased body weight gain correlated with decreased food consumption.
- The only treatment-related non-neoplastic microscopic finding was an increased incidence and severity of focal basophilic hypertrophy of acinar cells in the parotid salivary glands at doses ≥ 18 µg/kg/d. While there was no dose response with regard to incidence or severity in treated males, there was a weak dose response with regard to incidence or severity in treated females.
- None of the tumors observed was statistically significant or dose-related.
- Based on the conditions and findings of the study, daily SC injections of AC2993 at doses ≤ 250 µg/kg/d (95X MRHD, AUC) for 98 weeks in male mice and 96 weeks in female mice did not demonstrate any carcinogenic findings.

Adequacy of the carcinogenicity study and appropriateness of the test model:
CD-1 mice were dosed once daily by subcutaneous administration of AC2993 at doses of 18, 70 and 250 µg/kg/d for 96 weeks (females) and 98 weeks (males). The test model (CD-1 mouse) is appropriate because the mouse is a universal model routinely used for evaluating the toxicity and carcinogenicity of various classes of chemicals and for which there is a large historical database. The study was adequate because the doses evaluated provided adequate exposure multiples (12-95X) of the MRHD based on AUC; cumulative survival was greater in the treated groups relative to control; there was no significant change in mean body weight of treated groups relative to controls over the 2 year period. While the mice remained on study, and scheduled observations were continued until scheduled euthanasia after Week 104, treatment was discontinued after 96 weeks of dosing for the females (25/65 survival at 0 (Control 2) and 250 µg/kg/d, and after 98 weeks of dosing for the males (25/65 survival at 0 (Control 1) and 18 µg/kg/d based on reduced survival in both sexes and ECAC's recommendation. It is not clear from the individual animal histopathology data what caused the death in the early decedents. The sponsor does not know what caused the death of the early decedents either. However, the sponsor disclosed that survival rates are lower in mouse carcinogenicity studies by subcutaneous injection compared to oral gavage studies.

Evaluation of tumor findings:

- None of the tumors observed was statistically significant or dose-related.

Study no.: REST01053
Volume # and page #: N/A.
Conducting laboratory and location: (b) (4) (b) (4).
Date of study initiation: April 27, 2001.
GLP compliance: Yes.
QA report: yes (X) no ( )
Drug, lot #, and % purity: Lot # 01-0102TP, 95% pure; Lot # 00-0606TP, 93% pure.
CAC concurrence: Executive CAC did not concur with the doses selected for the mouse because of the exposure extrapolation approach used. However ECAC indicated that if the exposure margins projected were achieved, the study could be considered adequate. There was further concern that the volume necessary to deliver the proposed dose might exceed a maximum feasible dose based on the toxicity findings in the control and HD groups. The doses evaluated led to multiples of 12X, 28X and 95X the

MRHD (10 µg BID = 2076 pg.h/ml) based on AUC. The dose volumes used (947, 1400 and 3205 µl/kg for LD, MD and HD respectively; 3205 µl/kg for the control groups) did not appear to have exceeded the maximum feasible dose because survival in the MD and HD groups were greater relative to the LD group that received a lower dose volume. Survival in the control groups (given a higher dose volume) was also slightly higher relative to survival in the LD group (given a lower dose volume).

Methods

Doses: 0, 0, 18, 70, 250 µg/kg/d.
Basis of dose selection: AUC.
Species/strain: Mouse/CD-1.
Number/sex/group (main study): 65/sex/goup.
Route, formulation, volume: Subcutaneous injection; 947, 1400, 3205 µl/kg for LD, MD and HD groups. Controls received a dose volume of 3205 µl/kg.
Frequency of dosing: Once daily.
Satellite groups used for toxicokinetics or special groups: Some of the surviving animals in the main study group were used for TK.
Age: 7 to 8 weeks at study initiation.
Animal housing: Mice were individually housed in suspended, stainless steel, wire-mesh type cages in an environmentally-controlled room.
Restriction paradigm for dietary restriction studies: N/A.
Drug stability/homogeneity: Sponsor stated the test article was stable and homogeneous at the concentrations evaluated in the carcinogenicity study.
Dual controls employed: Yes.
Interim sacrifices: N/A.
Deviations from original study protocol: None.

Observation times

Mortality: Daily.
Clinical signs: Daily.
Body weights: Measured predose, weekly during the first 16 weeks of the study, and every 4 weeks thereafter.
Food consumption: Measured weekly during the first 16 weeks of the study, and every 4 weeks thereafter.
Histopathology: Peer review: yes (X), no ( )
Toxicokinetics: Blood samples for TK were collected during week 96 for females and week 98 for males from the first 5 surviving mice/sex/control groups, and from the first 10 surviving mice/treatment groups.

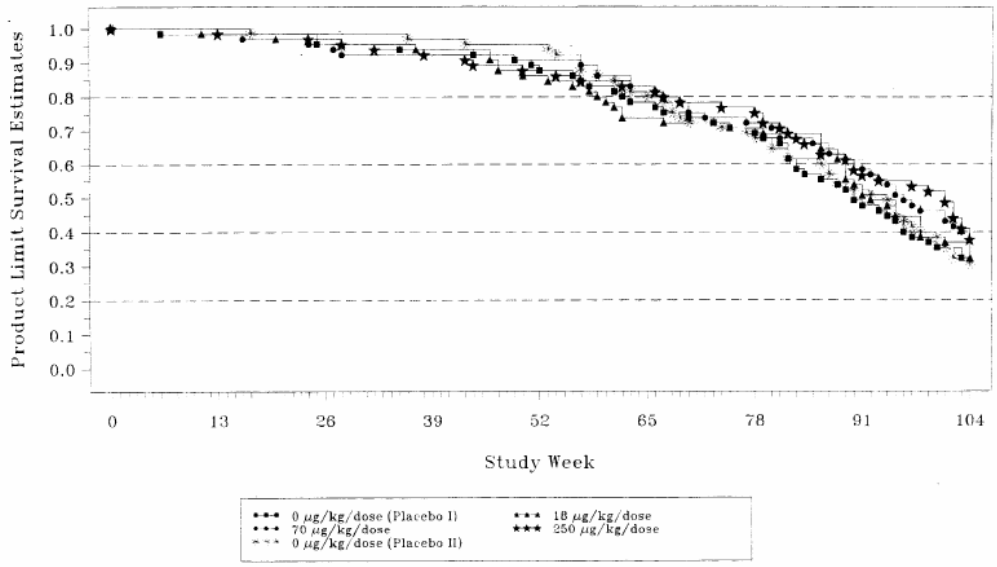
Results

Mortality:

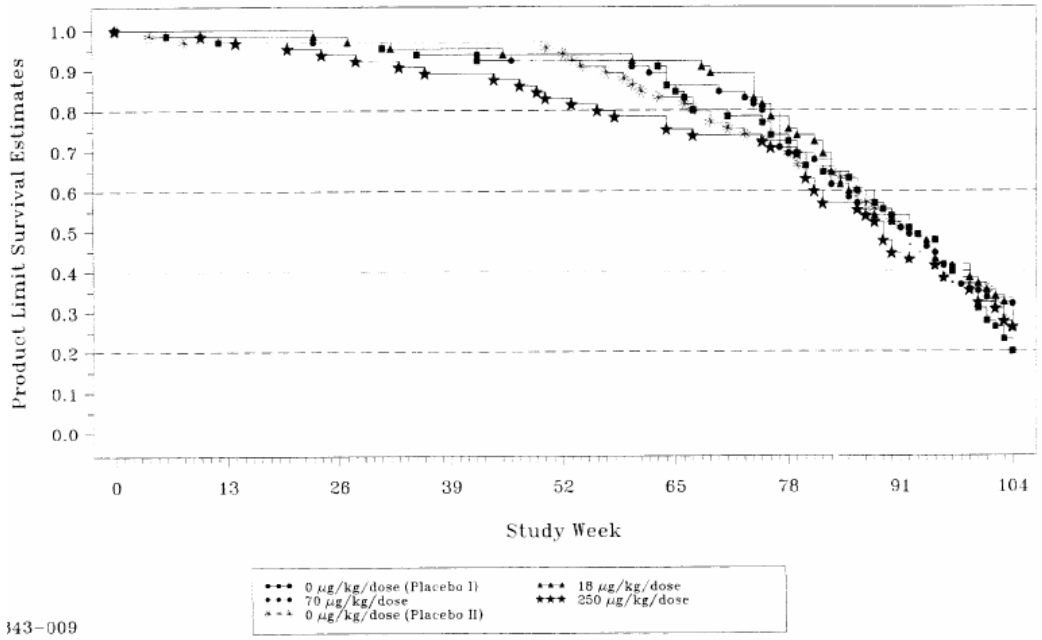
Dose (µg/kg/d)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
# of Animals Start of treatment	65	65	65	65	65	65	65	65	65	65
Died/sacrificed moribund	41	51	28	35	28	22	29	30	42	51
Scheduled sacrifice	24	14	37*	30**	37**	43**	36*	35*	23	14
Cummulative Survival (%)	37	23	57*	46**	59**	66**	57**	54**	39	22

* $p<0.05$; ** $p<0.01$

Survival Curves - Males



Survival Curves - Females



Clinical signs: No treatment-related clinical findings were observed during the study.

Body weights: (g)

Dose (µg/kg/d)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
Week 1	295	205	280**	205	275**	205	274**	203	290	202
Week 104	666	428	567**	383	558**	394	546**	373*	655	428
Body wt. gain	371	223	287**	178	283**	189	272**	170	365	226
% Decrease	-	-	23	20	24	15	27	24	-	-

* p< 0.05; ** p< 0.01

Food consumption: (g/day)

Dose (µg/kg/d)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
Week 1	25	19	22**	17**	21**	16**	20**	15**	25	18
Week 104	28	24	25*	21	24**	23	25*	22	27	24
% Decrease	-	-	11	13	14	4	11	8	-	-

* p< 0.05

Gross pathology: There were no treatment-related gross findings except for the gross findings noted at the injection sites. These findings were attributed to the trauma caused by repeated injection. Incidence of macroscopic findings were similar between treated and control groups.

Summary of Macroscopic Observations - Females

Dose (µg/kg/d)	Severity	0_1		0_2		18		70		250	
Sex		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
# of Animals Examined		41	24	42	23	28	37	28	37	29	36
Injection site, Left flank		1	0	2	0	1	0	0	0	4	0
Discoloration, red	Mild	1	0	1	0	0	0	0	0	4	0
	Moderate	0	0	0	0	1	0	0	0	0	0
	Severe	0	0	1	0	0	0	0	0	0	0
Ulcer/erosion	Moderate	0	0			0	0	1	0	0	0
Scab		4	0	4	0	2	0	0	0	3	0
	Mild	3	0	4	0	2	0	0	0	3	0
	Severe	1	0	0	0	0	0	0	0	0	0
Injection site, Left shoulder		1	0	4	0	1	0	3	0	2	0
Discoloration, red	Mild	1	0	1	0	0	0	3	0	2	0
	Moderate	0	0	3	0	1	0	0	0	0	0
Mass	Present	0	0	0	0	1	0	0	0	0	0
Injection site, Right flank		1	0	0	0	0	0	0	0	2	0
Discoloration, red	Mild	1	0	0	0	0	0	0	0	2	0
Scab	Mild	3	0	0	0	1	0	0	0	2	0
Injection site, Right shoulder		2	0	2	0	2	0	0	0	2	0
Discoloration, red	Minimal	1	0	0	0	0	0	0	0	0	0
	Mild	1	0	1	0	1	0	0	0	2	0
	Moderate	0	0	1	0	1	0	0	0	0	0
Scab	Mild			1	1						
	Moderate	3	0	0	0	1	0	0	0	0	0
Kidneys		2	0	3	0	2	0	5	1	6	0
Granular surface	Minimal	0	0	0	0	0	0	1	0	1	0
	Mild	2	0	3	0	1	0	2	1	4	0
	Moderate	0	0	0	0	1	0	2	0	1	0

DOS – Died or euthanized on study; SNC = Scheduled necropsy

Summary of Macroscopic Observations - Males

Dose (µg/kg/d)	Severity	0_1		0_2		18		70		250	
Sex		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
# of Animals Examined		51	14	51	14	35	30	22	43	30	35
Injection site, Left flank						1				1	
Discoloration, red	Minimal	0	0	0	0		0	0	0	1	0
	Mild					1					
Scab	Minimal	0	0	1	0	0	0	1	0	0	0
	Mild	0	0			0	0	0	0	1	0
Injection site, Left shoulder				2				1		1	
Scab	Minimal	0	0	1	0	0	0	1	0	0	0
	Mild	0	0	1	0	0	0	0	0	1	0
Injection site, Right flank						2		2		1	
Discoloration, red	Minimal	0	0	0	0	0	0	1	0	0	0
	Mild	0	0	0	0	2	0	0	0	1	0
	Moderate	0	0	0	0	0	0	1	0	0	0
Ulcer/erosion	Severe	0	0	0	0	0	0	0	0	1	0
Injection site, Right shoulder										2	

Discoloration, red	Minimal	0	0	0	0	0	0	0	0	1	0
	Mild	0	0	0	0	0	0	0	0	1	0
Lung		1	0	1	0	3	0	2	1	1	3
Discoloration, red	Mild	1	0	0	0	3	0	0	1	1	3
	Moderate	0	0	1	0	0	0	2	0	0	0

DOS – Died or euthanized on study; SNC = Scheduled necropsy

Histopathology:

Non-neoplastic:

104 Weeks Carcinogenicity Study – Summary of Non-Neoplastic Lesions

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
Non-Neoplastic Findings:										
Parotid salivary gland										
Hypertrophy, basophilic, focal										
minimal	1	4	14	14	14	9	14	17	3	2
mild	0	0	9	14	15	18	10	11	0	0
moderate	0	0	6	4	2	8	5	12	0	0
severe	0	0	0	0	1	0	3	2	0	0
total	1	4	29	32	32	35	32	42	3	2

N/A = not assayed or measured
- = No noteworthy findings or findings not different from controls
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).

The only treatment-related microscopic finding in male and female mice was increased incidence and severity of focal basophilic hypertrophy of acinar cells in the parotid salivary glands. While such foci were seen at a low incidence in the control groups (one male and four females in control group 1, and three males and two females in control group II), the incidence was greatly increased in all treated groups of both sexes. In treated males, there was no dose response with regard to incidence or severity, while in treated females there was a weak dose response with regard to incidence and severity. Sponsor stated that the basophilic foci were small, usually occupying a small portion of a lobule. Affected cells were enlarged by increased amounts of vesicular basophilic cytoplasm. As the number of lobules affected increased, and/or the number of small foci per lobule increased, the grade increased. No other significant microscopic changes were detected in the parotid salivary glands.

Neoplastic:

104 Weeks Carcinogenicity Study – Summary of Neoplastic Lesions

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
Clinical Observations:	-	-	-	-	-	-	-	-	-	-
Number of Animals with Neoplastic Lesions										
Adrenals glands										
Adenoma, subcapsular, bn, 1°	0	1	1	0	2	1	1	0	1	0
Pheochromocytoma, bn, 1°	0	0	0	1	0	0	0	0	0	0
Pheochromocytoma, mal, 1°	0	0	0	1	0	0	0	0	0	0
Brain										
Astrocytoma, mal, mc	0	0	0	0	1	0	0	0	0	0
Oligodendroglioma, mal, 1°	0	0	0	0	0	0	0	1	0	0
Epididymides										
Adenoma, interstitial cell, bn, 1°	0	NA	0	NA	1	NA	0	NA	0	NA
Schwanoma, bn	0		0		0		1		0	
Harderian glands										
Adenoma, bn, 1°	0	0	0	0	0	1	0	0	0	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test;
Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

104 Weeks Carcinogenicity Study – Summary of Neoplastic Lesions

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Injection site, left flank										
Fibrosarcoma, mal, 1°	0	0	1	0	0	1	0	0	0	0
Liposarcoma, mal, 1°	0	1	0	0	0	0	0	0	0	0
Injection site, left shoulder										
Fibrous histiocytoma, mal, 1°	0	0	0	1	0	0	0	0	0	0
Injection site, right flank	0	0	0	0	0	0	0	0	0	0
Injection site, right shoulder	0	0	0	0	0	0	0	0	0	0
Kidneys										
Adenoma, tubular cell, bn, 1°	0	0	0	0	0	0	1	0	1	0
Liver										
Adenoma, hepatocell, bn, 1°	7	1	8	2	5	1	7	1	4	1
Carcinoma, hepatocell, mal, 1°	2	0	3	0	1	0	2	1	4	0
Hemangioma, bn, 1°	1	0	0	1	0	0	0	0	0	0
Hemangiosarcoma, mal, 1°	4	0	0	0	2	2	2	0	2	1
Lung										
Adenoma, BA, bn,1°	13	11	9	10	14	8	13	6	11	12
Carcinoma, BA, mal, 1°	4	1	3	5	1	0	4	3	3	5
Mammary glands										
Adenocarcinoma, mal, 1°	0	1	0	1	0	0	0	0	0	0
Mesentery/peritoneum										
Hibernoma, bn, 1°	0	0	0	0	0	0	0	0	1	0
Multicentric neoplasm										
Leukemia, granulocytic, mal, mc	0	0	0	0	1	0	0	0	0	0
Lymphoma, mal, mc	4	6	4	8	3	6	1	8	5	4
Sarcoma, undiff, mal, 2°	0	0	0	0	0	0	1	0	1	0
Sarcoma, histiocytic, mal, mc	0	4	0	10	0	5	1	1	1	5
Carcinoma, 1° unknown, mal	0	0	0	1	0	0	0	0	0	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Ovary	NA		NA		NA		NA		NA	
Adenoma, tubulostromal, bn, 1°		0		0		0		0		1
Cystadenoma, bn, 1°		1		0		0		1		0
Leiomyosarcoma, mal, 1°		0		0		0		1		0
Sex-cord/stromal tumor, bn, 1°		1		0		1		0		3
Pancreas										
Adenoma, islet cell, bn, 1°	0	0	0	1	0	0	0	0	0	0
Pituitary gland										
Adenoma, pars distalis, bn, 1°	0	1	0	1	0	3	2	1	0	1
Adenoma, pars intermedia, bn, 1°	0	0	0	1	0	0	0	0	0	0
Seminal vesicles		NA		NA		NA		NA		NA
Hemangiosarcoma, mal 1°	0		0		1		0		0	
Skeletal muscle										
Hemangiosarcoma, mal, 1°	0	1	0	1	0	0	0	0	0	0
Skin, all										
Fibrosarcoma, mal, 1°	0	1	0	0	0	2	0	0	1	1
Hemangiosarcoma, mal, 1°	0	0	0	0	0	0	1	1	0	0
Sarcoma, undiff, mal, 1°	0	3	0	0	1	0	1	1	4	1
Carcinoma, basosquamous, mal, 1°	0	1	0	0	0	0	0	0	0	0
Carcinoma, squamous, mal, 1°	0	1	0	0	0	0	0	0	0	0
Keratoacanthoma, bn, 1°	0	0	0	0	0	0	0	0	0	1
Leiomyosarcoma, mal, 1°	0	1	0	0	0	0	0	0	0	0
Liposarcoma, mal, 1°	0	0	0	1	0	0	0	0	0	0
Fibrous histiocytoma, mal, 1°	0	0	0	1	0	0	0	0	0	0
Small intestine, all										
Adenocarcinoma, mal, 1°	0	0	0	0	1	0	0	0	1	0
Fibrosarcoma, mal, 1°	0	0	0	0	0	0	0	1	0	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

104 Weeks Carcinogenicity Study – Summary of Neoplastic Lesions

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	M	F	M	F	M	F	M	F	M	F
Spleen										
Hemangioma, bn, 1°	0	0	1	1	0	1	0	0	0	0
Hemangiosarcoma, mal, 1°	3	1	1	0	1	0	0	1	1	1
Stomach										
Osteosarcoma	0	0	0	0	0	0	1	0	0	0
Thoracic cavity										
Osteoma, bn, 1°	0	0	0	1	0	0	0	0	0	0
Thyroid										
Adenoma, follicular cell, bn, 1°	0	0	0	0	0	0	1	0	0	0
Carcinoma, follicular cell, mal, 1°	1	0	0	0	0	0	0	0	0	0
Urinary bladder										
Hemangioma, bn, 1°	0	0	0	0	0	0	1	0	0	0
Mesenchymal tumor, bn, 1°	0	0	1	0	0	0	0	0	0	1
Papilloma, transitional cell, bn, 1°	0	0	0	0	0	0	0	1	1	0
Uterus and Cervix	NA		NA		NA		NA		NA	
Adenocarcinoma, mal, 1°		1		1		0		0		1
Adenoma, bn, 1°		0		0		1		0		0
Fibroma, bn, 1°		0		0		1		0		0
Fibrosarcoma, mal, 1°		1		0		0		0		0
Granular cell tumor, bn, 1°		0		0		2		1		0
Hemangioma, bn, 1°		0		1		2		0		0
Hemangiosarcoma, mal, 1°		0		1		0		1		1
Leiomyoma, bn, 1°		2		1		0		3		0
Leiomyosarcoma, mal, 1°		1		0		3		1		0
Sarcoma, stromal, mal, 1°		4		1		0		5		3
Vagina										
Sarcoma, stromal, mal, 1°	NA	1	NA	0	NA	0	NA	0	NA	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test;
Tumor Analysis: Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

Toxicokinetics: Based on bolus SC TK from 91-day mouse study.

Dose (µg/kg/d)	0 (Control 1)	18	70	250	0 (Control 2)
AUC_{0-6h} (ph.h/ml)	M + F	M + F	M + F	M + F	M + F
Day 1	N/A	10,113	32,508	123,241	N/A
Day 91	N/A	25,425	58,403	197,295	N/A
C_{30min} (pg/ml)	< 10	22,177	77,814	231,460	< 10

Total daily $AUC_{(0-10hr)}$ for the MRHD (10 µg BID = 20 µg/day) = 2076 pg.h/ml

2.6.6.6 Reproductive and developmental toxicology

Fertility and Early Embryonic Development

2.6.6.6.1 Study title: Subcutaneous Fertility and General Reproduction Toxicity Study of AC2993 in Mice.

Key study findings:

- 1/25 males dosed 6 µg/kg/day was found dead on study day 7 (DS 7). Sponsor stated that the demise of this animal occurred after a tonic flexor convulsion.
- Body weight gain was significantly increased by 1.2-fold and 1.3-fold in MD and HD males relative to control. Pre-cohabitation body weight gain was significantly increased in treated females by 2-fold (LD), 4-fold (MD) and 3-fold (HD) relative to control.
- AC2993 neither affected mating nor fertility in males. The number of males that mated as well as fertility index were similar in both control and treated males.
- There was a dose-dependent decrease in number of motile sperm, however, the decrement was not significantly different from control.

- Absolute weight of seminal vesicle with fluid was significantly increased in LD and HD males whereas weight of the seminal vesicle without fluid was only significantly increased in LD males. Relative weight of the prostate was significantly increased in HD males relative to control.
- AC2993 did not affect mating, fertility or estrous cycling in treated females. The numbers of females that mated, percent of females pregnant as well as fertility index were similar in both control and treated females.
- The number of viable embryos and post-implantations loss was increased in treated females relative to control. The changes observed were not statistically significant.
- Post-implantation loss was increased by 2 to 3-fold in treated mice relative to control, but the differences were not statistically significant.
- There were no effects on mating and fertility parameters at the HD. NOAEL for mating and fertility > 760 µg/kg/d.

Study no.: REST01001.
Volume # and page #: N/A.
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: March 19, 2001.
GLP compliance: Yes (USA, UK and Japan).
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 00-0606 TP, Purity 92.9%.

Methods

Doses: 3, 34 and 380 µg/kg BID i.e. 6, 68 and 760 µg/kg/day.
Species/strain: Mouse/CD-1.
Number/sex/group: 25/sex/group.
Route, formulation, volume, and infusion rate: Subcutaneous injection; 4875 µl/kg (control and HD), 600 µl/kg (LD) and 1800 µg/kg (MD).
Satellite groups used for toxicokinetics: None. TK was not conducted.
Study design: Male mice were administered the test article and/or vehicle twice daily beginning 28 days before cohabitation and continuing through the day before sacrifice. The cohabitation period consisted of a maximum of 21 days. Female mice were administered the test article and/or vehicle twice daily beginning 15 days before cohabitation (maximum of 21 days) and continuing through GD 7. Dosage volumes were adjusted daily for body weight changes and given at approximately the same time each day. The two daily injections were separated by 11 to 13 hours.

Estrous cycling was evaluated daily by examination of vaginal cytology for 14 days before initiation of administration and for 14 days beginning with the day after the first administration and then until spermatozoa were observed in a smear of the vaginal contents and/or a copulatory plug was observed in situ during the cohabitation period.

All female mice were sacrificed on GD 13 or estimated GD 13, cesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Uteri of apparently non-pregnant mice were examined while being placed between glass plates to confirm pregnancy status. The number of corpora lutea in each ovary was recorded. The uterus of each mouse was excised and examined for pregnancy, number and distribution of implantation sites and viable and nonviable embryos.

All surviving male mice were sacrificed at the completion of the cohabitation period and pregnancy evaluation of the respective females. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The following organs were individually weighed: right testis, left

testis, left epididymis (whole and cauda), right epididymis, seminal vesicles (with and without fluid) and prostate. A portion of the left cauda epididymis was used for evaluation of cauda epididymal sperm concentration and motility using computer-assisted sperm analysis (CASA).

Parameters and endpoints evaluated:

Mortality: Daily.
Body weight: weekly
Food consumption: weekly.
Necropsy: After completion of the cohabitation period, all surviving male mice were sacrificed and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. To assess the potential toxicity of the test article on the male reproductive system, reproductive organs were weighed and retained for possible histopathological evaluation and sperm evaluations.
Organ weights: right testis, left testis, left epididymis (whole and cauda), right epididymis, seminal vesicles (with and without fluid) and prostate.
Semen Evaluation: A portion of the left cauda epididymis was used for evaluation of cauda epididyrnal sperm concentration and motility using computer-assisted sperm analysis (CASA). Motility was evaluated by (b) (4) using a sample collected from the left cauda epididymis. A homogenate was prepared for evaluation (b) (4) to determine sperm concentration (sperm per gram of tissue weight).
Histopathology: The remaining left epididymis (corpus and caput) were retained in neutral buffered 10% formalin for possible further histopathological evaluation. The remaining portion of the left epididymis, right epididymis, prostate and seminal vesicles were fixed in neutral buffered 10% formalin for possible histopathological evaluation. The testes were fixed in Bouin's solution for 48 to 96 hours and then retained in neutral buffered 10% formalin for possible histopathological evaluation.

Results

Mortality: 1/25 males dosed 6 µg/kg/day was found dead on study day 7 (DS 7). Sponsor stated that the demise of this animal occurred after a tonic flexor convulsion. Body weight gain and feed consumption were unremarkable. All tissues appeared normal at necropsy.

Clinical signs: Empty cells indicate zero incidence.

Dose (µg/kg/day)	**0**	**6**	**68**	**760**
Sex	**M**	**M**	**M**	**M**
Scab at dosage area	20/25	9/25	16/25	23/25
Chromodacryorrhea				2/25
Missing tail tip			1/25	1/25
Portion of tail black			1/25	1/25
Lacrimation				1/25
Back: ulceration				1/25
Abdominal distension				1/25
Ptosis				1/25
Tip of tail black			2/25	1/25
Convulsion: tonic flexor		1/25		

Body weight: (g) – Body weight of females is unremarkable.

Dose (µg/kg/day)	**0**	**6**	**68**	**760**
Sex	**M**	**M**	**M**	**M**
Day 1	33.7	33.7	33.2	33.6
Termination	40.6	41.9*	41.6	42.9**
Body wt. gain (g)	6.9 ± 1.6	8.2 ± 2.0	8.4 ± 2.6*	9.3 ± 2.7**
Body wt. gain (%)	21	24	25	28

*** $p \leq 0.05$; ** $p \leq 0.01$**

Food consumption: Unremarkable.

Toxicokinetics: No data.

Necropsy:
Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Summary of Mating and Fertility in Males

DOSAGE GROUP DOSAGE (MCG/KG/DAY)		I 0 (VEHICLE)	II 6	III 68	IV 760
MICE IN COHABITATION	N	25	24a	25	25
DAYS IN COHABITATION b,c	MEAN±S.D.	3.0 ± 1.8	3.0 ± 1.8 [23]	2.3 ± 1.3	3.3 ± 2.4
MICE THAT MATED c	N(%)	25(100.0)	24(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX d,e	N/N (%)	24/ 25 (96.0)	23/ 24 (95.8)	23/ 25 (92.0)	24/ 25 (96.0)
MICE WITH CONFIRMED MATING DATES c	N	25	23	23	23
MATED WITH FIRST FEMALE f					
DAYS 1-7	N(%)	24(96.0)	22(95.6)	23(100.0)	22(95.6)
DAYS 8-14	N(%)	1(4.0)	1(4.3)	0(0.0)	1(4.3)
MATED WITH SECOND FEMALE f					
DAYS 15-21	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
MICE PREGNANT/MICE IN COHABITATION e	N/N (%)	24/ 25 (96.0)	23/ 24 (95.8)	23/ 25 (92.0)	24/ 25 (96.0)

[ ] = NUMBER OF VALUES AVERAGED
a. Mouse 9234 was found dead on day 7 of study; values excluded from group averages and statistical analyses.
b. Restricted to mice with a confirmed mating date and mice that did not mate.
c. Includes only one mating for each male mouse.
d. Number of pregnancies/number of mice that mated.
e. Includes only one pregnancy for each mouse that impregnated more than one female mouse.
f. Restricted to mice with a confirmed mating date.

Summary of Mating and Fertility in Males Contd.

DOSAGE GROUP DOSAGE (MCG/KG/DAY)		I 0 (VEHICLE)	II 6	III 68	IV 760
MICE TESTED	N	25	24a	25	25
TERMINAL BODY WEIGHT	MEAN±S.D.	40.6 ± 1.8	41.9 ± 1.8*	41.6 ± 3.2	42.9 ± 1.5**
EPIDIDYMIS LEFT	MEAN±S.D.	0.0549 ±0.0044	0.0576 ±0.0032	0.0554 ±0.0062	0.0568 ±0.0055
CAUDA EPIDIDYMIS LEFT	MEAN±S.D.	0.0216 ±0.0027	0.0236 ±0.0025	0.0225 ±0.0032	0.0230 ±0.0027
TESTIS LEFT	MEAN±S.D.	0.1275 ±0.0143	0.1363 ±0.0178	0.1273 ±0.0185	0.1291 ±0.0180
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	0.3609 ±0.0638 [24]b	0.4182 ±0.0731**	0.3936 ±0.0674 [23]b	0.4056 ±0.0731* [24]b
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.2042 ±0.0377	0.2383 ±0.0520**	0.2206 ±0.0418	0.2218 ±0.0307
EPIDIDYMIS RIGHT	MEAN±S.D.	0.0554 ±0.0048	0.0578 ±0.0052	0.0545 ±0.0076	0.0570 ±0.0053
TESTIS RIGHT	MEAN±S.D.	0.1334 ±0.0160	0.1440 ±0.0185	0.1293 ±0.0286	0.1362 ±0.0205
PROSTATE	MEAN±S.D.	0.0448 ±0.0127 [24]b	0.0370 ±0.0176	0.0402 ±0.0152	0.0353 ±0.0083

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED.
a. Rat 9234 was found dead on day 7 of study; values excluded from group averages and statistical analyses.
b. Excludes values for mice that had organs damaged (weight affected) or weights not recorded.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Summary of Mating and Fertility in Males Contd.

DOSAGE GROUP DOSAGE (MCG/KG/DAY)		I 0 (VEHICLE)	II 6	III 68	IV 760
MICE TESTED	N	25	24a	25	25
TERMINAL BODY WEIGHT	MEAN±S.D.	40.6 ± 1.8	41.9 ± 1.8*	41.6 ± 3.2	42.9 ± 1.5**
EPIDIDYMIS LEFT b	MEAN±S.D.	135.342 ±11.266	137.699 ± 9.461	133.633 ±13.227	132.466 ±12.885
CAUDA EPIDIDYMIS LEFT b	MEAN±S.D.	53.182 ± 6.039	56.303 ± 6.443	54.168 ± 7.285	53.685 ± 7.015
TESTIS LEFT	MEAN±S.D.	0.314 ± 0.040	0.325 ± 0.046	0.307 ± 0.048	0.300 ± 0.040
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	0.886 ± 0.144 [24]c	0.995 ± 0.155	0.951 ± 0.168 [23]c	0.945 ± 0.168 [24]c
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.501 ± 0.084	0.567 ± 0.113	0.530 ± 0.088	0.517 ± 0.070
EPIDIDYMIS RIGHT b	MEAN±S.D.	136.660 ±12.949	138.286 ±13.878	131.306 ±17.735	132.970 ±12.115
TESTIS RIGHT	MEAN±S.D.	0.328 ± 0.044	0.344 ± 0.047	0.311 ± 0.070	0.318 ± 0.047
PROSTATE b	MEAN±S.D.	110.486 ±31.470 [24]c	89.546 ±47.155	97.042 ±36.838	82.248 ±18.827**

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.
[ ] = NUMBER OF VALUES AVERAGED.
a. Rat 9234 was found dead on day 7 of study; values excluded from group averages and statistical analyses.
b. Value was multiplied by 1000.
c. Excludes values for mice that had organs damaged (weight affected) or weights not recorded.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Summary of Mating and Fertility in Males Contd.

DOSAGE GROUP DOSAGE (MCG/KG/DAY)		I 0 (VEHICLE)	II 6	III 68	IV 760
MICE TESTED	N	25	24a	25	25
NUMBER MOTILE	MEAN±S.D.	433.2 ± 171.9	403.4 ± 138.1	395.4 ± 191.2	344.9 ± 137.4
MOTILE PERCENT	MEAN±S.D.	90.0 ± 5.9	89.6 ± 13.0	89.6 ± 10.4	91.1 ± 5.9
STATIC COUNT (NONMOTILE)	MEAN±S.D.	44.6 ± 22.4	44.5 ± 60.2	45.7 ± 46.3	30.9 ± 19.3*
TOTAL COUNT b	MEAN±S.D.	477.7 ± 173.9	448.0 ± 126.1	441.1 ± 216.6	375.8 ± 138.3
SPERM COUNT c	MEAN±S.D.	56.1 ± 17.4	56.1 ± 17.3	49.9 ± 22.7	54.3 ± 13.9
DENSITY d	MEAN±S.D.	1510.10 ± 452.77	1383.82 ± 419.56	1259.79 ± 443.00	1367.94 ± 317.18

a. Excludes values for mice that died.
b. Sum of number motile and static count. Groups of five fields were evaluated until a sperm count of at least 200 was achieved or 20 fields were evaluated.
c. Sperm count used in the calculation of sperm density. Ten fields were evaluated.
d. The sperm density was calculated by dividing the sperm count by the volume in the image area (34.3×10^{-6} mL), multiplying by 2 (dilution factor) and multiplying by 10^{6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table B15 for the weight of the left cauda epididymis) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

Summary of Mating and Fertility, Estrous Cycling and Days in Cohabitation – Females

DOSAGE GROUP DOSAGE (MCG/KG/DAY)a		I 0 (VEHICLE)	II 6	III 68	IV 760
ESTROUS CYCLING OBSERVATIONS					
MICE EVALUATED	N	25	25	25	25
PREDOSAGE ESTROUS CYCLING					
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	2.7 ± 0.6	2.8 ± 0.6	2.7 ± 0.7	2.6 ± 0.7
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	0	1	1	2
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	0	0	0	0
PRECOHABITATION ESTROUS CYCLING					
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	3.4 ± 0.9	3.4 ± 0.6	3.0 ± 0.9	3.0 ± 1.0
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	0	0	0	2
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	0	0	0	2

a. Dosage occurred on day 1 of study through day 7 of presumed gestation.

Summary of Mating and Fertility, Estrous Cycling and Days in Cohabitation – Females Contd.

DOSAGE GROUP DOSAGE (MCG/KG/DAY)a		I 0 (VEHICLE)	II 6	III 68	IV 760
MATING OBSERVATIONS					
MICE IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION b	MEAN±S.D.	3.0 ± 1.8	3.0 ± 1.8 [24]	2.3 ± 1.3 [23]	3.3 ± 2.4 [23]
MICE THAT MATED	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX c	N/N (%)	24/ 25 (96.0)	24/ 25 (96.0)	23/ 25 (92.0)	24/ 25 (96.0)
MICE WITH CONFIRMED MATING DATES	N	25	24	23	23
MATED BY FIRST MALE d					
DAYS 1-7	N(%)	24(96.0)	23(95.8)	23(100.0)	22(95.6)
DAYS 8-14	N(%)	1(4.0)	1(4.2)	0(0.0)	1(4.3)
MICE PREGNANT/MICE IN COHABITATION	N/N (%)	24/ 25 (96.0)	24/ 25 (96.0)	23/ 25 (92.0)	24/ 25 (96.0)

a. Dosage occurred on day 1 of study through day 7 of presumed gestation.
b. Restricted to mice with a confirmed mating date and mice that did not mate.
c. Number of pregnancies/number of mice that mated.
d. Restricted to mice with a confirmed mating date.

Summary of Mating and Fertility, Estrous Cycling and Days in Cohabitation – Females Contd.

DOSAGE GROUP DOSAGE (MCG/KG/DAY)a		I 0 (VEHICLE)	II 6	III 68	IV 760
MICE TESTED	N	25	25	25	25
PREGNANT	N(%)	24(96.0)	24(96.0)	23(92.0)	24(96.0)
MICE PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION b	N	24	24	23	24
CORPORA LUTEA	MEAN±S.D.	14.1 ± 1.5	14.7 ± 2.2	14.2 ± 1.9	13.8 ± 3.5
IMPLANTATIONS	MEAN±S.D.	13.0 ± 1.2	13.8 ± 1.8	13.3 ± 1.5	12.6 ± 1.7
VIABLE EMBRYOS	N	305	309	293	287
	MEAN±S.D.	12.7 ± 1.3	12.9 ± 2.2	12.7 ± 1.7	12.0 ± 1.8
NONVIABLE EMBRYOS	N	7	22	14	16
	MEAN±S.D.	0.3 ± 0.6	0.9 ± 1.6	0.6 ± 0.8	0.7 ± 1.0
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	6(25.0)	10(41.7)	11(47.8)	10(41.7)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE EMBRYOS	N(%)	24(100.0)	24(100.0)	23(100.0)	24(100.0)
PLACENTAE APPEARED NORMAL	N(%)	24(100.0)	24(100.0)	23(100.0)	24(100.0)
PREIMPLANTATION LOSS	MEAN±S.D.	7.4 ± 8.5	23.7 ± 26.0	5.7 ± 5.9	6.2 ± 11.1
POSTIMPLANTATION LOSS	MEAN±S.D.	2.2 ± 4.1	6.5 ± 11.1	4.6 ± 5.7	5.2 ± 7.5

PREIMPLANTATION LOSS = (NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS)/NUMBER OF CORPORA LUTEA X 100
POSTIMPLANTATION LOSS = (NUMBER OF IMPLANTATIONS - NUMBER OF LIVE EMBRYOS)/NUMBER OF IMPLANTATIONS X 100
a. Dosage occurred on day 1 of study through day 7 of gestation.
b. Includes values for mice without a confirmed mating date.

Summary of Mating and Fertility, Estrous Cycling and Days in Cohabitation – Females Contd.

DOSAGE GROUP DOSAGE (MCG/KG/DAY)a		I 0 (VEHICLE)	II 6	III 68	IV 760
MICE EXAMINED b	N	25	25	25	25
MORTALITY	N	0	0	0	0
APPEARED NORMAL	N	24	25	24	25
SPLEEN: LARGE	N	1	0	0	0
HEART: LEFT VENTRICLE, THREE WHITE RAISED AREAS	N	0	0	1	0

a. Dosage occurred on day 1 of study through day 7 of presumed gestation.
b. Refer to the individual clinical observations table (Table C13) for external observations confirmed at necropsy.

Embryo-Fetal Development

2.6.6.6.2 Study title: Developmental Toxicity Study of Subcutaneously Administered AC2993 in Mice (Segment II Teratology Study).

Key study findings:

- 1/25 female mice (# 251) in the 68 µg/kg/day dose group aborted on gestation day 15 (GD 15) and was sacrificed on GD 16. The litter consisted of 13 late resorptions. 1/25 female mice (# 321) in the 760 µg/kg/day dose group aborted on GD 16 and was sacrificed on GD 17. The litter consisted of 11 dead fetuses. One fetus had a cleft palate: all other fetuses appeared normal at gross external and soft tissue or skeletal examination.
- 1/25 female mice (# 255) in the 68 µg/kg/day dose group prematurely delivered on GD 17 and was sacrificed. The litter consisted of 10 live pups and 1 presumed cannibalized pup. One of the live pups was partially cannibalized. 1/5 females in the toxicokinetic 760 µg/kg/day dose group prematurely

delivered on GD 17. 1/25 female mice (# 287) in the 460 µg/kg/day dose group prematurely delivered on GD 17 and was sacrificed. All tissues examined appeared normal at necropsy. The litter consisted of 13 dead pups. One pup was partially cannibalized; all other pups appeared normal at gross external and soft skeletal examination. The cause of abortion or premature delivery is not clear.

- Food consumption was slightly decreased in all treated dams relative to control during the treatment period (GD 6-16). The changes in food consumption were reflected in body weight changes of the dams. Sponsor stated that this observation is consistent with the reported pharmacological activity of exendin-4 of slowing gastric emptying and reducing feed consumption.
- Number of implantations, litter sizes and live fetuses were significantly decreased in the 460 µg/kg/day group relative to control.
- Male and female fetal body weights decreased with increasing dose, achieving statistical significance at doses ≥ 460 µg/kg/day in males and at doses ≥ 68 µg/kg/day in females (poor nutritional status in dams fetal developmental toxicity).
- The incidence of wavy ribs in the litter and fetuses of the 760 µg/kg/day group were significantly increased by 11.8% (0.31% = historical control mean for litter; 0-4.8% = range) and 2.8% (0.05% = historical control mean for fetuses; 0-0.9% = range) respectively, relative to control. Sponsor stated that the higher incidence of reversible delayed ossification of ribs (i.e., wavy ribs) in the HD group is due to the slow development of the fetuses due to the decreased nutritional state of the dams.
- A slight but significant increase in ossification of the thoracic vertebra (13.43% compared to historical control mean of 13.39%), and decrease in ossification of the lumbar vertebra (5.57% compared to historical control mean of 5.60%) were observed at 460 µg/kg/day relative to control. Ossification sites in the rib pairs were also slightly but significantly increased at 6 (13.37%) and 460 (13.39%) µg/kg/day relative to control. Historical control mean = 13.32%.
- Five fetuses from the treated group and two from the control group had multiple findings. Cleft palate with or without hole was a common finding. In addition, some fetuses had interfrontal ossification site, cervical ribs and wavy ribs. Since the incidence of these findings were not dose-related, it is not clear if they are treatment-related or not.
- The TK data showed that the potential of AC2993 to cross the placental barrier is very low in mice. Therefore the fetal findings observed may be a consequence of maternal toxicity.
- The maternal NOAEL is = 6 µg/kg/day (3X MRHD, AUC) based on abortion. Fetal NOAEL is 6 µg/kg/day based on dose-related decrease in body weights of fetuses.

Study no.: REST99060R1
Volume # and page #: N/A.
Conducting laboratory and location: (b) (4)
Date of study initiation: February 3, 2000.
GLP compliance: Yes.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Lot # 991002 TP, 94.3% pure.

Methods

Doses: 6, 68, 460, 760 µg/kg/day. The drug was administered (at 3, 34 230 and 380 µg/kg, BID) twice daily on gestation days 6 through 15 (period of organogenesis). Each administration was separated by 11 to 13 hours. TK mice were given same doses twice daily on gestation days 6 through 18. Each administration was separated by 11 to 13 hours.
Species/strain: Mouse/Crl:CD-1®(ICR)BR.
Number/sex/group: 25 pregnant mice/group (main study).
Route, formulation, volume, and infusion rate: Subcutaneous injection. Please see study design for dose volumes. Test article was provided as 0.3 mg/ml formulated drug product. Each is a 1 ml

single dose, sterile formulation in 30mM acetate buffer pH 4.5 with mannitol added as an iso-osmolality modifer.
Satellite groups used for toxicokinetics: 5 pregnant mice/group.
Study design:

Dosage Group	Dosage[a]		Concentration (mg/mL)	Dosage Volume (mcL/kg)	Number of Mice	Assigned Numbers
	Daily (mcg/kg/day)	Individual (mcg/kg/dose)				
I	0 (Vehicle)	0 (Vehicle)	0	4,875	25	201 - 204, 2001[b], 2002[b], 207-225
II	6	3	0.005	600	25	226 - 250
III	68	34	0.019	1,790	25	251 - 275
IV	460	230	0.078	2,945	25	276 - 300
V	760	380	0.078	4,875	25	301 - 325
VI	0 (Vehicle)	0 (Vehicle)	0	4,875	5	326 - 330
VII	6	3	0.005	600	5	331 - 335
VIII	68	34	0.019	1,790	5	336 - 340
IX	460	230	0.078	2,945	5	341 - 345
X	760	380	0.078	4,875	5	50

a. It was assumed that the AC2993 (0.3 mg/mL) in the supplied stock preparation was 100% active for the purpose of dosage calculations.
b. Mice 205 and 206 were replaced by mice 2001 and 2002, respectively, on DG 6 because of weight loss between DGs 0 and 6.

Parameters and endpoints evaluated

Clinical signs: Daily.
Body weight: Daily.
Food consumption: Daily.
Terminal examination of females: All mice assigned to the main study (Groups I through V) were sacrificed on gestation day 18, Caesarean-sectioned, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Uteri from mice that appeared nonpregnant were examined while being pressed between glass plates to confirm the absence of implantation sites. The number of corpora lutea in each ovary was recorded. The uterus of each mouse was excised and examined for pregnancy, number and distribution of implantations, live and dead fetuses and early and late resorptions. Each fetus was removed from the uterus, weighed and examined for sex and gross external alterations. Live fetuses were sacrificed by an intraperitoneal injection of euthanasia solution.

Approximately one-half of the fetuses in each litter were examined for soft tissue alterations. The remaining fetuses in each litter were eviscerated, cleared, stained with alizarin red S and examined for skeletal alterations. Following completion of all blood sample collections from animals in the satellite portion of the study (Groups VI through X), carcasses and fetuses were discarded without further evaluation.

Mice that were sacrificed because of abortion were examined on the day the observation was made. The mice were examined for gross lesions. Pregnancy status and uterine contents were recorded. Aborted fetuses, delivered pups and/or conceptuses in utero were examined using the same methods described for term fetuses.

Toxicokinetics: Approximately 1.5 hours after the morning administration on GD 18, mice assigned to the TK group were sacrificed and blood collected. The uterus of each female mouse was excised and fetuses were removed. Blood samples were collected from each fetus and pooled (per litter).

Results

Mortality (dams): None.

Clinical signs (dams):
Abortions: 1/25 females in the 68 µg/kg/day dose group aborted on gestation day 15 (GD 15) and was sacrificed on GD 16. Adverse clinical observations after aborting included ungroomed coat and dehydration on GD 16. Body weight gains and feed consumption values were comparable to other mice in this group. All tissues examined appeared normal at necropsy. The litter consisted of 13 late resorptions.

1/25 female in the 760 µg/kg/day dose group aborted on GD 16 and was sacrificed on GD 17. The only other adverse clinical observation was scabs at the injection site on GD 12. Body weight gains and feed consumption values were comparable to other mice in this group. All tissues examined appeared normal at necropsy. The litter consisted of 11 dead fetuses. One fetus had a cleft palate: all other fetuses appeared normal at gross external and soft tissue or skeletal examination.

1/5 females in each of the 0 (Vehicle), 6 and 760 µg/kg/day dose groups was not pregnant.

Premature Deliveries: 1/5 females in the toxicokinetic 760 µg/kg/day dose group prematurely delivered on GD 17. 1/25 females in the 68 µg/kg/day dosage group prematurely delivered on GD 17 and was sacrificed. No additional adverse clinical observations occurred during the study. Body weight gains and feed consumption values were comparable to other mice in this group. All tissues examined appeared normal at necropsy. The litter consisted of 10 live pups and 1 presumed cannibalized pup. One of the live pups was partially cannibalized; all other pups appeared normal at gross external and soft or skeletal examination.

1/25 females in the 460 mcg/kg/day dose group prematurely delivered on GD 17 and was sacrificed. The only other adverse clinical observation was scabs at the injection site on GDs 13 to 17. Body weight gains and feed consumption values were comparable to other mice in this group. All tissues examined appeared normal at necropsy. The litter consisted of 13 dead pups. One pup was partially cannibalized; all other pups appeared normal at gross external and soft skeletal examination.

Body weight (dams): No treatment-related effects on body weight.

Period (Study Days)	Change in Body Weight				
	0 mcg/kg/day	6 mcg/kg/day	68 mcg/kg/day	460 mcg/kg/day	760 mcg/kg/day
6 to 9	+5.9%	+5.3%	+5.2%	+2.8%	+1.4%
6 to 16	+57.9%	+57.9%	+60.0%	+53.3%	+55.6%
6 to 18	+82.4%	+78.6%	+82.4%	+73.7%	+80.2%

Food consumption (dams):

Period (Study Days)	Absolute Feed Consumption Relative to Controls				
	0 mcg/kg/day	6 mcg/kg/day	68 mcg/kg/day	460 mcg/kg/day	760 mcg/kg/day
0 to 6	100%	95.4%	100%	100%	97.7%
6 to 9	100%	89.6%	79.2%	66.7%	64.6%
6 to 16	100%	94.1%	90.2%	82.4%	86.3%
6 to 18	100%	96.2%	94.3%	86.8%	90.6%

Toxicokinetics: This TK data was adopted from the 91-Day mouse toxicity study with BID dosing.

Dose (µg/kg BID)	3	34	230	380
AUC_{0-12hr} (pg.h/ml)	3485	51,389	252,080	539,949
Total Daily Dose (µg/kg/d)	6	68	460	760
Total Daily AUC_{0-12hr} (pg.h/ml)	6970	102,778	504,160	1,079,898

Total daily $AUC_{(0-10hr)}$ for the MRHD (10 µg BID = 20 µg/day) = 2076 pg.h/ml

AC2993 BID DOSE (mcg/kg)	N (Dams)	Mean Plasma Concentration (pg/ml)	N (Fetal)	Mean Fetal Plasma Concentration (pg/ml)	Mean Relative Distribution (Fetal ÷ Maternal)	Samples with Relative Distribution of < 0.01
3	5	665	4	18	0.027	3
34	5	20,870	5	162	0.008	3
230	5	194,087	3	3,987	0.021	0
380	5	11,126,136	3	7,584	0.001	2

Potential of AC2993 to Cross the Placenta:

The TK data show that the potential of AC2993 to cross the placental barrier is very low in the mouse (i.e., mean ratios of fetal plasma concentrations of AC2993 ÷ maternal plasma concentrations of AC2993 ranged from 0.001 to 0.027). Sponsor stated that a large variation in plasma drug levels was present in both dams and fetuses. Some control fetal plasma has detectable drug levels. LLQ = 2.5 pg/ml.

Terminal and Necroscopic evaluations:

DAMS

Mice pregnant and Caesarean sectioned on GD 18.

Dose µ/kg/day	0	6	68	460	760
Pregnant	24 (96%)	21 (84%)	23 (92%)	22 (88%)	19 (76%)
Prematurely delivered	0 (0.0%)	0 (0.0%)	1 (4.3%)	1 (4.5%)	1 (5.3%)
Aborted	0 (0.0%)	0 (0.0%)	1 (4.3%)	0 (0.0%)	1 (5.3%)
Corpora lutea	14.1 ± 1.6	13.3 ± 2.0	13.6 ± 1.5	12.9 ± 2.0	14.3 ± 1.9
Implantations	13.0 ± 1.4	12.3 ± 1.8	12.7 ± 1.3	11.8 ± 1.6*	13.2 ± 1.4
Litter sizes	12.7 ± 1.4	11.4 ± 2.2	12.2 ± 1.3	11.0 ± 1.6**	12.3 ± 1.4
Live fetuses	12.6 ± 1.5	11.4 ± 2.2	12.2 ± 1.4	11.0 ± 1.6*	12.2 ± 1.4
Dead fetuses	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2
Dams with resorptions	7 (29.1%)	12 (57.1%)	9 (42.8%)	11 (52.4%)	8 (47%)
Dams with late resorptions /litter	0.1 ± 0.3	0.1 ± 0.4	0.2 ± 0.4	0.2 ± 0.5	0.4 ± 0.7
Dams with viable fetuses	24(100%)	21(100%)	21(100%)	21(100%)	17(100%)
Live fetal body wt (g/litter)	1.27 ± 0.08	1.27 ± 0.10	1.22 ± 0.07	1.17 ± 0.07**	1.11 ± 0.06**
Male fetuses	1.29 ± 0.10	1.30 ± 0.09	1.24 ± 0.08	1.20 ± 0.09**	1.13 ± 0.07**
Female fetuses	1.25 ± 0.08	1.24 ± 0.11	1.19 ± 0.09*	1.14 ± 0.07**	1.07 ± 0.07**
% Dead or resorbed conceptus/litter	3.4 ± 5.3	7.7 ± 9.3	3.7 ± 4.8	6.3 ± 7.4	6.8 ± 8.8

***p<0.05; **P<0.01**

Terminal and Necroscopic evaluations:

FETUSES

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FETAL ALTERATIONS SUMMARY
FETAL GROSS EXTERNAL ALTERATIONS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MCG/KG/DAY)a		0 (VEHICLE)	6	68	460	760
LITTERS EVALUATED	N	24	21	21	21	17
FETUSES EVALUATED	N	304	239	257	232	209
LIVE	N	302	239	256	231	208
DEAD	N	2b	0	1b	1b	1b
PALATE: CLEFT						
LITTER INCIDENCE	N(%)	2(8.3)	4(19.0)	2(9.5)	2(9.5)	3(17.6)
FETAL INCIDENCE	N(%)	4(1.3)	5(2.1)	2(0.8)	3(1.3)	7(3.4)
BODY: HERNIA						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.8)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.4)	0(0.0)
EYE: LIDS OPENED						
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	1(5.9)
FETAL INCIDENCE	N(%)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	1(0.5)

a. Dosage occurred on days 6 through 15 of gestation.
b. Dead fetuses were excluded from group averages and statistical analyses. Observations for these conceptuses are cited on Table 21.

FETAL VISCERAL ALTERATIONS

Dose (mcg/kg/d) a	0	6	68	460	760
Litters evaluated	24	21	21	21	17
Fetuses evaluated	146	115	121	110	100
Live	146	115	121	110	99
Dead	0	0	0	0	1b
Palate: contained a hole (Malformation)					
Litter incidence	1(4.2%)	2(9.5%)	0(0.0%)	1(4.8%)	0(0.0%)
Fetal incidence	1(0.7%)	1(1.7%)	0(0.0%)	1(0.9%)	0(0.0%)
Vessels: Umbilical artery descended to the left of urinary bladder (Variation)					
Litter incidence	5(20.8%)	0(0.0%)	7(33.3%)	5(23.8%)	6(35.3%)
Fetal incidence	6(4.1%)	0(0.0%)	9(7.4%)	5(5.5%)	7(7.1%)

a. Dosage occurred on days 6 through 15 of gestation.
b. Dead fetuses were excluded from group averages and statistical analyses

Fetuses with multiple findings:

Mouse #-Fetus #	Dose (mcg/kg/d)	Findings
2001-5	0	Cleft palate with hole, Interfrontal ossification site, cervical ribs
2002-1	0	Cleft palate, Interfrontal ossification site
243-4	6	Cleft palate with hole, cervical ribs
243-9	6	Cleft palate, cervical ribs
298-10	460	Cleft palate, Interfrontal ossification site
282-9	460	Hernia in right flank, manubrium fused to 1st sternal centra, bifid xiphoid.
327-7	760	Cleft palate, Interfrontal ossification site, wavy ribs
327-9	760	Cleft palate, wavy ribs

FETAL SKELETAL ALTERATIONS

Dose mcg/kd/day	0	6	68	460	760
Litters evaluated	24	21	21	21	17
Fetuses evaluated	304	239	257	232	209
Live fetuses	302	239	256	231	208
Dead fetuses	2	0	1	1	1
Ribs: Wavy Litter incidence	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (11.8%)**
Ribs: Wavy Fetal incidence	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (2.8%)**

Skull: Interfrontal ossification site Litter incidence	14(58.3%)	15(71.4%)	18(85.7%)	11(52.4%)	13(76.5%)
Skull: Interfrontal ossification site **Fetal incidence**	28(17.9%)	36(29.0%)	34(25.2%)	31(25.6%)	32(29.4%)
Cervical vertebra: cervical ribs present at 7th cervical vertebra					
Litter incidence	13(52.4%)	11(52.4%)	13(61.9%)	7(33.3%)	6(35.3%)
Fetal Incidence	24(15.4%)	20(16.1%)	27(20.0%)	12(9.9%)	10(9.2%)

***p<0.05; **P<0.01**

FETAL DELAYED OSSIFICATION SITES					
Dose µg/kd/day	**0**	**6**	**68**	**460**	**760**
Vertebrae Thoracic	13.22 ± 0.24	13.41 ± 0.34	13.23 ± 0.27	13.43 ± 0.30*	13.40 ± 0.32
Vertebrae Lumbar	5.78 ± 0.24	5.58 ± 0.35	5.77 ± 0.27	5.57 ± 0.30*	5.59 ± 0.32
Ribs (pairs)	13.17 ± 0.20	13.37 ± 0.33*	13.18 ± 0.21	13.39 ± 0.30*	13.32 ± 0.28

***p<0.05; **P<0.01**

2.6.6.6.3 Developmental Toxicity Study of Subcutaneously Administered AC2993 in Rabbits (Segment II Teratology Study).

Key study findings:

- 1/20 females in the 2 µg/kg/day dose group was found dead on the morning of GD 10 prior to dosing. 1/20 females in the 22 µg/kg/day dosage group was found dead on GD 19, approximately 13 hours after the last dose. The cause of death of these does was not addressed by the sponsor.
- 1/20 females in the 156 µg/kg/day dose group aborted on GD 21 and was sacrificed. 1/22 females in the 22 µg/kg/day dosage group prematurely delivered on GD 29 and was sacrificed.
- Decrement in body weight was –2.8% and –5.1% at 156 and 260 µg/kg/day respectively relative to control (+7.3%) for gestation days 6 through 19. From gestation days 19 through 29 (post dose period), body weight increased with increasing dose and treated group values were greater than that of control. However, the overall body weight change in the treated groups decreased in a somewhat dose-dependent manner. This may relate to the decreased food consumption.
- Food consumption was significantly decreased during gestation days 6 to 9 and throughout the dosing period (GDs 6-19). After the dosing period (GDs 19-29), food consumption in the treated groups increased with increasing dose and the increments were generally greater than that of control except for the LD group.
- Some fetuses were observed with multiple findings (umbilical hernia with angulated hyoid, or with fused sternal centra, unossified pubis and absence of intermediate lung lobe) at doses ≥ 22 µg/kg/d.
- Treatment-related effects that were dose-dependent or showed a trend towards dose-dependency include dead/resorbed conceptuses/litter, umbilical hernia, angulated hyoid, fused sternal centra (litter) and ossification sites/fetus/litter for thoracic vertebra. For small gall bladder (fetus) and ossification sites/fetus/litter for lumbar vertebra, the incidence of these findings decreased with increasing dose. It is not clear if the incidence of fused ribs (litter) is treatment related or not because it was not observed in the HD group.
- The TK data show that the potential of AC2993 to cross the placental barrier is very low in the rabbit.
- Maternal NOAEL < 0.2 (0.2X MRHD, AUC) since mortality was observed in 1/20 does each in the 0.2 and 22 µg/kg/d groups. Doses of 22 µg/kg/day and higher also caused dose-related decreased weight gain during the dosing period. The developmental NOAEL was 0.2 µg/kg/day (0.2X MRHD, AUC) based on the higher incidence of dead/resorbed conceptuses/litter, fetal umbilical hernia, small gall bladder, angulated hyoid, fused sternal centra, decreased ossification of the lumbar vertebra at higher doses.

Study no.: REST99061R2
Volume # and page #: N/A.
Conducting laboratory and location: (b) (4)

Date of study initiation: February 6, 2000.
GLP compliance: Yes.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Lot # 991002TP, 94.3% pure.

Methods

Doses: 0.1, 11, 78, 130 µg/kg BID (0.2, 22, 156 and 260 µg/kg/d) on GDs 6 through18 (groups I-V) and on GDs 6 through 24 (groups VI-X).
Species/strain: Rabbit/New Zealand white.
Number/sex/group: 20 pregnant females/group (main study).
Route, formulation, volume, and infusion rate: Subcutaneous injection. See study design for dose volumes.
Satellite groups used for toxicokinetics: 5 pregnant females/group.
Study design:

Dosage Group	Dosage[a]		Concentration (mg/mL)	Dosage Volume (mcL/kg)	Number of Rabbits	Assigned Numbers
	Daily (mcg/kg/day)	Individual (mcg/kg/dose)				
I	0 (Vehicle)	0 (Vehicle)	0	433	20	2601 - 2620
II	0.2	0.1	0.01	10	20	2621 - 2640
III	22	11	0.3	36.5	20	2641 - 2660
IV	156	78	0.3	260	20	2661 - 2680
V	260	130	0.3	433	20	2681 - 2700
VI	0 (Vehicle)	0 (Vehicle)	0	433	5	2575 - 2579
VII	0.2	0.1	0.01	10	5	2580 - 2584
VIII	22	11	0.3	36.5	5	2585 - 2589
IX	156	78	0.3	260	5	2590 - 2594
X	260	130	0.3	433	5	2595 - 2599

a. It was assumed that the AC2993 (0.3 mg/mL) in the supplied stock preparation was 100% active for the purpose of dosage calculations.

Parameters and endpoints evaluated

Clinical signs: Daily
Body weight: Daily.
Food consumption: Daily.
Terminal examination of females: Surviving rabbits assigned to the main study (Groups I through V) were sacrificed on GD 29. The rabbits were Caesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Gross lesions were preserved for possible future evaluation.

The number of corpora lutea in each ovary was recorded. The uterus was excised and examined for pregnancy, number and distribution of implantations, live and dead fetuses and early and late resorptions. The fetuses were removed from the uterus. Each fetus was subsequently weighed and examined for gross external alterations. Live fetuses were sacrificed. All fetuses were examined internally to identify sex. Visceral alterations and cavitated organs were evaluated by dissection. The brains were cross-sectioned (a single cross section was made between the parietal and the frontal bones) and examined in situ. The fetuses in each lifter were examined for skeletal alterations after staining with alizarin red S.

Skeletal preparations were retained in glycerin with thymol added as a preservative. Late resorptions and dead fetuses were examined to the extent possible, using the same methods described for term fetuses. Fetal gross lesions were preserved in neutral buffered 10% formalin for possible future evaluation.

Rabbits that died or were sacrificed because of abortion or premature delivery were examined for the cause of death on the day the observation was made. Pregnancy status and uterine contents were recorded. Fetuses were examined to the extent possible, using the same methods described for term fetuses. Rabbits assigned to toxicokinetic evaluation (Groups VI though X) were sacrificed on GD 24. Live fetuses were sacrificed and following completion of blood sample collections, the carcasses and fetuses were discarded without evaluation.
Toxicokinetics: Approximately 1.5 hours after the morning administration on GD 24, the female rabbits assigned to the toxicokinetic sample collections (Groups VI through IX) were sacrificed, and blood was collected. The uterus of each female rabbit assigned to the TK group was excised, and the fetuses were removed, rinsed with warm saline and towel-dried. Blood samples were collected from each fetus via decapitation and pooled (per litter).

Results

Mortality (dams): 1/20 in the 0.2 µg/kg/day dose group was found dead on the morning of GD 10 prior to dosing. This doe appeared normal, gained weight and had normal feed consumption values prior to death. All tissues examined appeared normal at necropsy. The litter consisted of nine dead embryos that appeared normal for their developmental ages. No cause of death could be determined.

1/20 in the 22 µg/kg/day dose group was found dead on GD 19, approximately 13 hours after the last dose. This doe had scant feces on GDs 15, 17 and 18 and rigidity, yellow dried perioral substance and, ungroomed coat on GDs 17 and 18. It lost weight and had severely decreased feed consumption from GD 13 until death. All tissues examined appeared normal at necropsy. The litter consisted of nine dead fetuses that appeared normal for their developmental ages. Sponsor stated that the early gestational age precluded soft tissue and skeletal evaluations.

Clinical signs (dams): **Abortions:** 1/20 in the 156 µg/kg/day dose group aborted on GD 21 and was sacrificed. Adverse clinical observations consisted of scant feces on GDs 8, 10 to 16, 20 and 21 and no feces on GDs 17 to 19. The doe lost weight and had severely reduced feed consumption from GD 7. All tissues examined appeared normal at necropsy. The litter consisted of six fetuses, one early and four late resorptions. All fetuses appeared normal at gross external evaluation; early gestational age and/or autolysis precluded soft tissue and skeletal evaluations.

Premature Deliveries: 1/22 in the 22 µg/kg/day dose group prematurely delivered on GD 29 and was sacrificed. Adverse clinical observations occurred only on GD 29, red substance in the cage pan. Body weight gains and feed consumption values were unremarkable. At necropsy, the doe had a red substance in the stomach, presumed to be ingested blood. All tissues examined appeared normal at necropsy. The litter consisted of two pups. One pup was dead but appeared normal at gross examination and the other was partially cannibalized.

Body weight (dams): (kg)

Period (Study Days)	Percent Change in Body Weights				
	0 mcg/kg/day	0.2 mcg/kg/day	22 mcg/kg/day	156 mcg/kg/day	260 mcg/kg/day
6 to 19	+7.3%	+5.9%	+2.2%	-2.8%	-5.1%
19 to 24	+2.9%	+3.7%	+4.4%	+7.5%	+9.6%
19 to 29	+4.5%	+4.2%	+6.3%	+9.5%	+12.6%
6 to 29	+12.1%	+10.4%	+8.6%	+6.5%	+7.1%

Food consumption (dams): (g/day)

Period (Study Days)	Absolute Food Consumption Relative to Controls				
	0 mcg/kg/day	0.2 mcg/kg/day	22 mcg/kg/day	156 mcg/kg/day	260 mcg/kg/day
6 to 9	100%	99.2%	30.4%	12.2%	8.9%
6 to 19	100%	93.8%	54.6%	40.9%	32.8%
19 to 29	100%	99.2%	110.4%	114.5%	119.0%
6 to 29	100%	95.9%	75.6%	70.4%	65.4%

Toxicokinetics: Pregnant rabbits show non-linear PK. Water consumption is dramatically reduced and it is suspected that clearance of exenatide is markedly reduced.

Dose (µg/kg BID)	**0.1**	**11**	**78**	**130**
$AUC_{0\text{-}12hr}$ (pg.h/ml)	228	214,883	1,486,667	3,610,750
Total Daily Dose (µg/kg/d)	**0.2**	**22**	**156**	**260**
Total Daily AUC (pg.h/ml)	456	429,766	2,973,334	7,221,500

Total daily $AUC_{(0\text{-}10hr)}$ for the MRHD (10 µg BID = 20 µg/day) = 2076 pg.h/ml

AC2993 BID DOSE (mcg/kg)	N (Dams)	Mean Plasma Concentration (pg/ml)	N (Fetal)	Mean Fetal Plasma Concentration (pg/ml)	Mean Relative Distribution (Fetal ÷ Maternal)	Samples with Relative Distribution of < 0.01
1	5	27	3	< Low Std.	0	3
11	5	6690	2	62	0.009	1
78	5	368,211	3	467	0.001	3
130	5	431,670	3	806	0.002	3

Potential of AC2993 to Cross the Placenta:

The TK data show that the potential of AC2993 to cross the placental barrier is very low in the Rabbit (i.e., mean ratios of fetal plasma concentrations of AC2993 ÷ maternal plasma concentrations of AC2993 ranged from 0 to 0.009). Sponsor stated that a large variation in plasma drug levels is present in both dams and fetuses. Some control fetal plasma has detectable drug levels. LLQ = 2.5 pg/ml.

Terminal and necroscopic evaluations: C-section data

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MCG/KG/DAY)a		0 (VEHICLE)	0.2	22	156	260
RABBITS TESTED	N	20	20	20	20	20
PREGNANT	N(%)	19(95.0)	20(100.0)	19(95.0)	20(100.0)	18(90.0)
FOUND DEAD	N(%)	0(0.0)	1(5.0)	1(5.3)	0(0.0)	0(0.0)
ABORTED	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
DELIVERED	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	19	19	17	19	18
CORPORA LUTEA	MEAN±S.D.	10.4 ± 2.4	10.1 ± 2.1	9.2 ± 2.1	10.5 ± 2.1	10.5 ± 2.0
IMPLANTATIONS	MEAN±S.D.	8.8 ± 2.1	8.8 ± 2.2	7.6 ± 2.4	8.3 ± 1.9	9.1 ± 2.1
LITTER SIZES	MEAN±S.D.	8.8 ± 2.1	8.6 ± 2.2	7.4 ± 2.7	7.5 ± 2.3	8.0 ± 2.2
LIVE FETUSES	N	167	162	126	142	144
	MEAN±S.D.	8.8 ± 2.1	8.5 ± 2.3	7.4 ± 2.7	7.5 ± 2.3	8.0 ± 2.2
DEAD FETUSES	N	0	1	0	0	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RESORPTIONS	MEAN±S.D.	0.0 ± 0.0	0.2 ± 0.5	0.2 ± 0.6	0.8 ± 1.4**	1.1 ± 1.0**
EARLY RESORPTIONS	N	0	0	2	5	13
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.3 ± 0.7	0.7 ± 0.9**
LATE RESORPTIONS	N	0	4	2	11	7
	MEAN±S.D.	0.0 ± 0.0	0.2 ± 0.5	0.1 ± 0.3	0.6 ± 1.1*	0.4 ± 0.8
DOES WITH ANY RESORPTIONS	N(%)	0(0.0)	3(15.8)	3(17.6)	7(36.8)**	11(61.1)**

a. Dosage occurred on days 6 through 18 of gestation.
* Significantly different from the vehicle control group value ($p<0.05$).
** Significantly different from the vehicle control group value ($p\leq0.01$).

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Summary of C-Section Data Contd.

DOSAGE GROUP DOSAGE (MCG/KG/DAY)a		I 0 (VEHICLE)	II 0.2	III 22	IV 156	V 260
RABBITS TESTED	N	20	20	20	20	20
PREGNANT	N(%)	19(95.0)	20(100.0)	19(95.0)	20(100.0)	18(90.0)
FOUND DEAD	N(%)	0(0.0)	1(5.0)	1(5.3)	0(0.0)	0(0.0)
ABORTED	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
DELIVERED	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	19	19	17	19	18
DOES WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	19(100.0)	19(100.0)	17(100.0)	19(100.0)	18(100.0)
PLACENTAE APPEARED NORMAL	N(%)	19(100.0)	19(100.0)	17(100.0)	19(100.0)	18(100.0)

a. Dosage occurred on days 6 through 18 of gestation.

Summary of Caesarean-Delivered Fetuses

DOSAGE GROUP DOSAGE (MCG/KG/DAY)a		I 0 (VEHICLE)	II 0.2	III 22	IV 156	V 260
LITTERS WITH ONE OR MORE LIVE FETUSES	N	19	19	17	19	18
IMPLANTATIONS	MEAN±S.D.	8.8 ± 2.1	8.8 ± 2.2	7.6 ± 2.4	8.3 ± 1.9	9.1 ± 2.1
LIVE FETUSES	N	167	162	126	142	144
	MEAN±S.D.	8.8 ± 2.1	8.5 ± 2.3	7.4 ± 2.7	7.5 ± 2.3	8.0 ± 2.2
LIVE MALE FETUSES	N	91	66	63	71	65
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	53.4 ± 14.0	40.0 ± 20.4	47.2 ± 24.5	49.7 ± 19.3	43.9 ± 16.4
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	43.46 ± 5.14	42.58 ± 4.86	43.61 ± 5.47	41.50 ± 4.69	41.57 ± 6.30
MALE FETUSES	MEAN±S.D.	43.59 ± 4.93	44.18 ± 4.37 [18]b	43.86 ± 2.85 [15]c,d	41.17 ± 5.75	40.84 ± 7.45
FEMALE FETUSES	MEAN±S.D.	43.17 ± 5.94	41.19 ± 5.98	42.75 ± 6.13 [16]e	40.73 ± 4.70	41.38 ± 7.06
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	0.0 ± 0.0	3.1 ± 6.4	5.9 ± 14.4	10.1 ± 17.5*	12.7 ± 13.1**

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 18 of gestation.
b. Litter 2631 had no male fetuses.
c. Litter 2645 had no male fetuses.
d. Litter 2648 had no male fetuses.
e. Litter 2657 had no female fetuses.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Fetuses:

FETAL ALTERATIONS

DOSAGE GROUP DOSAGE (MCG/KG/DAY)a		I 0 (VEHICLE)	II 0.2	III 22	IV 156	V 260
LITTERS EVALUATED	N	19	19	17	19	18
FETUSES EVALUATED	N	167	163	126	142	144
LIVE	N	167	162	126	142	144
DEAD	N	0	1b	0	0	0
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	9(47.4)	7(36.8)	10(58.8)	12(63.2)	13(72.2)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	12(7.2)	12(7.4)	22(17.5)*	34(23.9)**	34(23.6)**
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	8.2 ± 12.0	7.4 ± 13.3	24.2 ± 29.9	22.0 ± 23.1	23.2 ± 24.0

a. Dosage occurred on days 6 through 18 of gestation.
b. Dead fetus was excluded from group averages and statistical analyses; adverse observations for these conceptuses are cited on Table 22.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

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FETUSES WITH GROSS EXTERNAL ALTERATIONS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MCG/KG/DAY)a		0 (VEHICLE)	0.2	22	156	260
LITTERS EVALUATED	N	19	19	17	19	18
FETUSES EVALUATED	N	167	163	126	142	144
LIVE	N	167	162	126	142	144
DEAD	N	0	1b	0	0	0
BODY: UMBILICAL HERNIA						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	2(11.8)	2(10.5)	6(33.3)**
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	2(1.6)	8(5.6)*	17(11.8)**

a. Dosage occurred on days 6 through 18 of gestation.
b. Dead fetus was excluded from group averages and statistical analyses; adverse observations for these conceptuses are cited on Table 22.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

FETAL VISCERAL ALTERATIONS

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MCG/KG/DAY)a		0 (VEHICLE)	0.2	22	156	260
LITTERS EVALUATED	N	19	19	17	19	18
FETUSES EVALUATED	N	167	163	126	142	144
LIVE	N	167	162	126	142	144
DEAD	N	0	1b	0	0	0
EYES: CIRCUMCORNEAL HEMORRHAGE						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.9)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	3(2.4)**	0(0.0)	0(0.0)
LUNGS: INTERMEDIATE LOBE ABSENT						
LITTER INCIDENCE	N(%)	2(10.5)	3(15.8)	1(5.9)	1(5.3)	3(16.7)
FETAL INCIDENCE	N(%)	5(3.0)	3(1.8)	2(1.6)c,d	1(0.7)	3(2.1)
KIDNEYS: DILATION, PELVIS						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	2(1.4)	0(0.0)
INTESTINES: PROTRUDES THROUGH UMBILICAL OPENING						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	2(11.8)	2(10.5)	6(33.3)**
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	2(1.6)	8(5.6)*	17(11.8)**
GALLBLADDER: ABSENT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	3(17.6)**	0(0.0)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	3(2.4)**c	0(0.0)	1(0.7)
GALLBLADDER: SMALL						
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)	3(17.6)	3(15.8)	2(11.1)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)	7(5.6)**d	5(3.5)**	4(2.8)**

a. Dosage occurred on days 6 through 18 of gestation.
b. Dead fetus was excluded from group averages and statistical analyses; adverse observations for these conceptuses are cited on Table 22.
c. Fetus 2646-3 had other soft tissue alterations.
d. Fetus 2646-5 had other soft tissue alterations.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

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FETAL SKELETAL ALTERATIONS

DOSAGE GROUP DOSAGE (MCG/KG/DAY)a		I 0 (VEHICLE)	II 0.2	III 22	IV 156	V 260
LITTERS EVALUATED	N	19	19	17	19	18
FETUSES EVALUATED	N	167	163	126	142	144
LIVE	N	167	162	126	142	144
DEAD	N	0	1b	0	0	0
SKULL - IRREGULAR OSSIFICATION: c (SUMMARIZATION OF ALL IRREGULAR OSSIFICATION OF THE SKULL d; INDIVIDUAL SUBCATEGORIES CITED BELOW)						
LITTER INCIDENCE	N(%)	4(21.0)	1(5.3)	1(5.9)	3(15.8)	3(16.7)
FETAL INCIDENCE	N(%)	4(2.4)	1(0.6)	1(0.8)	4(2.8)	3(2.1)
SKULL: NASALS, CONTAINED AN INTERNASAL						
LITTER INCIDENCE	N(%)	1(5.3)	0(0.0)	0(0.0)	1(5.3)	1(5.6)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	1(0.7)	1(0.7)n
SKULL: NASALS, MIDLINE SUTURE DISPLACED						
LITTER INCIDENCE	N(%)	2(10.5)	0(0.0)	0(0.0)	2(10.5)	1(5.6)
FETAL INCIDENCE	N(%)	2(1.2)	0(0.0)	0(0.0)	3(2.1)	1(0.7)
SKULL: NASALS, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)j
SKULL: NASAL, CONTAINED AN INTRANASAL						
LITTER INCIDENCE	N(%)	1(5.3)	0(0.0)	1(5.9)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	1(0.8)	0(0.0)	0(0.0)
SKULL: FRONTALS, IRREGULAR SUTURE						
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	0(0.0)	0(0.0)

See footnotes on the last page of this table

FETAL SKELETAL ALTERATIONS Contd.

DOSAGE GROUP DOSAGE (MCG/KG/DAY)a		I 0 (VEHICLE)	II 0.2	III 22	IV 156	V 260
LITTERS EVALUATED	N	19	19	17	19	18
FETUSES EVALUATED	N	167	163	126	142	144
LIVE	N	167	162	126	142	144
DEAD	N	0	1b	0	0	0
HYOID: ALA, ANGULATED						
LITTER INCIDENCE	N(%)	2(10.5)	2(10.5)	6(35.3)	4(21.0)	8(44.4)
FETAL INCIDENCE	N(%)	2(1.2)	3(1.8)	7(5.6)**	7(4.9)**	11(7.6)**j-m
CERVICAL VERTEBRAE: CENTRUM, MISALIGNED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.9)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)	0(0.0)	0(0.0)
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA						
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
THORACIC VERTEBRAE: HEMIVERTEBRA						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	3(15.8)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	3(2.1)e,g,h	1(0.7)m
THORACIC VERTEBRAE: ARCH, SMALL						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m
THORACIC VERTEBRAE: CENTRUM, BIFID						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m
THORACIC VERTEBRAE: CENTRA, FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)e	0(0.0)
THORACIC VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)e	0(0.0)
CAUDAL VERTEBRAE: MISALIGNED						
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)	0(0.0)	1(5.3)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)	0(0.0)	1(0.7)i	1(0.7)n

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FETAL SKELETAL ALTERATIONS Contd.

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MCG/KG/DAY)a		0 (VEHICLE)	0.2	22	156	260
LITTERS EVALUATED	N	19	19	17	19	18
FETUSES EVALUATED	N	167	163	126	142	144
LIVE	N	167	162	126	142	144
DEAD	N	0	1b	0	0	0
CAUDAL VERTEBRAE: FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)i	1(0.7)
RIBS: SPLIT						
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	2(10.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	2(1.4)e,h	0(0.0)
RIBS: FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	4(21.0)**	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	4(2.8)**e,g-i	1(0.7)m
RIBS: TWO SEGMENTS						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)h	0(0.0)
RIBS: INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)j
RIBS: THICKENED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)f	0(0.0)
RIBS: PROXIMATE						
LITTER INCIDENCE	N(%)	1(5.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
RIBS: BROAD						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m
STERNAL CENTRA: INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)l

See footnotes on the last page of this table

FETAL SKELETAL ALTERATIONS Contd.

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MCG/KG/DAY)a		0 (VEHICLE)	0.2	22	156	260
LITTERS EVALUATED	N	19	19	17	19	18
FETUSES EVALUATED	N	167	163	126	142	144
LIVE	N	167	162	126	142	144
DEAD	N	0	1b	0	0	0
STERNAL CENTRA: FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	4(21.0)**	4(22.2)**
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	5(3.5)**f-h	4(2.8)**k,n
STERNAL CENTRA: ASYMMETRIC						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)f	1(0.7)n
SCAPULAE: ALA, WAVY						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)j
PELVIS: PUBIS, NOT OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)

a. Dosage occurred on days 6 through 18 of gestation.
b. Dead fetus was excluded from group averages and statistical analyses; adverse observations for these conceptuses are cited on Table 22.
c. Fetuses with alterations of the skull and/or hyoid are not separately identified in this summarization, except when alterations of other ossification sites were also present.
d. Includes all alterations noted for the skull except hyoid, ala, angulated. This category is excluded because this alteration does not result from irregular ossification.
e. Fetus 2662-10 had other skeletal alterations.
f. Fetus 2664-2 had other skeletal alterations.
g. Fetus 2665-4 had other skeletal alterations.
h. Fetus 2672-3 had other skeletal alterations.
i. Fetus 2675-3 had other skeletal alterations.
j. Fetus 2683-10 had other skeletal alterations.
k. Fetus 2686-1 had other skeletal alterations.
l. Fetus 2688-7 had other skeletal alterations.
m. Fetus 2693-8 had other skeletal alterations.
n. Fetus 2696-6 had other skeletal alterations.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

FETAL OSSIFICATION SITES

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MCG/KG/DAY)a		0 (VEHICLE)	0.2	22	156	260
LITTERS EXAMINED	N	19	19	17	19	18
FETUSES EXAMINED	N	167	162	126	142	144
OSSIFICATION SITES PER FETUS PER LITTER						
HYOID	MEAN±S.D.	1.00 ± 0.00	0.99 ± 0.04	0.99 ± 0.03	1.00 ± 0.00	0.99 ± 0.05
VERTEBRAE						
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	12.51 ± 0.32	12.55 ± 0.29	12.80 ± 0.28**	12.84 ± 0.22**	12.90 ± 0.14**
LUMBAR	MEAN±S.D.	6.48 ± 0.32	6.43 ± 0.30	6.19 ± 0.28**	6.16 ± 0.22**	6.09 ± 0.14**
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	16.85 ± 0.42	16.88 ± 0.35	17.08 ± 0.33	16.98 ± 0.40	17.14 ± 0.56
RIBS (PAIRS)	MEAN±S.D.	12.47 ± 0.30	12.49 ± 0.27	12.73 ± 0.29*	13.10 ± 1.49**	12.84 ± 0.20**
STERNUM						
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	3.89 ± 0.16	3.89 ± 0.15	3.99 ± 0.03	3.96 ± 0.10	3.95 ± 0.13
XIPHOID	MEAN±S.D.	0.97 ± 0.08	0.99 ± 0.03	0.95 ± 0.19	0.89 ± 0.17	0.93 ± 0.13
FORELIMB b						
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	5.00 ± 0.00	4.98 ± 0.04	4.97 ± 0.10	4.96 ± 0.10	4.93 ± 0.15
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	13.97 ± 0.06	13.89 ± 0.16	13.78 ± 0.34	13.77 ± 0.36	13.75 ± 0.59
HINDLIMB b						
TARSALS	MEAN±S.D.	2.00 ± 0.00	2.00 ± 0.00	1.99 ± 0.05	2.00 ± 0.00	1.99 ± 0.05
METATARSALS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
PHALANGES	MEAN±S.D.	12.00 ± 0.00	12.00 ± 0.00	11.99 ± 0.05	12.00 ± 0.00	11.94 ± 0.24

a. Dosage occurred on days 6 through 18 of gestation.
b. Calculated as average per limb.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

Fetuses with multiple findings

Dam-Fetus #	Dose (mcg/kg/d)	Findings
2645-5	22	Umbilical hernia, angulated hyoid
2679-8	156	Umbilical hernia, angulated hyoid
2686-2	260	Umbilical hernia, unossified pubis
2686-5	260	Umbilical hernia, fused sternal centra
2687-3	260	Umbilical hernia, angulated hyoid, absent intermediate lobe of lung
2688-4	760	Umbilical hernia, angulated hyoid

Table shows doses at which incidence (%) was statistically significant relative to control.

OBSERVATION	INCIDENCE (%) AT INDICATED DOSE (mcg/kg/d)					HISTORICAL CONTROL DATA
	0	2	22	156	260	Mean % (range %)
Dead/resorbed conceptuses/litter	0.0			10.1	12.7	3.7 (0-22.2)
Umbilical hernia (ltter)	0.0				33.3	0.28 (0-5.3)
Umbilical hernia (fetus)	0.0		1.6	5.6	11.8	0.03 (0-0.6)
Circumcorneal hemorrhage (fetus)	0.0		2.4			0.22 (0-1.3)
Small gall bladder (fetus)	0.0		5.6	3.5	2.8	0.10 (0-1.7)
Angulated hyoid (fetus)	1.2		5.6	4.9	7.6	2.06 (0-6.4)
Fused ribs (litter)	0.0			21		2.81 (0-21.4)
Fused sternal centra (litter)	0.0			21	22.2	9.97 (0-25.0)
Fused sternal centra (fetus)	0.0			3.5		1.65 (0-4.4)
Thoracic vertebra: Ossification sites/fetus/litter	12.5		12.8	12.8	12.9	12.6 (12.47-12.82)
Lumbar vertebra: Ossification sites/fetus/litter	6.48		6.19	6.16	6.09	6.39 (6.18-6.53)
Rib pairs: fetal ossification sites	12.47		12.73	13.1	12.8	12.53 (13.39-12.71)

2.6.6.6.4 Study title: Comparative Evaluation of the Effects on Normal Development and Growth of the Embryo and Fetus in Rabbits of Subcutaneously Administered AC2993 at Doses that Cause Depression in Feed Consumption and Matched Pair Fed (PF) Animals.

The purpose of this study was to determine if effects observed in fetuses in a previous Segment II reproduction study are explainable as the result of the compromised nutritional state of the does due to reduced feed consumption.

Key study findings:

- One out of 20 HD does was found dead on GD 17. This was considered drug-related since it occurred at the HD. This doe lost weight and feed and water consumption were reduced.
- Two MD does aborted in GDs 20 and 21. One HD doe aborted on GD 21 as well. These does lost weight and feed and water consumption were reduced. All tissues appeared normal at necropsy.
- Reversible and dose-dependent decreases in mean body weight was noted in the drug treated groups relative to control (non pair-fed) during GDs 6-18. Decreases were observed in the vehicle-treated pair fed groups. The decreased body weight gain correlated with the decreased food consumption noted in both drug-treated and vehicle-treated pair fed groups during GDs 6-18. However, drug treated groups have greater decrease in weight compared to pair-fed controls despite both groups having equivalent food. This suggest drug toxicity.
- Reversible and dose-dependent decreases in water consumption was noted in the drug treated groups but not in the vehicle-treated pair fed groups during GDs 6-18.
- Lymphocyte count was decreased in the drug-treated groups relative to controls.
- Slight but reversible increase in serum glucose levels were noted in the vehicle-treated pair fed groups on GD 9. Reversible increases in lactate levels were observed in the vehicle-treated pair fed animals that matched the MD and HD groups.
- Reversible and dose-dependent increases in β-hydroxybutyric acid (a marker of starvation) was observed in the MD and HD treated groups (on GD 9) as well as the vehicle-treated pair fed animals that matched the HD group. By GD 29 (post treatment), β-hydroxybutyric acid levels in the MD and HD groups as well as the vehicle-treated pair fed animals that matched the HD group were 2X lower relative to that of control (non pair-fed). Dose-dependent and reversible decreases in serum potassium levels (another marker of starvation) was noted in all drug-treated groups on GD 9 as well as the vehicle-treated pair fed animals that matched the HD group. Total protein was slightly decreased in the HD group as well as the vehicle-treated pair fed groups on GD 18. Albumin was decreased in the HD group (GD 18) as well as in the vehicle-treated pair fed animals that matched the HD group.
- There were no significant drug-related effects on number of corpora lutea, implantations, litter size, live fetuses, and fetal weight. Incidence of resorptions was increased (not SS) 3 to 2-fold in MD and HD does.
- An increased incidence of umbilical hernia was observed in fetuses from MD (0.7%) and HD (6.2%) does. The incidence in fetuses from the MD and HD does is greater than the historical control mean (0.05%). While the incidence of this finding in fetuses from HD does exceeds the range (0 – 0.7%), the incidence in fetuses from MD does is equal to the higher end of the range. The incidence of umbilical hernia was also increased in litters from MD (5.9%) and HD (29.4%) does. Both incidences are greater than the historical control mean (0.4%). While the incidence in MD litters falls within the historical control range (0 – 6.2%), the incidence in HD litters exceeds the range.
- An increased incidence of bifid thoracic vertebrae-centrum was observed in fetuses from MD (0.7%) and HD (0.8%) does. The incidence in fetuses from the MD and HD does is greater than the historical control mean (0.08%). While the incidence of this finding in fetuses from HD does equals the range higher end of the (0 – 0.8%), the incidence in fetuses from MD does falls within

the range. The incidence of bifid thoracic vertebrae-centrum was also increased in litters from MD (5.9%) and HD (5.9%) does. Both incidences are greater than the historical control mean (0.68%) but equals the higher end of the range (0 – 5.9%). The incidence of fused thoracic vertebrae centrum was increased in fetuses from MD (0.7%) and HD (0.8%) does. These values are greater than the historical control mean (0.16%) but falls within the range (0 – 1.1%). Similarly increased incidence of this finding was noted in litters from MD (5.9%) and HD (5.9%) does. These values are greater than the historical control mean (1.35%) but falls within the range (0 – 10.5%). The incidence of unilateral ossification of thoracic vertebrae centrum was increased in fetuses from MD (1.4%) and HD (0.8%) does. These values are greater than historical control mean (0.06%) but falls with in the range (0 – 3.8%). Similarly increased incidence of this finding was noted in litters from MD (11.8%) and HD (5.9%) does. These values are greater than the historical control mean (0.54%) but falls within the range (0 – 20%). The incidences of split, fused and irregularly shaped/wavy ribs in both litters and fetuses are greater than their historical control means. The incidences of these findings falls within their respective ranges except for the fetal and liter incidences of incompletely ossified sternal centra and wavy ribs that is greater than the range. The incidence of unossified pubis was increased in fetuses and litters from HD does. These values are greater than their historical control means and ranges. Increased incidence of ossification sites (not dose-dependent) of the thoracic vertebrae and rib pairs were observed in fetuses from LD and HD does as well as in fetuses from the vehicle-treated pair-fed groups. Incidence of ossification sites of the lumbar vertebrae were decreased in fetuses from drug-treated does as well as in fetuses from the vehicle-treated pair-fed groups.

- Maternal NOAEL is 2 µg/kg/d (12X MRHD, AUC) based on the decreases in body weight, food and water consumption, mortality at HD, and abortion at doses ≥ 22 µg/kg/d. Fetal NOAEL is 2 µg/kg/d (12X MRHD, AUC) based on increased resorptions and fetal skeletal anomalies at doses ≥ 22 µg/kg/d.

Study no.: REST02022.
Volume # and page #: N/A.
Conducting laboratory and location: (b) (4)
Date of study initiation: March 31, 2002.
GLP compliance: Yes.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Lot #s 00-0605 TP, 92% pure; and 01-0102 TP, 95.1% pure.

Methods

Doses: 1, 11 and 130 µg/kg BID (giving total daily doses of 2, 22 and 260 µg/kg/d).
Species/strain: Rabbit/NZW.
Number/sex/group: 20/females/group.
Route, formulation, volume, and infusion rate: Subcutaneous injection. See study design for dose volumes.
Satellite groups used for toxicokinetics: None.

Study design:

Part	Dosage Group	Dosage[a] Per Injection (mcg/kg/dose)	Dosage[a] Per Day (mcg/kg/day)	Concentration (mg/mL)	Dosage Volume (mcL/kg)	Number of Rabbits	Assigned Rabbit Numbers
A	I	0 (Placebo)	0 (Placebo)	0	433	20	9320 - 9340
A	II	1	2	0.1	10	20	9341 - 9360
A	III	11	22	0.3	36.5	20	9361 - 9380
A	IV	130	260	0.3	433	20	9381 - 9400
B	V	0 (Placebo)	0 (Placebo)	0	10	20	9601 - 9620
B	VI	0 (Placebo)	0 (Placebo)	0	36.5	20	9621 - 9631, 888[b], 9633 - 9640
B	VII	0 (Placebo)	0 (Placebo)	0	433	20	9641 - 9660

The test article was considered 100% active/pure for the purpose of dosage calculations.

a. The test article and/or vehicle was administered twice daily; the two daily injections were separated by 11 to 13 hours.

b. Rabbit 9632 aborted and was sacrificed on DG 4 (14 April 2002) and was replaced with rabbit 888.

One-hundred and forty New Zealand White rabbits were randomly assigned to seven dosage groups (Groups I through VII), 20 rabbits per group. Formulations of the test article, AC2993 for injection, and/or the vehicle, were administered subcutaneously twice daily (BID) to these female rabbits on days 6 through 18 of presumed gestation (GDs 6 through 18) at dosages of 0, 1, 11, and 130 µg/kg BID (total doses of 2, 22 and 260 µg/kg/day) for Groups I through IV, respectively. Rabbits in Groups V, VI and VII were pair fed to match the feed consumption in the groups administered 2 (Group II), 22 (Group III) and 260 µg/kg/day (Group IV), respectively. Groups V through VII were administered the vehicle. The dosage volumes were 433 (Groups I, IV and VII), 10 (Groups I1 and V) and 36.5 (Groups Ill and VI) µl/kg, adjusted daily on the basis of the individual body weights recorded immediately before administration of the test article and/or vehicle.

On GDs 6, 7, 8, 9, 10, 11, 12, 14, 16 and 18 (three hours after the first daily dosage) and on GDs *5,* 19, 20, 22, 24, 26 and 29 (approximately the same time each day as the GD 6 through 18 samples are collected) blood samples were collected from at least 12 rabbits per dose group. As soon as possible following blood collection at each time point, a sample of whole blood was assayed for glucose and lactate. On GDs 4, 9, 18, 22 and the day of scheduled sacrifice (GD 29), whole blood samples were collected from each of the rabbits for hematological and clinical biochemical evaluation.

The surviving rabbits were Caesarean-sectioned on GD 29 and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number of corpora lutea in each ovary was recorded. The uterus was excised and examined for pregnancy, number and distribution of implantations, early and late resorptions and live and dead fetuses. Uteri from does that appeared nonpregnant were examined while being pressed between glass plates to confirm the absence of implantation sites. All fetuses were weighed and examined for gross external alterations. All fetuses were examined internally to identify sex. The fetuses were examined for skeletal alterations after staining with alizarin red S.

Parameters and endpoints evaluated:
Clinical signs: Twice daily.
Body weight: Daily.
Food consumption: Twice daily.
Water consumption: Twice daily.

Hematology: On GDs 4, 9, 18, 22 and the day of scheduled sacrifice (GD 29), whole blood samples were collected from each of the rabbits for hematological evaluation.
Clinical chemistry: On GDs 4, 9, 18, 22 and the day of scheduled sacrifice (GD 29), whole blood samples were collected from each of the rabbits for clinical chemistry evaluations. On GDs 6, 7, 8, 9, 10, 11, 12, 14, 16 and 18 (three hours after the first daily dosage) and on GDs 5, 19, 20, 22, 24, 26 and 29 (approximately the same time each day as the GD 6 through 18 samples are collected) blood samples were collected from at least 12 rabbits per dosage group. As soon as possible following blood collection at each time point, a sample of whole blood was assayed for glucose and lactate. The percent change from the predosage concentration was calculated.
Terminal examination of females: All surviving rabbits were sacrificed on GD 29. Rabbits were Caesarean-sectioned and the thoracic, abdominal and pelvic viscera were examined for gross lesions. Uteri of apparently non-pregnant does were examined while being pressed between glass plates to confirm the absence of implantation sites. The number of corpora lutea in each ovary was recorded. The uterus of each rabbit was excised and examined for pregnancy, number and distribution of implantation sites, early and late resorptions and live and dead fetuses.

The fetuses were weighed, examined for gross external alterations and individually identified with a tag noting study number, litter number and uterine distribution. Live fetuses were sacrificed. All fetuses were examined internally to identify sex. Cavitated organs were evaluated in all fetuses by dissection. A single cross-section was made between the parietal and the frontal bones, and the brain was examined *in situ.* All fetuses were examined for skeletal alterations after staining with alizarin red S.

Rabbits that died or were sacrificed because of abortion or premature delivery were examined for the cause of death or moribund condition on the day the observation was made. Pregnancy status and uterine contents were recorded. Aborted fetuses and/or delivered pups were examined to the extent possible, using the same methods described for term fetuses.

Toxicokinetics: Not conducted.

Results

Mortality (dams): One 260 µg/kg/day dosage group doe was found dead on GD 17. The death was considered related to the test article because it occurred in the HD group. All other does survived to scheduled sacrifice.

Dose (µg/kg/d)	0	2	22	260	0 PF with 2	0 PF with 22	0 PF with 260
# of Females	**20**	**20**	**20**	**20**	**20**	**20**	**20**
# Died	0	0	0	1(GD 17)	0	0	0

Clinical signs (dams): Two 22 µg/kg/day (GDs 20, 21) and one 260 µg/kg/day (GD 21) dose group does aborted and one 2 µg/kg/day (GD 29) dose group doe delivered before scheduled sacrifice.

Dose (µg/kg/d)	0	2	22	260	0 PF with 2	0 PF with 22	0 PF with 260
# of Females	**20**	**20**	**20**	**20**	**20**	**20**	**20**
Scant feces	1/20	20/20	19/20	20/20	6/20	19/20	20/20
No feces	0/20	0/20	9/20	13/20	0/20	2/20	1/20
No urine	0/20	0/20	6/20	4/20	0/20	0/20	0/20
Pregnant (%)	95	100	95	95	95	90	100
Abortions (%)	0	0	10	5	0	0	0
Premature Deliveries (%)	0	5	0	0	0	0	0

2 µg/kg/day Dose Group: Doe 9352 delivered and was sacrificed on GD 29. This doe had scant feces on DGs 8, 11 and 16. This doe lost weight and feed and water consumption values were reduced after GD 26. All tissues appeared normal at necropsy. The litter consisted of two pups and nine fetuses; all appeared normal for their developmental ages at gross external, soft tissue and skeletal evaluation.

22 µg/kg/day Dose Group: Doe 9371 aborted and was sacrificed on GD 2 1. This doe had scant feces on GDs 7 to 14 and 19, no urine in the cage pan on GDs 15, 17 and 18 and no feces in the cage pan on GDs 15 to18. This doe generally lost weight and feed and water consumption values were severely reduced between GDs 6 and 17. All tissues appeared normal at necropsy. The litter consisted of seven late resorptions. Doe 9372 aborted and was sacrificed on GD 20. This doe had scant feces on GDs 7 to 9, 12 and 17 to 18, no feces in the cage pan on GDs 10 to 11, 13 to 16 and 19 to 20 and no urine in the cage pan on GD 19. This doe generally lost weight, feed consumption was severely reduced and water consumption was reduced after GD 6. All tissues appeared normal at necropsy. The litter consisted of seven late resorptions.

260 µg/kg/day Dose Group: Doe 9381 in the 260 µg/kg/day dosage group was found dead approximately 9 hours after the first dose on GD 17; a total of 23 doses were administered. This doe had scant feces on GDs 8 to 10 and 15 to 17, no feces in the cage pan on GDs 11 to 14 and emaciation and dehydration on GD 17. This doe lost weight and had severely reduced feed and water consumption after GD 6. All tissues appeared normal at necropsy. The litter consisted of 11 embryos; early developmental age precluded evaluation of the embryos.

Doe 9388 aborted and was sacrificed on GD 21. This doe had scant feces on GDs 6 to 10, 13 to 16 and 20 to 2 1, no feces in the cage pan on GDs 11 to 12 and 17 to 19 and no urine in the cage pan on GDs 18 and 20. This doe generally lost weight and had severely reduced feed and water consumption after GD 6. All tissues appeared normal at necropsy. The litter consisted of one early and 10 late resorptions.

Body weight (dams): (kg)

Percent Change in Mean Body Weights							
Dose (µg/kg/d)	**0**	**2**	**22**	**260**	**0 PF with 2**	**0 PF with 22**	**0 PF with 260**
# of Females	**20**	**20**	**20**	**20**	**20**	**20**	**20**
GD 6	3.43	3.52	3.54	3.48	3.68*	3.68**	3.69**
GD 18	3.64	3.54	3.43	3.27**	3.71	3.65	3.61
% Δ GD 6-18	6.12	0.01	-3.11	-6.03	0.80	-0.08	-2.17
GD 29	3.84	3.76	3.77	3.68	3.92	3.91	3.85

Food consumption (dams):

Absolute Feed Consumption Relative to Controls(%)							
Dose (µg/kg/d)	**0**	**2**	**22**	**260**	**0 PF with 2**	**0 PF with 22**	**0 PF with 260**
# of Females	**20**	**20**	**20**	**20**	**20**	**20**	**20**
GD 6 – 19	100	70**	48**	30**	69**	46**	28**
GD 19 – 29	100	105	110**	112**	96	103	110
GD 6 - 29	100	83**	76**	64**	80**	69**	61**

** p<0.01

Water consumption: ** p<0.01

Absolute Water Consumption Relative to Controls (%)							
Dose (µg/kg/d)	**0**	**2**	**22**	**260**	**0 PF with 2**	**0 PF with 22**	**0 PF with 260**
# of Females	**20**	**20**	**20**	**20**	**20**	**20**	**20**
GD 6 – 19	100	86	67**	41**	92	103	100
GD 19 – 29	100	105	114	119	91	101	108
GD 6 - 29	100	95	90	73**	91	102	100

Hematology:

Dose (µg/kg/d)	0	2	22	260	0 PF = 2	0 PF = 22	0 PF = 260
# of Females	**20**	**20**	**20**	**20**	**20**	**20**	**20**
Lymphocytes (10^3/mm^3) GD 9	7.8	5.8**	4.8**	4.9**	7.0	7.9	8.1

** p<0.01

Clinical chemistry:

Daily Dose (µg/kg/day)	0 (Control)	2	22	260	0 (PF=2)	0 (PF=22)	0 (PF=260)
Number of Females	20	20	20	20	20	20	20
Serum Chemistry							
Glucose DG 9 (mg/dL)	121	124	121	123	133**	133**	134**
Glucose DG 18 (mg/dL)	126	131	128	126	124	123	123
Glucose DG 29 (mg/dL)	122	124	124	127	111	115	113
Lactate DG 9 (mg/dL)	23.68	21.25	20.78	24.29	23.54	24.58	29.86
Lactate DG 18 (mg/dL)	26.13	25.78	23.95	26.38	38.61	41.00*	51.68**
Lactate DG 29 (mg/dL)	20.98	38.32	24.42	20.96	38.94	31.90	30.70
BHBA DG 9 (mg/dL)	0.91	1.14	2.67**	2.82**	0.77	0.85	2.33**
BHBA DG 18 (mg/dL)	1.03	1.58	1.95	1.72	1.10	1.16	1.00
BHBA DG 29 (mg/dL)	4.07	3.63	2.08*	1.94**	2.98	2.57	2.22*
Potassium DG 9 (mmol/L)	4.7	4.3**	4.0**	3.8**	4.8	4.6	4.4*
Potassium DG 18 (mmol/L)	4.8	4.2**	4.3**	4.3*	4.8	4.9	5.0
Potassium DG 29 (mmol/L)	4.3	4.3	4.4	4.4	4.6	4.5	4.6
Total protein DG 9 (g/dL)	5.7	5.8	5.9	5.9	5.6	5.6	5.8
Total protein DG 18 (g/dL)	5.7	5.7	5.6	5.2**	5.5*	5.4**	5.1**
Total protein DG 29 (g/dL)	4.7	4.7	5.0	4.9	4.6	4.6	4.8
Albumin DG 9 (g/dL)	4.3	4.4	4.3	4.3	4.3	4.3	4.3
Albumin DG 18 (g/dL)	4.2	4.3	4.2	3.8**	4.1	4.1	3.8**
Albumin DG 29 (g/dL)	3.3	3.4	3.5	3.5	3.3	3.4	3.5

BID = Dose divided and administered twice daily N/A = Not assayed or measured No.= Number DG = Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings PF = Pair-fed to match respective exenatide-treated group
C = cervical T = thoracic Lu = Lumbar Cau = Caudal S = Sacral BHBA = β-hydroxybutyric acid
* = p < 0.05 ** = p < 0.01

Toxicokinetics: TK was not conducted in this study. The data provided below was adopted from previous study.

Dose (µg/kg BID)	1	11	130
AUC_{0-12hr} (pg.h/ml)	12,164	214,883	3,610,750
Total Daily Dose (µg/kg/d)	**2**	**22**	**260**
Total Daily AUC (pg.h/ml)	24,328	429,766	7,221,500

Total daily $AUC_{(0-10hr)}$ for the MRHD (10 µg BID = 20 µg/day) = 2076 pg.h/ml

EFFECTS ON EMBRYO-FETAL DEVELOPMENT

Terminal and Necroscopic Evaluations: Does

Daily Dose (µg/kg/day)	0 (Control)	2	22	260	0 (PF=2)	0 (PF=22)	0 (PF=260)
Number of Females	20	20	20	20	20	20	20
Mean No. Corpora Lutea	10.6	10.3	11.0	10.6	10.3	10.2	10.0
Mean No. Implantations	8.7	7.7	9.2	8.6	9.7	9.4	9.3
Mean Litter Sizes	8.3	7.5	8.0	7.6	9.1	8.6	8.8
Mean Live Fetuses/Litter	8.3	7.5	8.0	7.6	9.0	8.6	8.8
Mean Resorptions	0.4	0.3	1.2	1.0	0.6	0.8	0.5
Early Resorptions	0.3	0.1	0.8	0.6	0.4	0.3	0.2
Late Resorptions	0.1	0.2	0.4	0.4	0.2	0.6	0.2
Mean Live Fetal Body Weight/Litter(g)							
Male	44.30	45.53	40.72	41.22	44.96	44.55	42.92
Female	43.65	43.29	40.24	39.43	44.86	42.72	41.43
Mean Percent Male Fetuses	48.6	46.6	45.2	53.3	55.4	50.6	47.8

BID = Dose divided and administered twice daily; N/A Not assayed or measured; No. Number ;
DG = Presumed Day of Gestation (starting on Day 0); PF Pair-fed to match respective exenatide-treated group; * p<0.05; ** p<0.01

Fetuses

Fetal External and Visceral Anomalies

Daily Dose (µg/kg/day)	0 (Control)	2	22	260	0 (PF=2)	0 (PF=22)	0 (PF=260)
Number of Females	20	20	20	20	20	20	20
Fetal Anomalies:							
Gross External % (litter/fetal):							
Umbilical Hernia	0.0/0.0	0.0/0.0	5.9/0.7	29.4/6.2	0.0/0.0	0.0/0.0	0.0/0.0
Visceral Anomalies % (litter/fetal):							
Eyes-Circumcorneal Hemorrhage	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	5.3/0.6	5.6/0.6	0.0/0.0
Eyes-Microphthalmia	5.3/0.6	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Heart-Septal Defect	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Vessels-Positional changes (all)	10.5/1.9	0.0/0.0	11.8/1.5	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Lung-Interm. Lobe Absent	5.3/0.6	5.3/0.7	23.5/2.9	5.9/0.8	5.3/0.6	11.1/1.3	5.0/0.6
Lung-Large	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Kidney-Absent	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Kidney-Dilation of Pelvis	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Intestine-Protrude, umbilical	0.0/0.0	0.0/0.0	5.9/0.7	29.4/6.2	0.0/0.0	0.0/0.0	0.0/0.0
Gallbladder-Absent	5.3/0.6	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Gallbladder-Small	5.3/0.6	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Adrenal-Misplaced	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.0/0.6

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- = No noteworthy findings PF = Pair-fed to match respective exenatide-treated group
* = p < 0.05 ** = p < 0.01

Historical Control Data	Fetal Incidence (%)	Fetal Range (%)	Litter Incidence (%)	Litter Range (%)
Umbilical hernia	0.05	0 – 0.7	0.4	0 – 6.2

Fetal Skeletal Anomalies

Daily Dose (µg/kg/day)	0 (Control)	2	22	260	0 (PF=2)	0 (PF=22)	0 (PF=260)
Number of Females	20	20	20	20	20	20	20
Skeletal Anomalies % (litter/fetal):							
Skull-Irregular ossification (all)	0.0/0.0	5.3/1.4	5.9/0.7	5.9/0.8	5.3/0.6	0.0/0.0	10.0/1.1
Hyoid: Ala, angulated	0.0/0.0	21.0/3.5	17.6/2.2	11.8/1.5	5.3/0.6	5.6/1.3	10.0/1.7
C. Vertebrae-C6 present	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
C. Vertebrae-C. rib at C7	0.0/0.0	5.3/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
C. Vertebrae-Centra fused	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.0/0.6
T. Vertebrae-Hemivertebrae	0.0/0.0	5.3/0.7	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
T. Vertebrae-Arch fused	5.3/0.6	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
T.Vertebrae-Centrum not ossified	5.3/0.6	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
T. Vertebrae-Extra ossification	0.0/0.0	0.0/0.0	0.0/0.0	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
T. Vertebrae-Centrum, bifid	0.0/0.0	0.0/0.0	5.9/0.7	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
T. Vertebrae-Centrum fused	0.0/0.0	0.0/0.0	5.9/0.7	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
T. Vertebrae-Unilat. ossification	0.0/0.0	0.0/0.0	11.8/1.4	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
T. Vertebrae-Arch small	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Lu. Vertebrae-Arch large	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
S. Vertebrae-Fused	0.0/0.0	0.0/0.0	5.9/1.4	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
S. Vertebrae-Arch large	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Cau. Vertebrae-Misaligned	0.0/0.0	5.3/0.7	5.9/0.7	0.0/0.0	5.3/0.6	16.7/2.6**	0.0/0.0
Cau. Vertebrae-Fused	0.0/0.0	0.0/0.0	5.9/1.4	0.0/0.0	0.0/0.0	0.0/0.0	5.0/0.6
Cau. Vertebrae-Cau2 present	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Cau. Vertebrae-Small	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Cau. Vertebrae-Cau9 present	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0

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- = No noteworthy findings PF = Pair-fed to match respective exenatide-treated group
C = cervical T = thoracic Lu = Lumbar Cau = Caudal S = Sacral BHBA = β-hydroxybutyric acid
* = p < 0.05 ** = p < 0.01

Historical Control Data	Fetal Incidence (%)	Fetal Range (%)	Litter Incidence (%)	Litter Range (%)
T. Vertebrae-extra ossification	No data	No data	No data	No data
T. Vertebrae-centrum, bifid	0.08	0 – 0.80	0.68	0 – 5.9
T. Vertebrae-centrum, fused	0.16	0 – 1.10	1.35	0 – 10.5
T. Vertebrae-unilateral, ossification	0.06	0 – 3.80	0.54	0 – 20.0
Hyoid: Ala, angulated	1.76	0 – 6.4	12.70	0 – 31.6

Fetal Skeletal Anomalies Contd.

Daily Dose (µg/kg/day)	0 (Control)	2	22	260	0 (PF=2)	0 (PF=22)	0 (PF=260)
Number of Females	20	20	20	20	20	20	20
Skeletal Anomalies (CONTINUED) % (litter/fetal):							
Ribs-Thickened	5.3/0.6	0.0/0.0	5.9/0.7	0.0/0.0	5.3/0.6	0.0/0.0	0.0/0.0
Ribs-Split	0.0/0.0	0.0/0.0	5.9/0.7	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
Ribs-Fused	5.3/0.6	0.0/0.0	11.8/1.4	5.9/1.5	0.0/0.0	0.0/0.0	5.0/0.6
Ribs-Short	0.0/0.0	0.0/0.0	0.0/0.0	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
Ribs-Irregularly shaped	0.0/0.0	0.0/0.0	5.9/0.7	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
Ribs-Broad	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Ribs-Thin	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Ribs-Small	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.3/0.6	0.0/0.0	0.0/0.0
Manubrium-Fused	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Sternal Centra-Incomplete ossification	0.0/0.0	0.0/0.0	11.8/1.4	11.8/1.5	5.3/1.2	0.0/0.0	0.0/0.0
Sternal Centra-Fused	5.3/0.6	10.5/1.4	23.5/5.8	5.9/2.3	21.0/2.3	16.7/1.9	15.0/3.4
Sternal Centra-Asymmetric	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Sternal Centra-Irregular shape	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Scapula-Irregular shape	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Pelvis-Pubis not ossified	0.0/0.0	0.0/0.0	0.0/0.0	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0

Fetal Ossification Sites

Daily Dose (µg/kg/day)	0 (Control)	2	22	260	0 (PF=2)	0 (PF=22)	0 (PF=260)
Number of Females	20	20	20	20	20	20	20
Notable Ossification Sites (no./fetus/litter)							
Vertebrae, thoracic	12.55	12.80**	12.71	12.85**	12.74*	12.74*	12.82**
Vertebrae, lumbar	6.44	6.20*	6.27	6.14**	6.26*	6.24*	6.17**
Ribs, pairs	12.49	12.73*	12.65	12.78**	12.67*	12.67*	12.73**
Total Affected Fetuses (%):	7.0	9.2	19.0**	13.1*	7.6	7.7	10.3

*** p<0.05; ** p<0.01**

Historical Control Data	Fetal Incidence (%)	Fetal Range (%)	Litter Incidence (%)	Litter Range (%)
Ribs-split	0.13	0 – 1.40	0.95	0 – 10.5
Ribs-fused	0.17	0 – 3.8	1.49	0 – 20.0
Ribs-irregularly shaped/wavy	0.02	0 – 0.6	0.14	0 – 5.3
Sternal centra-incomplete ossification	0.24	0 – 1.20	1.89	0 – 10.5
Pelvis-pubis not ossified	0.03	0 – 0.6	0.27	0 – 5.3

2.6.6.6.5 Study title: The Toxicokinetics of AC2993 and the Pharmacodynamics of Plasma Glucose in Pregnant Rabbits Administered AC2993 by Subcutaneous Injection

The purposes of this study were: 1) to determine the plasma concentration of AC2993; and 2) to evaluate the AC2993-related changes in plasma glucose in pregnant rabbits as a function of AC2993 dosage.

Key study findings:

- Body weight gain was suppressed at doses ≥ 22 µg/kg/d. This correlated with the decreased food and water consumption.
- Food consumption was significantly decreased at doses ≥ 22 µg/kg/d and showed a dose-dependent effect. Water consumption also decreased dose-dependently being significant at doses ≥ 156 µg/kg/d.
- β-hydroxy butyrate (a marker of starvation) was increased by 1 to 5-fold (not DD) in all treated groups relative to control. However the differences were not significant relative to control.
- Average glucose levels were significantly reduced ($p \leq 0.05$) in the 156 and 260 µg/kg/day dosage groups on GD 9 at 5 hours post-dose but the reductions were not strictly dose dependent. Average

glucose levels and the percent changes in glucose from 0 minutes post-dose were generally comparable among the six dose groups at all other time-points tested on GDs 6, 9, 12, 18 and 19; no toxicologically important differences occurred.

- Average lactate levels and the percent changes in lactate from 0 minutes post-dose were generally comparable among the six dose groups at all time-points tested on GDs 6, 9, 12, 18 and 19; no toxicologically important differences occurred. Significant differences ($p \leq 0.05$ to $p \leq 0.01$) in the lactate levels were considered unrelated to treatment because they were not dose dependent. Lactate levels were significantly increased ($p \leq 0.05$ to $p \leq 0.01$) in the 0.2 and 2 µg/kg/day dose groups on GD 18 at 15 minutes post-dose and in the 2 µg/kg/day dose group on GD 19.
- Weights of the liver were significantly decreased in the 156 and 260 µg/kg/day dose groups relative to control. This may be due to the decreased body weights noted in these groups.
- Fetal resorption was increased by 3 to 4-fold (not SS) at doses ≥ 22 µg/kg/day.
- Plasma concentrations of AC2993 (AUC and Cmax) increased with increasing dose. Rabbit TK indicates greater exposure than in mice, rats or monkeys at a similar dose or NOAEL. Decreased water consumption coincides with the unusual increased exposure. The sponsor suggests that since exenatide is cleared by the kidney, impaired clearance in the pregnant rabbit may explain the sensitivity to toxicity.

Study no.: REST02021.
Volume # and page #: N/A.
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: May 7, 2002.
GLP compliance: Yes.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Lot # 00-0605TP, 94% pure; 00-0606TP, 93% pure; 00-0102TP, 95% pure.
Methods

Doses: 0.1, 1, 11, 78, 130 µg/kg BID (total daily doses of 0.2, 2, 22, 156 and 260 µg/kg/d).
Species/strain: Rabbit/NZW.
Number/sex/group: 5 pregnant females/group.
Route, formulation, volume, and infusion rate: Subcutaneous injection. See study design for dose volumes.
Satellite groups used for toxicokinetics: All animals were used for TK as well.
Study design: Thirty NZW rabbits were randomly assigned to six dosage groups (Groups I through VI), five rabbits per group. AC2993 for Injection, and/or the vehicle were administered subcutaneously twice daily to these female rabbits on GDs 6 through 19 at doses of 0, 0.2, 2, 22, 156 and 260 mcg/kg/day for Groups I through VI, respectively.

Dosage Group	Dosage[a]		Concentration (mg/mL)	Dosage Volume (mcL/kg)	Number of Rabbits	Assigned Rabbit Numbers
	Per Injection (mcg/kg/dose)	Per Day (mcg/kg/day)				
I	0 (Placebo)	0 (Placebo)	0	433	5	9801-9805
II	0.1	0.2	0.01[b]	10	5	9806-9810
III	1	2	0.1	10	5	9811-9815
IV	11	22	0.3	36.5	5	9816-9820
V	78	156	0.3	260	5	9821-9825
VI	130	260	0.3	433	5	9826-9830

a. The test article was considered 100% active for the purpose of dosage calculations. The test article and/or vehicle was administered twice daily; the two daily injections were separated by 11 to 13 hours.
b. Corrected for loss due to dilution of 8 ± 1 mcg/mL.

Parameters and endpoints evaluated:

Mortality: Daily.

Clinical signs: Daily.
Body weight: Daily.
Food consumption: Daily.
Water consumption: Daily.
Hematology: Blood samples for hematology evaluation were collected on GD 19 after the morning dose.
Clinical chemistry: Blood samples for clinical chemistry evaluation were collected on GD 19 after the morning dose.
Toxicokinetics: On GDs 6, 9, 12 and 18, blood samples were collected from each rabbit before and after the morning dose. Samples were collected prior to the morning dosage (t=0) and at approximately 15 minutes, 30 minutes, 45 minutes, 1, 2, 5, 8 and 12 hours post-dose after the first daily dosage. The 12-hour sample was collected prior to the second daily dose (afternoon).
Necropsy: All rabbits were sacrificed on GD 19. Rabbits were Caesarean-sectioned and the thoracic, abdominal and pelvic viscera were examined for gross lesions. The gravid uterus was excised and weighed. The following organs were individually weighed (paired organs were weighed as pairs): brain, heart, liver, kidneys and ovaries, and retained in neutral buffered 10% formalin.

The number of corpora lutea in each ovary was recorded. The uterus of each rabbit was examined for pregnancy, number and distribution of implantation sites, early and late resorptions and live and dead fetuses.

Following sacrifice of the does on GD 19, amniotic fluid was collected and the amount was recorded. The fluid was transferred to a tube labeled with the study number, animal number, dosage group/level, the date, the fetus number and the nature of the specimen (i.e., amniotic fluid).

Results

Mortality (dams): None.

Clinical signs (dams):

Dose (µg/kg/d)	0	0.2	2	22	156	260
Scant feces	0/5	0/5	2/5	3/5**	5/5**	5/5**

** p<0.01

Body weight (dams): (kg)

Dose (µg/kg/d)	0	0.2	2	22	156	260
GD 6	3.51	3.35	3.45	3.34	3.39	3.48
GD 19	3.57	3.49	3.46	3.22	3.00	3.13
Wt. gain	0.06	0.14	0.01	-0.12	-0.39	-0.35
% Δ in B. wt	1.7	4.2	3.0	-3.6	-11.5	-10.0

Food consumption (dams): Absolute Food Consumption Relative to Control -g/day

Dose (µg/kg/d)	0	0.2	2	22	156	260
GD 6 - 19	159	149	96*	86**	45**	48**
% Δ	100	93.7	60.4	54.0	28.3	30.2

* p<0.05; ** p<0.01

Water consumption: Absolute Water Consumption Relative to Control -g/day

Dose (µg/kg/d)	0	0.2	2	22	156	260
GD 6 - 19	304	392	240	207	146*	143*
% Δ	100	129	79	68	48*	47*

* p<0.05

Hematology: No treatment-related changes.

Clinical chemistry:

Dose (µg/kg/d)	0	0.2	2	22	156	260
Glucos. (mg/dl)	100	95	94	100	100	116**
BHBA (mg/dl)	1.01±0.023	1.22±0.29	1.25±0.50	2.48±3.78	4.54±4.97	3.74±6.60

** $p<0.01$; BBHA = β-hydroxy butyrate

Glucose: See summary data for details.
Average glucose levels were comparable among the six dosage groups on GD 6 before dosage administration (0 minutes post-dose). The average glucose levels were significantly reduced ($p\leq0.05$) in the 156 and 260 µg/kg/day dosage groups on GD 9 at 5 hours post-dose but the reductions were not strictly dose dependent. Average glucose levels and the percent changes in glucose from 0 minutes post-dose were generally comparable among the six dosage groups at all other time-points tested on GDs 6, 9, 12, 18 and 19; no toxicologically important differences occurred. All other significant differences ($p\leq0.05$ to $p\leq0.01$) in the glucose levels or the percent changes in glucose from 0 minutes post-dose were considered unrelated to the test article because they were not dosage dependent.

Lactate: See summary tables for details.
Average lactate levels were comparable among the six dosage groups on GD 6 before dosage administration (0 minutes post-dose). Average lactate levels and the percent changes in lactate from 0 minutes post-dose were generally comparable among the six dosage groups at all time-points tested on GDs 6, 9, 12, 18 and 19; no toxicologically important differences occurred. Significant differences ($p\leq0.05$ to $p\leq0.01$) in the lactate levels were considered unrelated to the test article because they were not dosage dependent. Lactate levels were significantly increased ($p\leq0.05$ to $p\leq0.01$) in the 0.2 and 2 meg/kg/day dosage groups on GD 18 at 15 minutes post-dose and in the 2 µg/kg/day dosage group on GD 19.

Serum Glucose Results

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DAY 9 OF GESTATION

DOSAGE GROUP		I	II	III	IV	V	VI
DOSAGE (MCG/KG/DAY)a		0 (PLACEBO)	0.2	2	22	156	260
RABBITS TESTED	N	5	5	5	5	5	5
GLUCOSE (mg/dL)							
0 MIN. (PREDOSE)	MEAN ± S.D.	124.04 ± 40.78	107.62 ± 7.43	93.04 ± 13.56	95.72 ± 5.50 [4]b	99.95 ± 7.79 [4]b	94.98 ± 13.88
15 MIN.	MEAN ± S.D.	110.94 ± 19.73	89.34 ± 9.69	103.80 ± 29.25	92.80 ± 5.74 [4]b	98.92 ± 4.84 [4]b	98.04 ± 8.72
30 MIN.	MEAN ± S.D.	109.68 ± 17.13	90.26 ± 7.69	100.32 ± 44.18	83.22 ± 1.87 [4]b	95.25 ± 6.54 [4]b	93.80 ± 13.07
45 MIN.	MEAN ± S.D.	112.04 ± 11.14	96.46 ± 8.38	100.22 ± 36.06	83.95 ± 7.53 [4]b	95.60 ± 7.81 [4]b	95.74 ± 12.62
1 HR.	MEAN ± S.D.	113.80 ± 7.66	100.60 ± 10.16	96.44 ± 37.40	91.68 ± 7.70	96.45 ± 7.74 [4]b	92.74 ± 13.03
2 HR.	MEAN ± S.D.	104.80 ± 1.48	103.94 ± 9.45	89.74 ± 15.46	89.38 ± 6.14 [4]b	91.35 ± 9.75 [4]b	93.35 ± 10.55 [4]b
5 HR.	MEAN ± S.D.	103.95 ± 8.46 [4]b	102.76 ± 7.35	95.08 ± 6.94 [4]b	92.28 ± 8.55 [4]b	88.00 ± 7.03* [4]b	90.66 ± 7.04*
8 HR.	MEAN ± S.D.	101.60 ± 8.10	99.68 ± 3.85	96.38 ± 6.02 [4]b	96.68 ± 2.00 [4]b	101.88 ± 8.66 [4]b	93.18 ± 2.81
12 HR.	MEAN ± S.D.	103.90 ± 5.55	99.82 ± 4.84 [4]b	95.48 ± 5.13 [4]b	101.56 ± 3.11	104.00 ± 3.46 [3]b	98.98 ± 6.58
% CHANGE FROM 0 MIN (PREDOSE)							
15 MIN.	MEAN ± S.D.	-6.20 ± 20.44	-17.01 ± 6.22	10.14 ± 14.60	-2.67 ± 10.40 [4]b	-0.32 ± 12.22 [4]b	4.21 ± 9.86
30 MIN.	MEAN ± S.D.	-7.89 ± 15.51	-15.91 ± 7.72	4.93 ± 28.55	-12.85 ± 5.20 [4]b	-4.03 ± 12.54 [4]b	-0.11 ± 15.73
45 MIN.	MEAN ± S.D.	-4.95 ± 18.24	-10.21 ± 7.83	5.47 ± 20.58	-12.39 ± 4.28 [4]b	-3.84 ± 11.53 [4]b	2.22 ± 17.42
1 HR.	MEAN ± S.D.	-2.51 ± 22.53	-6.44 ± 8.21	1.20 ± 21.65	-6.70 ± 11.18 [4]b	-2.59 ± 15.60 [4]b	-1.11 ± 16.53
2 HR.	MEAN ± S.D.	-8.99 ± 25.28	-3.14 ± 10.09	-3.14 ± 14.44	-6.30 ± 9.64 [4]b	-7.91 ± 14.43 [4]b	-6.83 ± 11.43 [4]b
5 HR.	MEAN ± S.D.	-1.78 ± 14.63 [4]b	-4.46 ± 3.84	2.23 ± 9.08 [4]b	-3.65 ± 5.87 [4]b	11.52 ± 10.57 [4]b	1.94 ± 23.72
8 HR.	MEAN ± S.D.	-13.42 ± 17.96	-7.09 ± 6.20	3.82 ± 10.97 [4]b	1.26 ± 6.62 [4]b	2.10 ± 7.78 [4]b	0.40 ± 19.77
12 HR.	MEAN ± S.D.	-10.12 ± 24.13	-8.02 ± 4.61 [4]b	3.21 ± 14.04 [4]b	5.92 ± 5.66 [4]b	9.50 ± 12.09 [2]b	6.58 ± 22.06

MIN. = MINUTES POSTDOSE HR. = HOUR(S) POSTDOSE
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 19 of gestation.
b. Excludes values in which blood was not collected due to condition of ear as well as those that were not recorded or appeared incorrectly recorded.
* Significantly different from the Group I value ($p\leq0.05$).

Serum Glucose Results Contd.

DAY 12 OF GESTATION

DOSAGE GROUP		I	II	III	IV	V	VI
DOSAGE (MCG/KG/DAY)a		0 (PLACEBO)	0.2	2	22	156	260
RABBITS TESTED	N	5	5	5	5	5	5
GLUCOSE (mg/dL)							
0 MIN. (PREDOSE)	MEAN ± S.D.	96.68 ± 7.56 [4]b	99.68 ± 11.95 [4]b	113.97 ± 24.30 [3]b	100.10 ± 5.16 [3]b	103.50 ± 3.54 [2]b	102.48 ± 17.45 [4]b
15 MIN.	MEAN ± S.D.	102.40 ± 7.13 [4]b	92.30 ± 14.56 [4]b	85.60 ± 6.33 [3]b	94.87 ± 2.32 [3]b	101.40 ± 3.68 [2]b	93.38 ± 7.49 [4]b
30 MIN.	MEAN ± S.D.	99.08 ± 8.14 [4]b	94.22 ± 9.11 [4]b	79.40 ± 4.92** [3]b	90.47 ± 3.26 [3]b	104.50 ± 9.19 [2]b	94.00 ± 3.70 [4]b
45 MIN.	MEAN ± S.D.	103.42 ± 7.53 [4]b	93.95 ± 6.99 [4]b	84.03 ± 4.82* [3]b	102.90 ± 11.51 [3]b	107.50 ± 10.61 [2]b	98.00 ± 6.68 [4]b
1 HR.	MEAN ± S.D.	102.25 ± 4.57 [4]b	96.80 ± 9.27 [4]b	91.47 ± 9.24 [3]b	92.40 ± 5.55 [3]b	113.95 ± 21.28 [2]b	97.48 ± 7.17 [4]b
2 HR.	MEAN ± S.D.	99.58 ± 5.48 [4]b	102.32 ± 10.01 [4]b	87.43 ± 4.31 [3]b	90.13 ± 3.72 [3]b	105.25 ± 10.96 [2]b	95.85 ± 4.62 [4]b
5 HR.	MEAN ± S.D.	94.48 ± 5.23 [4]b	102.30 ± 10.54 [4]b	95.80 ± 3.90 [3]b	101.93 ± 8.05 [3]b	96.70 ± 0.00 [2]b	91.35 ± 6.99 [4]b
8 HR.	MEAN ± S.D.	94.98 ± 4.26 [4]b	96.60 ± 6.88 [4]b	95.37 ± 2.87 [3]b	102.67 ± 0.58 [3]b	108.00 ± 2.83** [2]b	102.00 ± 2.71 [4]b
12 HR.	MEAN ± S.D.	95.70 ± 5.13 [4]b	94.55 ± 9.96 [4]b	98.17 ± 5.06 [3]b	99.77 ± 1.36 [3]b	104.50 ± 2.12 [2]b	101.42 ± 7.73 [4]b
% CHANGE FROM 0 MIN (PREDOSE)							
15 MIN.	MEAN ± S.D.	6.04 ± 4.48 [4]b	-7.56 ± 6.51 [4]b	-22.44 ± 18.20** [3]b	-5.13 ± 2.99 [3]b	-2.03 ± 0.21 [2]b	-7.55 ± 11.43 [4]b
30 MIN.	MEAN ± S.D.	2.68 ± 8.03 [4]b	-5.16 ± 6.50 [4]b	-27.98 ± 16.96 [3]b	-9.36 ± 7.70 [3]b	0.87 ± 5.44 [2]b	6.39 ± 15.37 [4]b
45 MIN.	MEAN ± S.D.	7.14 ± 5.92 [4]b	-5.33 ± 5.67 [4]b	-23.92 ± 17.08** [3]b	2.61 ± 6.53 [3]b	3.75 ± 6.70 [2]b	-2.60 ± 15.40 [4]b
1 HR.	MEAN ± S.D.	6.02 ± 5.04 [4]b	-2.62 ± 5.22 [4]b	-17.96 ± 15.13 [3]b	-7.36 ± 9.96 [3]b	9.81 ± 16.81 [2]b	-2.89 ± 17.75 [4]b
2 HR.	MEAN ± S.D.	3.29 ± 6.90 [4]b	2.87 ± 3.32 [4]b	-21.43 ± 13.55** [3]b	-9.92 ± 1.42 [3]b	1.57 ± 7.12 [2]b	-4.65 ± 14.62 [4]b
5 HR.	MEAN ± S.D.	-2.09 ± 4.63 [4]b	2.81 ± 3.37 [4]b	-13.22 ± 19.18 [3]b	1.88 ± 7.44 [3]b	-6.52 ± 3.19 [2]b	-9.53 ± 10.90 [4]b
8 HR.	MEAN ± S.D.	-1.48 ± 5.56 [4]b	-2.58 ± 7.13 [4]b	-13.74 ± 18.18 [3]b	2.73 ± 5.00 [3]b	4.36 ± 0.83 [2]b	1.90 ± 18.68 [4]b
12 HR.	MEAN ± S.D.	-0.82 ± 4.29 [4]b	-4.59 ± 11.06 [4]b	-11.25 ± 18.88 [3]b	-0.12 ± 6.19 [3]b	0.99 ± 1.40 [2]b	0.35 ± 11.10 [4]b

MIN. = MINUTES POSTDOSE HR. = HOUR(S) POSTDOSE
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 19 of gestation.
b. Excludes values in which blood was not collected due to condition of ear as well as those that were not recorded or appeared incorrectly recorded.
* Significantly different from the Group I value (p≤0.05).
** Significantly different from the Group I value (p≤0.01).

DAY 18 OF GESTATION

DOSAGE GROUP		I	II	III	IV	V	VI
DOSAGE (MCG/KG/DAY)a		0 (PLACEBO)	0.2	2	22	156	260
RABBITS TESTED	N	5	5	5	5	5	5
GLUCOSE (mg/dL)							
0 MIN. (PREDOSE)	MEAN ± S.D.	88.20 ± 5.89	91.42 ± 7.37	86.10 ± 7.74 [4]b	92.88 ± 3.02	86.68 ± 6.54	84.87 ± 3.20 [3]b
15 MIN.	MEAN ± S.D.	97.26 ± 9.64	87.14 ± 11.65	76.62 ± 2.00** [4]b	89.12 ± 9.82	83.36 ± 5.66*	84.48 ± 10.34 [4]b
30 MIN.	MEAN ± S.D.	93.86 ± 7.61	84.26 ± 5.40	82.20 ± 6.48 [4]b	85.00 ± 6.45	86.40 ± 12.32	90.48 ± 6.44 [4]b
45 MIN.	MEAN ± S.D.	91.60 ± 7.23	85.62 ± 5.00	82.60 ± 12.06	81.88 ± 5.65	85.40 ± 7.86	85.42 ± 11.48
1 HR.	MEAN ± S.D.	89.52 ± 6.67	95.16 ± 13.04	86.30 ± 8.50	87.82 ± 5.38	91.78 ± 13.66	89.30 ± 7.90
2 HR.	MEAN ± S.D.	97.16 ± 5.72	92.76 ± 5.84	86.72 ± 7.86	84.68 ± 4.00	90.76 ± 13.92	89.32 ± 6.31
5 HR.	MEAN ± S.D.	96.76 ± 7.61	88.48 ± 8.89	87.70 ± 3.02 [4]b	92.40 ± 5.34	83.35 ± 8.40 [4]b	91.43 ± 1.58 [3]b
8 HR.	MEAN ± S.D.	92.32 ± 4.47	96.26 ± 2.84	88.32 ± 6.87	91.86 ± 8.67	89.88 ± 5.86	94.17 ± 3.53 [3]b
12 HR.	MEAN ± S.D.	94.55 ± 9.12 [2]b	95.33 ± 7.28 [3]b	78.50 ± 0.00 [1]b	---------- [0]b	91.95 ± 9.40 [2]b	---------- [0]b
% CHANGE FROM 0 MIN (PREDOSE)							
15 MIN.	MEAN ± S.D.	10.23 ± 7.12	-4.68 ± 10.33	-10.57 ± 6.64 [4]b	-3.90 ± 11.66	-3.71 ± 4.14	0.77 ± 16.70 [3]b
30 MIN.	MEAN ± S.D.	6.61 ± 8.88	-7.49 ± 7.73	-4.03 ± 10.82 [4]b	-8.37 ± 8.10	-0.49 ± 9.82	7.30 ± 5.03 [3]b
45 MIN.	MEAN ± S.D.	3.94 ± 6.56	-6.00 ± 7.16*	-9.76 ± 4.14** [4]b	-11.63 ± 8.62**	1.56 ± 2.83	6.55 ± 6.09 [3]b
1 HR.	MEAN ± S.D.	1.56 ± 5.20	4.33 ± 14.40	-3.19 ± 9.70 [4]b	-5.28 ± 7.91	5.62 ± 10.26	11.66 ± 6.18 [3]b
2 HR.	MEAN ± S.D.	10.80 ± 12.92	1.62 ± 3.43	-1.10 ± 7.26 [4]b	-8.66 ± 6.98	4.46 ± 11.05	7.33 ± 9.73 [3]b
5 HR.	MEAN ± S.D.	10.07 ± 11.02	-2.99 ± 9.62	2.35 ± 7.91 [4]b	-0.50 ± 5.11	-6.91 ± 6.71 [4]b	8.85 ± 4.97 [2]b
8 HR.	MEAN ± S.D.	5.06 ± 8.79	5.93 ± 10.26	1.00 ± 4.20 [4]b	-1.00 ± 10.24	4.02 ± 8.05	9.91 ± 0.53 [2]b
12 HR.	MEAN ± S.D.	14.68 ± 5.86 [2]b	10.55 ± 9.56 [3]b	-2.85 ± 0.00 [1]b	---------- [0]b	5.57 ± 11.90 [2]b	---------- [0]b

MIN. = MINUTES POSTDOSE HR. = HOUR(S) POSTDOSE
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 19 of gestation.
b. Excludes values in which blood was not collected due to condition of ear as well as those that were not recorded or appeared incorrectly recorded.
* Significantly different from the Group I value (p≤0.05).
** Significantly different from the Group I value (p≤0.01).

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Serum Glucose Results Contd.

DAY 19 OF GESTATION							
DOSAGE GROUP		I	II	III	IV	V	VI
DOSAGE (MCG/KG/DAY)a		0 (PLACEBO)	0.2	2	22	156	260
RABBITS TESTED	N	5	5	5	5	5	5
GLUCOSE (mg/dL)							
FOLLOWING DOSAGE ADMINISTRATION	MEAN ± S.D.	87.00 ± 4.98	81.62 ± 6.43	83.18 ± 6.57	82.56 ± 2.20	82.28 ± 2.82	88.06 ± 5.22

MIN. = MINUTES POSTDOSE HR. = HOUR(S) POSTDOSE

a. Dosage occurred on days 6 through 19 of gestation.

Serum Lactate Results

DAY 6 OF GESTATION							
DOSAGE GROUP		I	II	III	IV	V	VI
DOSAGE (MCG/KG/DAY)a		0 (PLACEBO)	0.2	2	22	156	260
RABBITS TESTED	N	5	5	5	5	5	5
LACTATE (mg/dL)							
0 MIN. (PREDOSE)	MEAN ± S.D.	15.32 ± 9.26 [4]b	24.86 ± 9.47	19.21 ± 14.28	24.68 ± 15.29	14.72 ± 2.70 [4]b	16.56 ± 4.91
15 MIN.	MEAN ± S.D.	25.42 ± 19.67	42.70 ± 25.00	28.46 ± 15.31	40.68 ± 20.65	31.76 ± 16.26	23.76 ± 10.29
30 MIN.	MEAN ± S.D.	14.94 ± 4.56	33.62 ± 16.58 [4]b	26.56 ± 18.90	35.60 ± 21.01 [4]b	29.54 ± 15.68	22.52 ± 1.28 [4]b
45 MIN.	MEAN ± S.D.	24.34 ± 31.55	37.35 ± 24.89 [4]b	29.56 ± 20.66	41.75 ± 20.64 [4]b	24.72 ± 7.07	27.55 ± 3.26 [4]b
1 HR.	MEAN ± S.D.	17.14 ± 13.75	33.02 ± 19.86	27.08 ± 14.42	45.65 ± 23.45 [4]b	34.86 ± 19.89	22.74 ± 7.62
2 HR.	MEAN ± S.D.	13.54 ± 5.66	34.12 ± 21.04	46.25 ± 25.22 [4]b	25.42 ± 7.17 [4]b	24.95 ± 13.96 [4]b	40.36 ± 27.60
5 HR.	MEAN ± S.D.	11.03 ± 4.68	30.66 ± 22.25	13.72 ± 7.43	29.42 ± 14.40 [4]b	37.00 ± 31.09	13.10 ± 2.25
8 HR.	MEAN ± S.D.	26.15 ± 23.24	41.75 ± 23.29 [4]b	41.65 ± 5.90 [4]b	35.80 ± 21.70	24.88 ± 18.34	28.00 ± 14.30 [4]b
12 HR.	MEAN ± S.D.	33.04 ± 27.67	44.10 ± 28.29 [4]b	30.71 ± 19.36	16.98 ± 9.10	12.60 ± 2.69 [2]b	26.32 ± 31.68
% CHANGE FROM 0 MIN (PREDOSE)							
15 MIN.	MEAN ± S.D.	29.16 ± 78.23 [4]b	79.61 ± 117.28	73.33 ± 61.16	74.78 ± 35.57	72.16 ± 49.55 [4]b	51.57 ± 63.70
30 MIN.	MEAN ± S.D.	0.18 ± 32.02 [4]b	41.49 ± 72.74 [4]b	64.80 ± 97.05	81.70 ± 25.25 [4]b	55.40 ± 25.20 [4]b	55.48 ± 55.73 [4]b
45 MIN.	MEAN ± S.D.	45.92 ± 93.67 [4]b	69.97 ± 108.42 [4]b	74.74 ± 67.44	121.52 ± 39.27 [4]b	54.13 ± 35.28 [4]b	89.74 ± 67.87 [4]b
1 HR.	MEAN ± S.D.	11.92 ± 22.54 [4]b	42.41 ± 90.05	68.83 ± 65.77	154.01 ± 153.45 [4]b	81.65 ± 51.56 [4]b	43.10 ± 44.99
2 HR.	MEAN ± S.D.	1.13 ± 53.01 [4]b	36.24 ± 58.39	323.04 ± 566.04 [4]b	62.21 ± 100.80 [4]b	64.16 ± 82.07 [4]b	202.14 ± 275.14
5 HR.	MEAN ± S.D.	-30.60 ± 23.63 [4]b	11.62 ± 42.89	-15.42 ± 33.63	41.38 ± 45.57 [4]b	72.42 ± 102.70 [4]b	-14.46 ± 30.95
8 HR.	MEAN ± S.D.	94.48 ± 197.72 [4]b	116.20 ± 141.42 [4]b	220.71 ± 218.67 [4]b	90.47 ± 132.05	19.37 ± 41.92 [4]b	77.69 ± 50.28 [4]b
12 HR.	MEAN ± S.D.	88.38 ± 143.54 [4]b	78.84 ± 93.87 [4]b	76.90 ± 110.24	-11.84 ± 46.79	-22.32 ± 0.99 [2]b	112.03 ± 314.51

MIN. = MINUTES POSTDOSE HR. = HOUR(S) POSTDOSE

[ ] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on days 6 through 19 of gestation.

b. Excludes values in which blood was not collected due to condition of ear as well as those that were not recorded or appeared incorrectly recorded.

Serum Lactate Results Contd.

DAY 9 OF GESTATION

DOSAGE GROUP		I	II	III	IV	V	VI
DOSAGE (MCG/KG/DAY)a		0 (PLACEBO)	0.2	2	22	156	260
RABBITS TESTED	N	5	5	5	5	5	5
LACTATE (mg/dL)							
0 MIN. (PREDOSE)	MEAN ± S.D.	17.78 ± 8.00 [4]b	45.26 ± 34.77	35.60 ± 14.08 [4]b	18.92 ± 8.70 [4]b	23.82 ± 15.09 [4]b	32.56 ± 29.34
15 MIN.	MEAN ± S.D.	20.12 ± 12.39	34.92 ± 17.61	24.40 ± 8.39	25.48 ± 7.70 [4]b	37.48 ± 29.57 [4]b	18.69 ± 8.38
30 MIN.	MEAN ± S.D.	13.22 ± 6.08	41.70 ± 30.03	17.28 ± 5.37	17.85 ± 5.57 [4]b	30.55 ± 23.39 [4]b	15.66 ± 6.07
45 MIN.	MEAN ± S.D.	17.52 ± 5.69	33.00 ± 19.74	21.28 ± 9.60	16.52 ± 5.12 [4]b	24.82 ± 14.16 [4]b	15.20 ± 5.48
1 HR.	MEAN ± S.D.	17.00 ± 5.10	34.18 ± 23.24	24.02 ± 10.53	39.88 ± 29.66	27.18 ± 25.43	22.20 ± 6.98
2 HR.	MEAN ± S.D.	11.86 ± 3.72	17.32 ± 11.60 [4]b	24.05 ± 16.68	29.15 ± 33.16 [4]b	28.84 ± 32.49 [4]b	10.49 ± 4.49 [4]b
5 HR.	MEAN ± S.D.	23.60 ± 23.11	20.93 ± 14.82	21.68 ± 13.53 [4]b	25.90 ± 11.57 [4]b	18.69 ± 15.84 [4]b	11.04 ± 5.82
8 HR.	MEAN ± S.D.	26.58 ± 17.32	22.15 ± 16.44 [4]b	18.99 ± 13.77 [4]b	17.45 ± 5.52 [4]b	15.80 ± 7.58 [4]b	16.83 ± 8.46
12 HR.	MEAN ± S.D.	28.22 ± 18.02	24.20 ± 18.88 [4]b	27.95 ± 17.58 [4]b	21.30 ± 8.91	35.47 ± 37.06 [3]b	27.04 ± 14.09
% CHANGE FROM 0 MIN (PREDOSE)							
15 MIN.	MEAN ± S.D.	-10.38 ± 19.96 [4]b	29.22 ± 106.38	-29.29 ± 16.81 [4]b	44.00 ± 44.92 [4]b	65.62 ± 98.32 [4]b	-12.35 ± 55.75
30 MIN.	MEAN ± S.D.	-32.46 ± 24.07 [4]b	21.04 ± 69.11	-50.71 ± 7.76 [4]b	-0.46 ± 27.21 [4]b	34.97 ± 73.82 [4]b	-17.06 ± 58.64
45 MIN.	MEAN ± S.D.	0.78 ± 37.68 [4]b	9.48 ± 69.96	-41.61 ± 13.43 [4]b	-8.77 ± 13.40 [4]b	16.95 ± 50.23 [4]b	20.01 ± 60.43
1 HR.	MEAN ± S.D.	-4.48 ± 28.26 [4]b	10.71 ± 71.56	-39.07 ± 25.41 [4]b	66.44 ± 125.84 [4]b	46.48 ± 88.18 [4]b	42.91 ± 127.05
2 HR.	MEAN ± S.D.	-27.40 ± 26.57 [4]b	-27.64 ± 49.03 [4]b	-42.53 ± 28.28 [4]b	25.90 ± 82.02 [4]b	28.92 ± 116.41 [4]b	-24.16 ± 52.20 [4]b
5 HR.	MEAN ± S.D.	49.92 ± 162.16 [4]b	-37.64 ± 39.22	-23.75 ± 35.32 [3]b	56.14 ± 79.18 [4]b	-21.91 ± 50.20 [4]b	-48.50 ± 29.69
8 HR.	MEAN ± S.D.	151.38 ± 260.73 [4]b	-2.14 ± 79.26 [4]b	-50.09 ± 34.87 [3]b	0.13 ± 36.30 [4]b	-27.52 ± 15.67 [4]b	-19.97 ± 61.40
12 HR.	MEAN ± S.D.	24.52 ± 38.22 [4]b	7.82 ± 89.81 [4]b	-5.51 ± 72.28 [3]b	-0.02 ± 24.81 [4]b	28.58 ± 77.49 [2]b	28.00 ± 99.48

MIN. = MINUTES POSTDOSE HR. = HOUR(S) POSTDOSE
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 19 of gestation.
b. Excludes values in which blood was not collected due to condition of ear as well as those that were not recorded or appeared incorrectly recorded.

DAY 12 OF GESTATION

DOSAGE GROUP		I	II	III	IV	V	VI
DOSAGE (MCG/KG/DAY)a		0 (PLACEBO)	0.2	2	22	156	260
RABBITS TESTED	N	5	5	5	5	5	5
LACTATE (mg/dL)							
0 MIN. (PREDOSE)	MEAN ± S.D.	41.52 ± 18.04 [4]b	62.48 ± 26.01 [4]b	20.40 ± 0.00 [1]b	45.97 ± 20.03 [3]b	22.60 ± 3.25 [2]b	50.25 ± 10.68 [2]b
15 MIN.	MEAN ± S.D.	11.67 ± 3.43 [4]b	19.58 ± 8.42 [4]b	39.23 ± 34.48 [3]b	36.70 ± 14.91 [3]b	20.30 ± 0.71 [2]b	24.82 ± 4.75 [4]b
30 MIN.	MEAN ± S.D.	9.88 ± 2.22 [4]b	21.45 ± 5.78 [4]b	27.03 ± 18.77 [3]b	26.73 ± 17.17 [3]b	20.80 ± 0.56 [2]b	19.90 ± 8.28 [4]b
45 MIN.	MEAN ± S.D.	12.40 ± 1.36 [4]b	15.21 ± 5.51 [4]b	22.50 ± 7.71 [3]b	16.40 ± 1.27 [2]	24.85 ± 7.00 [2]b	16.10 ± 7.35 [4]b
1 HR.	MEAN ± S.D.	12.95 ± 3.02 [4]b	16.72 ± 6.48 [4]b	15.95 ± 7.71 [2]	27.50 ± 21.39 [3]b	26.70 ± 8.91 [2]b	17.90 ± 13.84 [4]b
2 HR.	MEAN ± S.D.	8.36 ± 2.00 [4]b	26.16 ± 29.50 [4]b	9.75 ± 2.78 [3]b	14.16 ± 11.34 [3]b	16.30 ± 2.40 [2]b	10.48 ± 3.51 [4]b
5 HR.	MEAN ± S.D.	12.78 ± 1.43 [4]b	27.48 ± 10.13 [4]b	11.87 ± 8.10 [3]b	25.58 ± 18.76 [3]b	9.80 ± 0.57 [2]b	8.15 ± 4.48 [4]b
8 HR.	MEAN ± S.D.	17.58 ± 7.44 [4]b	35.20 ± 24.43 [4]b	29.33 ± 30.86 [3]b	23.47 ± 1.14 [3]b	19.65 ± 9.26 [2]b	19.68 ± 4.85 [4]b
12 HR.	MEAN ± S.D.	14.60 ± 7.62 [4]b	25.02 ± 16.15 [4]b	15.29 ± 6.43 [3]b	20.04 ± 9.91 [3]b	13.55 ± 2.47 [2]b	16.52 ± 6.10 [4]b
% CHANGE FROM 0 MIN (PREDOSE)							
15 MIN.	MEAN ± S.D.	-68.60 ± 13.66 [4]b	-58.25 ± 36.20 [4]b	-42.65 ± 0.00 [1]b	-6.78 ± 57.86 [3]b	-9.46 ± 9.90 [2]b	-47.88 ± 5.45 [2]b
30 MIN.	MEAN ± S.D.	-73.17 ± 10.07 [4]b	-55.68 ± 32.87 [4]b	-40.69 ± 0.00 [1]b	-37.05 ± 34.61 [3]b	-7.18 ± 10.86 [2]b	-44.75 ± 19.90 [2]b
45 MIN.	MEAN ± S.D.	-66.05 ± 12.69 [4]b	-64.64 ± 35.75 [4]b	-17.16 ± 0.00 [1]b	-48.61 ± 29.69 [2]	8.85 ± 15.31 [2]b	-56.98 ± 1.55 [2]b
1 HR.	MEAN ± S.D.	-64.84 ± 15.00 [4]b	-67.05 ± 21.26 [4]b	-48.53 ± 0.00 [1]b	-41.16 ± 27.26 [3]b	16.51 ± 22.65** [2]b	-46.19 ± 15.16 [2]b
2 HR.	MEAN ± S.D.	-77.37 ± 8.26 [4]b	-55.12 ± 42.24 [4]b	-67.84 ± 0.00 [1]b	-71.74 ± 12.52 [3]b	-26.35 ± 21.24 [2]b	-78.41 ± 5.19 [2]b
5 HR.	MEAN ± S.D.	-65.83 ± 10.47 [4]b	-48.32 ± 27.47 [4]b	-67.35 ± 0.00 [1]b	-46.30 ± 31.10 [3]b	-56.02 ± 8.86 [2]b	-81.21 ± 9.64 [2]b
8 HR.	MEAN ± S.D.	-48.48 ± 33.21 [4]b	-41.95 ± 25.96 [4]b	-28.43 ± 0.00 [1]b	-38.87 ± 35.59 [3].	-15.12 ± 28.77 [2]b	-51.66 ± 14.77 [2]b
12 HR.	MEAN ± S.D.	-61.68 ± 19.14 [4]b	-58.18 ± 21.30 [4]b	-51.62 ± 0.00 [1]b	-56.31 ± 12.11 [3]b	-38.62 ± 19.78 [2]b	-68.38 ± 8.62 [2]b

MIN. = MINUTES POSTDOSE HR. = HOUR(S) POSTDOSE
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 19 of gestation.
b. Excludes values in which blood was not collected due to condition of ear as well as those that were not recorded or appeared incorrectly recorded.
** Significantly different from the Group I value ($p \leq 0.01$).

Serum Lactate Results Contd.

DAY 18 OF GESTATION

DOSAGE GROUP		I	II	III	IV	V	VI
DOSAGE (MCG/KG/DAY)a		0 (PLACEBO)	0.2	2	22	156	260
RABBITS TESTED	N	5	5	5	5	5	5
LACTATE (mg/dL)							
0 MIN. (PREDOSE)	MEAN ± S.D.	31.66 ± 26.14	39.45 ± 16.93 [4]b	49.62 ± 10.86 [4]b	32.88 ± 13.04	32.84 ± 18.21	43.37 ± 16.79 [3]b
15 MIN.	MEAN ± S.D.	10.44 ± 2.39	25.30 ± 7.49*	37.30 ± 12.43** [4]b	21.62 ± 2.53	20.36 ± 10.33	20.52 ± 10.15 [4]b
30 MIN.	MEAN ± S.D.	11.71 ± 3.20	30.84 ± 12.32	29.90 ± 7.33 [3]b	21.92 ± 7.43	27.59 ± 16.32	38.88 ± 33.03 [4]b
45 MIN.	MEAN ± S.D.	14.98 ± 6.68	23.97 ± 18.33	47.42 ± 30.76	20.04 ± 6.85	26.20 ± 18.74	30.98 ± 30.83
1 HR.	MEAN ± S.D.	15.03 ± 10.18	18.88 ± 5.40 [4]b	32.02 ± 12.16 [4]b	33.64 ± 20.84	15.30 ± 7.72 [3]b	29.21 ± 20.49
2 HR.	MEAN ± S.D.	15.80 ± 11.80	16.20 ± 11.90	30.36 ± 15.34	21.67 ± 14.50	23.55 ± 16.40	29.92 ± 37.19
5 HR.	MEAN ± S.D.	20.20 ± 6.69	36.94 ± 27.32	50.75 ± 23.26 [4]b	21.42 ± 5.25	30.40 ± 37.43 [4]b	12.28 ± 4.44 [3]b
8 HR.	MEAN ± S.D.	16.72 ± 6.54	18.73 ± 11.10	40.42 ± 28.93	35.00 ± 31.11	30.66 ± 25.99	8.44 ± 1.98 [3]b
12 HR.	MEAN ± S.D.	9.68 ± 1.87 [2]b	32.40 ± 25.21 [3]b	22.90 ± 0.00 [1]b	---------- [0]b	27.80 ± 23.33 [2]b	---------- [0]b
% CHANGE FROM 0 MIN (PREDOSE)							
15 MIN.	MEAN ± S.D.	-56.12 ± 18.59	-34.59 ± 25.49 [4]b	-24.87 ± 22.54 [4]b	-25.59 ± 29.40	-37.25 ± 12.95	-53.09 ± 8.83 [3]b
30 MIN.	MEAN ± S.D.	-48.35 ± 31.13	-25.73 ± 30.71 [4]b	-33.57 ± 12.53 [3]b	-24.12 ± 38.71	-15.71 ± 36.82	2.10 ± 31.95 [3]b
45 MIN.	MEAN ± S.D.	-26.34 ± 65.14	-56.30 ± 15.89 [4]b	-26.46 ± 36.11 [4]b	-30.48 ± 39.02	-24.64 ± 29.12	-28.28 ± 55.18 [3]b
1 HR.	MEAN ± S.D.	-47.39 ± 11.97	-47.17 ± 20.82 [4]b	-35.90 ± 16.49 [4]b	-1.63 ± 35.75	40.25 ± 17.47 [3]b	-38.75 ± 19.25 [3]b
2 HR.	MEAN ± S.D.	-38.55 ± 35.50	-67.47 ± 17.14 [4]b	-50.75 ± 15.62 [4]b	-38.62 ± 25.00	-31.34 ± 33.50	-69.59 ± 8.53 [3]b
5 HR.	MEAN ± S.D.	-5.87 ± 56.43	-13.40 ± 80.32 [4]b	0.36 ± 38.53 [4]b	-29.15 ± 26.13	-34.57 ± 46.33 [4]b	-56.12 ± 0.60 [2]b
8 HR.	MEAN ± S.D.	-27.00 ± 38.68	-57.54 ± 22.73 [4]b	-4.68 ± 72.06 [4]b	14.35 ± 98.38	-1.02 ± 85.40	71.82 ± 0.33 [2]b
12 HR.	MEAN ± S.D.	-45.66 ± 2.11 [2]b	-52.66 ± 32.09 [2]b	-47.84 ± 0.00 [1]b	---------- [0]b	47.79 ± 23.74 [2]b	---------- [0]b

MIN. = MINUTES POSTDOSE HR. = HOUR(S) POSTDOSE
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 19 of gestation.
b. Excludes values in which blood was not collected due to condition of ear as well as those that were not recorded or appeared incorrectly recorded.
* Significantly different from the Group I value (p≤0.05).
** Significantly different from the Group I value (p≤0.01).

Serum Lactate Results Contd.

DAY 19 OF GESTATION

DOSAGE GROUP		I	II	III	IV	V	VI
DOSAGE (MCG/KG/DAY)a		0 (PLACEBO)	0.2	2	22	156	260
RABBITS TESTED	N	5	5	5	5	5	5
LACTATE (mg/dL)							
FOLLOWING DOSAGE ADMINISTRATION	MEAN ± S.D.	38.44 ± 12.59	34.40 ± 13.73	67.76 ± 25.76*	42.50 ± 9.58	42.84 ± 19.19	59.02 ± 19.50

MIN. = MINUTES POSTDOSE HR. = HOUR(S) POSTDOSE
a. Dosage occurred on days 6 through 19 of gestation.
* Significantly different from the Group I value (p≤0.05).

Organ weights:

Dose (µg/kg/d)	0	0.2	2	22	156	260
Liver (g)	103	113	106	88	68*	75*
Liver/body wt.	3.0	3.2	3.1	2.7	2.2*	2.4*

* p<0.05

Terminal and Necroscopic Evaluations:

BEST POSSIBLE COPY

EMBRYO-FETAL DEVELOPMENT

CAESAREAN SECTIONING AND LITTER OBSERVATION

DOSAGE GROUP DOSAGE (MCG/KG/DAY)a		I 0 (PLACEBO)	II 0.2	III 2	IV 22	V 156	VI 260
RABBITS TESTED	N	5	5	5	5	5	5
PREGNANT	N(%)	5(100.0)	5(100.0)	5(100.0)	5(100.0)	5(100.0)	5(100.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 19 OF GESTATION	N	5	5	5	5	5	5
CORPORA LUTEA	MEAN±S.D.	9.8 ± 1.5	10.4 ± 1.1	9.0 ± 1.9	10.2 ± 1.9	10.6 ± 1.5	10.0 ± 1.6
IMPLANTATIONS	MEAN±S.D.	7.8 ± 1.1	10.0 ± 1.4	8.0 ± 2.8	7.6 ± 2.8	9.6 ± 2.4	8.2 ± 2.3
LITTER SIZES	MEAN±S.D.	7.4 ± 1.1	9.6 ± 1.1	7.6 ± 2.8	6.4 ± 2.6	8.6 ± 1.5	6.6 ± 3.0
LIVE FETUSES	N	37	48	38	32	43	33
	MEAN±S.D.	7.4 ± 1.1	9.6 ± 1.1	7.6 ± 2.8	6.4 ± 2.6	8.6 ± 1.5	6.6 ± 3.0
DEAD FETUSES	N	0	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.4 ± 0.5	0.4 ± 0.5	0.4 ± 0.9	1.2 ± 1.6	1.0 ± 2.2	1.6 ± 1.8
EARLY RESORPTIONS	N	1	1	0	4	0	7
	MEAN±S.D.	0.2 ± 0.4	0.2 ± 0.4	0.0 ± 0.0	0.8 ± 0.8	0.0 ± 0.0	1.4 ± 1.9
LATE RESORPTIONS	N	1	1	2	2	5	1
	MEAN±S.D.	0.2 ± 0.4	0.2 ± 0.4	0.4 ± 0.9	0.4 ± 0.9	1.0 ± 2.2	0.2 ± 0.4
DOES WITH ANY RESORPTIONS	N(%)	2(40.0)	2(40.0)	1(20.0)	3(60.0)	1(20.0)	3(60.0)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	5(100.0)	5(100.0)	5(100.0)	5(100.0)	5(100.0)	5(100.0)
PLACENTAE APPEARED NORMAL	N(%)	5(100.0)	5(100.0)	5(100.0)	5(100.0)	5(100.0)	5(100.0)
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	5.0 ± 6.8	3.7 ± 5.0	4.4 ± 9.9	13.7 ± 18.2	7.7 ± 17.2	21.2 ± 28.2

a. Dosage occurred on days 6 through 19 of gestation.

Toxicokinetics:

Dose (µg/kg BID)	AUC (pg·hr/mL)				Mean
	Day 6	Day 9	Day 12	Day 18	
0.1	185	210	215	303	228
1	2476	34600	6073	5506	4,685 (a)
11	95648	330854	201650	231379	214,883
78	1283000	1896000		1281000	1,486,667
130	2769000	4044000	4125000	3505000	3,610,750

(a) Mean does not include day 9 value

Dose (µg/kg BID)	Cmax (pg/mL)				Mean
	Day 6	Day 9	Day 12	Day 18	
0.1	246	212	303	362	281
1	2214	21702	6174	4597	4,328 (a)
11	63532	185503	265190	233616	186,960
78	586450	1078000		668397	777,616
130	1831000	3059000	1115000	2109000	2,028,500

(a) Mean does not include day 9 value

Dose (µg/kg BID)	Tmax (hr)				Mean
	Day 6	Day 9	Day 12	Day 18	
0.1	0.25	0.25	0.25	0.25	0.25
1	0.5	0.75	0.5	0.5	0.50 (a)
11	0.75	0.75	0.75	0.5	0.69
78	1	0.75		0.75	0.83
130	0.75	0.75	1	0.5	0.75
(a) Mean does not include day 9 value					

The primary purposes of this study were to determine the systemic load of AC2993 in pregnant rabbits at the same SC BID doses of AC2993 used in two previous developmental toxicity studies and to define pharmacodynamics of blood glucose in these animals. Secondary objectives were to confirm, in the same study using all of the doses used in the two previous rabbit developmental toxicity studies, the effects of AC2993 on feed and water consumption, impact in a plasma indicator of starvation (β-hydroxybutyrate) and body weight. The plasma concentrations of AC2993 as measured by AUC and Cmax increased with dose of peptide while Tmax remained relatively constant over the BID dose range of 0.1 µg/kg to 130 µg/kg (e.g., AUC values ranged from 228 pg.h/ml to 3,611,000 pg.h/ml).

In contrast to what was expected, there was not a clear and consistent AC2993-related pattern of blood glucose depression, a known pharmacological action of the peptide. The Sponsor has conducted a PK/PD study in non-pregnant female rabbits and showed that rabbits can exhibit AC2993-dependent decreases in plasma glucose concentrations. An explanation for not seeing such a pattern of glucose change in the current study could be the confounding relationship between the effect of AC2993 to lower glucose and the glucose increases associated with the stress of handling and b1eeding the animals in the Sponsor's study were sedated to reduce stress while they were not sedated in the current study so as not to compromise the pregnancy. Thus, one is unable to make a conclusion regarding the effect of AC2993 on blood glucose concentrations in pregnant rabbits.

The doses of AC2993 at SC BID doses of 0.1, 1, 11, 78 and 130 µg/kg used in the current study were previously used in two other developmental toxicity studies in rabbits. The same dose-related patterns of depressed feed consumption, depressed water consumption and weight loss observed in these two studies were also observed in the current study. In addition, a serum indicator of starvation (β-hydroxybutyrate) was observed to increase with dose as was observed in the other study where it was measured. In addition, there was a dose-related decrease in liver weight that is probably associated with the depressed nutritional state of the does.

Prenatal and Postnatal Development

2.6.6.6.6 Developmental and Perinatal Reproduction Toxicity Study of AC2993 in Mice, Including a Postnatal Behavioral/Function Evaluation (Segment III Study).

Key study findings:

- 1/25 female mice died at all dose levels. The HD female died while delivering a litter. Sponsor stated that the death may be drug-related because it occurred in the HD group and the other mice in this dose group had increased incidences of stillbirths and pup deaths on day 1 of lactation. Although the cause of death could not be determined, it is likely that the deaths in the 6 and 68 mg/kg/day dose groups were also drug-related.

- Maternal (F0) body weight gain decreased in a dose-dependent manner by 6%, 12% and 18% for the LD, MD and HD dams respectively during gestation. During lactation, body weight gain was significantly increased in the MD and HD groups by 169% and 167% respectively.
- Maternal (F0) Food consumption was significantly decreased in MD and HD animals by 11% relative to control during GD 15-18. This may explain the decreased body weight gain observed during gestation. Food consumption was also decreased during lactation by 16% in the HD group.
- F0 Dams delivering stillborn pups was significantly increased in the HD group (24%) relative to control. Dams with all pups dying during days 1-4 postpartum was significantly increased in the HD group (12%) relative to control. Implantation sites were decreased by 29% (NS) in the HD group relative to control.
- Number of live birth was significantly decreased in the HD group (92%) relative to control (100%). Still birth was significantly increased by 6% in the HD group relative to control (0%).
- Pups found dead/presumed cannibalized was significantly increased in the HD group (3%) on day 1 postpartum. Similar increases were observed in the LD (3.2%) and HD groups (5.5%) relative to control during days 2-4 postpartum, and in the MD group (4.5%) during days 8-14 postpartum. Viability index was slightly but significantly decreased in the HD group (92%) relative to control (99%). Lactation index was also slightly but significantly decreased in the MD group (92%) relative to control (97%).
- Number of surviving pups/litter was significantly decreased in the HD group by 25% relative to control during days 1-14 postpartum.
- Pup weight/litter decreased significantly in the HD group on days 1-7 postpartum and on PPD 7 postpartum in the MD group.
- Postweaning body weight was slightly but significantly decreased in the HD F1 males by 5% from day 1 post weaning to terminal sacrifice. Postweaning body weight was also slightly but significantly decreased in the HD F1 females by 5% during precohabitation, on GD 0 and on GD 18 relative to control.
- Total number of pups delivered was decreased by 29% in the HD group. Live-born pups was slightly but significantly decreased by 8% whereas still-born pups was significantly increased by 6% in the HD group relative to control.
- Maternal administration of the test article at doses as high as 760 µg/kg/d (HD) did not affect short or long term memory in the F1 generation mice. No significant differences in the number of trials to criterion, or in latency time occurred among the groups tested in the passive avoidance paradigm. No mice failed to learn.
- There were no treatment-related effects on corpora lutea, implantations, litter sizes and resorptions in cesarean-sectioned F1 females.
- 1/297 (0.3%) LD F2 fetuses had a cleft palate (within historical range: 0-1.2%). 1/268 MD F2 fetuses had exencephaly, opened eyelids and a cleft snout. Litter and fetal incidences of forked tail tip and flexed (downward) hindlimb were slightly increased (not statistically significant) in F2 litters/fetuses of HD F1 parents.
- The maternal (F0) NOAEL < 6µg/kg/d (3X MRHD, AUC) due to mortality at doses ≥ 6 µg/kg/d. NOAEL for fetal viability and growth is 6 µg/kg/d (3X MRHD, AUC) because the 68 µg/kg/d (MD) and 760 µg/kg/d (HD) dose groups caused reduced pup body weights preweaning and the HD increased perinatal mortality and reduced body weight gains postweaning.

Study no.: REST00150R1.
Volume # and page #: N/A.
Conducting laboratory and location: (b) (4) (b) (4).
Date of study initiation: July 19, 2000.
GLP compliance: Yes (USA, Japan, UK)

QA reports: Yes (X) no ( )
Drug, lot #, and % purity: Lot # 991002 TP, 94.3% pure.

Methods

Doses: 3, 34 380 µg/kg BID giving total doses of 6, 68 and 760 µg/kg/day.
Species/strain: Mouse/CD-1.
Number/sex/group: 25 female mice/group.
Route, formulation, volume, and infusion rate: Subcutaneous injection. Please see study design for dose volumes. Test article was provided as 0.3 mg/ml formulated drug product. Each is a 1 ml single dose, sterile formulation in 30mM acetate buffer pH 4.5 with mannitol added as an iso-osmolality modifer.
Satellite groups used for toxicokinetics: 3 female mice/group.
Study design:
Parental Females (F0): Presumed-pregnant female mice (25 females/group) were dosed with 3, 34 and 380 µg/kg BID giving total doses of 6, 68 and 760 µg/kg/day AC2993. The doses were administered by subcutaneous injection on Day 6 of gestation (GD 6) through day 20 of lactation (DL 20, mice that delivered a litter) or GD 22 (mice that did not deliver a litter). Three additional females/group were used for toxicokinetics. The female mice were evaluated for adverse clinical signs during parturition, duration of gestation, litter size and pup viability at birth. Maternal behavior was evaluated on DLs 1, 4, 7, 14 and 21.

F1 Generation: F1 generation pups were not directly given test article or vehicle, but were possibly exposed to the test article or vehicle during maternal gestation (in utero exposure) or via maternal milk during the lactation period. Litters were observed for dead pups at least twice daily. Clinical observations were recorded once daily during the pre-weaning period (DLs 1 to 21). Pup body weights were recorded on DLs 1 (birth), 4, 7, 14 and 21. At weaning on DL 21, 25 male and 25 female pups/group were chosen for continued evaluation. F1 generation animals were evaluated for growth, development and behavior and for the ability to mate and reproduce. The fetuses of F1 dams were examined on GD 18 for abnormalities.

F1 Generation Litters – Pre-weaning Observations:
DL 1 was defined as the day of birth and is also the first day on which all pups in a litter were individually weighed (pup body weights were recorded after all pups in a litter were delivered and groomed by the dam). Litters were observed for dead pups at least twice daily. The pups in each litter were counted once daily. Clinical observations were recorded once daily during the preweaning period (DLs 1 to 21). Pup body weights were recorded on DLs 1 (birth), 4, 7, 14 and 21.

F1 Generation Mice – Post-weaning Observations:
All F1 generation male and female mice were observed for viability at least twice each day of the study. These mice were also examined for clinical observations and general appearance once weekly during the post-weaning period. Body weights for male mice were recorded weekly during the post-weaning period and at sacrifice. Body weights for female mice were recorded weekly during the post-weaning period and on GDs 0, 6, 12 and 18. Female mice were evaluated for the age of vaginal patency, beginning on day 27 postpartum. Male mice were evaluated for the age of preputial separation, beginning on day 27 postpartum.

Beginning at day 23 ±1 postpartum, one male rat and one female mouse from each litter, where possible, were evaluated (b) (4)

(b) (4)

[redacted]

Each mouse was tested twice. The test sessions were separated by a one-week interval, and the criterion is the same for both days of testing. Dosage groups were compared for the following dependent measures: the number of trials to the criterion in the first session (this measure was used to compare groups for overall learning performance), the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the first test session (this measure was used to compare groups for activity levels and exploratory tendencies in a novel environment), the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 2 in the first test session (this measure was used to compare groups for short-term retention, the number of trials to the criterion in the second test session) (this measure was used to compare groups for long-term retention) and the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the second session (this value was another indication of long-term retention).

At approximately 90 days of age, the F1 generation mice within each dosage group were assigned to cohabitation, one male mouse per female mouse, based on a random unit table, with the exclusion of sibling matings. The cohabitation period consisted of a maximum of 6 days. All female mice were removed from cohabitation daily. Female mice not observed to have had a copulatory plug in situ were returned to cohabitation in the evening. Female mice with a copulatory plug observed in situ were considered to be at GD 0 and assigned to individual housing.

Parameters and endpoints evaluated:
Clinical signs: Daily.
Body weight: Weekly.
Food consumption: Daily.
Terminal Examination:
After completion of the 21-day postpartum period, F0 generation female mice assigned to the main study were sacrificed and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites were recorded. All pups culled on DL 21 were sacrificed and examined for gross lesions. Necropsy included a single cross section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for hydrocephaly.

Gross Necropsy
F0 Generation Dams: Following completion of milk sample collection on DL 14, mice assigned for TK evaluation were sacrificed and plasma was collected. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites

were recorded. Gross lesions were retained in neutral buffered 10% formalin for possible future evaluation.

After completion of the 21-day postpartum period, F0 generation female mice assigned to the main study were sacrificed, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites ware recorded. Gross lesions were retained in neutral buffered 10% formalin for possible future evaluation.

Mice that did not deliver a litter were sacrificed on GD 23 and examined for gross lesions. The uterus was examined while being pressed between glass plates to confirm the absence of implantation sites.

Dams with no surviving pups were sacrificed after the last pup was found dead or missing, presumed cannibalized. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites were recorded.

Mice that died were examined for the cause of death on the day the observation was made. The mice were examined for gross lesions. Pregnancy status and uterine contents of female mice were recorded. Delivered pups were examined. Uteri of apparently nonpregnant mice were examined while being pressed between glass plates to confirm the absence of implantation sites.

F1 Generation Litters/Mice:
Surviving male mice were sacrificed after completion of the 14-day cohabitation period. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Testes and epididymides of male mice were excised and paired organ weights were recorded. The epididimides were retained in neutral buffered 10% formalin. The testes were fixed in Bouin's solution for 48 to 96 hours and then retained in neutral buffered 10% formalin.

Surviving female mice were sacrificed on GD 18, Caesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Female mice without a confirmed date of mating were sacrificed on an estimated GD 16. Uteri of apparently nonpregnant mice were examined while being pressed between glass plates to confirm the absence of implantation sites. Gross lesions were retained in neutral buffered 10% formalin for possible future evaluation.

The mice were examined for number and distribution of corpora lutea, implantation sites, placentae that appeared abnormal (size, color or shape), live and dead fetuses (a live fetus was defined as one that responded to stimuli; a dead fetus was defined as a term fetus that did not respond to stimuli and that was not markedly autolyzed; dead fetuses demonstrating marked to extreme autolysis were considered to be late resorptions), and early and late resorptions (a conceptus was defined as a late resorption if it was grossly evident that organogenesis had occurred; if this was not the case, the conceptus was defined as an early resorption). Each fetus was weighed and examined for sex and gross external alterations. Live fetuses were sacrificed by an intraperitoneal injection (b) (4) Fetuses were tagged with identification noting study number, litter number, uterine distribution and fixative, and retained for possible future evaluation. Approximately one-half of the fetuses in each litter were retained in Bouin's solution; the remaining fetuses were retained in alcohol.

Mice that died or were sacrificed because of moribund condition or delivery were examined for the cause of death or moribund condition on the day the observation was made. The mice were examined for gross lesions. Testes and epididymides of male mice were excised and paired organ weights were recorded. The epididymides were retained in neutral buffered 10% formalin. The

testes were fixed in Bouin's solution for 48 to 96 hours and then retained in neutral buffered 10% formalin. Pregnancy status and uterine contents of female mice were recorded. Delivered fetuses were examined to the extent possible, using the same methods described for term fetuses.

Toxicokinetics: 3 dams/group. Approximately 1 hr after administration of the first dose on DL 14, milk samples were collected. The dams were sacrificed following completion of milk collection. Following sacrifice, blood samples were collected from each dam for analysis of AC2993 concentrations.

Results

F_0:

Mortality: Main Study Group

Dose (µg/kg/day)	0	6	68	760
Deaths	0/25	1/25 (LD 11)	1/25 (LD 16)	1/25 (LD 1)

1/25 HD mice died while delivering a litter. Sponsor stated that the death may be related to administration of the test article because it occurred in the HD group and the other mice in this dose group had increased incidences of stillbirths and pup deaths on day 1 of lactation. Although the cause of death could not be determined, the deaths in the 6 and 68 µg/kg/day dosage groups were considered treatment-related. All tissues appeared normal at necropsy.

TK Group

Dose (µg/kg day)	0	6	68	760
Deaths	2/3	0/3	0/3	0/3

Sponsor stated that the mice in the control group died during milk collection on day 14 of lactation.

Clinical signs: There were no treatment-related clinical signs.

F0 GENERATION FEMALE MICE BODY WEIGHTS (g)

GESTATION				
Dose (µg/kg day)	**0**	**6**	**68**	**760**
GD 6	30.6	30.0	30.2	30.2
GD 18	55.6	53.5	53.1	50.6**
Wt. gain	25	23.5	22.9	20.4
% Decrease in wt. gain	0	6	12	18

** p<0.01

LACTATION				
Dose (µg/kg day)	**0**	**6**	**68**	**760**
DL 1	35.4	35.3	34.8	34.9
DL 21	40.8	42.6	43.9	43.9
Wt. Gain	5.4	7.3	9.1**	9.0*
% Increase in wt. gain	0	135	169	167

* p<0.05; ** p<0.01

Food consumption: g/day

GESTATION				
Dose (µg/kg day)	**0**	**6**	**68**	**760**
Days 6 - 9	6.5	6.0	6.1	6.1
Days 15 - 18	8.7	8.1	7.7**	7.7**
Gain in food intake	2.2	2.1	1.6	1.6
% decrement in food intake	0	5	27	27

** p<0.01

LACTATION				
Dose (µg/kg day)	**0**	**6**	**68**	**760**
Days 1 - 4	13.9	13.0	12.2*	11.2**
Days 10-14	20.3	19.7	18.5	17.1**
Gain in food intake	6.4	6.7	6.3	5.9
% decrement in food intake	0	0	1.6	7.8

* p<0.05; ** p<0.01

Mating/Fertility Data: F0 Generation Female Mice.

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DOSAGE GROUP DOSAGE (MCG/KG/DAY) a		I 0 (VEHICLE)	II 6	III 68	IV 760
MICE ASSIGNED TO NATURAL DELIVERY	N	28	28	28	28
PREGNANT	N(%)	24(87.5)	27(96.4)	25(89.3)	21(75.0)
DELIVERED LITTERS	N(%)	24(100.0)	27(100.0)	25(100.0)	20(95.2)b
INCLUDED IN ANALYSES	N	21c	24c	22c	17c
DURATION OF GESTATION d	MEAN±S.D.	19.4 ± 0.5	19.4 ± 0.5	19.2 ± 0.5	19.5 ± 0.5
IMPLANTATION SITES	N	285	304	258	203
PER DELIVERED LITTER	MEAN±S.D.	13.6 ± 1.7	12.7 ± 2.0	12.3 ± 2.7 [21]e	11.9 ± 2.2
DAMS WITH STILLBORN PUPS	N(%)	0(0.0)	1(4.2)	0(0.0)	4(23.5)**
DAMS WITH NO LIVEBORN PUPS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
GESTATION INDEX f	%	100.0	100.0	100.0	100.0
	N/N	21/ 21	24/ 24	22/ 22	17/ 17
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	2(11.8)**
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 6 of gestation through day 20 of lactation.
b. Excludes values for mouse 205, which was found dead on day 18 of gestation before completion of delivery.
c. Excludes values for mice that were assigned to toxicokinetic evaluation.
d. Calculated as the time (in days) elapsed between confirmed mating (arbitrarily defined as day 0) and the time (in days) the first pup was delivered.
e. Excludes a value that was not recorded.
f. Number of mice with live offspring/number of pregnant mice.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

F_0 necropsy: Unremarkable.

F_1 physical development:

Litter Observations (Naturally delivered pups) – F1 Generation Litters

DOSAGE GROUP DOSAGE (MCG/KG/DAY)a		I 0 (VEHICLE)	II 6	III 68	IV 760
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	21b	24b	22b	17b
PUPS DELIVERED (TOTAL)	N	256	281	249	183
	MEAN±S.D.	12.2 ± 1.7	11.7 ± 2.4	11.3 ± 2.8	10.8 ± 2.1
LIVEBORN	MEAN±S.D.	12.2 ± 1.7	11.7 ± 2.4	11.3 ± 2.8	9.9 ± 3.3
	N(%)	256(100.0)	280(99.6)	249(100.0)	169(92.3)**
STILLBORN	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.6 ± 1.6
	N(%)	0(0.0)	1(0.4)	0(0.0)	11(6.0)**
UNKNOWN VITAL STATUS	N	0	0	0	3
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED					
DAY 1	N/N(%)	0/256(0.0)	1/280(0.4)	0/249(0.0)	5/169(3.0)**
DAYS 2- 4	N/N(%)	3/256(1.2)	9/279(3.2)*	3/249(1.2)	9/164(5.5)**
DAYS 5- 7	N/N(%)	3/253(1.2)	1/270(0.4)	1/246(0.4)	0/155(0.0)
DAYS 8-14	N/N(%)	1/250(0.4)	1/269(0.4)	11/245(4.5)**	0/155(0.0)
DAYS 15-21	N/N(%)	4/249(1.6)	2/268(0.7)	7/234(3.0)	0/155(0.0)
VIABILITY INDEX c	%	98.8	96.4	98.8	91.7
	N/N	253/256	270/280	246/249	155/169**
LACTATION INDEX d	%	96.8	98.5	92.3	100.0
	N/N	245/253	266/270	227/246**	155/155

DAY(S) = DAY(S) POSTPARTUM
a. Dosage occurred on day 6 of gestation through day 20 of lactation.
b. Excludes values for mice that were assigned to toxicokinetic evaluation.
c. Number of live pups on day 4 postpartum/Number of liveborn pups on day 1 postpartum.
d. Number of live pups on day 21 postpartum/Number of live pups on day 4 postpartum.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Litter Observations (Naturally delivered pups) – F1 Generation Litters

DOSAGE GROUP DOSAGE (MCG/KG/DAY)a		I 0 (VEHICLE)	II 6	III 68	IV 760
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	21b	24b	22b	17b
SURVIVING PUPS/LITTER c					
DAY 1	MEAN±S.D.	12.2 ± 1.7	11.7 ± 2.4	11.3 ± 2.8	9.9 ± 3.3
DAY 4	MEAN±S.D.	12.0 ± 2.0	11.2 ± 2.4	11.2 ± 2.6	9.1 ± 3.8**
DAY 7	MEAN±S.D.	11.9 ± 1.9	11.2 ± 2.4	11.1 ± 2.6	9.1 ± 3.8*
DAY 14	MEAN±S.D.	11.8 ± 1.8	11.3 ± 2.4 [23]d	10.6 ± 2.8	9.1 ± 3.8*
DAY 21	MEAN±S.D.	11.7 ± 1.9	11.2 ± 2.4 [23]d	10.2 ± 3.4 [21]d	9.1 ± 3.8
PERCENT MALE PUPS PER NUMBER OF PUPS SEXED					
DAY 1	MEAN±S.D.	47.6 ± 13.1	48.7 ± 16.5	48.3 ± 16.6	54.7 ± 20.9
DAY 4	MEAN±S.D.	47.2 ± 13.2	49.3 ± 17.2	48.1 ± 16.7	56.5 ± 21.6 [15]e
DAY 7	MEAN±S.D.	47.4 ± 13.2	49.1 ± 17.4	48.2 ± 16.6	56.5 ± 21.6 [15]e
DAY 14	MEAN±S.D.	47.6 ± 13.4	48.1 ± 17.4 [23]d	49.5 ± 16.7	56.5 ± 21.6 [15]e
DAY 21	MEAN±S.D.	46.8 ± 13.4	47.8 ± 17.2 [23]d	50.6 ± 16.2 [20]d,e	56.5 ± 21.6 [15]e

[ ] = NUMBER OF VALUES AVERAGED
DAY = DAY POSTPARTUM
a. Dosage occurred on day 6 of gestation through day 20 of lactation.
b. Excludes values for mice that were assigned to toxicokinetic evaluation.
c. Average number of live pups per litter, including litters with no surviving pups.
d. Excludes values for mice that were found dead.
e. Excludes values for dams that had no surviving pups.

Litter Observations (Naturally delivered pups) – F1 Generation Litters

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCG/KG/DAY)a		0 (VEHICLE)	6	68	760
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	21b	24b	22b	17b
LIVE LITTER SIZE AT WEIGHING					
DAY 1	MEAN±S.D.	12.2 ± 1.7	11.6 ± 2.4	11.3 ± 2.8	10.9 ± 1.8 [15]c
DAY 4	MEAN±S.D.	12.0 ± 2.0	11.2 ± 2.4	11.2 ± 2.6	10.3 ± 1.8 [15]c
DAY 7	MEAN±S.D.	11.9 ± 1.9	11.2 ± 2.4	11.1 ± 2.6	10.3 ± 1.8 [15]c
DAY 14	MEAN±S.D.	11.8 ± 1.8	11.3 ± 2.4 [23]d	10.6 ± 2.8	10.3 ± 1.8 [15]c
DAY 21	MEAN±S.D.	11.7 ± 1.9	11.2 ± 2.4 [23]d	10.8 ± 2.6 [20]c,d	10.3 ± 1.8 [15]c
PUP WEIGHT/LITTER (GRAMS)					
DAY 1	MEAN±S.D.	1.6 ± 0.1	1.6 ± 0.2	1.6 ± 0.2	1.3 ± 0.1** [15]c
DAY 4	MEAN±S.D.	2.5 ± 0.3	2.4 ± 0.3	2.3 ± 0.4	2.0 ± 0.3** [15]c
DAY 7	MEAN±S.D.	3.9 ± 0.5	3.7 ± 0.6	3.5 ± 0.6*	3.2 ± 0.4** [15]c
DAY 14	MEAN±S.D.	6.5 ± 1.3	6.3 ± 1.0 [23]d	5.8 ± 1.4	5.8 ± 0.8 [15]c
DAY 21	MEAN±S.D.	8.9 ± 2.4	9.0 ± 1.9 [23]d	8.4 ± 2.2 [20]c,d	7.8 ± 1.5 [15]c

[ ] = NUMBER OF VALUES AVERAGED
DAY = DAY POSTPARTUM
a. Dosage occurred on day 6 of gestation through day 20 of lactation.
b. Excludes values for mice that were assigned to toxicokinetic evaluation.
c. Excludes values for dams that had no surviving pups.
d. Excludes values for mice that were found dead.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Clinical Signs: Clinical observation from birth to day 21 postpartum

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	6	68	760
LITTERS EXAMINED (N)		21b	24b	22b	17b
TRANSIENT CLINICAL OBSERVATIONS: c		TOTAL FREQUENCY (DAYS X PUPS)/LITTERS WITH OBSERVATIONS			
COLD TO TOUCH	N/N	12/2	1/1	13/5	3/3
CHEST: SCAB	N/N	0/0	0/0	0/0	7/1
PORTION OF TAIL BLACK	N/N	13/1	39/1	7/1	3/1

a. Dosage occurred on day 6 of gestation through day 20 of lactation.
b. Excludes values for mice that were assigned to toxicokinetic evaluation.
c. Tabulation restricted to adverse observations; all other pups appeared normal.

F_1 necropsy:
F1 Generation pups (Preweaning).

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MCG/KG/DAY)a		0 (VEHICLE)	6	68	760
LITTERS EXAMINED	N	21	24	21b	17
TOTAL PUPS STILLBORN OR FOUND DEAD c,d	N	2	5	10**	10**
STILLBORN	N	0	1	0	7**
FOUND DEAD	N	2	4	10**	3
NO MILK IN STOMACH e	N(%)	2(100.0)	3(75.0)	10(100.0)	1(33.3)**
PUPS SACRIFICED AND NECROPSIED ON DAY 7 OR 21 POSTPARTUM d					
LITTERS EVALUATED	N	21	23	20	15
PUPS EVALUATED	N	196	208	165	101
APPEARED NORMAL					
LITTER INCIDENCE	N(%)	21(100.0)	23(100.0)	20(100.0)	15(100.0)
PUP INCIDENCE	N(%)	196(100.0)	208(100.0)	165(100.0)	101(100.0)

a. Dosage occurred on day 6 of gestation through day 20 of lactation.
b. Excludes values for litter 157; pup necropsy observations were not recorded.
c. Restricted to pups in which complete necropsies were performed. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded evaluation.
d. Refer to the individual pup clinical observation table (Table B24) for external observations confirmed at necropsy.
e. Analysis restricted to pups found dead and necropsied.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Cesarean-Sectioning Observations – F1 Generation Female Mice

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MCG/KG/DAY)		0 (VEHICLE)	6	68	760
MICE TESTED	N	24a	25	25	24b
PREGNANT	N(%)	19(79.2)	22(88.0)	24(96.0)	22(91.7)
DELIVERED	N(%)	0(0.0)	0(0.0)	1(4.0)	0(0.0)
MICE PREGNANT AND CAESAREAN-SECTIONED ON DAY 18 OF GESTATION	N	18c,d	22c	21d	21d
CORPORA LUTEA	MEAN±S.D.	15.3 ± 1.6	16.0 ± 1.9	15.1 ± 2.0	15.4 ± 2.5
IMPLANTATIONS	MEAN±S.D.	14.4 ± 1.7	14.7 ± 1.9	14.1 ± 1.4	14.3 ± 2.1
LITTER SIZES	MEAN±S.D.	13.0 ± 1.9	13.5 ± 1.8	12.5 ± 2.6	13.2 ± 2.5
LIVE FETUSES	N	235	296	259	277
	MEAN±S.D.	13.0 ± 1.9	13.4 ± 1.8	12.3 ± 2.6	13.2 ± 2.5
DEAD FETUSES	N	0	1	4	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.2	0.2 ± 0.7	0.0 ± 0.0
RESORPTIONS	MEAN±S.D.	1.3 ± 1.4	1.2 ± 1.3	1.6 ± 2.0	1.1 ± 1.4
EARLY RESORPTIONS	N	19	22	30	21
	MEAN±S.D.	1.0 ± 1.2	1.0 ± 1.3	1.4 ± 1.9	1.0 ± 1.3
LATE RESORPTIONS	N	5	4	3	2
	MEAN±S.D.	0.3 ± 0.6	0.2 ± 0.5	0.1 ± 0.4	0.1 ± 0.3
MICE WITH ANY RESORPTIONS	N(%)	12(66.7)	13(59.1)	13(61.9)	12(57.1)
MICE WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
MICE WITH VIABLE FETUSES	N(%)	18(100.0)	22(100.0)	21(100.0)	21(100.0)
PLACENTAE APPEARED NORMAL	N(%)	18(100.0)	22(100.0)	21(100.0)	21(100.0)

a. Excludes values for mouse 425, which was found dead on day 2 postweaning.
b. Excludes values for mouse 477, which delivered a litter on day 37 postweaning (prior to cohabitation).
c. Includes values for mice that did not have a confirmed mating date.
d. Excludes values for mice with unilateral pregnancies consisting of four or less conceptuses.

For peri-postnatal development studies:

In-life observations: Offspring

Mortality: F1 Generation (Postweaning):

Dose (µg/kg/d)	**0**		**6**		**68**		**760**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality	**2/25**	**1/25**	**1/25**		**1/25**		**1/25**	**1/25**
Moribund sacrifice							1/25 (PWD 2)	1/25 (DG 6)
Found Dead	1/25 (PWD 2)	1/25 (PWD 2)	1/25 (PWD 29)		1/25 (PWD 25)			
Accidental Death	1/25 (PWD 9)							
Emaciation								1/25
Dehydration								1/25
Tissues Appeared Normal	25/25	25/25	25/25	25/25	25/25	24/25	25/25	25/25

(PWD) = Day Postweaning on which death occurred; (DG) = Day of gestation on which death occurred

Clinical signs:

Dose (µg/kg/d)	**0**		**6**		**68**		**760**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Bent tail	5/25	2/25	4/25	2/25	7/25	2/25	9/25	5/25

Body weight: (g) Postweaning – Males *** p < 0.05; ** p < 0.01**

Dose (µg/kg/d)	**0**	**6**	**68**	**760**
Day 1	11	11	10	9*
Precohabitation	37	37	37	34**
Terminal body wt.	37	37	37	35*
% Decrement in body wt	0	0	0	5

For males, absolute and relative weights of the testes and epididymides in treated groups were not significantly different from those of control.

F1 Generation – Female body wt. (g)

Dose (µg/kg/d)	0	6	68	760
Day 1	10	10	9	9
Precohabitation	30	30	29	28**
Gestation Day 0	30	30	29	28**
Gestation Day 18	65	64	61*	62*
% Decrement in body wt	0	2	6	5

*** p < 0.05; ** p < 0.01**

Food Consumption: No data.

F1 Generation Sexual Maturation: Average number of days postpartum that the prepuce was observed to be separated or the vagina patent.

Dose (µg/kg/d)	0	6	68	760
Preputial Separation	29	30	31	31
Vaginal patency	32	32	32	32

F_1 behavioral evaluation:

F1 Generation: Passive Avoidance Performance

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MCG/KG/DAY)		0 (VEHICLE)	6	68	760
MALE MICE:					
SESSION 1a	N	21	22	20	14b
TRIALS TO CRITERION	MEAN±S.D.	3.6 ± 1.1	4.3 ± 1.1	4.4 ± 1.4	4.5 ± 1.4
LATENCY TRIAL 1c	MEAN±S.D.	14.5 ± 14.1	8.4 ± 4.7	14.2 ± 17.1	12.9 ± 16.6
LATENCY TRIAL 2c	MEAN±S.D.	48.7 ± 18.5	39.6 ± 22.5	44.7 ± 23.8	36.4 ± 23.7
FAILED TO LEARN d	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2a	N	19e	21b	20	14b
TRIALS TO CRITERION	MEAN±S.D.	2.9 ± 0.9	3.3 ± 1.6	2.6 ± 0.8	3.2 ± 2.3
LATENCY TRIAL 1c	MEAN±S.D.	41.4 ± 25.4	35.8 ± 25.6	43.9 ± 25.3	39.1 ± 25.5
FEMALE MICE:					
SESSION 1a	N	21	23	19b	14
TRIALS TO CRITERION	MEAN±S.D.	4.0 ± 0.9	3.9 ± 1.2	3.9 ± 0.8	4.0 ± 1.2
LATENCY TRIAL 1c	MEAN±S.D.	9.6 ± 13.5	10.0 ± 8.3	9.1 ± 8.4	9.3 ± 15.1
LATENCY TRIAL 2c	MEAN±S.D.	41.7 ± 24.3	45.0 ± 22.0	37.0 ± 25.2	38.2 ± 24.9
FAILED TO LEARN d	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2a	N	20e	23	19b	14
TRIALS TO CRITERION	MEAN±S.D.	2.9 ± 0.8	2.9 ± 0.7	3.1 ± 1.4	2.6 ± 1.1
LATENCY TRIAL 1c	MEAN±S.D.	44.7 ± 23.0	29.6 ± 26.2	35.3 ± 25.9	43.8 ± 23.0

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. Excludes values for mice that were tested to the incorrect criteria.
c. The latency was recorded in seconds.
d. Number of mice that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these mice were excluded from group averages and statistical analyses.
e. Excludes values for mice that were found dead or had an accidental death.

F_1 reproduction:

F1 Generation: Mating and Fertility – Male Mice

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MCG/KG/DAY)		I 0 (VEHICLE)	II 6	III 68	IV 760
MICE IN COHABITATION	N	23a	24a	24a	24a
DAYS IN COHABITATION b	MEAN±S.D.	2.4 ± 1.3 [22]	2.2 ± 1.5 [23]	2.3 ± 1.2 [23]c	2.1 ± 1.2
MICE THAT MATED d	N(%)	23(100.0)	24(100.0)	24(100.0)	24(100.0)
FERTILITY INDEX e,f	N/N (%)	18/23 (78.3)	21/24 (87.5)	23/24 (95.8)	22/24 (91.7)
MICE WITH CONFIRMED MATING DATES	N	22	23	23c	24
MATED WITH FIRST FEMALE g DAYS 1-7	N(%)	22(100.0)	23(100.0)	23(100.0)c	24(100.0)
MICE PREGNANT/MICE IN COHABITATION g	N/N (%)	18/23 (78.3)	21/24 (87.5)	23/24 (95.8)	22/24 (91.7)

[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for mice that were found dead, moribund sacrificed or had an accidental death.
b. Restricted to mice with a confirmed mating date and mice that did not mate.
c. Excludes values for mouse 362; the mating date was incorrectly identified.
d. Includes only one mating for each male mouse.
e. Number of pregnancies/number of mice that mated.
f. Includes only one pregnancy for each mouse that impregnated more than one female mouse.
g. Restricted to mice with a confirmed mating date.

F_2 findings:

Litter Observations (Cesarean-Delivered Fetuses) – F2 Generation Litters

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MCG/KG/DAY)		I 0 (VEHICLE)	II 6	III 68	IV 760
LITTERS WITH ONE OR MORE LIVE FETUSES	N	18a,b	22a	21b	21b
IMPLANTATIONS	MEAN±S.D.	14.4 ± 1.7	14.7 ± 1.9	14.1 ± 1.4	14.3 ± 2.1
LIVE FETUSES	N	235	296	259	277
	MEAN±S.D.	13.0 ± 1.9	13.4 ± 1.8	12.3 ± 2.6	13.2 ± 2.5
LIVE MALE FETUSES	N	118	148	136	147
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	50.2 ± 14.6	50.3 ± 13.3	51.9 ± 17.2	52.8 ± 15.8
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	1.36 ± 0.08 [17]c	1.32 ± 0.09 [21]c	1.34 ± 0.09	1.38 ± 0.10
MALE FETUSES	MEAN±S.D.	1.39 ± 0.10 [17]c	1.35 ± 0.10 [21]c	1.35 ± 0.10	1.40 ± 0.11
FEMALE FETUSES	MEAN±S.D.	1.33 ± 0.07 [17]c	1.30 ± 0.08 [21]c	1.33 ± 0.09	1.36 ± 0.08
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	9.1 ± 9.8	8.0 ± 8.5	12.9 ± 14.6	7.9 ± 9.6

[ ] = NUMBER OF VALUES AVERAGED
a. Includes values for litters in which the mouse did not have a confirmed mating date.
b. Excludes values for litters that were unilateral pregnancies consisting of four or less conceptuses.
c. Excludes values for litters in which the mouse did not have a confirmed mating date.

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Fetal Gross External Alterations – F2 Generation Litters/Fetuses

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MCG/KG/DAY)		0 (VEHICLE)	6	68	760
LITTERS EVALUATED	N	19a,b	22a	23b,c	22b
FETUSES EVALUATED	N	237	297	268	279
LIVE	N	237	296	264	279
DEAD	N	0	1d	4d	0
HEAD: EXENCEPHALY					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.4)e	0(0.0)
EYES: LID, OPENED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.4)e	0(0.0)
SNOUT: CLEFT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.4)e	0(0.0)
PALATE: CLEFT					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.3)	0(0.0)	0(0.0)
TAIL: TIP, FORKED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.5)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.4)
LEFT HINDLIMB: FLEXED DOWNWARD					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.5)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.4)

a. Includes values for litters in which the mouse did not have a confirmed mating date.
b. Includes values for litters which were unilateral pregnancies consisting of four or less conceptuses.
c. Excludes values for litter 462; the mouse had an incorrectly identified mating date.
d. Dead fetuses were excluded from group averages and statistical analyses.
e. Fetus 461-6 had other gross external alterations.

Toxicokinetics:

Mean AC2993 Concentration (pg/ml) in Plasma and Milk

Dose (µg/kg/d)	ANIMAL #	PLASMA	MILK
0	107	< Low Std	652.1
	106	< Low Std	782.9
	110	< Low Std	187.5
6	139	< Low Std	1,031.3
	145	< Low Std	952.0
	146	1,658.4	871.3
68	170	8,072.5	979.1
	171	6,594.4	1,322.4
	175	7,357.8	75,693.0
760	196	163,982.5	3,871.0
	198	259,995.0	7,207.8
	201	161,001.0	5,882.0

Please note that AC2993 was observed in the milk of vehicle control animals that were not dosed with the drug. Sponsor did not offer any explanation as to how this might have occurred.

2.6.6.7 Local tolerance

Local tolerance of exenatide was conducted as part of the pivotal repeat-dose studies with durations in mice up to 182 days, rats up to 28 days, and monkeys up to 273 days. Local tolerance was evaluated by clinical observations and morphologic pathology of injection sites. A tabulated summary of local tolerance studies and effects observed are provided in the following tables.

LOCAL TOLERANCE STUDIES AND RESULTS

Species/ Strain	Method of Administration	Duration of Dosing (Days)	Formulation Control Article/ Test Article	Number per Group (Main Study)	Noteworthy Findings
Mouse/ CRL:CD-1	SC BID rotated through 4 sites	28	AC-2993-F12/ AC-2993-F7 (exenatide compared from 3 manufacturers including Star, Bachem, and Mallinckrodt)	10 M 10 F	No effect due to exenatide. Localized trace to mild inflammatory, hemorrhagic, fibrotic, exudative, and degenerative changes were noted in both vehicle and exenatide-treated groups in a minority of injection sites.
Mouse/ CRL:CD-1	SC BID rotated through 4 sites	91	PBO-F11/ AC-2993-F4	20-21 M 20-21 F	No effect due to exenatide. Localized inflammatory, hemorrhagic, fibrotic, exudative, and degenerative changes were noted among vehicle and exenatide-treated groups given the highest dose volume of 4.9 mL/kg, but not at 0.6 or 1.8 mL/kg.
Mouse/ CRL:CD-1 (ICR) BR	SC BID rotated through 4 sites	182	AC-2993-F12/ AC-2993-F7	20 M+F Vehicle 25 M+F Test Article	No effect due to exenatide. Localized trace to mild inflammatory, hemorrhagic, fibrotic, exudative, and degenerative changes were noted in both vehicle and exenatide-treated groups with a trend of increasing incidence correlating with increasing dose volume (0.9<3.1<4.9 mL/kg).
Rat/ Sprague-Dawley Crl:CD	SC once daily at a single site	28	PBO-F10/ AC-2993-F1, AC-2993-F2	10 M 10 F	No effect due to exenatide. Localized minimal to moderate inflammatory, hemorrhagic, fibrotic, exudative, and degenerative changes changes were noted among vehicle and exenatide-treated groups given the highest dose volume of 1.0 mL/kg, but not at 0.03 or 0.3 mL/kg.

SC = Subcutaneous BID = Dose divided and administered twice daily M = Male F = Female

Species/ Strain	Method of Administration	Duration of Dosing (Days)	Formulation Control Article/ Test Article	Number per Group (Main Study)	Noteworthy Findings
Monkey/ *Macaca fascicularis*	SC once daily rotated through 6 sites	28	PBO-F10/ AC-2993-F1, AC-2993-F2	3 M 3 F	No effect due to exenatide. Localized minimal to moderate inflammatory, hemorrhagic, exudative, and degenerative changes were noted among vehicle and exenatide-treated groups at similar incidence among the vehicle and exenatide-treated groups.
Monkey/ *Macaca fascicularis*	SC BID rotated through 8 sites	91	PBO-F11/ AC-2993-F4	4 M 4 F	No effect due to exenatide. Localized minimal to moderate inflammatory, hemorrhagic, fibrotic, exudative, and degenerative changes were noted among vehicle and exenatide-treated groups at similar incidence among the vehicle and exenatide-treated groups.
Monkey/ *Macaca fascicularis*	SC BID rotated through 6 sites	273	AC-2993-F12/ AC-2993-F7	6 M 6 F	No effect due to exenatide. Localized minimal to moderate inflammatory, hemorrhagic, fibrotic, exudative, and degenerative changes were noted among vehicle and exenatide-treated with a trend of increasing incidence correlating with increasing dose volume (0.02<0.09<0.25 mL/kg).

SC = Subcutaneous BID = Dose divided and administered twice daily M = Male F = Female

Mouse: The local tolerance of exenatide SC injection was assessed by clinical observations and morphologic pathology of the injection sites in repeat-dose toxicity studies with BID dosing over 28, 91, and 182 days duration. Exenatide and vehicle formulations were similar in the three studies but differed in the dilutions employed. Formulations contained 0 or 0.3 mg/ml exenatide in the 28-day study; 0, 0.005,

0.019, or 0.078 mg/ml exenatide in the 91-day study; and 0, 0.01, 0.019, or 0.078 mg/ml exenatide in the 182-day study. Metacresol concentrations were 2.20 mg/ml in the 28-day study, 3.00 mg/ml in the 91-day study and 2.20 mg/ml in the 182-day study. There were no exenatide-related effects on local tolerance in studies up to 182 days duration. There were no exenatide-related changes in clinical observations or morphologic pathology observations; events common to both vehicle- and exenatide-treated groups included the presence of localized redness, alopecia, or exudate. Local microscopic changes in all studies were common to both vehicle- and exenatide-treated groups, consisting of localized, inflammatory, hemorrhagic, fibrotic, exudative, or degenerative changes, or epithelial hyperplasia/acanthosis of trace-to-mild severity. Severity and incidence of the morphologic changes were related to the volume of either vehicle or exenatide injection, which in the 182-day study was up to 4.9 ml/kg/injection. The resulting volume of injection of about 170 µl/injection in a 35 g mouse, is 4.3 times the proposed human dose of 40 µl. The morphologic changes in each study were consistent with the consequences of repeated injection trauma and were not attributed to any exenatide-related effects. The results in the general toxicity studies were replicated in the mouse carcinogenicity study, where no local, exenatide-related effects were noted following once-daily, SC injection for up to 98 weeks.

Rat: The local tolerance of exenatide SC injection was assessed by clinical observations and morphologic pathology of the injection sites in a repeat-dose toxicity study, once-daily injection, of 28 days duration. Formulations contained exenatide concentrations of 0, 0.30, or 1.00 g/l but did not contain metacresol. There were no exenatide-related effects on local tolerance following 28 days of dosing. There were no exenatide-related changes in clinical observations or morphologic pathology; events common to both vehicle- and exenatide-treated groups included the presence of localized redness. Local microscopic changes in all studies were limited to the vehicle and high-dose, exenatide-treated groups, both treated at 1.0 ml/kg/injection. The resulting volume of injection was about 350 µl in a 350-g rat, is 8.8 times the proposed human dose of 40 µl. Microscopic changes across the treatment groups included localized inflammatory, hemorrhagic, fibrotic, exudative, or degenerative changes of minimal to moderate severity. The morphologic changes were the consequences of repeated injection trauma and were not attributed to any exenatide-related effects. The results in the general toxicity studies were replicated in the rat carcinogenicity study, where no local, exenatide-related effects were noted following once-daily, SC injection for up to 2 years.

Monkey: The local tolerance of exenatide SC injection was assessed by clinical observations and morphologic pathology of the injection sites in repeat-dose toxicity studies with once to BID dosing over 28, 91 and 273 days duration. Exenatide and vehicle formulations were similar in the three studies, except for dilution employed and the presence/absence of metacresol. Formulations contained 0, 0.3, or 1.0 mg/ml exenatide in the 28-day study, 0, 0.05, 0.1, or 0.3 mg/ml exenatide in the 91-day study, and 0, 0.05, 0.1, and 0.3 mg/ml exenatide in the 273-day study. Metacresol concentrations were 0.00 mg/ml in the 28-day study, 3.00 mg/ml in the 91-day study, and 2.20 mg/ml in the 273-day study. There were no exenatide-related effects on local tolerance in studies up to 273 days duration. There were no exenatide-related changes in clinical observations or morphologic pathology; events common to both vehicle- and exenatide-treated groups included the presence of localized redness or exudate. Local microscopic changes in all studies were common to both vehicle- and exenatide-treated groups, consisting of localized, inflammatory, hemorrhagic, fibrotic, exudative, or degenerative changes of trace-to-mild severity. Severity and incidence of microscopic changes were mostly related to the volume of either vehicle or exenatide injection, which in the 273-day study were up to 0.25 ml/kg/injection. The resulting volume of injection of about 750 µl in a 3-kg monkey, is 18.8 times the proposed 40-µL human dose. The morphologic changes in each study were consistent with the consequences of repeated injection trauma and were not attributed to any exenatide-related effects.

Conclusion: Exenatide (drug substance) was well tolerated, lacking exenatide-related local effects, when administered as a SC injection in repeat-dose studies of up to 98 weeks in mice once daily, 104 weeks in

rats once daily, and 273 days in monkeys BID. Exenatide formulations (drug products) were well tolerated, with changes limited to those normally expected from repeated injections, when administered as a SC injection in repeat-dose studies of up to 98 weeks in mice once daily, 104 weeks in rats once daily, and 273 days in monkeys BID. Events common to both vehicle- and exenatide-treated groups included the presence of localized redness or exudates. Local microscopic changes in all studies were common to both vehicle- and exenatide-treated groups, consisting of localized, inflammatory, hemorrhagic, fibrotic, exudative, or degenerative changes of trace-to-moderate severity.

2.6.6.8 Special Toxicology Studies - Antigenicity

Study title: **Neutralizing Anti-Exendin-4 Antibody Production in NIH Swiss Mice**

Key study findings:

- The animals treated with exendin-4 showed a consistent drop in plasma glucose levels an hour after IP administration regardless of the duration of weekly treatment with exendin-4, GLP-1, or vehicle.
- There was no reduction in biological activity of exendin-4, as measured by the glucose lowering effect, in any treatment group.
- No measurable anti-exendin-4 antibody titers were established with the treatment of exendin-4 for up to 8 weeks.

Study no.: REST98145

Methods

Species/strain: Mouse/Non-diabetic NIHSw.

Doses employed: Please see study design.

Route of Administration: I. P. injection.

Rationale: Exendin-4 is a synthetic preparation of the natural sequence of the peptide isolated from the salivary secretions of the Gila monster lizard, *Heloderma suspectum*. Because no mammalian homolog of the peptide has been identified (except the glucagon-like peptide 1 i.e. GLP-1 to which it has approximately 50% sequence homology), it has been presumed that the molecule is foreign to mammals and that production of antibody is likely to defeat its therapeutic use. Hence this study was conducted to determine the biological activity of the drug and to determine the presence of anti-exendin and anti GLP-1 antibody by ELISA.

Number of animals/sex/dosing group: Please see study design.

Study design: Mice were deprived of food for 2 hrs, lightly anesthetized and blood was collected to determine baseline plasma glucose, anti-exendin and anti GLP-1 antibody levels. The animals were then injected I.P. with either saline (100 µl), exendin-4 (1 µg/100 µl per animal) or GLP-1 (100 µg/100 µl per animal). One hour after initial glucose sampling and injections, animals are again lightly anesthetized and 50 µl of blood collected for plasma glucose concentration. By this schedule, there was one week after peptide injection before sampling for antibody.

Weeks	Group 1	Group 2	Group 3	Group 4	Activity
0	GLP-1 100 µg	GLP-1 100 µg	Exendin 1 µg	Saline	a
1	GLP-1 100 µg	GLP-1 100 µg	Exendin 1 µg	Saline	b
2	GLP-1 100 µg	GLP-1 100 µg	Exendin 1 µg	Saline	b
3	GLP-1 100 µg	GLP-1 100 µg	Exendin 1 µg	Saline	b
4	GLP-1 100 µg	Exendin 1 µg	Exendin 1 µg	Saline	a
5	GLP-1 100 µg	Exendin 1 µg	Exendin 1 µg	Saline	b
6	GLP-1 100 µg	Exendin 1 µg	Exendin 1 µg	Saline	b
7	GLP-1 100 µg	Exendin 1 µg	Exendin 1 µg	Saline	b
8	GLP-1 100 µg	Exendin 1 µg	Exendin 1 µg	Exendin 1 µg	a
8 + 2 days	Exendin 1 µg	Exendin 1 µg	Exendin 1 µg	Exendin 1 µg	a

a: glucose sample at t=0, antibody at t-0, inject peptide, glucose sample at t=1 hr.
b: inject peptide only.

Results: % change in plasma glucose level, 1 hr after I.P. injection was measured at week 0, week 4, week 8 and week 8+2days for each group of animals.

Weeks	Group 1	Group 2	Group 3	Group 4
0	-2.94 ± 3.50	-2.83 ± 5.38	-12.25 ± 4.18	18.26 ± 4.04
4	-9.36 ± 6.30	-12.40 ± 3.91	-19.25 ± 4.08	2.85 ± 3.35
8	-8.57 ± 4.27	-20.62 ± 3.01	-25.28 ± 3.4	-23.0 ± 2.92
8 + 2 days	-33.75 ± 3.26	-32.94 ± 3.36	-30.67 ± 4.17	-29.3 ± 3.20

Group 1 (treated with GLP-1 for 8 weeks, exendin-4 week 8+2 days)
Group 2 (treated with GLP-1 for 1st 4 weeks, exendin-4 week 5 through 8+2 days)
Group 3 (treated with exendin-4 for 8 weeks)
Group 4 (treated with saline for 7 weeks, exendin-4 on week 8 and 8+2 days)

Glucose Levels: At week 0 the group of animals treated with vehicle (saline) showed an increase in plasma glucose levels 1 hour post-injection (18.3 %) compared to GLP-1 treated groups (-2.9% and -2.8 %) and the exendin-4 treated group (-12.3%). As treatment progressed, animals treated with exendin-4 consistently showed a decrease in plasma glucose level 1 hour after I.P. injection; and this decrease in plasma glucose due to exertdin-4 was seen in all groups of animals, regardless of their treatment during the previous weeks with either GLP-1, combination of GLP-I and exertdin-4, or saline. Thus, there was no reduction in biological action of exendin-4, as measured by the glucose lowering activity, in any treatment group.

Plasma antibody: Plasma antibody titers for anti-exendin-4 activity were also evaluated in all animals using ELISA at week 0, week 4, week 8 and week 8+2 days. Only two animals showed a positive response to a presence of anti-exendin-4 antibody and both of these animals had been treated with GLP-1. None of the animals treated with exendin-4 showed titers for anti-exendin-4 antibody.

Conclusion: Based on the biological response (glucose lowering 1 hour after peptide injection) in the NIH Swiss mice, no diminishing effect is seen over time. The animals treated with exendin-4 showed a consistent drop in plasma glucose levels an hour after peripheral administration regardless of the duration of weekly treatment with exendin-4, GLP-1, or vehicle. In this study, no measurable anti-exendin-4 antibody titers were established with the treatment of exendin-4 for up to 8 weeks.

Study Title: Effects of Anti-AC2993 Antibodies on Plasma Toxicokinetics, Body Weight Changes and Histological Change in Cynomolgus Monkey Administered AC2993 BID by Subcutaneous Injection for 9 Months

Key study findings:

- There was no effect of antibody formation on decreased body weight gain in the treated groups.
- There was no effect of antibody formation on increased pancreas islet cellularity.
- Except for one, monkeys with an antibody titer >125 exhibited a larger plasma exenatide AUC value at sample days 90, 180 and 273 relative to the AUC value on day 1.
- Anti-AC2993 antibodies were not neutralizing with regard to the biological responses evaluated in this study.

Study no.: REST02136
Methods
Species/strain: Monkey/Cynomolgus.
Doses employed: Please see study design.
Route of Administration: Subcutaneous injection.
Rationale: To investigate the potential impact of antibody formation on the toxicokinetics, body weight gains and histological changes in a 9-month toxicity study, where cynomolgus monkeys were exposed to

AC2993 by twice daily subcutaneous. Plasma antibodies to AC2993 were detected using a sensitive ELISA method. Antibody titers were evaluated using plasma samples collected just prior to necropsy after 9 months of dosing.
Number of animals/sex/dosing group: 6/sex/group.
Study design: 6 Monkeys/sex/group were dosed by subcutaneous administration of AC2993 at 1.1, 9 and 75 µg/kg BID for 9 months (273 Days). Prior to necropsy on Day 275, blood samples were collected to determine anti-AC2993 antibodies using ELISA, and evaluation of toxicokinetics. The primary objective was to determine if anti-AC2993 antibodies were formed and whether the antibodies have a neutralizing effect on AC2993 by evaluating the effect of the antibodies on body weight changes, systemic exposure to AC2993 and on pancreas islet cellularity.

Results
Summary of Anti-AC2993 Antibody Results: A summary of the anti-AC2993 antibody results from the ELISA method evaluation of the plasma samples collected prestudy and after 9 months of exposure to AC2993 is shown in Table 1. With the exception of one female (FN15711) in the LD group with a titer of 625, all animals that had SDscore ≥ 3 had titers between 5 and 125.

Table 1: Summary of Assay Results for Anti-AC2993 Antibodies in Cynomolgus Monkeys Exposed to AC2993 for 9 Months

BID Dose of AC2993	Animal Number	Pre-Study SD Score	Pre-Study Titer	Day-275 SD Score	Day-275 Titer
1.1 µg/kg	FN15707M	NEG	ND	3.0	5
1.1 µg/kg	FN15710M	NEG	ND	NEG	ND
1.1 µg/kg	FN15734M	NEG	ND	48.8	125
1.1 µg/kg	FN15735M	NEG	ND	NEG	ND
1.1 µg/kg	FN15737M	43.8	25	142.8	125
1.1 µg/kg	FN15746M	NEG	ND	22.6	25
1.1 µg/kg	FN14937F	NEG	ND	147.2	125
1.1 µg/kg	FN15711F	NEG	ND	191.0	625
1.1 µg/kg	FN15715F	NEG	ND	NEG	ND
1.1 µg/kg	FN15728F	NEG	ND	114.2	125
1.1 µg/kg	FN15729F	44.8	25	41.2	25
1.1 µg/kg	FN15742F	4.2	5	3.0	5
9 µg/kg	FN15708M	NEG	ND	50.3	25
9 µg/kg	FN15709M	17.8	5	16.1	25
9 µg/kg	FN15722M	NEG	ND	80.1	125
9 µg/kg	FN15733M	NEG	ND	29.3	25
9 µg/kg	FN15740M	NEG	ND	66.1	125
9 µg/kg	FN15741M	NEG	ND	170.7	125
9 µg/kg	F4288CQF	NEG	ND	7.0	5
9 µg/kg	FN15703F	NEG	ND	NEG	ND
9 µg/kg	FN15713F	NEG	ND	73.5	125
9 µg/kg	FN15723F	NEG	ND	NEG	ND
9 µg/kg	FN15726F	NEG	ND	6.7	5
9 µg/kg	FN15731F	NEG	ND	NEG	ND
75 µg/kg	FN14300M	NEG	ND	NEG	ND
75 µg/kg	FN15702M	NEG	ND	50.3	125
75 µg/kg	FN15705M	NEG	ND	28.0	25
75 µg/kg	FN15714M	NEG	ND	42.5	125
75 µg/kg	FN15736M	NEG	ND	NEG	ND
75 µg/kg	FN15744M	NEG	ND	3.9	5
75 µg/kg	FN14007F	NEG	ND	8.2	5
75 µg/kg	FN14015F	NEG	ND	28.0	25
75 µg/kg	FN15701F	NEG	ND	NEG	ND
75 µg/kg	FN15712F	NEG	ND	NEG	ND
75 µg/kg	FN15717F	7.6	>25	6.7	5
75 µg/kg	FN15718F	NEG	ND	34.2	25

NEG = SDscore <3 or less, ND = not determined since SDscore <3
Titer = Greatest serial dilution (1:5, 1:125: 1:625, etc.) of plasma that was made with measurable SDscore ≥3

Summary of Toxicokinetics: As part of the chronic monkey study, timed, serial plasma samples were collected after the morning dose on days 1, 90, 180 and 273. They were assayed for AC2993 concentration using a validated immunoenzymetric assay (IEMA). In summary, the mean AUC values for all animals within a dose group increased with the number of doses received.
Interpretation of Toxicokinetics with Consideration for Anti-AC2993 Antibodies: Some individual animals exhibited an increase in AUC values at day 90, 180 and/or 273 relative to the day-1 AUC values

while others did not appear to increase with length of exposure to AC2993. Therefore, animals were grouped by visual inspection into two groups: 1) those with a plasma profile of AC2993 concentration vs. time which appeared to give a constant AUC over the study (i.e., AUC on days 1, 90, 180 and 273 approximately equal) or 2) those with a plasma profile of AC2993 concentration vs. time which appeared to be greater at days 90, 180 and/or 273 than at day 1. Toxicokinetic graphs for individual animals by dose group were provided but not include in this review. The graphs were evaluated with regard to the end-of-the-study titer values of the animals. All but two animals [FN15705M (titer = 25) and FN14015F (titer = 25) both at 150 µg/kg/day] that were judged to have had an increase in AUC values over time exhibited antibody titers ≥125. Only one animal (FN15702M at 150 µg/kg/day) had a titer of 125, but no change in AUC over time. The relationship of titer to changes in AUC values are summarized by dose group and sex in Table 2. Ten of eleven animals with a titer ≥125 exhibited a change in AUC value over time. Twenty-three of twenty-five animals with a titer <125 did not exhibit a change in AUC value over time. Thus, the antibody titer correlated with a change or lack of change in AUC value over the length of dose administration in 33 of 36 animals.

Table 2: Summary of the Relationship between Change in Plasma AUC Values With Length of Dose Administration in Monkeys and the Assay Results for Anti-AC2993 Antibody Titers

Dose (µg/kg/day) & Sex	Anti-AC2993 Antibody ELISA Results Titer ≥125		Anti-AC2993 Antibody ELISA Results Titer <125	
	Number of Monkeys With Change in AUC[1]	Number of Monkeys With No Change in AUC[2]	Number of Monkeys With Change in AUC[1]	Number of Monkeys With No Change in AUC[2]
2.2 Males	2	0	0	4
2.2 Females	3	0	0	3
18 Males	3	0	0	3
18 Females	1	0	0	5
150 Males	1	1	1	3
150 Females	0	0	1	5

[1] Change in AUC means the plasma AUC for AC2993 was judged to be greater on samples days 90, 180 and/or 273 that on day 1
[2] No Change in AUC means the plasma AUC for AC2993 was judged not to be greater on samples days 90, 180 and/or 273 that on day 1

Definition of Antibody Positive: With one exception, animals with an antibody titer >125 exhibited a larger plasma AC2993 AUC value at sample days 90, 180 and/or 273 relative to the AUC value on day 1. Based on this evaluation, an antibody titer >125 caused a change in plasma pharmacokinetics, probably by slowing renal clearance due to increased plasma protein binding. This is considered a "positive" titer for causing a measurable pharmacokinetic change in the animals.

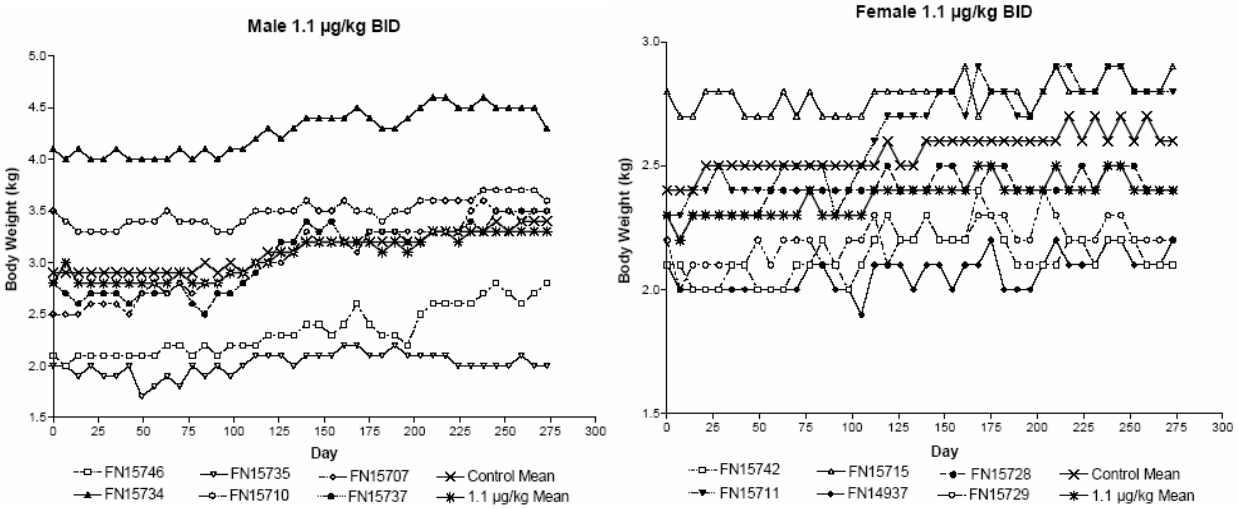
AUC DATA From Animals That Were Judged To Have No Change Over Time

BID Dose (µg/kg)	Day	AUC			AUC ÷ Dose		
		AUC Mean ± SD	N	SEM	AUC/DOSE Mean ± SD	N	SEM
1.1	1	5682 ± 2771	7	1047	5165 ± 2519	7	952
	90	5419 ± 1998	7	755	4926 ± 1816	7	687
	180	7135 ± 3521	7	1331	6486 ± 3201	7	1210
	273	6848 ± 2720	7	1028	6225 ± 2472	7	934
9	1	58707 ± 10860	7	4105	6523 ± 1207	7	456
	90	47572 ± 26046	8	9209	5286 ± 2894	8	1023
	180	57740 ± 27247	8	9634	6416 ± 3027	8	1070
	273	53493 ± 19732	8	6976	5944 ± 2192	8	775
75	1	501199 ± 121917	9	40639	6683 ± 1626	9	542
	90	560983 ± 302266	9	100755	7480 ± 4030	9	1343
	180	489431 ± 200486	9	66829	6526 ± 2673	9	891
	273	460514 ± 236938	9	78979	6140 ± 3159	9	1053

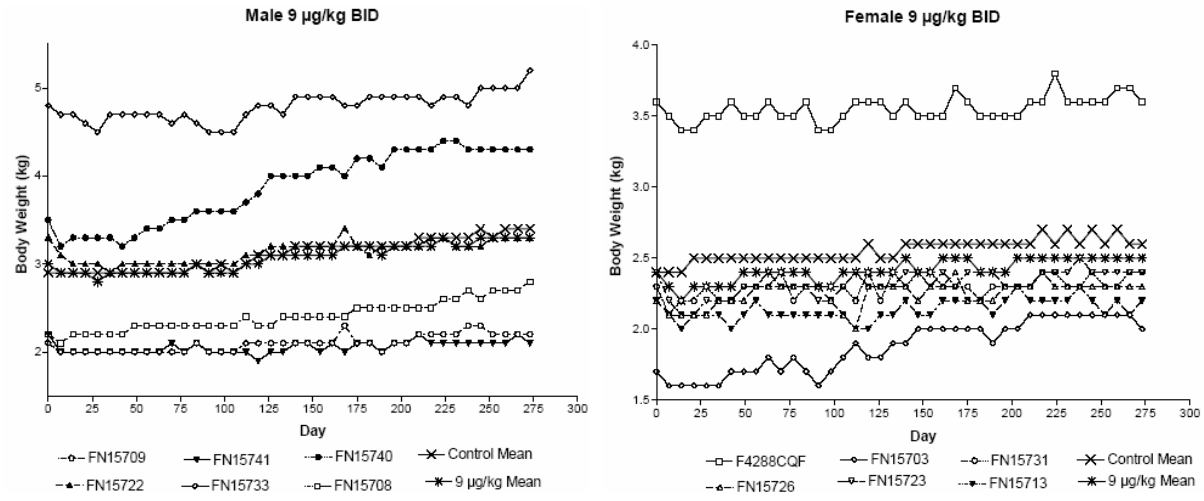
Effect of Anti-AC2993 Antibody Formation on Body Weights: Antibodies formed against a peptide may neutralize the biological activity of the peptide. In the 9-month toxicity study of AC2993, the changes in

body weight over time of the animals appeared to be effected by AC2993 (Figures 1 - 6). An evaluation was done on individual animals to determine if the formation of anti-AC2993 antibodies caused a change in the pattern of depressed body weight gains that would indicate a neutralization of this biological expression of AC2993 activity. Data related to body weight are shown in Figures 1 - 6. In the group of male monkeys administered 1.1 µg/kg BID (Figure 1), monkey FN15737 (titer = 125) exhibited an increase in body weight starting approximately day 100. In contrast monkey FN15734 (titer = 125) did not change his pattern of weight gain. In females administered 1.1 µg/kg BID (Figure 2), monkey FN15711 (titer = 625), exhibited an increase in body weight around day 100, while monkeys FN15728 (titer = 125) and FN14937 (titer = 125) did not change their pattern of weight gain. Male monkey FN15740 (titer = 125) administered 9 µg/kg BID showed an increase in body weight gain beginning approximately day 50 while monkeys FN15741 (titer = 125) and FN15722 (titer = 125) did not change their pattern of weight gain (Figure 3). In females at 9 µg/kg BID (Figure 4), one monkey (FN15703) exhibited a change in the weight gain pattern and this animal was negative for the presence of anti-AC2993 antibodies. An initial pattern of weight loss was observed at the 75 µg/kg BID dose in both males and females. Male FN15705 (titer = 25) showed a recovery in body weight gain around day 125, while monkey FN15714 (titer = 125) showed no change (Figure 5). In females administered 75 µg/kg (Figure 6), one animal (FN14015) was antibody positive (titer = 25) and she maintained her pattern of weight change throughout the study. Thus, no consistent pattern between anti-AC2993 antibody formation and changes in body weight gains or loss could be discerned in this study.

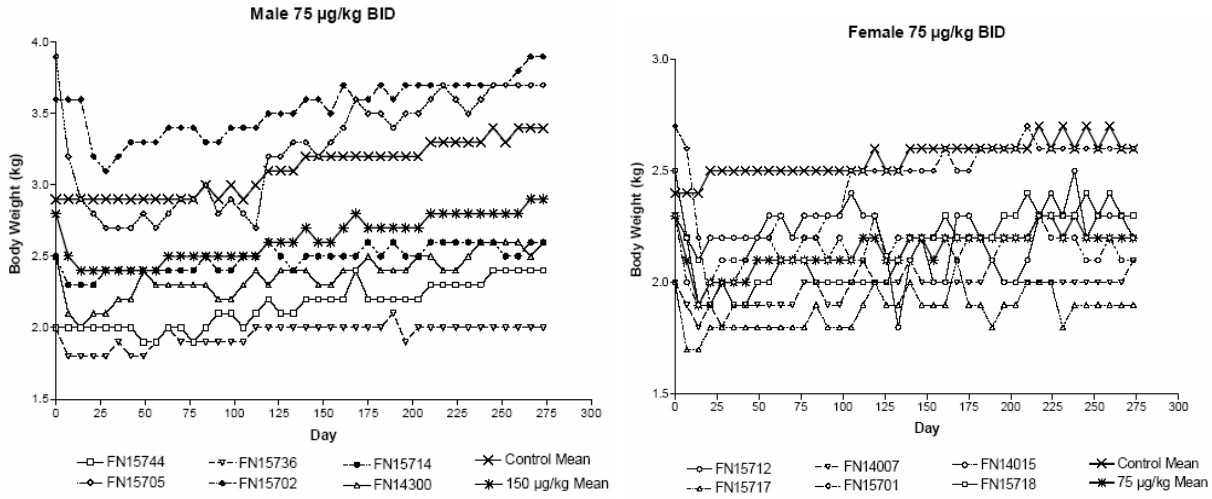
Figures 1 & 2: Body Weights in Male (Fig. 1) and Female (Fig. 2) Monkeys Administered 1.1 µg/kg BID (open symbols = animals with antibody titer <125, closed symbols = animals with antibody titer >125)



Figures 3 & 4: Body Weights in Male (Fig. 3) and Female (Fig. 4) Monkeys Administered 9 µg/kg BID (open symbols = animals with antibody titer <125, closed symbols = animals with antibody titer >125)



Figures 5 & 6: Body Weights in Male (Fig. 5) and Female (Fig. 6) Monkeys Administered 75 µg/kg BID (open symbols = animals with antibody titer <125, closed symbols = animals with antibody titer >125)



Effect of Anti-AC2993 Antibody Formation on Histological Change: The only histological change observed in the 9-month toxicity study of AC2993 in monkeys that appeared related to AC2993 was in the pancreas: increased islet cellularity. Table 3 is a summary of these findings.

Table 3: Summary of Histological Change of Increased Islet Cellularity in Cynomolgus Monkeys Exposed to AC2993 for 273 Days

Group 1 (0 µg/kg/day)		Group 2 (2.2 µg/kg/day)		Group 3 (18 µg/kg/day)		Group 4 (150 µg/kg/day)	
Animal No. & Sex	Histology Grade	Animal No.	Histology Grade	Animal No.	Histology Grade	Animal No.	Histology Grade
FN15727M	NR[1]	FN15746M	NR	FN15709M	NR	FN15714M	NR
FN15724M	NR	FN15707M	NR	FN15722M	NR	FN15744M	NR
FN15706M	NR	FN15734M	NR	FN15740M	NR	FN15705M	NR
FN15721M	NR	FN15710M	NR	FN15733M	NR	FN15736M	mild
FN15738M	NR	FN15735M	NR	FN15741M	NR	FN15702M	mild
FN15739M	NR	FN15737M	NR	FN15708M	NR	FN14300M	NR
FN14912F	NR	FN15728F	NR	FN15726F	NR	FN15712F	NR
FN15716F	NR	FN15711F	NR	FN15731F	NR	FN15717F	NR
FN15725F	NR	FN15742F	NR	F4288CQF	NR	FN14015F	NR
FN15743F	NR	FN14937F	NR	FN15723F	NR	FN15701F	minimal
FN15745F	NR	FN15729F	NR	FN15713F	NR	FN15718F	mild
FN15732F	NR	FN15715F	NR	FN15703F	NR	FN14007F	mild

[1]Not Remarkable with regard to hypercellular islet

No animals in the two lower dose groups (2.2 and 18 µg/kg/day) were found to have increase islet cellularity and thus, no relationships to antibody formation at these doses for the presence or lack of this histological change could be discerned. At 150 µg/kg/day, two males exhibited the histological change. One (FN15702) had a titer = 125 while the other (FN15736) had no detectable antibodies. The other three males were negative for the histological change and their titers ranged from negative to 125. In the females, the three animals with the histological change had titers that ranged from negative to 25 and those that did not exhibit the histological change had titers that ranged from negative to 25. Thus, there was no obvious relationship between the formation of anti-AC2993 antibodies and pancreatic change.

Discussion

Plasma antibodies to AC2993 in cynomolgus monkeys exposed to AC2993 by twice-daily subcutaneous injections were detected using a sensitive ELISA method. The potential impact of antibody formation on the toxicokinetics, body weight gains and histological change in this study were evaluated. An effect of antibody formation on depressed body weight gain and on increased pancreas islet cellularity could not be discerned. Based on these two biological responses, the anti-AC2993 antibodies were not neutralizing. Animals with an antibody titer >125 tended to exhibit a greater plasma AC2993 AUC value at sample days 90, 180 and/or 273 relative to the AUC value on day 1. Evaluation of the TK data indicated that, in monkeys, a titer >125 is a good predictor for change in plasma TK (i.e., 33 of 36 animals correctly predicted change or no change in AUC values over time). Thus, in the 9-month toxicity study, the following is concluded with regard to positive animals in each dose group (Table 4):

Table 4: Anti-AC2993 Antibody "Positive" Monkeys (Titer >125) Based on ELISA Results of Plasma Samples Obtained After 9 Months of BID Subcutaneous Administration

Dose (µg/kg BID)	Males + Females	Number Antibody Positive	% Antibody Positive
0	12	0	0
1.1	12	5	42
9	12	4	33
75	12	2	17

What is the cause of increased AUC in some animals that developed anti-AC2993 antibodies? Sponsor stated that a definitive answer cannot be provided at this time. However, two key assumptions were proposed as an explanation. The assumptions are: 1) the IEMA method used to measure plasma AC2993 measures total AC2993 and not just free AC2993 and 2) clearance in the monkey is, like the rat, primarily by renal glomerular filtration. Therefore, the apparent increase in AUC in antibody positive animals is

explained by increase in the plasma protein binding in the presence of antibodies and the corresponding decrease in renal clearance. Since only free AC2993 can be elimination by glomerular filtration, plasma clearance is less efficient in a monkey when antibody titer >125 result in an increase in plasma protein binding and the AUC increases.

Conclusion:
An effect of antibody formation on depressed body weight gain and on increased pancreas islet cellularity could not be discerned. Thus, with regard to these two biological responses, the anti-AC2993 antibodies were not neutralizing.

Antigenicity in Other Species (See also individual toxicology studies)
Antigenicity of exenatide was evaluated by analysis of anti-exenatide antibody by ELISA in repeat-dose toxicity studies in mice, rats, and monkeys for up to 182, 91, and 273 days, respectively. Anti-exenatide antibody was also assessed following 36 weeks of once-daily treatment days in rats during a carcinogenicity study. In addition to anti-exenatide antibody, injection site observations and morphologic pathology were performed as part of pivotal repeat-dose toxicity or carcinogenicity studies in mice, rats, and monkeys for up to 96-98 weeks, 104 weeks, and 39 weeks, respectively. Finally, anti-exenatide antibody was measured following SC BID treatment in mice for 28 days with exenatide from three manufacturers, Star, Bachem, and Mallinckrodt.

Mouse
The antigenicity of exenatide was assessed by analysis of clinical observations, morphologic pathology of the injection sites, and anti-exenatide antibody in repeat-dose toxicity studies with BID dosing over 28, 91, and 182 days duration. In addition, injection sites were assessed following 96 to 98 weeks dosing in a 2-year carcinogenicity study. There were no exenatide-related changes in injection site clinical observations or morphologic pathology; events noted were common to both vehicle- and exenatide-treated groups. Severity and incidence of injection site microscopic changes were related to the volume of either vehicle or exenatide injection. The morphologic changes in each study were attributed to repeated injection trauma but not attributed to any exenatide-related effects. Anti-exenatide antibody, while uncommon in the three studies, was present in few mice at very low titers (1:5, to 1:25), and was not dose-dependent, as summarized in Table 1.

Table 1: Anti-exenatide Antibody-positive Incidence in Mice

Study	Treatment Duration (Weeks)	Treatment Frequency	Dose (µg/kg/day)	Anti-Exenatide Antibody Positive/ Total Tested
REST02075 [a]	4	BID	0	0/20
			760 (Star)	2/20
			760 (Bachem)	0/20
			760 (Mallinckrodt)	0/19
REST99051 [b]	13	BID	0	0/37
			6	0/4
			68	0/4
			760	0/4
REST00119 [c]	26	BID	0	2/33
			18	0/18
			116	2/20
			760	0/17

BID = Dose divided and administered twice daily.
[a] Antibody data reported in REST03032, Section 4.2.3.2.1.2.
[b] Antibody data reported in REST01152, Section 4.2.3.2.2.4.
[c] Antibody data reported in REST01165, Section 4.2.3.2.4.3.

The low titer antibody response along with similar incidence of mice scoring positive between vehicle and exenatide-treated groups following treatment up to 26 weeks indicate exenatide is very weakly

antigenic in mice, if at all. The lack of exenatide-related effects at injection sites indicates no local antigenic or immune-mediated tissue reaction. Among the exenatide lots produced by the different manufacturers of drug substance, Star, Bachem, or Mallinckrodt, the lot manufactured by Star was weakly antigenic.

Rat

The antigenicity of exenatide was assessed in a repeat-dose toxicity study using once-daily, SC injection for 91 days. Antigenicity in the rat was also assessed in the carcinogenicity study by clinical observations, morphologic pathology of the injection sites following treatment for 104 weeks, and anti-exenatide antibody following treatment for 36 weeks with once-daily dosing. There were no exenatide-related changes in injection site clinical observations or morphologic pathology. Events noted were common to both vehicle- and exenatide-treated groups. Severity and incidence of injection site morphologic changes tended to be related to the volume of either vehicle or exenatide injection. The morphologic changes in each study were attributed to repeated injection trauma and not to any exenatide-related effects. Anti-exenatide antibody was uncommon in the three studies, present in few rats at very low titers (≤1:25) and lacking dose-response relationship, as summarized in Table 2.

Table 2: Anti-Exenatide Antibody-Positive Incidence in Rats

Study	Treatment Duration (Weeks)	Treatment Frequency	Dose (µg/kg/day)	Anti-Exenatide Antibody Positive/ Total Tested
REST02246R1 [a]	13	Daily	0	1/24
			18	1/18
			70	1/15
			250	3/17
REST01052 [b]	36	Daily	0	5/78
			18	2/40
			70	3/40
			250	3/40

[a] Antibody data reported separately in REST03282, Section 4.2.3.2.6.3, assay only was non-GLP.
[b] Antibody data reported separately in REST02132R1, Section 4.2.3.4.2.1, assay only was non-GLP.

The low titer antibody response along with similar incidence of rats scoring positive between vehicle and exenatide-treated groups following treatment up to 36 weeks indicate exenatide is very weakly antigenic in rats. The lack of exenatide-related effects on injection sites indicates no local antigenic or immune-mediated reaction following treatment up to 104 weeks.

Monkey

Anti-exenatide antibody formation was assessed in repeat-dose toxicity studies with once-daily dosing over 28 days or BID dosing over 91 days and 273 days duration. There were no exenatide-related changes in injection site clinical observations or morphologic pathology. Events were common to both vehicle- and exenatide-treated groups. The morphologic changes in each study were attributed to repeated injection trauma and not any exenatide-related effects. Although many exenatide-treated monkeys were anti-exenatide antibody positive, there were no anaphylactic reactions for up to 273 days. Additionally, there was no apparent evidence of autoimmune or antibody-antigen complex-related pathology, such as autoimmune or delayed-type hypersensitivity changes (dermal reactions, arthritis, anemia or aplasias, mucocutaneous reactions) or antibody-antigen complex-related pathology (arthritis, nephropathies), or other immune response-related pathology following dosing up to 273 days. Unlike rodents, anti-exenatide antibody formation was more common among monkeys. Anti-exenatide antibody-positive monkeys generally had low titers of ≤1:125, except a single female with 1:625 at 2.2 µg/kg/day exenatide for 273 days. No dose-response relationship on incidence or titer was apparent, as summarized in Table 3.

Table 3: Anti-Exenatide Antibody-Positive Incidence Monkeys

Study	Treatment Duration (Weeks)	Treatment Frequency	Dose (µg/kg/day)	Anti-Exenatide Antibody Positive Total Tested
REST98079 [a]	4	Daily	0	0/6
			10	2/6
			100	0/6
			1000	2/6
REST99050R1 [b]	13	BID	0	2/44
			1.2	3/8
			13.4	2/8
			150	4/8
REST00120R1 [c]	39	BID	0	0/12
			2.2	9/12
			18	9/12
			150	8/12

BID = Dose divided and administered twice daily.
[a] Antibody data reported in REST98059, Section 4.2.3.2.8.2.
[b] Antibody data reported in REST01150, Section 4.2.3.2.9.4, including assays of 36 additional naïve monkeys.
[c] Antibody data reported in REST01190, Section 4.2.3.2.10.3.

The low titer of antibody response, but higher (up to 75%) incidence following treatment up to 39 weeks indicate exenatide, was antigenic in monkeys. In the 273-day study, monkeys with an antibody titers ≥1:125 measured at Day 275 demonstrated disproportionately higher plasma exenatide AUC values at 90, 180, and 273 days of treatment, compared to AUC values on the first day of treatment. Since renal clearance has been demonstrated to be the major component of exenatide disposition, these data suggested decreased elimination due to antibody binding was likely responsible for the altered toxicokinetics. However, exenatide-related effects on body weight and focal pancreatic islet cell hypercellularity in monkeys were not affected by the presence of anti-exenatide antibody. These data demonstrated that while anti-exenatide antibody altered the pharmacokinetics of exenatide, it was not neutralizing in monkeys. The lack of exenatide-related effects at injection sites indicated that no local antigenic or immune-mediated tissue reaction were associated with the presence of anti-exenatide antibody.

Conclusions

Exenatide was very weakly antigenic or non-antigenic in rodents and antigenic in monkeys. Anti-exenatide antibodies were noted following 1 month of treatment, and were present following 9 months of treatment, resulting in 8 months of exposure to anti-exenatide antibody in monkeys. The formation of anti-exenatide antibody in monkeys was not dose-dependent. The presence of anti-exenatide antibody at titers ≥1:125 resulted in altered pharmacokinetics in monkeys but was not neutralizing. There were no apparent adverse effects of anti-exenatide antibody formation in monkeys such as injection sites reactions, anaphylaxis, delayed-type hypersensitivity, autoimmune (dermal reactions, arthritis, anemia or aplasias, mucocutaneous reactions) or antibody-antigen-complex-related pathology (arthritis, nephropathies).

IMPURITIES AND DEGRADATION PRODUCTS

Studies were not performed with specific exenatide-related impurities. A 28-day toxicity study conducted in mice and genotoxicity studies (bacterial reverse mutagenesis and in vitro chromosomal aberration) compared lots of exenatide drug substance with their respective impurities from different manufacturers (Star, Bachem, Mallinckrodt). There were no toxicologically important differences in the effects of exenatide from the three manufacturers.

To assess the potential toxicity of degradation products in exenatide drug product, the sponsor conducted a 28-day study in CD-1 to evaluate the toxicity of heat-degraded (H-D) AC2993 following twice daily subcutaneous administration. A 0.1 mg/ ml formulation of AC2993 was exposed to heat (50°C) in order to simulate an improper storage temperature of product. The formulated AC2993 before heating had a

purity of 92% to 94% while the formulated AC2993 after heating (HD-AC2993) had a purity of 76% to 77%. No treatment-related differences were observed between AC2993 and H-D AC2993 with respect to survival, clinical findings, body weights, food consumption, ophthalmology, hematology, clinical chemistry, organ weights, and macroscopic and microscopic pathology. Both treated groups exhibited a slight increase in body weight, more prominent in the H-D AC2993 group (females), and a slight decrease in food consumption, more prominent in the AC2993 group (males and females) relative to control. AC2993 and H-D AC2993 were well tolerated by the mice and no substantive differences were observed for the test substances when compared to each other or to control. The toxicokinetics of AC2993 showed that the plasma concentration profiles of AC2993 in animals administered AC2993 and HD-AC2993 were similar with regards to Tmax, Cmax and AUC values.

TK Data following 28-Days Treatment with AC2993 and Heat-Degraded AC2993

Dose Group	T_{max} (hr)	C_{max} (pg/ml)	$AUC_{0-4\ hr}$ (pg.hr/ml)
AC2993	0.5	392,251	301,298
Heat-Degraded AC2993	0.5	489,809	356,018

2.6.6.9 Discussion and Conclusions

In single dose studies, the median lethal dose values for exenatide in mice (IV), rats (SC), and monkeys (SC) were >1500 µg/kg, >30,000 µg/kg, and >5000 µg/kg, respectively.

Following subacute exposure (~ 1 month) to exenatide, mean body weight of rats and monkeys (but not mice) was decreased due to decreased food consumption. Weight of the thymus decreased in monkeys dosed 1000 µg/kg/d (3592X MRHD, AUC) which correlated with the lymphoid depletion observed microscopically. Increased incidence of basophilic foci in the parotid salivary gland (minimal severity) was observed in mice dosed 760 µg/kg/d (520X MRHD, AUC). Very low titers of anti-exenatide antibody were evident in 2/20 (titers ≤ 1:25) mice treated with exenatide manufactured by Star. Anti-exenatide antibody was not evident in mice treated with exenatide manufactured by Bachem or Mallinckrodt. In monkeys, very low titers (<1:5) of anti-exenatide antibody were observed at doses ≥ 10 µg/kg/d (19X MRHD, AUC). Anti-exenatide antibody detection was not performed in the rat study.

Subchronic exposure (91 days) of mice to exenatide at doses of 3, 34 and 380 µg/kg BID was well tolerated. A high incidence of basophilic foci in the parotid salivary gland (minimal to mild severity) was observed in mice at doses ≥ 6 µg/kg/d (3X MRHD, AUC). A high incidence of mandibular lymph node hemorrhage was observed in mice dosed 706 µg/kg/d (520X MRHD, AUC). In another subchronic (91 days) toxicity study, mice dosed with exenatide at 18, 70 and 250 µg/kg QD followed by a 30-day recovery period showed reversible increases in incidence of basophilic foci in the parotid salivary gland at doses ≥ 18 µg/kg/d (12X MRHD, AUC). Subchronic exposure (91 days) of rats and monkeys to exenatide caused decreased body weight gain which correlated with decreased food consumption. Reversible decreases in body weight gain were observed in rats at doses ≥ 18 µg/kg/d (5X MRHD, AUC). Decreased body weight gain was observed in monkeys at doses ≥ 0.6 µg/kg BID (3X MRHD, AUC). Reversibility was not assessed in monkeys. A low incidence and minimal severity of basophilic foci (reversible) was observed in female rats at 18 µg/kg/d (5X MRHD, AUC) and 250 µg/kg/d (129X MRHD, AUC). At the end of the recovery period, relatively high incidences of vacuolar change (adrenal gland), lymphocyte infiltration (pancreas), and a low incidence of basophilic foci (parotid salivary gland) with minimal severity were noted in 250 µg/kg/d (129X MRHD, AUC) male rats. NOAEL could not be established in the rat study since microscopic evaluation was performed on only a few selected organs/tissues. In the monkey, the target organs toxicity of minimal to mild severity were observed in the lung (inflammation, hemorrhage, syncytial giant cells), endometrium (hemorrhage) pancreas (hypercellular islet) and stomach (focal inflammation) at doses ≥ 6.7 µg/kg BID (65X MRHD, AUC). NOAEL for the monkey study was 0.6 µg/kg BID (3X MRHD, AUC). The potential of exenatide to elicit an immune response in rats was

low. In monkeys, 5% of control animals tested positive for anti-exenatide antibodies compared to 38%, 25% and 50% for the 0.6, 6.7 or 75 μg/kg BID groups respectively. There was to be a treatment-related increase in percentage of animals that tested positive suggesting that the drug may be antigenic to monkey. However, the positive finding in some control animals (which may be due to contamination or background error) undermines the accuracy of this study. Moreover, with the exception of one 75 μg/kg BID (1004X MRHD, AUC) animal that had an antibody titer of 125, the rest of the treated animals had antibody titer of 25 regardless of treatment group. Systemic exposure increased with dose in the monkey suggesting that the anti-exenatide antibody formed is not neutralizing.

Chronic toxicity studies in mice (182 days) and monkeys (273 days) showed no treatment-related effects on body weight/body weight gain in the mouse but body weight gain decreased dose-dependently in treated monkeys. The target organs of toxicity in the mouse include the eye (retinal atrophy, corneal mineralization, cataract), testis (degeneration of seminiferous tubules), parotid salivary gland (basophilia), bone marrow (hyperplasia) and injection sites (inflammation, hemorrhage, fibrosis, epithelial hyperplasia). Except for the basophilia observed at all doses in the parotid salivary gland, most of the remaining toxicities were limited to the HD of 380 μg/kg BID (520X MRHD, AUC) group. Anti-exenatide antibody reactivity was not different between control and exenatide-treated mice. NOAEL could not be established because of the ophthalmology findings, tissue reaction at the injection sites and the parotid gland basophilia observed at all doses. In monkeys, the target organs of toxicity include the brain (mononuclear cell infiltration, hemorrhage), thyroid (follicular distension, epithelial degeneration - males), adrenal gland (mineralization - males, nodular hypertrophy – female), kidney (tubular dilatation - males), heart (mononuclear cell infiltration - males), skeletal muscle (lymphoid cell infiltrate - males), pancreas (vacuolation, fibrosis, mononuclear cell infiltrate, hypercellular islet – males and females), sciatic nerve (fibrosis - male), uterus (protein deposits - females), stomach (lymphoid hyperplasia, lymphoplasmacytic infiltrate), colon (cystic dilatation), cecum (pigmented macrophages), jejunum (cytoplasmic vacuolation), rectum (inflammation)- all the GI lesions were observed in females except for the pigmented macrophages observed in a HD males; injection sites (epidermal hyperplasia – males). Most of the toxic effects occurred in the 9 μg/kg BID (1360X MRHD, AUC) and 75 μg/kg BID (994X MRHD, AUC) groups. NOAEL was 1.1 μg/kg BID (8X MRHD, AUC) based on histopathology. One of 12 control monkeys compared to 9/12 monkeys each receiving 1.1 μg/kg/BID and 9.0 μg/kg/BID and 8 /12 monkeys receiving 75 μg/kg/BID were found positive for anti-exenatide antibody. The anti-exenatide antibody was be neutralizing at 75 μg/kg BID (994X MRHD, AUC) due to the decreased systemic exposure relative to systemic exposure at 9 μg/kg BID (1360X MRHD, AUC).

Reproductive toxicology: The potential of exenatide to cause reproductive or developmental toxicity was evaluated in mice and rabbits. In fertility and general reproductive toxicity studies, male and female mice were dosed at 3, 34 and 380 μg /kg BID (3X, 50X and 520X MRHD, AUC). There were no treatment-related effects on mating and fertility in both sexes or estrous cycling in treated females. There was a dose-dependent decrease (not SS) in number of motile sperm by 7%, 8% and 20% at 3, 34 and 380 μg/kg BID respectively. There were dose-dependent decreases (not SS) in number of corpora lutea, implantations and viable embryos in treated females relative to control. Post-implantation loss was increased by 2 to 3-fold (not dose-related) in treated mice relative to control, but the differences were not significant relative to control. A decrease in relative weight of the prostate was observed in males dosed 380 μg/kg BID. NOAEL for mating and fertility is 380 μg/kg BID (520X MRHD, AUC).

In a mouse teratology study, exenatide doses of 3, 34, 230 and 380 μg/kg BID (3X, 50X, 243X and 520X MRHD, AUC) were administered subcutaneously to pregnant mice on GDs 6 through 15. In addition, extra pregnant mice were exposed to the same doses of exenatide and used to assess the extent of placental transfer. Food consumption was decreased in all treated dams relative to control. One out of 25 female mice each dosed 34 μg/kg BID (50X MRHD) and 380 μg/kg BID (520X MRHD) aborted on GDs 15 and 16 respectively. 1/25 female mice in the 34, 230 and 380 μg/kg BID groups prematurely delivered.

In addition, one female in the toxicokinetic 380 μg/kg BID (520X MRHD) group delivered prematurely. Food consumption was slightly but significantly decreased by 13% and 9% in the 230 μg/kg BID and 380 μg/kg BID dose groups respectively relative to control. Number of implantations, litter sizes and live fetuses were significantly decreased in the 230 μg/kg BID group relative to control. Male fetal body weights showed decrements with increasing dose, achieving statistical significance at doses ≥ 230 μg/kg BID. Female fetal body weights also showed decrements with increasing dose, achieving statistical significance at doses ≥ 68 μg/kg BID. Five fetuses from the treated group and two from the control group had multiple findings. Cleft palate with/without hole was a common finding. In addition, some fetuses had interfrontal ossification site, cervical ribs and wavy ribs. Since the incidence of the multiple findings was greater in the treated group compared to control, it may be treatment-related. The findings that occurred at 230 μg/kg BID (243X MRHD) and 380 μg/kg BID (520X MRHD) may not be that concerning since they occurred at higher multiples of the MRHD and occurred at doses above the maternal NOAEL, 3 μg/kg BID (3X MRHD). The incidence of wavy ribs in the litter and fetuses were significantly increased at 380 μg/kg BID group relative to control and the increments were greater than their historical control means. Sponsor stated that the higher incidence of reversible delayed ossification of ribs (i.e. wavy ribs) in the 380 μg/kg BID group is due to the slowed development of the fetuses as a result of the decreased nutritional state of the dams. The TK data showed that the potential of exenatide to cross the placental barrier is very low in mice. Maternal NOAEL is 3 μg/kg BID (3X MRHD) based on the abortions observed. Developmental NOAEL is also 3μg/kg BID (3X MRHD) based on dose-related lower body weights in fetuses at higher doses, cleft palate and wavy ribs. Since the potential of exenatide to cross the placental barrier is very low, the fetal findings observed may be a consequence of the dose-related reduced nutritional state of the dams during gestation or maternal toxicity. Sponsor stated that dams with compromised nutritional state during organogenesis, produced fetuses with decreased body weights and delays in normal fetal maturation (e.g., wavy ribs).

In a rabbit teratology study, timed pregnant female rabbits were dosed subcutaneously at 0.1, 11, 78 and 130 μg/kg BID resulting in total daily doses of 0.2 (0.2X), 22 (207X), 156 (1432X), or 260 μg/kg/day (3479X MRHD, AUC). A satellite group of 25 female rabbits were exposed to the same doses of exenatide and used to assess the extent of placental transfer. One out of 20 females in the 0.2 μg/kg/day (0.2X MRHD) dose group was found dead on the morning of GD 10 prior to dosing. One of 20 females in the 22 μg/kg/day (207X MRHD) dosage group was found dead on GD 19, approximately 13 hours after the last dose. Sponsor stated that the cause of death could not be determined since all tissues examined appeared normal at necropsy. One of 20 females in the 156 μg/kg/day (1432X MRHD) dosage group aborted on GD 21 and was sacrificed. Another 1/20 females in the 22 μg/kg/day (207X MRHD) dose group prematurely delivered on GD 29 and was sacrificed. These events were considered unrelated to the test article because they were not dose-dependent, the death of one doe appeared to be related to an injury, and the abortion and delivery of a single doe in a study is within the historical control incidence for the testing facility. Body weight gain was significantly decreased in all treated groups in a dose-dependent manner relative to control (GDs 6-19). The decreased body weight gain during the treatment period (GDs 6-19) correlated with the decreased food consumption observed. The decreased food consumption may be due to the pharmacological activity of the drug.

Fetal and litter incidence of umbilical hernia (intestines protruding through the umbilical opening) were significantly increased in the 260 μg/kg/day group (3479X MRHD). The increment is greater than the historical control mean. Fetal incidence of circumcorneal hemorrhage was significantly increased at 22, μg/kg/day (207X MRHD) by 2.4% relative to control. This increment is greater than the historical control mean (0.22%). The significance of this finding is not clear since it was not observed at 156 and 260 μg/kg/day. Fetal incidence of small gall bladder was significantly increased at 22, 156 and 260 μg/kg/day by 5.6%, 3.5% and 2.8% respectively relative to control. These increments are greater than the historical

control mean (0.10%). Fetal incidence of angulated hyoid and fetal ossification sites per fetus per were significantly increased at doses ≥ 22 μg/kg/day relative to control. The increments are greater than the historical control means. Incidence of mean fetal ossification sites per fetus per litter in the lumbar vertebra was slightly but significantly decreased with increasing dose at 22, 156 and 260 μg/kg/day relative to control. The decrements at 22 (6.19%), 156 (6.16%) and 260 (6.09%) μg/kg/day dose groups were less than the historical control mean (6.39%). Incidence of fetal ossification sites in the rib pairs were slightly but significantly increased in all treated groups relative to control. The increments are greater than the historical control mean. Some fetuses were observed with multiple findings (umbilical hernia with angulated hyoid, or with fused sternal centra, unossified pubis and absence of intermediate lung lobe) at doses ≥ 22 μg/kg/day (207X MRHD). Since these multiple findings were not observed in control fetuses, they are likely to be treatment related. Maternal NOAEL = 0.2 μg/kg/day (0.2X MRHD) based on dose-related decrease in weight gain during the dosage period. The developmental NOAEL is also 0.2 μg/kg/day (0.2X MRHD) based on the developmental toxicity (higher incidence of umbilical hernia, small gall bladder, angulated hyoid, delayed ossifications and fused sternal centra). The potential of exenatide to cross the placental barrier is very low. Therefore the fetal findings observed may be a consequence of the reduced nutritional state of the dams during gestation or maternal toxicity. Sponsor stated that dams with compromised nutritional state during organogenesis, produce fetuses with decreased body weights and delays in normal fetal maturation (e.g., resorptions, umbilical hernia and delays in ossifications).

Another rabbit teratology study was performed to better define the NOAEL with regard to fetal effects and to clarify the role of exenatide-related decreases in food consumption and body weight on developmental effecs. In this study, pregnant rabbits were administered 1, 11 and 130 μg/kg BID SC exenatide resulting in total daily doses of 2 (12X MRHD) , 22 9207X MRHD), and 260 μg/kg/day (3479X MRHD). Three additional groups were pair-fed (fed the same average daily amount of food) to match the three respective exenatide-dosed groups. Rabbits that were administered exenatide exhibited profound, dose-related decreases in food and water consumption and loss in body weight. Clinical indicators of starvation (β-hydroxybuterate and K) and body weight loss were more pronounced in the exenatide-treated groups than in the pair-fed groups. Based on the severity of the body weight loss and anorexia, the MTD in pregnant rabbits was exceeded at doses ≥22 μg/kg/day exenatide. As in the previous rabbit study, developmental toxicity occurred only at doses ≥22 μg/kg/day exenatide, doses that exceeded the MTD in pregnant rabbits. None of the fetuses from pair-fed dams and from the dams administered 2 μg/kg/day exenatide had umbilical hernias. Skeletal variations were present in similar incidences in both exenatide and pair-fed groups, suggesting these effects were a consequence of compromised maternal condition. Thus, exenatide was not a developmental toxicant in rabbits; the NOEL for developmental toxicity was 2 μg/kg/day exenatide (12X MRHD).

In a developmental and perinatal/postnatal reproduction toxicity study, pregnant mice were administered exenatide at doses of 3, 34 and 380 μg/kg BID SC resulting in total daily doses of 6 (3X MRHD), 68 (50X MRHD) and 760 μg/kg/d (520X MRHD). One of 25 (F0) female mice died at all dose levels. The HD (520X MRHD) female died while delivering a litter. The HD death might be drug-related because it occurred in the HD group and the other mice in this dose group had increased incidences of stillbirths and pup deaths on LD1 (Lactation Day 1). Although the cause of death could not be determined, sponsor indicated that the deaths in the 6 (3X MRHD) and 68 μg/kg/day (50X MRHD) dose groups were not considered drug-related because the incidences were not dose-dependent. F0 Dams delivering stillborn pups was significantly increased in the 760 μg/kg/day group (24%) relative to control (0%). Dams with all pups dying during days 1-4 postpartum was also significantly increased in the 760 μg/kg/day group (12%) relative to control (0%). Number of live birth was significantly decreased in the 760 μg/kg/day group (92%) relative to control (100%). Still birth was significantly increased in the HD group (6%) relative to control (0%).

F1 pups found dead/presumed cannibalized was significantly increased in the 6 µg/kg/day (3.2%) and 760 µg/kg/day groups (5.5%) relative to control during days 1-4 postpartum, and in the 68 µg/kg/day group (4.5%) during days 8-14 postpartum. Two of 25 (control) and 1/25 F1 generation males each in the 6, 68 and 760 µg/kg/day respectively, and 1/25 F1 generation female in the control and 760 µg/kg/day maternal dose groups died prior to scheduled sacrifice. These deaths were not considered related to exenatide because the incidences were not dose-dependent. All tissues appeared normal at necropsy. Viability index, surviving pups/litter, and pup weight/litter were significantly decreased in the 760 µg/kg/day group relative to control. Post-weaning body weight was also slightly but significantly decreased in the 760 µg/kg/day F1 females during precohabitation, on GD 0 and on GD 18 relative to control. There were no treatment-related effects on corpora lutea, implantations, litter sizes and resorptions in cesarean-sectioned F1 females. 1/297 LD F2 fetuses had a cleft palate. 1/268 MD F2 fetuses had exencephaly, opened eyelids and a cleft snout. Litter and fetal incidences of forked tail tip and flexed (downward) hindlimb were slightly increased (not SS) in F2 litters/fetuses of HD F1 parents.

Maternal administration of exenatide at doses as high as 760 µg/kg/d did not affect the day of preputial separation or day of vaginal patency in the F1 generation mice, learning or memory, mating or fertility, cesarean-sectioning parameters or the incidence of fetal alterations in F2 generation mice. The maternal (F0) NOAEL < 6 µg/kg/d (<3X MRHD) due to mortality at doses ≥ 6 µg/kg/d. NOAEL for fetal viability and growth is 6 µg/kg/d (3X MRHD) because the 68 µg/kg/d (50X MRHD) and 760 µg/kg/d (520X MRHD) dose groups caused reduced pup body weights preweaning and the 760 µg/kg/day increased perinatal mortality and reduced body weight gains postweaning.

In an anti-exenatide antibody study in NIH Swiss mice, no measurable anti-exenatide antibody titers were established with the treatment of exenatide for up to 8 weeks. The results indicate that no neutralizing antibodies are formed since treatment with exenatide showed consistent drop in glucose levels. In monkeys exposed to exenatide for 9 months, there were no effects of antibody formation on decreased body weight gain and increased pancreas islet cellularity in the treated groups. Except for one, monkeys with antibody titer >125 exhibited a larger plasma exenatide AUC value at sample days 90, 180 and 273 relative to the AUC value on day 1. Based on this evaluation, an antibody titer >125 caused a change in plasma pharmacokinetics, probably by slowing renal clearance due to increased plasma protein binding. The anti-exenatide antibody may be neutralizing at 75 µg/kg BID (994X MRHD, AUC) due to the decreased systemic exposure relative to systemic exposure at 9 µg/kg BID (1360X MRHD, AUC).

Overal, exenatide was weakly antigenic or non-antigenic in rodents but antigenic in monkeys. Sponsor stated that there were no apparent adverse effects of anti-exenatide antibody formation in monkeys such as injection sites reactions, anaphylaxis, delayed-type hypersensitivity, autoimmune (dermal reactions, arthritis, anemia or aplasias, mucocutaneous reactions) or antibody-antigen-complex-related pathology (arthritis, nephropathies).

A 28-Day study in CD-1 mice which evaluated the toxicity of AC2993 and heat-degraded AC2993 showed no treatment-related differences between AC2993 and heat-degraded AC2993 with respect to survival, clinical findings, body weights, food consumption, ophthalmology, hematology, clinical chemistry, organ weights, and macroscopic and microscopic pathology.

Toxicology conclusions

Exenatide caused no lethality and minimal toxic responses when administered as a single, IV dose in mice at doses up to 1500 µg/kg, as a SC dose in rats up to 30,000 µg/kg, and as a SC dose in monkeys up to 5000 µg/kg. Exenatide caused minimal toxicity following SC dosing in repeat-dose toxicity studies in mice at ≤760 µg/kg/day for up to 182 days, rats at ≤250 µg/kg/day for up to 91 days, and monkeys at ≤150 µg/kg/day for up to 273 days.

Exenatide-related effects demonstrated most consistently in rats and monkeys (not mice) were decreased food consumption and correlative decrease in body weight/body weight gain. Effects on body weight and food consumption were related to the known pharmacologic effects of exenatide. Treatment in rats at ≥18 µg/kg/day (5X MRHD) and monkeys at ≥13.4 µg/kg/day (131X MRHD) decreased body weight/body weight gain and food consumption. Conversely, exenatide treatment in mice generally tended to mildly elevate body weight and food consumption, but these effects subsided with chronic dosing. The two most notable exenatide-related microscopic pathology changes were basophilic foci in the parotid salivary gland of mice and focal islet cell hypercellularity in the pancreas of monkeys. Basophilic foci in the parotid salivary gland were noted in mice at ≥18 µg/kg/day exenatide (10X MRHD) at 91 and 182 days, and at 760 µg/kg/day exenatide (520X MRHD) at 28 days. Reversibility of these lesions was demonstrated in mice treated for 91 days and allowed a 30-day recovery period following completion of exenatide treatment. These lesions were of minimal to moderate severity. Basophilic foci were noted in all exenatide-treated groups of mice at ≥18 µg/kg/day exenatide (10X MRHD) in the 2-year carcinogenicity. However, despite the lesion's relatively common occurrence (~ 45% to 65% across all exenatide-treated groups) there were no exenatide-related increases in salivary gland tumors and no exenatide-related adverse effects on survival. Sponsor stated that the physiologic significance of this lesion remains unclear, but the lack of any adverse or preneoplastic consequence of the lesion suggest that the basophilic foci of the parotid salivary gland is not a toxicologically important effect.

Focal, minimal-to-mild islet cell hypercellularity was noted in the pancreas of monkeys treated at 150 µg/kg/day exenatide (994 to 2007X MRHD) for 91 and 273 days. These monkeys had tremors, males had decreased body weight in addition to pancreatic islet hypercellularity. Islet cell hypercellularity was accompanied by increased staining with Gomori's Aldehyde Fuchsin, suggesting the hypercellularity was an increase in the β-cell population. No islet cell changes were noted in mice or rats. Sponsor stated that exenatide, exendin-4 (naturally occurring form of exenatide), GLP-1, and GLP-1 analogs have been demonstrated to increase β-cell mass both in vitro and in vivo. There were no changes in serum glucose noted in either study and no degenerative microscopic changes. There were no neoplastic changes in the pancreas of mice or rats treated with 250 µg/kg/day exenatide (>90X MRHD) in two-year carcinogenicity studies. Based on the minimal to mild severity and lack of adverse effects, these changes were considered a pharmacologic effect of exenatide, not toxicity. Thus, exenatide was generally well-tolerated in repeat-dose toxicity studies with durations of up to 182 days in mice, 91 days in rats, and 273 days in monkeys. Decreased body weight/food consumption was transient in rodents and occurred in monkeys (chronic dosing). Anti-exenatide antibody formation was observed in rodents and monkeys.

Exenatide was neither mutagenic nor clastogenic in the battery of genotoxicity studies conducted. Exenatide was not tumorigenic in mice when administered SC for up to 96 weeks (females) and 98 weeks (males) at doses resulting in exposures 129X human systemic exposure at 10 µg BID. Exenatide when administered SC for up to 104 weeks in rats at doses resulting in 95X the clinical exposure at 10 µg BID, was associated with increased incidence of thyroid C-cell adenoma in all drug treated females relative to controls. The incidence in HD females is 23% relative to controls (8% and 5% for control groups 1 and 2 respectively) and is greater than the sponsor's historical control mean (5%) and range (0-10%). The thyroid C-cell adenomas may have been drug related. Exenatide was devoid of mutagenic effects, with or without metabolic activation, in both in vitro (Ames bacterial reverse mutation, chromosomal aberration in mammalian cells) and in vivo (mouse micronucleus formation) assays.

Exenatide produced no impairment of fertility, sperm concentration, or sperm motility in male mice, or fertility or estrous cycling in female mice at doses up to 760 µg/kg/d resulting in exposures 260X the clinical exposure at 10 µg BID based on AUC. Exenatide was not teratogenic in mice at doses up to 6 µg/kg/d resulting in exposures 5X the clinical exposure and in rabbits at 12X the clinical exposure 10 µg BID. Higher exposures in rats and particularly rabbits resulted in maternal toxicity (death, weight loss, litter loss) which confounded the developmental assessment.

Exenatide was weakly antigenic in rodents (mouse & rat) and monkeys. The antibodies seem to be neutralizing at 75 µg/kg BID (994X MRHD, AUC) due to the decreased systemic exposure relative to systemic exposure at 9 µg/kg BID (1360X MRHD, AUC). In the chronic monkey study, exenatide exposure increased with titer ≥ 1:125 but body weight effects were still seen suggesting an effect on exenatide antibody complex excretion rather than neutralizing antibody formation. A 28-Day study in CD-1 mice which evaluated the toxicity of AC2993 and heat-degraded AC2993 showed no treatment-related differences between AC2993 and heat-degraded AC2993 with regards to toxicity but there were differences in antibody formation depending on manufacturer (i.e. Star) and impurities profile for each.

2.6.6.10 Tables and Figures

Tables and figures were presented with their respective individual studies.

2.6.7 TOXICOLOGY TABULATED SUMMARY

2.6.7.1 Single-Dose Toxicity

Species/ Strain	Method of Administration/ Vehicle/Formulation	Doses (µg/kg)	Number and Sex per Group	Observed Maximum Nonlethal Dose (µg/kg)	Approximate Lethal Dose (µg/kg)	Noteworthy Findings	Study Number
Mouse/ ICR	Intravenous injection/ Aqueous saline/ Prepared at test site	0, 30, 300, 1500	10M except motor activity at 8M	1500	>1500	No lethality or signs of serious toxicity at any dose. ≥300 µg/kg: decreased grip strength, limb tone ≥30 µg/kg: transient decreases in spontaneous motor activity	REST98095 [a] Section 4.2.1.3.1 (non-GLP)
Rat/ Sprague-Dawley Crl:CD	Subcutaneous injection/ AC-2993-F1, AC-2993-F2	Rising-dose 100, 300, 1000, 3000, 10,000, 30,000 Single-dose 30, 300, 3000	2M, 2F 5M, 5F	30,000 3000	>30,000 >3000	No lethality or signs of serious toxicity at any dose. ≥10,000 µg/kg: hunched posture, staining of fur, piloerection 3000 µg/kg: reduced body weights compared to lowest dose	REST98098 Section 4.2.3.1.1 (GLP)
Cynomolgus monkey/ *Macaca fascicularis*	Subcutaneous injection/ AC-2993-F1, AC-2993-F2	Rising-dose 100, 300, 1000, 3000, 5000	1M, 1F (2M, 2F total, with 1/sex at each rising dose)	5000	>5000	No lethality or sign of serious toxicity. ≥3000 µg/kg: reduced food consumption	REST98099R1 [b] Section 4.2.3.1.2 (GLP)

M = Male F = Female
[a] Data derived from single-dose neurobehavioral pharmacology study.
[b] Study conducted in two phases, including rising single-dose toxicity and 5 day repeat-dose toxicity. Data from repeat-dose toxicity summarized in Section 2.6.7.6 Repeat-dose Toxicity-Non-Pivotal.

2.6.7.2 Repeat-dose Toxicity – Pivotal Studies

Test Article: Exenatide

Report Title: Toxicity Evaluation of AC2993 From Three Different Suppliers When Administered Subcutaneously Twice Daily for 28 Days to CD-1 Mice
Species/Strain: Mice/ Crl:CD-1 (ICR) BR **Duration of Dosing:** 28 days **Study No.:** REST02075
Initial Age: 6 weeks **Duration of Recovery:** 0 days **Location in CTD:** Section 4.2.3.2.1
Date of First Dose: 14May2002 **Method of Administration:** Subcutaneous, BID **GLP Compliance:** GLP
Vehicle/Formulation: AC-2993-F12 /AC-2993-F7
Special Features: Comparison of potential toxicity of exenatide from three manufacturers
No Observed Adverse Effect Level: N/A

Daily Dose (µg/kg/day)	0 (Control)		760 (Star)		760 (Bachem)		760 (Mallinckrodt)	
Number of Animals	M	F	M	F	M	F	M	F
Main study	10	10	10	10	10	10	10	10
Toxicokinetics only	10	10	10	10	10	10	10	10
Toxicokinetics:[a]	N/A							
Noteworthy Findings								
Died or Sacrificed Moribund:	0	0	0	0	0	0	0	0
Mean Body Weight (g):								
Week 4	33.56	27.82	34.46	28.51	34.81	28.46	34.90	27.82
Mean Food Consumption (g):								
Week 1	6.85	5.67	6.59	8.19*	8.83*	8.02*	10.45**	10.93**
Week 4	5.66	5.57	6.24	5.69	5.87	5.56	6.27	5.79
Clinical Observations (incidence):								
Hair discolored, yellow	0	0	3	0	3	0	1	0
Unkempt appearance	0	0	2	0	2	0	2	0

BID Dose divide and administered twice daily N/A not assayed
- = No noteworthy findings or finding not different from controls
* – p < 0.05 ** – p < 0.01 One-way ANOVA with Dunnett's t-test
[a] REST03031, Section 4.2.3.2.1.1. Single time point was assessed only to verify exposure to exenatide, no other calculations or analyses were performed.
[b] REST03032, Section 4.2.3.2.1.2.

2.6.7.2 Contd.

Daily Dose (µg/kg/day)	0 (Control)		760 (Star)		760 (Bachem)		760 (Mallinckrodt)	
Number of Animals	M	F	M	F	M	F	M	F
Main study	10	10	10	10	10	10	10	10
Toxicokinetics only	10	10	10	10	10	10	10	10
Ophthalmology:	-	-	-	-	-	-	-	-
Hematology (n = 5):								
Lymphocytes (1000/µL)	6.7	4.4	2.6	5.1	4.5	3.2	3.6	5.1
Clinical Chemistry:	-	-	-	-	-	-	-	-
Organ Weights:	-	-	-	-	-	-	-	-
Macroscopic Pathology:	-	-	-	-	-	-	-	-
Injection site	-	-	-	-	-	-	-	-
Microscopic Pathology:								
Basophilic foci, parotid salivary gland								
-trace	0	0	3	9	5	7	8	9
Injection sites	-	-	-	-	-	-	-	-
Anti-Exenatide Antibody:[b]								
Positive Titer 1:5	0	0	1	0	0	0	0	0
Positive Tier 1:25	0	0	1	0	0	0	0	0

BID = Dose divide and administered twice daily N/A = not assayed
- = No noteworthy findings or finding not different from controls
* = $p < 0.05$ ** = $p < 0.01$ One-way ANOVA with Dunnett's t-test
[a] REST03031, Section 4.2.3.2.1.1. Single time point was assessed only to verify exposure to exenatide, no other calculations or analyses were performed.
[b] REST03032, Section 4.2.3.2.1.2

2.6.7.3

Report Title: A 91-Day Toxicity Study of AC2993 Administered BID by Subcutaneous Injection to Mice
Species/Strain: Mice/Crl:CD-1 **Duration of Dosing:** 91 days **Study No.:** REST99051
Initial Age: 7-8 weeks **Duration of Recovery:** 0 **Location in CTD:** Section 4.2.3.2.2
Date of First Dose: 01Feb2000 **Method of Administration:** Subcutaneous injection, BID **GLP Compliance:** GLP
Vehicle/Formulation: PBO-F11/AC-2993-F4
Special Features: None
No Observed Adverse Effect Level: 760 µg/kg/day

Daily Dose (µg/kg/day)	0 (Control)		6		68		760	
Number of Animals:	M:	F:	M:	F:	M:	F:	M:	F:
Main Study	20	20	21	21	21	21	21	21
Toxicokinetics only	0	0	54	54	54	54	54	54
Toxicokinetics:[a]								
AUC $_{0\text{-}6h}$ Day 91 (pg•h/mL)	N/A	N/A	3426	3250	56,699	45,974	633,253	476,576
Noteworthy Findings								
Died or Sacrificed Moribund:	3	0	5	1	4	0	0	4
Mean Body Weight (Week 13) (g):	37.8	29.9	37.8	32.2*	37.6	31.7*	37.0	32.3*
Mean Food Consumption (g/day):								
Week 1	6.0	5.3	5.8	5.2	5.6	5.0	5.4*	4.9
Week 13	5.8	5.6	6.2	5.6	5.8	5.8	5.9	5.9
Clinical Observations:	-	-	-	-	-	-	-	-
Ophthalmology:	-	-	-	-	-	-	-	-
Clinical Chemistry:								
Triglycerides (mg/dL)	186	161	143	118	137	97*	111*	91*
Hematology:	-	-	-	-	-	-	-	-
Urinalysis:[b]	-	-	-	-	-	-	-	-
Organ Weights:	-	-	-	-	-	-	-	-
Macroscopic Pathology:								
Injection site lesions/focus including exudative, superficial scabs	9	6	1	0	1	5	7	7
Microscopic Pathology:								
Basophilic foci, parotid salivary gland [c]								
-trace	0	1	10	12	16	18	15	16
-mild	0	0	0	0	3	2	2	2
Anti-Exenatide Antibody:[d]								
Positive Titer/Total Assayed	0/17	0/20	0/2	0/2	0/1	0/3	0/3	0/1

BID = Dose divided and administered twice daily N/A = not assayed
- = No noteworthy findings
* = $p < 0.05$ ** = $p < 0.01$ One-way ANOVA with Dunnett's t-test
[a] REST00248, Section 4.2.3.2.2.3.
[b] Limited number of samples were collected in this study.
[c] Includes additional histopathological assessment recorded in REST02199, Section 4.2.3.2.2.1.
[d] REST01152, Section 4.2.3.2.2.4.

2.6.7.4

Report Title: Subcutaneous Toxicokinetic Study of AC2993 in CD-1 Mice With Selective Measurements of Biological Response With a 91-Day Exposure

Species/Strain: Mice/Crl:CD-1 (ICR) BR **Duration of Dosing:** 91 days **Study No.:** REST02325R1

Initial Weight: Males 25.6-29.6 g Females 21.3-25.0 g **Duration of Recovery:** 30 days **Location in CTD:** Section 4.2.3.2.3

Date of First Dose: 14JAN2003 **Method of Administration:** Subcutaneous injection once daily

Vehicle/Formulation: PBO-F12/AC-2993-F7 **GLP Compliance:** GLP

Special Features: Primary objective of study was to determine toxicokinetics in mice following 90 days dosing with additional endpoints to assess body weight, food consumption, water consumption, and reversibility of parotid salivary gland microscopic changes.

No Observed Adverse Effect Level: 250 µg/kg/day

Daily Dose (µg/kg/day)	0 (Control)		18		70		250	
Number of Animals:	M:	F:	M:	F:	M:	F:	M:	F:
Main Study	20	20	10	10	10	10	20	20
Toxicokinetics	0	0	40	40	40	40	40	40
Toxicokinetics[a] AUC_{0-6h} (pg•h/mL):								
Day1	N/A		10,113		32,508		123,241	
Day 91	N/A		25,425		58,403		197,295	
Noteworthy Findings								
Died or Sacrificed Moribund:	1	0	0	0	0	0	1	0
Mean Body Weight (g):								
Week 1	28.44	23.09	28.92	24.29	28.65	22.90	28.26	24.34*
Week 4	33.60	27.34	34.16	29.04*	34.04	29.40*	34.36	29.26**
Week 13	37.34	30.54	37.54	32.40	36.98	32.98*	37.51	32.42*
Mean Food Consumption (g/day):	-	-	-	-	-	-	-	-
Mean Water Consumption (g/day):								
Week 4	-	8.46	-	8.66	-	9.83	-	10.25**
Week 13	-	8.32	-	8.68	-	10.84**	-	9.80
Number of Animals:	M:	F:	M:	F:	M:	F:	M:	F:
Main Study	20	20	10	10	10	10	20	20
Toxicokinetics	0	0	40	40	40	40	40	40
Clinical Observations:	-	-	-	-	-	-	-	-
Macroscopic Pathology:	-	-	-	-	-	-	-	-
Microscopic Pathology:								
Basophilic foci, parotid salivary gland								
-trace	0	0	5	7	5	5	2	4
-mild	0	0	2	2	2	5	4	4
-moderate	0	0	0	0	1	0	0	1
Recovery Period (Number Animals):	10	10	0	0	0	0	10	10
Mean Body Weight Week 17 (g):	38.04	32.19	N/A	N/A	N/A	N/A	39.22	33.98
Mean Food Consumption (g/day):	-	-	N/A	N/A	N/A	N/A	-	-
Mean Water Consumption Week 17 (g/day)	-	8.16	N/A	N/A	N/A	N/A	-	9.63*
Microscopic Pathology:								
Basophilic foci, parotid salivary gland								
-trace	0	0	N/A	N/A	N/A	N/A	0	1
-mild	0	0	N/A	N/A	N/A	N/A	0	0
-moderate	0	0	N/A	N/A	N/A	N/A	0	0

N/A = not assayed or measured
- = No noteworthy findings or findings not different from controls.
* = $p < 0.05$ ** = $p < 0.01$ Dunnett's t-test
[a] REST03288, Section 4.2.3.2.3.2. Male and female values combined.

2.6.7.5

Report Title: Toxicity Evaluation of AC2993 in CD-1 Mice When Administered Subcutaneously Twice Daily for 182 Consecutive Days
Species/Strain: Mice/Crl:CD-1 (ICR) **Duration of Dosing:** 182 days **Study No.:** REST00119
Initial Age: 6 weeks **Duration of Recovery:** 0 **Location in CTD:** Section 4.2.3.2.4
Date of First Dose: 08Nov2000 **Method of Administration:** Subcutaneous injection, BID **GLP Compliance:** GLP
Vehicle/Formulation: AC-2993-F12/AC-2993-F7
Special Features: None
No Observed Adverse Effect Level: 760 µg/kg/day

Daily Dose (µg/kg/day)	0 (Control)		18		116		760	
Number of Animals:	M:	F:	M:	F:	M:	F:	M:	F:
Main study	20	20	25	25	25	25	25	25
Toxicokinetics only	0	0	50	50	50	50	50	50
Toxicokinetics[a] (Day182): **AUC $_{0-6h}$ (pg•h/mL):**	N/A		10,562		54,789		538,670	
Noteworthy Findings								
Died or Sacrificed Moribund:	2	5	6	3	7	0	2	3
Body Weight (g):								
Week 13	-	30.01	-	31.62*	-	32.11**	-	32.76**
Week 26	-	32.34	-	33.96	-	33.81	-	34.00
Food Consumption:	-	-	-	-	-	-	-	-
Clinical Observations:	-	-	-	-	-	-	-	-
Ophthalmoscopy:	-	-	-	-	-	-	-	-
Clinical Chemistry:	-	-	-	-	-	-	-	-
Hematology:	-	-	-	-	-	-	-	-
Organ Weights:								
Heart/BW (%x10)	6.10	6.18	-	-	-	-	5.44*	5.36**
Pituitary (mg)	2	-	-	-	-	-	4**	-
Pituitary/BW (%x1000)	6.69	-	-	-	-	-	11.06**	-
Thyroid/parathyroid (mg)	7	7	-	-	-	-	9*	9**
Macroscopic Pathology:	-	-	-	-	-	-	-	-
Injection site	-	-	-	-	-	-	-	-
Microscopic Pathology:								
Basophilic foci, parotid salivary gland[c]								
-trace	0	0	13	16	16	12	12	10
-mild	0	0	7	5	0	6	6	12
-moderate	0	0	2	0	0	1	1	2
Injection sites	-	-	-	-	-	-	-	-
Anti-Exenatide Antibody:[d]								
Positive Titer 1:5/Total	0/18 (0%)	2/15 (13.3%)	0/9 (0%)	0/9 (0%)	0/8 (0%)	2/12 (16.7%)	0/8 (0%)	0/9 (0%)

BID – Dose divided and administered twice daily N/A – not assayed
- – No noteworthy findings.
* – p < 0.05 ** – p < 0.0 One-way ANOVA with Dunnett's t-test.
[a] REST01164, Section 4.2.3.2.4.2. Combined group male and female mean values shown.
[b] Sodium, potassium, and chloride assessed at 91 days, but not 182 days due to low sample volumes.
[c] Data also summarized in REST02199, Section 4.2.3.2.2.1.
[d] REST01165, Section 4.2.3.2.4.3. Samples from animal in main and/or toxicokinetic groups at indicated dose level.

2.6.7.6

Report Title: A 28-Day Toxicity Study of AC2993 Administered by Subcutaneous Injection to Rats
Species/Strain: Rats/ Sprague-Dawley Crl:CD **Duration of Dosing:** 28 days **Study No.:** REST98082
Initial Age: 6-7 weeks **Duration of Recovery:** 0 days **Location in CTD:** Section 4.2.3.2.5
Date of First Dose: 23Feb1998 **Method of Administration:** Subcutaneous injection once daily
Vehicle/Formulation: PBO-F10 /AC-2993-F1, AC-2993-F2 **GLP Compliance:** GLP
Special Features: None
No Observed Adverse Effect Level: 1000 µg/kg/day

Daily Dose (µg/kg/day)	0 (Control)		10		100		1000	
Number of Animals	M:	F:	M:	F:	M:	F:	M:	F:
Main study	10	10	10	10	10	10	10	10
Toxicokinetics only	0	0	24	24	24	24	24	24
Toxicokinetics[a] (Day 27): **AUC $_{0-6hr}$ (pg•h/mL)**	N/A		3674	2250	104,791	119,498	1,653,257	4,073,438
Noteworthy Findings								
Died or Sacrificed Moribund:	0	0	0	0	0	0	0	0
Mean Body Weight (g):								
Week 4	375.0	237.6	363.9	225.7	357.9	224.7	332.8*	215.6
Mean Food Consumption (g):								
Week 1	22.3	19.0	25.2	17.3	22.4	17.4	18.4*	13.8*
Week 4	30.3	24.7	29.4	20.3	27.1	21.5	25.8*	19.2*
Clinical Observations:								
Reduced activity postdose (days)	0	0	5.4	6.8	10.3	9.9	12.2	10.3
Salivation postdose (incidence)[b]	0	0	1	2	6	3	9	7
Number of Animals	M:	F:	M:	F:	M:	F:	M:	F:
Main study	10	10	10	10	10	10	10	10
Toxicokinetics only	0	0	24	24	24	24	24	24
Clinical Chemistry:	-	-	-	-	-	-	-	-
Hematology:	-	-	-	-	-	-	-	-
Mean Organ Weights :								
Adrenal Gland (g)	0.074	0.075	0.083	0.082	0.080	0.082	0.081	0.081
Adrenal Gland/BW (x1000)	0.202	0.330	0.236	0.379*	0.237	0.383*	0.256	0.405*
Macroscopic Pathology:	-	-	-	-	-	-	-	-
Injection site	-	-	-	-	-	-	-	-
Microscopic Pathology:								
Injection site	-	-	-	-	-	-	-	-

N/A – not assayed BW – body weight M – Male F – Female
- – No noteworthy findings or finding not different from controls.
* – p < 0.05 ** – p < 0.01 One-way ANOVA with Dunnett's t-test
[a] REST98080, Section 4.2.3.2.5.1.
[b] Main study animals only.

2.6.7.7

Report Title: Subcutaneous Toxicokinetic Study of AC2993 in Sprague-Dawley Rats With Selective Measurements of Biological Response With a 91-Day Exposure

Species/Strain: Rats/Crl:CD (SD)IGS BR **Duration of Dosing:** 91 days **Study No.:** REST02246R1

Initial Weight: Males 246-287 g Females 179-238 g **Duration of Recovery:** 30 days **Location in CTD:** Section 4.2.3.2.6

Date of First Dose: 17DEC2002 **Method of Administration:** Subcutaneous injection **GLP Compliance:** GLP [b]

Vehicle/Formulation: PBO-F12/AC-2993-F7

Special Features: Primary objective of study was to determine toxicokinetics in rats following 91 days dosing with additional endpoints to assess body weight, food consumption, water consumption, anti-exenatide antibody, and microscopic changes in limited tissues

No Observed Adverse Effect Level: 250 µg/kg/day

Daily Dose (µg/kg/day)	0 (Control)		18		70		250	
Number of Animals	M:	F:	M:	F:	M:	F:	M:	F:
Main study	20	20	10	10	10	10	20	20
Toxicokinetics	0	0	40	40	40	40	40	40
Toxicokinetics:[a] AUC $_{0\text{-}6h}$ (pg•h/mL);								
Day 1	N/A		20,188		45,619		201,764	
Day 91	N/A		10,178		48,554		268,094	
Noteworthy Findings								
Died or Sacrificed Moribund:	0	0	0	0	0	0	1	0
Mean Body Weight (g):								
Week 1	325.1	228.0	329.8	226.3	319.7	228.5	320.1	219.1
Week 4	414.1	263.4	397.1	260.8	384.0*	262.0	376.4**	250.8*
Week 13	527.9	306.7	474.5**	287.0	465.1**	288.4	454.5**	279.5**
Mean Food Consumption (g/day):								
Week 1	23.30	17.16	22.17	15.80*	20.07*	14.98**	19.06**	13.38**
Week 4	25.96	18.79	23.75	18.35	23.26**	16.77**	22.39**	16.94**
Week 13	26.59	19.88	25.84	19.21	24.79	18.11	23.89**	16.11**

Daily Dose (µg/kg/day)	0 (Control)		18		70		250	
Number of Animals	M:	F:	M:	F:	M:	F:	M:	F:
Main study	20	20	10	10	10	10	20	20
Toxicokinetics	0	0	40	40	40	40	40	40
Water Consumption (g/day):								
Week 1	30.11	25.62	40.55**	47.64**	40.77**	44.25**	40.96**	43.67**
Week 4	36.39	27.56	43.04	58.44**	50.79**	48.51**	55.64**	53.67**
Week 13	36.69	30.77	42.06	48.55**	47.44*	53.70**	50.17**	44.27**
Clinical Observations:	-	-	-	-	-	-	-	-
Macroscopic Pathology:	-	-	-	-	-	-	-	-
Organ Weights:								
Adrenal (mg)	68	69	76	84	87**	82**	82*	82*
Adrenal/BW (x1000)	12.8	22.8	16.4*	30.2*	18.9*	31.3**	18.1*	30.3*
Thyroid/Pthy (mg)	30	21	22**	20	25*	23	25*	20
Thyroid/Pthy/BW (x1000)	5.71	6.78	4.77*	7.16	5.32	8.63*	5.47	7.24
Microscopic Pathology:								
Basophilic foci, parotid salivary gland, trace	0	0	0	1	0	0	0	1
Anti-Exenatide Antibody [b]								
Titer 1:5/Total	0/10	0/14	0/8	0/10	0/6	1/9	2/8	1/9
Titer 1:25/Total	0/10	0/14	1/8	0/10	0/6	0/9	0/8	0/9
Titer 1:125/Total	1/10	0/14	0/8	0/10	0/6	0/9	0/8	0/9
Recovery Period Week 17	M:	F:	M:	F:	M:	F:	M:	F:
(Number Recovery Animals):	10	10	0	0	0	0	10	10
Body Weight (g):	552.8	312.8	N/A	N/A	N/A	N/A	510.2**	298.7
Food Consumption (g/day):	27.21	19.46	N/A	N/A	N/A	N/A	26.37	17.79
Water Consumption (g/day)	35.78	34.00	N/A	N/A	N/A	N/A	37.59	34.54
Organ Weights:								
Adrenal (mg)	57	67	N/A	N/A	N/A	N/A	63	70
Thyroid/Parathyroid (mg)	30	22	N/A	N/A	N/A	N/A	28	21
Microscopic Pathology:								
Basophilic foci, parotid salivary gland, trace	0	0	N/A	N/A	N/A	N/A	1	2

N/A = not assayed
- = No noteworthy findings or no difference from controls * = $p < 0.05$ ** = $p < 0.01$One-way ANOVA with Dunnett's t-test
[a] REST03286, Section 4.2.3.2.6.2. Male and female values combined.
[b] REST03282, Section 4.2.3.2.6.3. Total number assayed varied due to limited plasma volumes, assay only was non-GLP.

2.6.7.8

Report Title: A 28-Day Toxicity Study of AC2993 Administered by Subcutaneous Injection to Cynomolgus Monkeys
Species/Strain: Monkey/*Macaca fascicularis* **Duration of Dosing:** 28 days **Study No.:** REST98079
Initial Age: 21-25 months **Duration of Recovery:** 0 days **Location in CTD:** Section 4.2.3.2.8
Date of First Dose: 25Feb1998 **Method of Administration:** Subcutaneous injection once daily **GLP Compliance:** GLP
Vehicle/Formulation: PBO-F10/AC-2993-F1, AC-2993-F2
Special Features: None
No Observed Adverse Effect Level: 100 µg/kg/day

Daily Dose (µg/kg/day)	0 (Control)		10		100		1000	
Number of Animals	M:	F:	M:	F:	M:	F:	M:	F:
Main study	3	3	3	3	3	3	3	3
Toxicokinetics [a] (Day 27):								
AUC $_{0\text{-}12h}$ (pg•h/mL)	N/A	N/A	39,483	40,101	484,518	497,425	6,752,905	8,162,648
Noteworthy Findings								
Died or Sacrificed Moribund:	0	0	0	0	0	0	0	0
Body Weight:								
Week 4 (kg)	2.8	2.6	2.8	2.5	2.6	2.3	2.3*	2.1
Weight gain, Weeks 0–4 (kg)	-0.2	0.0	-0.1	-0.1	-0.3	-0.3	-0.6	-0.4
Food Consumption (biscuits/day):								
Pretest	10	13	-	-	13	13	11	9.7
Week 1	11.6	13.3	-	-	5.2	6.2	2.6*	3.6
Week 4	12.5	13.7	-	-	11.7	12.3	5.7*	7.3
Clinical Observations (incidence):								
Mucous membrane pallor	1/3	0/3	1/3	2/3	2/3	2/3	2/3	1/3
Scant feces	0/3	1/3	0/3	2/3	2/3	1/3	3/3	2/3
Vomit	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
Dehydration/Emaciated	0/3	0/3	0/3	1/3	0/3	1/3	0/3	2/3
Electrocardiography:	-	-	-	-	-	-	-	-
Clinical Chemistry:	-	-	-	-	-	-	-	-
Hematology:	-	-	-	-	-	-	-	-
Mean Organ Weights:								
Spleen (g)	5.83	-	-	-	-	-	3.29	-
Thymus (g)	4.39	2.95	-	-	-	-	2.27	1.24
Macroscopic Pathology:								
Small Thymus	0/3	0/3	0/3	0/3	0/3	0/3	1/3	1/3
Injection Site	-	-	-	-	-	-	-	-
Microscopic Pathology:								
Thymus								
-lymphoid depletion	0/3	0/3	0/3	0/3	0/3	0/3	1/3	3/3
Injection sites	-	-	-	-	-	-	-	-
Anti-Exenatide Antibody:[b]								
Positive /Total	0/3	0/3	0/3	2/3	0/3	0/3	1/3	1/3

N/A – not assayed M – Male F – Female
- – No noteworthy findings or differences from control.
* = $p < 0.05$ ** = $p < 0.01$ One-way ANOVA with Dunnett's t-test
[a] REST98081, Section 4.2.3.2.8.1.
[b] REST98059, Section 4.2.3.2.8.2.

2.6.7.9

Report Title: A 91-Day Toxicity Study of AC2993 Administered BID by Subcutaneous Injection to Cynomolgus Monkeys
Species/Strain: Monkey/*Macaca fascicularis* **Duration of Dosing:** 91 days **Study No.:** REST99050R1
Initial Age: 33-49 months **Duration of Recovery:** 0 days **Location in CTD:** Section 4.2.3.2.9
Date of First Dose: 15FEB2000 **Method of Administration:** Subcutaneous injection, BID **GLP Compliance:** GLP
Vehicle/Formulation: PBO-F11/AC-2993-F4
Special Features: Additional histopathological assessment of pancreas recorded in REST02103, Section 4.2.3.2.9.1
No Observed Adverse Effect Level: 150 µg/kg/day

Daily Dose (µg/kg/day)	0 (Control)		1.2		13.4		150	
Number of Animals	M:	F:	M:	F:	M:	F:	M:	F:
Main study	4	4	4	4	4	4	4	4
Toxicokinetics [a] (Week 13):								
AUC $_{0-12h}$ (pg•h/mL)	N/A		6347		135,776		2,084,084	
Noteworthy Findings								
Died or Sacrificed Moribund:	0	0	0	0	0	0	0	0
Mean Body Weight:								
Day -1 (kg)	3.5	2.6	3.0	2.5	3.2	2.5	3.0	2.6
Week 13 (kg)	4.0	2.8	3.2	2.6	3.4	2.6	3.0	2.4
Study weight gain (%)	14.3	7.7	6.7	4.0	6.3	4.0	0.0	-7.7
Food Consumption (biscuits/day):								
Pretreatment	7.8	5.1	-	-	8.4	5.0	8.2	6.1
Week 1	8.6	7.2	-	-	7.7	5.6	3.5*	4.6
Week 13	10.5	6.9	-	-	9.7	7.3	8.3	7.4
Clinical Observations:								
Inappetence (all signs)	1/4	3/4	2/4	2/4	2/4	3/4	4/4	4/4
Infrequent stool	0/4	1/4	0/4	1/4	1/4	1/4	2/4	2/4
Electrocardiography:	-	-	-	-	-	-	-	-
Ophthalmoscopy:	-	-	-	-	-	-	-	-
Clinical Chemistry:	-	-	-	-	-	-	-	-
Hematology:	-	-	-	-	-	-	-	-
Urinalysis:	-	-	-	-	-	-	-	-
Organ Weights:	-	-	-	-	-	-	-	-
Macroscopic Pathology:	-	-	-	-	-	-	-	-
Injection site	-	-	-	-	-	-	-	-
Microscopic Pathology:								
Pancreas, increased islet cellularity [b]								
-minimal	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
-mild	0/4	1/4	0/4	0/4	0/4	1/4	0/4	2/4
Injection sites	-	-	-	-	-	-	-	-
GAF-Positive Islet Cell Score:[b]	2.0 ± 0.8		2.4± 0.9		2.4± 0.7		2.3± 0.7	
Anti-Exenatide Antibody:[c]								
Positive Titer 1:5/Total	2/44		1/4	0/4	0/4	0/4	0/4	0/4
Positive Titer 1:25/Total	0/44		1/4	1/4	2/4	0/4	2/4	1/4
Positive Titer 1:125/Total	0/44		0/4	0/4	0/4	0/4	1/4	0/4

BID = Dose divided and administered twice daily N/A = not assayed - = No noteworthy findings or differences from controls
* = p < 0.05 ** = p < 0.01 One-way ANOVA with Dunnett's t-test.
[a] REST01134, Section 4.2.3.2.9.3. Combined group male and female mean values shown.
[b] Histopathological analysis in REST02103, Section 4.2.3.2.9.1. GAF = Gomori'sAldehyde Fuchsin Positive Islet Cells (1 = 30-50% ; 2 = 50-80%; and 3 = 80-100% of islet cells).
[c] REST01150, Section 4.2.3.2.9.4. Anti-exenatide antibody measured in 36 additional naïve animals to assess background, sexes were combined as the additional control animal sex was not identified.

2.6.7.8.0

Report Title: Toxicity Evaluation of AC2993 in Cynomolgus Monkeys When Administered Subcutaneously Twice Daily for 273 Consecutive Days
Species/Strain: Monkey/*Macaca fascicularis* **Duration of Dosing:** 273 days **Study No.:** REST00120R1
Initial Age: 2.8-7.3 years **Duration of Recovery:** 0 days **Location in CTD:** Section 4.2.3.2.10
Date of First Dose: 08NOV2000 **Method of Administration:** Subcutaneous injection BID **GLP Compliance:** GLP
Vehicle/Formulation: AC-2993-F12/AC-2993-F7
Special Features: Study incorporates effects of nine months treatment with exenatide on humoral immune response (anti-KLH antibody levels), and additional histopathological studies on pancreatic islet cellularity.
No Observed Adverse Effect Level: 150 µg/kg/day

Daily Dose (µg/kg/day)	0 (Control)		2.2		18		150	
Number of Animals	M:	F:	M:	F:	M:	F:	M:	F:
Main study	6	6	6	6	6	6	6	6
Toxicokinetics[a] AUC $_{0-12h}$ (pg•h/mL)								
Day 1	N/A		5121		61,019		500,354	
Day 90	N/A		8429		290,411		736,288	
Day 180	N/A		14,279		788,730		777,046	
Day 273	N/A		8317		1,411,201		1,031,391	
Noteworthy Findings								
Died or Sacrificed Moribund:	0	0	0	0	0	0	0	0
Body Weight (kg):								
Pretreatment	2.6		2.5		2.6		2.5	
Day 28	2.7		2.5		2.6		2.2*	
Day 91	2.7		2.5		2.6		2.3	
Day 273	3.0		2.9		2.9		2.5	

BID = Dose divided and administered twice daily N/A = not assayed M = Male F = Female
- = No noteworthy findings or finding not different from controls
* = p < 0.05 ** = p < 0.01 One-way ANOVA with Dunnett's t-test.
[a] REST01187R1, Section 4.2.3.2.10.2. Combined group male and female mean values shown.
[b] 3/6 males and 3/6 females from each group were tested.
[c] REST02103, Section 4.2.3.2.9.1. GAF Gomori's Aldehyde Fuchsin Positive Islet Cells (1 30-50% ; 2 50-80%; and 3 80-100% of islet cells).
[d] REST01190, Section 4.2.3.2.10.3.

2.6.7.8.0 Contd.

Daily Dose (µg/kg/day)	0 (Control)		2.2		18		150	
Number of Animals:	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6
Clinical Observation (Incidence):								
Reduced skin turgor	1/6	1/6	2/6	2/6	1/6	2/6	3/6	5/6
Tremors, generalized	0/6	0/6	0/6	0/6	0/6	0/6	0/6	2/6
Electrocardiography								
Qualitative	-	-	-	-	-	-	-	-
QT interval	-	-	-	-	-	-	-	-
RR interval	-	-	-	-	-	-	-	-
QTc interval	-	-	-	-	-	-	-	-
Heart Rate	-	-	-	-	-	-	-	-
Ophthalmoscopy:	-	-	-	-	-	-	-	-
Clinical Chemistry:	-	-	-	-	-	-	-	-
Hematology:	-	-	-	-	-	-	-	-
Coagulation:	-	-	-	-	-	-	-	-
Urinalysis:	-	-	-	-	-	-	-	-
Anti-KLH Antibody Response:[b]								
Animals with titer ≥25	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Animals with titer ≥125	2/3	3/3	3/3	3/3	2/3	3/3	3/3	3/3

Daily Dose (µg/kg/day)	0 (Control)		2.2		18		150	
Number of Animals:	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6
Organ Weights:								
Thyroid-Pthy (g)	0.349		0.415		0.414		0.381	
Thyroid-Pthy/BW (x1000)	0.119		0.155		0.152		0.154*	
Macroscopic Pathology:	-	-	-	-	-	-	-	-
Injection site	-	-	-	-	-	-	-	-
Microscopic Pathology:								
Pancreas, islet hypercellularity								
-trace	0/6	0/6	0/6	0/6	0/6	0/6	0/6	1/6
-mild	0/6	0/6	0/6	0/6	0/6	0/6	2/6	2/6
Injection sites	-	-	-	-	-	-	-	-
GAF-Positive Islet Cell Score [c]	1.9 ± 0.7		2.0 ± 0.6		2.2 ± 0.4		2.4 ± 0.5	
Anti-Exenatide Antibody:[d]								
Negative	6/6	6/6	2/6	1/6	0/6	3/6	2/6	2/6
Positive Titer 1:5/Total	0/6	0/6	1/6	1/6	0/6	2/6	1/6	2/6
Positive Titer 1:25/Total	0/6	0/6	1/6	1/6	3/6	0/6	1/6	2/6
Positive Titer 1:125/Total	0/6	0/6	2/6	2/6	3/6	1/6	2/6	0/6
Positive Titer 1:625/Total	0/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6

BID = Dose divided and administered twice daily N/A = not assayed M = Male F = Female Pthy = parathyroids
- = No noteworthy findings or finding not different from controls
* = $p < 0.05$ ** = $p < 0.01$ One-way ANOVA with Dunnett's t-test
[a] REST01187R1, Section 4.2.3.2.10.2. Combined group male and female mean values shown.
[b] 3/6 males and 3/6 females from each group were tested.
[c] REST02103, Section 4.2.3.2.9.1. GAF = Gomori's Aldehyde Fuchsin Positive Islet Cells (1 = 30-50% ; 2 = 50-80%; and 3 = 80-100% of islet cells).
[d] REST01190, Section 4.2.3.2.10.3.

2.6.7.8.1 GENETIC TOXICOLOGY (In Vitro)

Report Title: Mutagenicity Test With AC2993 in the *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay With a Confirmatory Assay
Test for Induction of: Reverse mutation in bacteria
Strains: *S. typhimurium* and *E. coli*
Metabolizing System: Aroclor 1254-induced liver S9 fraction (S9)
Test Article Vehicle: Deionized water
Treatment Method: Plate incorporation method
Special Features: None
Cytotoxic Effects: None
Genotoxic Effects: None
Number Independent Assays: 2
Number Replicate Cultures: 3
Study No.: REST98093
Location in CTD: Section 4.2.3.3.1
GLP Compliance: GLP
Date of Dosing: 30Jan98

Metabolic Activation	Test/Control Article	Dose Level (µg/plate)	Assay 1 Mean Revertant Colony Counts Per Plate (± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without S9	Vehicle Control	0	15 (2)	84 (4)	10 (2)	7 (1)	17 (2)
	Exenatide	33.3	14 (4)	89 (4)	8 (4)	6 (3)	10 (3)
		100	14 (8)	89 (8)	8 (2)	6 (2)	13 (5)
		333	14 (2)	85 (12)	14 (3)	9 (7)	19 (4)
		1000	14 (8)	78 (11)	12 (6)	6 (1)	14 (5)
		3330	16 (2)	91 (11)	7 (3)	7 (2)	18 (6)
		5000	14 (2)	92 (9)	9 (4)	5 (2)	19 (5)
	Positive Control		2NF 169 (28)	NAz 604 (60)	NAz 540 (44)	ICR 1004 (51)	4NQO 449 (63)
With S9	Vehicle Control	0	23 (3)	90 (16)	11 (2)	8 (4)	16 (5)
	Exenatide	33.3	26 (14)	97 (2)	12 (6)	9 (3)	12 (6)
		100	31 (9)	91 (0)	9 (2)	10 (2)	14 (2)
		333	24 (8)	102 (8)	9 (2)	7 (4)	16 (3)
		1000	29 (8)	94 (3)	14 (2)	10 (2)	16 (5)
		3330	32 (8)	104 (9)	7 (3)	12 (1)	15 (2)
		5000	38 (4)	104 (7)	13 (2)	10 (2)	15 (3)
	Positive Control		BaP 356 (82)	2AA2 969 (49)	2AA2 145 (8)	2AA2 169 (17)	2AA25 386 (26)

N/A = not applicable SD = standard deviation
ICR = ICR-191 2.0 µg/plate BaP = benzo(a)pyrene 2.5 µg/plate 2NF = 2-nitrofluorene 1.0 µg/plate
2AA2 = 2-aminoanthracene 2.5 µg/plate 2AA25 = 2-aminoanthracene 25 µg/plate NAz = sodium azide 2.0 µg/plate 4NQO = 4-nitroquinoline-*N*-oxide 1.0 µg/plate

Metabolic Activation	Test/Control Article	Dose Level (µg/plate)	Assay 2 Mean Revertant Colony Counts Per Plate (± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without S9	Vehicle Control	0	15 (3)	96 (4)	10 (0)	8 (5)	11 (2)
	Exenatide	33.3	19 (4)	101 (16)	9 (2)	7 (2)	13 (1)
		100	16 (3)	94 (16)	9 (2)	6 (1)	15 (3)
		333	26 (7)	93 (11)	10 (4)	5 (1)	12 (2)
		1000	21 (8)	98 (7)	9 (1)	7 (1)	15 (9)
		3330	23 (3)	92 (13)	14 (6)	7 (3)	14 (4)
		5000	21 (1)	83 (18)	10 (1)	7 (2)	13 (4)
	Positive Control		2NF 107 (6)	NAz 717 (36)	NAz 649 (20)	ICR 507 (91)	4NQO 210 (74)
With S9	Vehicle Control	0	23 (8)	90 (6)	12 (5)	10 (1)	12 (1)
	Exenatide	33.3	25 (4)	100 (11)	10 (1)	10 (2)	12 (3)
		100	24 (1)	96 (19)	15 (6)	5 (2)	12 (2)
		333	31 (8)	88 (7)	12 (2)	12 (5)	16 (4)
		1000	32 (4)	101 (20)	9 (5)	12 (1)	13 (4)
		3330	29 (4)	105 (5)	10 (1)	8 (4)	12 (6)
		5000	33 (4)	107 (17)	10 (5)	9 (1)	14 (3)
	Positive Control		BaP 392 (17)	2AA2 680 (148)	2AA2 137 (16)	2AA2 163 (18)	2AA25 355 (18)

N/A = not applicable SD = standard deviation
ICR = ICR-191 2.0 µg/plate BaP = benzo(a)pyrene 2.5 µg/plate 2NF = 2-nitrofluorene 1.0 µg/plate
2AA2 = 2-aminoanthracene 2.5 µg/plate 2AA25 = 2-aminoanthracene 25 µg/plate NAz = sodium azide 2.0 µg/plate 4NQO = 4-nitroquinoline-*N*-oxide 1.0 µg/plate

2.6.7.8.2

Report Title:	*Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay With a Confirmatory Assay With AC2993				
Test for Induction of:	Reverse mutation in bacteria	**Number Independent Assays:**	2	**Study No.:**	REST02099
Strains:	*S. typhimurium* and *E. coli*	**Number Replicate Cultures:**	3	**Location in CTD:**	Section 4.2.3.3.2
Metabolizing System:	Aroclor 1254-induced liver S9 fraction (S9)			**GLP Compliance:**	GLP
Test Article Vehicle:	Deionized water			**Date of Dosing:**	12Jun2002
Treatment Method:	Plate incorporation method				
Special Features:	Study performed to compare genotoxicity of exenatide from new manufacturer (Bachem)				
Cytotoxic Effects:	None				
Genotoxic Effects:	None				

Metabolic Activation	Test/Control Article	Dose Level (µg/plate)	Assay 1 Mean Revertant Colony Counts Per Plate (± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without S9	Vehicle Control	0	12 (7)	77 (10)	12 (4)	6 (4)	15 (4)
	Exenatide	33.3	12 (6)	92 (13)	8 (1)	5 (3)	16 (5)
		100	15 (4)	88 (13)	10 (5)	2 (1)	14 (3)
		333	14 (4)	89 (17)	12 (5)	8 (3)	17 (4)
		1000	11 (3)	93 (10)	9 (3)	5 (4)	10 (4)
		3330	10 (4)	89 (15)	9 (3)	4 (2)	13 (2)
		5000	12 (7)	100 (6)	9 (3)	10 (2)	14 (5)
	Positive Control		2NF 162 (7)	NAz 1069 (33)	NAz 735 (14)	ICR 520 (21)	4NQO 294 (45)
With S9	Vehicle Control	0	19 (4)	82 (10)	16 (1)	6 (4)	12 (4)
	Exenatide	33.3	24 (5)	93 (13)	6 (1)	8 (6)	18 (2)
		100	24 (5)	89 (14)	10 (4)	9 (1)	14 (3)
		333	25 (9)	100 (8)	9 (4)	10 (1)	19 (4)
		1000	17 (3)	82 (5)	11 (2)	7 (4)	13 (5)
		3330	22 (3)	110 (18)	10 (4)	9 (3)	14 (4)
		5000	31 (7)	139 (9)	9 (3)	7 (2)	11 (1)
	Positive Control		BaP 379 (21)	2AA2 1014 (109)	2AA2 79 (8)	2AA2 77 (20)	2AA25 350 (12)

SD = standard deviation
ICR = ICR-191 2.0 µg/plate BaP = benzo(a)pyrene 2.5 µg/plate 2NF = 2-nitrofluorene 1.0 µg/plate
2AA2 = 2-aminoanthracene 2.5 µg/plate 2AA25 = 2-aminoanthracene 25 µg/plate NAz = sodium azide 2.0 µg/plate 4NQO = 4-nitroquinoline-*N*-oxide 1.0 µg/plate

Metabolic Activation	Test/Control Article	Dose Level (µg/plate)	Assay 2 Mean Revertant Colony Counts (± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without S9	Vehicle Control	0	12 (3)	75 (14)	11 (6)	7 (1)	15 (6)
	Exenatide	33.3	9 (1)	74 (10)	15 (5)	9 (2)	16 (6)
		100	13 (1)	80 (6)	13 (3)	5 (2)	15 (3)
		333	15 (1)	79 (12)	11 (3)	7 (1)	19 (3)
		1000	16 (5)	79 (11)	16 (6)	9 (2)	19 (6)
		3330	13 (3)	75 (8)	10 (5)	4 (3)	15 (4)
		5000	18 (4)	82 (9)	11 (4)	7 (4)	14 (3)
	Positive Control		2NF 141 (19)	NAz 969 (44)	NAz 655 (98)	ICR 1783 (160)	4NQO 205 (24)
With S9	Vehicle Control	0	19 (8)	77 (8)	12 (3)	11 (6)	8 (7)
	Exenatide	33.3	33 (6)	82 (9)	13 (1)	13 (3)	8 (5)
		100	32 (3)	83 (12)	10 (5)	7 (3)	9 (5)
		333	27 (2)	85 (5)	9 (3)	9 (1)	8 (3)
		1000	27 (4)	80 (10)	15 (5)	10 (9)	9 (5)
		3330	30 (1)	94 (9)	8 (2)	10 (4)	6 (1)
		5000	40 (2)	89 (9)	11 (6)	8 (3)	9 (4)
	Positive Control		BaP 328 (18)	2AA2 588 (23)	2AA2 112 (6)	2AA2 116 (17)	2AA25 985 (47)

SD = standard deviation
ICR = ICR-191 2.0 µg/plate BaP = benzo(a)pyrene 2.5 µg/plate 2NF = 2-nitrofluorene 1.0 µg/plate
2AA2 = 2-aminoanthracene 2.5 µg/plate 2AA25 = 2-aminoanthracene 25 µg/plate NAz = sodium azide 2.0 µg/plate 4NQO = 4-nitroquinoline-*N*-oxide 1.0 µg/plate

2.6.7.8.3

Report Title: *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay With a Confirmatory Assay with AC2993
Test for Induction of: Reverse mutation in bacteria
Strains: *S. typhimurium* and *E. coli*
Metabolizing System: Aroclor 1254-induced liver S9 fraction (S9)
Test Article Vehicle: Deionized water
Treatment Method: Plate incorporation method
Special Features: Study performed to compare genotoxicity of exenatide from new manufacturer (Mallinckrodt)
Cytotoxic Effects: None
Genotoxic Effects: None
Number Independent Assays: 2
Number Replicate Cultures: 3
Study No.: REST02098
Location in CTD: Section 4.2.3.3.3
GLP Compliance: GLP
Date of Dosing: 12Jun2002

Metabolic Activation	Test/Control Article	Dose Level (µg/plate)	Assay 1 Mean Revertant Colony Counts Per Plate (± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without S9	Vehicle Control	0	18 (9)	71 (10)	11 (5)	8 (1)	11 (5)
	Exenatide	33.3	14 (2)	77 (12)	15 (4)	6 (6)	10 (4)
		100	20 (11)	90 (10)	14 (5)	7 (5)	16 (6)
		333	16 (6)	75 (17)	11 (3)	10 (6)	16 (3)
		1000	15 (3)	85 (11)	15 (7)	8 (2)	20 (2)
		3330	11 (4)	79 (6)	11 (6)	11 (4)	18 (10)
		5000	12 (3)	87 (18)	17 (2)	6 (2)	17 (3)
	Positive Control		2NF 208 (7)	NAz 1068 (108)	NAz 707 (34)	ICR 736 (193)	4NQO 246 (23)
With S9	Vehicle Control	0	25 (3)	87 (9)	13 (8)	8 (2)	21 (1)
	Exenatide	33.3	32 (8)	90 (4)	12 (6)	12 (3)	18 (2)
		100	28 (6)	96 (19)	13 (6)	12 (2)	22 (6)
		333	27 (6)	85 (18)	12 (4)	15 (7)	17 (7)
		1000	29 (1)	91 (3)	16 (2)	11 (5)	19 (2)
		3330	27 (5)	95 (4)	14 (5)	13 (3)	19 (1)
		5000	35 (13)	91 (12)	17 (3)	14 (3)	18 (1)
	Positive Control		BaP 352 (10)	2AA2 690 (198)	2AA2 127 (17)	2AA2 88 (3)	2AA25 619 (47)

SD = standard deviation
ICR = ICR-191 2.0 µg/plate BaP = benzo(a)pyrene 2.5 µg/plate 2NF = 2-nitrofluorene 1.0 µg/plate
2AA2 = 2-aminoanthracene 2.5 µg/plate 2AA25 = 2-aminoanthracene 25 µg/plate NAz = sodium azide 2.0 µg/plate 4NQO = 4-nitroquinoline-*N*-oxide 1.0 µg/plate

Metabolic Activation	Test/Control Article	Dose Level (µg/plate)	Assay 2 Mean Revertant Colony Counts Per Plate (± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without S9	Vehicle Control	0	15 (3)	66 (6)	14 (6)	6 (0)	20 (3)
	Exenatide	33.3	14 (4)	63 (4)	12 (3)	8 (4)	21 (4)
		100	13 (3)	69 (1)	15 (4)	8 (3)	16 (3)
		333	13 (1)	63 (9)	13 (3)	8 (3)	16 (8)
		1000	17 (3)	66 (9)	10 (1)	7 (4)	18 (7)
		3330	10 (5)	64 (12)	14 (5)	10 (8)	17 (2)
		5000	15 (5)	78 (14)	14 (6)	5 (3)	15 (2)
	Positive Control		2NF 199 (13)	NAz 844 (70)	NAz 627 (34)	ICR 1668 (66)	4NQO 121 (42)
With S9	Vehicle Control	0	23 (2)	64 (11)	14 (2)	8 (3)	17 (9)
	Exenatide	33.3	27 (8)	64 (7)	7 (3)	8 (3)	21 (6)
		100	22 (3)	59 (15)	13 (3)	9 (2)	16 (8)
		333	27 (7)	49 (3)	14 (3)	5 (2)	17 (4)
		1000	34 (10)	66 (5)	11 (3)	8 (3)	13 (3)
		3330	27 (7)	75 (5)	10 (1)	11 (4)	17 (2)
		5000	35 (6)	85 (8)	15 (1)	11 (5)	14 (4)
	Positive Control		BaP 263 (11)	2AA2 219 (12)	2AA2 103 (9)	2AA2 62 (8)	2AA25 561 (17)

SD = standard deviation
ICR = ICR-191 2.0 µg/plate BaP = benzo(a)pyrene 2.5 µg/plate 2NF = 2-nitrofluorene 1.0 µg/plate
2AA2 = 2-aminoanthracene 2.5 µg/plate 2AA25 = 2-aminoanthracene 25 µg/plate NAz = sodium azide 2.0 µg/plate 4NQO = 4-nitroquinoline-*N*-oxide 1.0 µg/plate

2.6.7.8.4

Report Title:	Mutagenicity Test on AC2993 Measuring Chromosomal Aberration in Chinese Hamster Ovary (CHO) Cells				
Test for Induction of:	Chromosomal Aberrations	**Number Independent Assays:**	2	**Study No.:**	REST98094
Cell Type:	Chinese Hamster Ovary (CHO)	**Number Replicate Cultures:**	2	**Location in CTD:**	Section 4.2.3.3.4
Metabolizing System:	Aroclor 1254-induced liver S9 fraction (S9)	**Control Article(s) Vehicle:**	DMSO	**GLP Compliance:**	GLP
Test Article Vehicle:	Deionized water			**Date of Dosing:**	04Feb1998
Treatment Method:	Assay 1 with 3 h treatment and 20 h total incubation ± S9; Assay 2 with 18 h (-S9) or 3 h (+S9) treatment and 20 h total incubation [t]				
Special Features:	None				
Cytotoxic Effects:	None				
Genotoxic Effects:	None				

Metabolic Activation	Test/Control Article	Dose Concentration (µg/mL)	Assay 1 (Total 200 Cells Counted)				
			Mitotic Index (%)	Endoreduplicated Cells (%)	Polyploid Cells (%)	Cells With Chromosomal Aberrations (%)	Cells With > 1 Chromosomal Aberrations (%)
Without S9	Negative (media)	0	8.2	0.0	0.5	1.0	0.5
	Vehicle Control	0	3.4	0.0	0.0	1.5	0.5
	Exenatide	625	6.7	0.0	1.0	1.5	0.0
		1250	6.6	0.0	0.5	1.5	0.5
		2500	3.6	0.0	1.5	2.0	0.5
		5000	11.5	0.0	0.0	0.5	0.0
	MMC (50 cells)	1.50	3.0	0.0	3.0	50.0*	32.0*
With S9	Negative (media)	0	9.0	2.5	0.5	2.5	0.5
	Vehicle Control	0	10.6	2.5	0.5	2.0	0.0
	Exenatide	625	4.6	5.5	0.5	4.5	0.5
		1250	10.1	1.0	0.0	0.5	0.0
		2500	6.3	0.5	1.0	2.5	0.0
		5000	13.9	4.5	0.5	0.5	0.0
	CP (50 cells)	5.00	8.9	0.0	1.5	62.0*	32.0*

[t] Times listed are approximate N/A = not applicable * Fisher's Exact Test p ≤ 0.01 MMC = mitomycin C CP = cyclophosphamide h = hour

Metabolic Activation	Test/Control Article	Dose Concentration (µg/mL)	Assay 2 (Total 200 Cells Counted)				
			Mitotic Index (%)	Endoreduplicated Cells (%)	Polyploid Cells (%)	Cells With Chromosomal Aberrations (%)	Cells With > 1 Chromosomal Aberrations (%)
Without S9	Negative (media)	0	6.6	0.0	1.5	1.0	0.0
	Vehicle Control	0	6.2	0.0	0.5	1.0	0.0
	Exenatide	625	7.8	0.0	1.0	1.5	0.0
		1250	8.1	0.0	1.0	0.5	0.0
		2500	8.7	0.0	0.0	1.5	0.0
		5000	7.6	0.0	1.0	0.0	0.0
	MMC (50 cells)	0.100	5.5	0.0	1.5	15.0*	2.5
With S9	Negative (media)	0	10.5	0.5	1.0	0.0	0.0
	Vehicle Control	0	11.3	0.5	1.0	1.5	0.0
	Exenatide	625	10.6	0.0	3.0	2.5	0.0
		1250	7.3	2.5	2.5	1.0	0.0
		2500	11.3	0.0	2.0	1.0	0.0
		5000	8.9	0.0	0.5	1.0	0.0
	CP (50 cells)	5.00	4.5	0.0	4.5	34.0*	10.0*

[t] Times listed are approximate N/A = not applicable * Fisher's Exact Test p ≤ 0.01 MMC = mitomycin C CP = cyclophosphamide h = hour

2.6.7.8.5

Report Title: Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells
Test for Induction of: Chromosomal Aberrations **Number Independent Assays:** 2 **Study No.:** REST02305
Cell Type: Chinese Hamster Ovary (CHO) **Number Replicate Cultures:** 2 **Location in CTD:** Section 4.2.3.3.5
Metabolizing System: Aroclor 1254-induced liver S9 fraction (S9) **GLP Compliance:** GLP
Test Article Vehicle: Deionized water **Date of Dosing:** 03Feb2003
Treatment Method: Assay 1 with 3 h treatment and 20 h total incubation ± S9; Assay 2 with 18 h (-S9) or 3 h (+S9) treatment and 20 h total incubation [t]
Special Features: Study performed to compare genotoxicity of exenatide from new manufacturer (Bachem)
Cytotoxic Effects: None
Genotoxic Effects: None

Metabolic Activation	Test/Control Article	Dose Concentration (µg/mL)	Assay 1 (Total 200 Cells Counted)				
			Mitotic Index (%)	Endoreduplicated Cells (%)	Polyploid Cells (%)	Cells With Chromosomal Aberrations (%)	Cells With Gaps or Chromosomal Aberrations (%)
Without S9	Negative (media)	0	13.7	0.0	0.0	0.0	1.0
	Vehicle Control	0	14.2	0.0	0.0	0.0	0.5
	Exenatide	625	-	0.0	0.0	0.5	0.5
		1250	-	0.5	0.0	0.5	0.5
		2500	-	0.0	0.0	0.0	0.0
		5000	19.3	0.0	0.0	0.5	1.0
	MMC (100 cells)	0.75	-	0.0	0.0	47.0*	48.0*
With S9	Negative (media)	0	11.5	1.0	0.0	1.0	1.0
	Vehicle Control	0	14.5	0.5	0.0	0.0	0.0
	Exenatide	625	-	0.0	0.5	0.5	1.0
		1250	-	1.0	0.0	0.5	0.5
		2500	15.9	1.0	0.0	0.0	0.0
		5000	12.7	0.0	0.0	1.0	1.0
	CP (100 cells)	7.50	-	0.0	2.0	50.0*	53.0*

[t] Times listed are approximate N/A = not applicable * Fisher's Exact Test p ≤ 0.01 MMC = mitomycin C CP = cyclophosphamide
- = not tested

Metabolic Activation	Test/Control Article	Dose Concentration (µg/mL)	Assay 2 (Total 200 Cells Counted)				
			Mitotic Index (%)	Endoreduplicated Cells (%)	Polyploid Cells (%)	Cells With Chromosomal Aberrations (%)	Cells With Gaps or Chromosomal Aberrations (%)
Without S9	Negative (media)	0	7.1	0.0	0.0	2.0	3.0
	Vehicle Control	0	14.0	0.0	0.0	0.0	2.0
	Exenatide	625	-	0.0	0.0	1.0	2.5
		1250	-	0.0	0.5	1.5	3.5
		2500	14.8	0.0	0.0	0.0	0.5
		5000	13.7	0.0	0.0	0.5	3.0
	MMC (100 cells)	0.200	-	0.0	0.0	71.0*	77.0*
With S9	Negative (media)	0	12.7	0.0	0.0	1.0	6.5
	Vehicle Control	0	12.3	0.0	0.0	3.0	5.5
	Exenatide	625	9.2	0.0	0.0	0.5	3.0
		1250	9.4	0.5	0.0	0.5	3.0
		2500	10.0	0.0	0.0	0.5	2.0
		5000	9.6	1.0	0.0	1.0	2.0
	CP (100 cells)	7.50	-	0.5	0.0	57.0*	61.0*

[t] Times listed are approximate N/A = not applicable * Fisher's Exact Test p ≤ 0.01 MMC = mitomycin C CP = cyclophosphamide
- = not tested

2.6.7.8.6

Report Title:	Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells				
Test for Induction of:	Chromosomal Aberrations	**Number Independent Assays:**	2	**Study No.:**	REST02304
Cell Type:	Chinese Hamster Ovary (CHO)	**Number Replicate Cultures:**	2	**Location in CTD:**	Section 4.2.3.3.6
Metabolizing System:	Aroclor 1254-induced liver S9 fraction (S9)			**GLP Compliance:**	GLP
Test Article Vehicle:	Deionized water			**Date of Dosing:**	05Feb2003
Treatment Method:	Assay 1 with 3 h treatment and 20 h total incubation ± S9; Assay 2 with 18 h (-S9) or 3 h (+S9) treatment and 20 h total incubation [t]				
Special Features:	Study performed to compare genotoxicity of exenatide from new manufacturer (Mallinckrodt)				
Cytotoxic Effects:	None				
Genotoxic Effects:	None				

Metabolic Activation	Test/Control Article	Dose Concentration (µg/mL)	Assay 1 (Total 200 Cells Counted)				
			Mitotic Index (%)	Endoreduplicated Cells (%)	Polyploid Cells (%)	Cells With Chromosomal Aberrations (%)	Cells With Gaps or Chromosomal Aberrations (%)
Without S9	Negative (media)	0	13.7	0.0	0.0	0.0	1.0
	Vehicle Control	0	14.2	0.0	0.0	0.0	0.5
	Exenatide	625	-	0.0	0.0	0.0	0.0
		1250	-	0.0	0.0	0.0	0.5
		2500	16.4	0.0	0.0	0.5	2.0
		5000	15.4	0.0	0.0	0.5	2.5
	MMC (100 cells)	0.75	-	0.0	0.0	47.0*	48.0*
With S9	Negative (media)	0	11.5	1.0	0.0	1.0	1.0
	Vehicle Control	0	14.5	0.5	0.0	0.0	0.0
	Exenatide	625	-	0.0	0.0	0.0	1.0
		1250	-	0.5	0.0	1.0	3.0
		2500	16.2	0.0	0.0	0.0	1.0
		5000	13.0	1.0	0.0	0.0	1.0
	CP (100 cells)	7.50	-	0.0	2.0	50.0*	53.0*

[t] Times listed are approximate N/A = not applicable * Fisher's Exact Test p ≤ 0.01 MMC = mitomycin C CP = cyclophosphamide
- = not tested

Metabolic Activation	Test/Control Article	Dose Concentration (µg/mL)	Assay 2 (Total 200 Cells Counted)				
			Mitotic Index (%)	Endoreduplicated Cells (%)	Polyploid Cells (%)	Cells With Chromosomal Aberrations (%)	Cells With Gaps or Chromosomal Aberrations (%)
Without S9	Negative (media)	0	7.1	0.0	0.0	2.0	3.0
	Vehicle Control	0	14.0	0.0	0.0	0.0	2.0
	Exenatide	625	-	0.0	0.0	0.0	2.0
		1250	-	0.0	0.0	0.0	1.0
		2500	-	0.0	0.0	1.0	2.5
		5000	14.1	0.0	0.0	0.0	1.5
	MMC (100 cells)	0.200	-	0.0	0.0	71.0*	77.0*
With S9	Negative (media)	0	12.7	0.0	0.0	1.0	6.5
	Vehicle Control	0	12.3	0.0	0.0	3.0	5.5
	Exenatide	625	-	0.0	0.0	0.5	2.5
		1250	-	0.0	0.5	0.0	2.0
		2500	-	0.0	0.0	0.0	2.0
		5000	13.5	0.0	0.0	0.5	2.0
	CP (100 cells)	7.50	-	0.5	0.0	57.0*	61.0*

[t] Times listed are approximate N/A = not applicable * Fisher's Exact Test p ≤ 0.01 MMC = mitomycin C CP = cyclophosphamide
- = not tested

2.6.7.9 GENETIC TOXICOLOGY (In Vivo)

Report Title:	In Vivo Mouse Micronucleus Assay With AC2993			**Study No.:**	REST00078
Test for Induction of:	Micronucleated Polychromatic Erythrocytes			**Location in CTD:**	Section 4.2.3.3.7
Species/Strain:	Mouse/Crl:CD-1 (ICR) BR			**GLP Compliance:**	GLP
Weight:	Approx. 27.6-33.4 g			**Date of Dosing:**	04April2000
Vehicle/Formulation:	PBO-F11/AC-2993-F4	**Treatment Schedule:**	Single dose	**Cells Evaluated:**	Bone marrow PCE
No. of Cells Analyzed/Animal:	2000 PCE/animal	**Sampling Times:**	24 h (34, 380 µg/kg), 24+48 h (0, 2000 µg/kg)		
Special Features:	None	**Method of Administration:**	SC injection (oral gavage CP)		
Toxic/Cytotoxic Effects:	No general or bone marrow cytotoxicity at doses up to 2000 µg/kg exenatide				
Genotoxic Effects:	None				
Evidence of Exposure:	Dosing records and dosing solution analysis				

Test Article	Dose (µg/kg)	No./Sex of Animals	Harvest Time (h)	% Micronucleated PCEs (± SE)	Ratio PCE/NCE (± SE)
Vehicle	0	6 M	24	0.09 (0.03)	0.57 (0.04)
Vehicle	0	6 M	48	0.03 (0.02)	0.53 (0.03)
Exenatide	34	6 M	24	0.06 (0.02)	0.88 (0.05)
Exenatide	380	6 M	24	0.03 (0.01)	0.66 (0.03)
Exenatide	2000	6 M	24	0.03 (0.02)	0.82 (0.07)
Exenatide	2000	6 M	48	0.04 (0.02)	0.45 (0.06)
CP	80,000	6 M	24	1.60 (0.31)**	0.71 (0.07)

h = hour SC = subcutaneous PCE = polychromatic erythrocyte NCE = normochromatic erythrocytes CP = cyclophosphamide M = males
ANOVA and Dunnett's t-Test * = $p < 0.05$ ** = $p < 0.01$ SE = standard error

2.6.7.10 CARCINOGENICITY

2.6.7.10.1 Mouse Carcinogenicity Study

Report Title:	104-Week Carcinogenicity Study of AC2993 Administered Subcutaneously in Mice				
Species/Strain:	Mice/Crl:CD-1 (ICR) BR	**Duration of Dosing:**	up to 98 weeks	**Study No.:**	REST01053
Initial Weight:	Males 24.6-29.7 g Females 21.4-26.4 g	**Duration of Postdose:**	None	**Location in CTD:**	Section 4.2.3.4.1
Date of First Dose:	09May2001	**Treatment of Controls:**	Vehicle injection	**GLP Compliance:**	GLP
Vehicle/Formulation:	AC2993-F12, PBO-F12/AC-2993-F7	**Method of Administration:**	Subcutaneous injection once daily		
Special Features:	Complete toxicokinetics from parallel study, REST02325R1, Section 4.2.3.2.3 (TK report REST03288, Section 4.2.3.2.3.2). Toxicokinetics from single time point during carcinogenicity study in REST04052 within this final report.				

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Toxicokinetics [a] $AUC_{0\text{-}6h}$ (pg•h/mL):										
Day 1	N/A		10,113		32,508		123,241		N/A	
Day 91	N/A		25,425		58,403		197,295		N/A	
Toxicokinetics [b] C_{30min} (pg/mL):	<10		22,177		77,814		231,460		<10	
Number of Animals										
Start of Treat:	65	65	65	65	65	65	65	65	65	65
Died/Sacrifice Moribund:	48	52	44	49	40	45	43	50	45	49
Scheduled Sacrifice:	17	13	21	16	25	20	22	15	20	16
Cumulative Survival (%):	32.31	20.00	32.31	26.15	40.00	30.77	36.92	26.15	30.77	26.15
Mean Body Weight (g):										
Week 1	30.40	25.61	30.71	25.78	30.93	25.86	30.92	26.21	30.25	25.05*
Week 52	42.65	34.49	42.52	36.19*	42.95	35.48	43.24	36.93**	41.66	34.80
Week 104	42.20	36.02	41.68	37.22	41.28	36.26	41.98	35.44	42.30	37.11
Mean Food Consumption (g/day):										
Week 1	7.09	6.40	7.45	7.97*	7.66*	7.70**	6.48**	6.29	6.55	7.27*
Week 52	7.05	6.64	6.33**	6.86	6.77	7.11	6.51*	6.95	6.75	6.54
Week 104	6.02	5.94	6.20	6.14	6.39	6.11	6.16	6.13	5.73	5.83

N/A = not assayed or measured
- = No noteworthy findings or findings not different from controls.
* = $p < 0.05$ ** = $p < 0.01$ Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
[a] REST03288, Section 4.2.3.2.3.2. Male and female values combined.
[b] 5/sex control groups and 10/sex exenatide-treated groups.

2.6.7.10.1 Contd.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Clinical Observations:	-	-	-	-	-	-	-	-	-	-
Hematology:										
Leukocytes (1000/mm3)	3.61	5.79	5.42*	5.46	5.94**	4.93	5.54*	5.16	4.87	6.56
Erythrocytes (million/mm3)	7.942	6.759	7.851	7.442	7.636	7.336	7.910	7.465	7.836	7.712*
Neutrophils (1000/µL)	1.236	2.730	2.542*	2.311	2.686**	1.758	2.069*	1.548	1.748*	2.714
Monocytes (1000/µL)	0.164	0.176	0.221	0.181	0.276**	0.181	0.236*	0.160	0.194	0.198*
Number of Animals with Neoplastic Lesions										
Adrenals glands										
Adenoma, subcapsular, bn, 1°	0	1	1	0	2	1	1	0	1	0
Pheochromocytoma, bn, 1°	0	0	0	1	0	0	0	0	0	0
Pheochromocytoma, mal, 1°	0	0	0	1	0	0	0	0	0	0
Brain										
Astrocytoma, mal, mc	0	0	0	0	1	0	0	0	0	0
Oligodendroglioma, mal, 1°	0	0	0	0	0	0	0	1	0	0
Epididymides										
Adenoma, interstitial cell, bn, 1°	0	NA	0	NA	1	NA	0	NA	0	NA
Schwanoma, bn	0		0		0		1		0	
Harderian glands										
Adenoma, bn, 1°	0	0	0	0	0	1	0	0	0	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test;
Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Injection site, left flank										
Fibrosarcoma, mal, 1°	0	0	1	0	0	1	0	0	0	0
Liposarcoma, mal, 1°	0	1	0	0	0	0	0	0	0	0
Injection site, left shoulder										
Fibrous histiocytoma, mal, 1°	0	0	0	1	0	0	0	0	0	0
Injection site, right flank	0	0	0	0	0	0	0	0	0	0
Injection site, right shoulder	0	0	0	0	0	0	0	0	0	0
Kidneys										
Adenoma, tubular cell, bn, 1°	0	0	0	0	0	0	1	0	1	0
Liver										
Adenoma, hepatocell, bn, 1°	7	1	8	2	5	1	7	1	4	1
Carcinoma, hepatocell, mal, 1°	2	0	3	0	1	0	2	1	4	0
Hemangioma, bn, 1°	1	0	0	1	0	0	0	0	0	0
Hemangiosarcoma, mal, 1°	4	0	0	0	2	2	2	0	2	1
Lung										
Adenoma, BA, bn,1°	13	11	9	10	14	8	13	6	11	12
Carcinoma, BA, mal, 1°	4	1	3	5	1	0	4	3	3	5
Mammary glands										
Adenocarcinoma, mal, 1°	0	1	0	1	0	0	0	0	0	0
Mesentery/peritoneum										
Hibernoma, bn, 1°	0	0	0	0	0	0	0	0	1	0
Multicentric neoplasm										
Leukemia, granulocytic, mal, mc	0	0	0	0	1	0	0	0	0	0
Lymphoma, mal, mc	4	6	4	8	3	6	1	8	5	4
Sarcoma, undiff, mal, 2°	0	0	0	0	0	0	1	0	1	0
Sarcoma, histiocytic, mal, mc	0	4	0	10	0	5	1	1	1	5
Carcinoma, 1° unknown, mal	0	0	0	1	0	0	0	0	0	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

2.6.7.10.1 Contd.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Ovary	NA		NA		NA		NA		NA	
Adenoma, tubulostromal, bn, 1°		0		0		0		0		1
Cystadenoma, bn, 1°		1		0		0		1		0
Leiomyosarcoma, mal, 1°		0		0		0		1		0
Sex-cord/stromal tumor, bn, 1°		1		0		1		0		3
Pancreas										
Adenoma, islet cell, bn, 1°	0	0	0	1	0	0	0	0	0	0
Pituitary gland										
Adenoma, pars distalis, bn, 1°	0	1	0	1	0	3	2	1	0	1
Adenoma, pars intermedia, bn, 1°	0	0	0	1	0	0	0	0	0	0
Seminal vesicles		NA		NA		NA		NA		NA
Hemangiosarcoma, mal 1°	0		0		1		0		0	
Skeletal muscle										
Hemangiosarcoma, mal, 1°	0	1	0	1	0	0	0	0	0	0
Skin, all										
Fibrosarcoma, mal, 1°	0	1	0	0	0	2	0	0	1	1
Hemangiosarcoma, mal, 1°	0	0	0	0	0	0	1	1	0	0
Sarcoma, undiff, mal, 1°	0	3	0	0	1	0	1	1	4	1
Carcinoma, basosquamous, mal, 1°	0	1	0	0	0	0	0	0	0	0
Carcinoma, squamous, mal, 1°	0	1	0	0	0	0	0	0	0	0
Keratoacanthoma, bn, 1°	0	0	0	0	0	0	0	0	0	1
Leiomyosarcoma, mal, 1°	0	1	0	0	0	0	0	0	0	0
Liposarcoma, mal, 1°	0	0	0	1	0	0	0	0	0	0
Fibrous histiocytoma, mal, 1°	0	0	0	1	0	0	0	0	0	0
Small intestine, all										
Adenocarcinoma, mal, 1°	0	0	0	0	1	0	0	0	1	0
Fibrosarcoma, mal, 1°	0	0	0	0	0	0	0	1	0	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test;
Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Spleen										
Hemangioma, bn, 1°	0	0	1	1	0	1	0	0	0	0
Hemangiosarcoma, mal, 1°	3	1	1	0	1	0	0	1	1	1
Stomach										
Osteosarcoma	0	0	0	0	0	0	1	0	0	0
Thoracic cavity										
Osteoma, bn, 1°	0	0	0	1	0	0	0	0	0	0
Thyroid										
Adenoma, follicular cell, bn, 1°	0	0	0	0	0	0	1	0	0	0
Carcinoma, follicular cell, mal, 1°	1	0	0	0	0	0	0	0	0	0
Urinary bladder										
Hemangioma, bn, 1°	0	0	0	0	0	0	1	0	0	0
Mesenchymal tumor, bn, 1°	0	0	1	0	0	0	0	0	0	1
Papilloma, transitional cell, bn, 1°	0	0	0	0	0	0	0	1	1	0
Uterus and Cervix	NA		NA		NA		NA		NA	
Adenocarcinoma, mal, 1°		1		1		0		0		1
Adenoma, bn, 1°		0		0		1		0		0
Fibroma, bn, 1°		0		0		1		0		0
Fibrosarcoma, mal, 1°		1		0		0		0		0
Granular cell tumor, bn, 1°		0		0		2		1		0
Hemangioma, bn, 1°		0		1		2		0		0
Hemangiosarcoma, mal, 1°		0		1		0		1		1
Leiomyoma, bn, 1°		2		1		0		3		0
Leiomyosarcoma, mal, 1°		1		0		3		1		0
Sarcoma, stromal, mal, 1°		4		1		0		5		3
Vagina										
Sarcoma, stromal, mal, 1°	NA	1	NA	0	NA	0	NA	0	NA	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test;
Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

2.6.7.10.1 Contd.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Non-Neoplastic Findings:										
Parotid salivary gland										
Hypertrophy, basophilic, focal										
minimal	1	4	14	14	14	9	14	17	3	2
mild	0	0	9	14	15	18	10	11	0	0
moderate	0	0	6	4	2	8	5	12	0	0
severe	0	0	0	0	1	0	3	2	0	0
total	1	4	29	32	32	35	32	42	3	2

N/A = not assayed or measured
- = No noteworthy findings or findings not different from controls
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).

2.6.7.10.2 Rat Carcinogenicity Study

Report Title:	104-Week Carcinogenicity Study of AC2993 Administered Subcutaneously in Rats				
Species/Strain:	**Rat/CD [Crl:CD (SD) IGS BR]**	**Duration of Dosing:**	up to 106 weeks	**Study No.:**	REST01052
Initial Weight:	Males 172-213 g Females 144-178 g	**Duration of Postdose:**	None	**Location in CTD:**	Section 4.2.3.4.2
Date of First Dose:	24May2001	**Treatment of Controls:**	Vehicle injection	**GLP Compliance:**	GLP [c]
Vehicle/Formulation:	AC-2993-F12, PBO-F12/AC-2993-F7	**Method of Administration:**	Subcutaneous injection once daily		
Special Features:	Complete toxicokinetics from parallel study, REST02246R1, Section 4.2.3.2.6 (TK report REST03286, Section 4.2.3.2.6.2). Toxicokinetics from single time point on Day 722 of carcinogenicity study in REST04053 within this final report.				

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Toxicokinetics [a] AUC $_{0\text{-}6h}$ (pg•h/mL):										
Day 1	N/A		20,188		45,619		201,764		N/A	
Day 91	N/A		10,178		48,554		268,094		N/A	
Toxicokinetics [b] C_{30min} Day 722 (pg/mL):	31		13,413		47,179		208,635		14	
Anti-exenatide antibody (number positive):[c]	2		2		3		3		3	
Number of Animals										
Start of Treat:	65	65	65	65	65	65	65	65	65	65
Died/Sacrifice Moribund:	41	51	28	35	28	22	29	30	42	51
Scheduled Sacrifice:	24	14	37*	30**	37**	43**	36*	35**	23	14
Cumulative Survival (%):	36.92	23.08	56.92*	46.15**	58.46**	66.15**	56.92*	53.85**	38.46	21.54
Mean Body Weight (g):										
Week 1	295.0	205.4	280.4**	204.9	274.9**	204.5	273.7**	203.2	290.0	201.6
Week 52	634.6	371.3	524.0**	314.5**	516.7**	317.6**	499.4**	312.0**	639.4	361.6
Week 104	665.8	428.3	567.1**	382.7	558.3**	393.5	546.1**	372.7*	654.5	428.1
Mean Food Consumption (g/day):										
Week 1	25.36	18.90	22.12**	16.57**	20.65**	15.65**	20.03**	15.01**	24.77	18.29
Week 52	28.82	23.35	25.56**	20.28**	24.77**	20.23**	24.85**	20.73**	27.23**	22.70
Week 104	28.17	24.05	25.00*	21.16	24.74**	22.87	25.15*	22.23	26.81	23.47

N/A = not assayed or measured * = p < 0.05** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
[a] REST03286, Section 4.2.3.2.6.2. Male and female values combined.
[b] 5/sex control groups and 10/sex exenatide-treated groups.
[c] REST02132R1, Section 4.2.3.4.2.1. Anti-exenatide antibody positive, all titers were ≤1:25, assay only was non-GLP.

2.6.7.10.2 Contd.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Clinical Observations:	-	-	-	-	-	-	-	-	-	-
Hematology:	-	-	-	-	-	-	-	-	-	-
Number of Animals with Neoplastic Lesions										
Adipose Tissues										
Lipoma, bn, 1°	0	0	0	0	0	0	1	0	0	0
Hibernoma, bn, 1°	0	0	0	1	0	0	0	0	0	0
Hibernoma, mal, 1°	0	0	0	1	0	0	0	0	0	0
Adrenals glands										
Adenoma, cortical, bn, 1°	0	4	1	1	1	4	4	3	2	1
Carcinoma, cortical, mal, 1°	0	2	3	0	0	0	0	0	0	0
Pheochromocytoma, bn, 1°	3	3	1	3	4	3	6	2	12	2
Pheochromocytoma, mal, 1°	0	0	1	0	0	0	0	1	0	0
Brain										
Astrocytoma, mal, 1°	1	0	2	0	0	0	1	0	1	0
Hemangiosarcoma, mal, 1°	1	0	0	0	0	0	0	0	0	0
Granular cell tumor, mal, 1°	0	0	0	0	0	0	0	1	0	0
Meningioma, bn, 1°	0	1	2	0	0	0	0	0	0	0
Oligodendroglioma, bn, 1°	0	0	0	1	0	0	0	0	0	0
Papilloma, choroid plexus,bn,1°	0	0	0	0	0	0	0	1	0	0
Reticulosis, mal, 1°	0	1	0	0	0	0	0	0	0	0
Cavity, abdominal or thoracic										
Rhabdomyosarcoma, mal, 1°	0	0	0	0	1	0	0	0	0	0
Sarcoma, undiff, mal, 1°	1	0	0	0	0	0	0	0	0	0
Hibernoma, mal	0	0	0	0	0	0	1	0	0	0
Neuroendocrine tumor, mal, 1°	0	0	0	1	0	0	0	0	0	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cell or cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Injection site, left flank	0	0	0	0	0	0	0	0	0	0
Injection site, left shoulder										
Sarcoma, undiff, mal, 1°	0	0	1	0	0	0	0	0	0	0
Injection site, right flank										
Fibrosarcoma, mal, 1°	0	0	0	0	0	0	0	0	1	0
Injection site, right shoulder										
Trichoepithelioma, bn, 1°	0	0	0	0	1	0	0	0	0	0
Kidneys										
Carcinoma, squamous cell, mal, 1°	0	0	1	0	0	0	0	0	0	0
Adenoma, tubular cell, bn, 1°	0	0	0	0	0	1	0	0	0	0
Carcinoma, tubular cell, mal, 1°	0	0	0	0	0	0	0	0	0	1
Lipoma, bn, 1°	0	0	0	0	0	0	0	0	0	1
Nephroblastoma, bn, 1°	0	1	0	0	0	0	0	0	0	0
Large intestine, cecum										
Fibroma, bn, 1°	0	0	0	0	0	0	0	0	0	1
Liver										
Adenoma, hepatocell, bn, 1°	0	2	0	1	1	1	2	1	2	2
Carcinoma, hepatocell, mal, 1°	0	1	0	0	1	0	1	1	0	0
Lymph nodes, all										
Hemangioma, bn 1°	0	0	0	0	1	0	0	0	0	0
Mammary glands										
Adenocarcinoma, mal, 1°	0	27	0	8	0	7	0	11	0	24
Adenoma, bn, 1°	0	4	0	3	0	1	0	1	0	1
Fibroadenoma, bn, 1°	0	26	0	12	0	17	2	13	0	21
Mediastinum										
Sarcoma, undiff, mal, 1°	0	0	0	0	0	0	0	0	1	0
Fibrosarcoma, mal, 1°	0	1	0	0	0	0	0	0	0	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

2.6.7.10.2 Contd.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Multicentric neoplasm										
Lymphoma, mal, mc	2	1	0	1	0	1	2	0	1	0
Mast cell tumor, mal, mc	0	0	0	0	0	0	0	0	0	1
Sarcoma, histiocytic, mal, mc	2	2	1	4	0	0	0	0	1	0
Ovary	NA		NA		NA		NA		NA	
Adenoma, tubulostromal, bn, 1°		0		0		1		0		0
Carcinoma, tubulostromal, mal, 1°		0		0		0		1		0
Sex-cord/stromal tumor, bn, 1°		1		1		1		0		1
Sex-cord/stromal tumor, mal, 1°		0		0		0		0		1
Pancreas										
Adenoma, acinar cell, bn, 1°	0	0	0	0	0	1	0	0	0	0
Adenoma, islet cell, bn, 1°	3	1	3	1	4	2	5	2	2	2
Carcinoma, acinar cell, mal, 1°	0	0	0	0	0	0	0	1	0	0
Carcinoma, islet cell, mal, 1°	1	1	0	0	0	0	0	0	0	3
Parathyroid glands										
Adenoma, bn, 1°	1	0	2	0	0	0	1	0	4	0
Pituitary gland										
Adenoma, pars distalis, bn, 1°	36	55	31	47	26	56	29	48	29	49
Adenoma, pars intermedia, bn, 1°	0	0	1	0	0	0	0	0	0	0
Carcinoma, pars distalis, mal, 1°	0	0	0	0	0	0	0	1	0	0
Primary site unknown										
Adenocarcinoma, mal, 1°	0	0	0	0	0	0	0	0	1	0
Carcinoma, squamous cell, mal, 1°	0	0	0	0	0	0	0	1	0	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test;
Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Skin, all										
Adenoma, basal cell, bn, 1°	0	0	0	0	0	0	0	0	0	1
Carcinoma, squamous cell, mal, 1°	0	0	1	1	0	1	0	1	1	0
Papilloma, squamous, bn, 1°	0	0	0	1	1	0	0	0	1	0
Fibroma, bn, 1°	3	0	0	0	3	0	3	0	2	0
Fibrosarcoma, mal, 1°	0	2	0	1	1	1	0	0	1	3
Hemangiosarcoma, mal, 1°	1	0	0	1	0	0	0	0	1	0
Keratoacanthoma, bn, 1°	0	0	0	1	0	0	0	0	0	0
Lipoma, bn, 1°	1	0	0	0	0	0	0	0	1	0
Sarcoma, undiff, mal, 1°	0	0	1	0	0	0	0	0	1	0
Sarcoma, histiocytic, mal, 1°	0	1	0	3	0	0	0	0	0	0
Small intestine, all										
Adenocarcinoma, mal, 1°	0	0	0	0	0	0	0	0	1	0
Leiomyoma, bn, 1°	0	0	0	0	0	1	0	0	0	0
Stomach										
Carcinoma, squamous, mal, 1°	0	0	1	0	0	0	0	0	0	0
Testes		NA		NA		NA		NA		NA
Adenoma, interstitial cell, bn, 1°	3		5		4		2		1	
Mesothelioma, mal, 1°	0		0		1		0		0	
Thymus gland										
Thymoma, bn, 1°	0	0	0	2	0	0	0	0	0	1
Thyroid gland										
Adenoma, c-cell, bn, 1°	8	5	10	9	15	7	10	15	10	3
Adenoma, follicular cell, bn, 1°	0	0	0	0	1	0	0	2	0	1
Carcinoma, c-cell, mal, 1°	0	0	0	0	0	0	0	0	1	0
Carcinoma, follicular cell, mal 1°	0	0	0	0	0	1	0	0	1	0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* = p < 0.05 ** = p < 0.01 Compared to Control 1+2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test;
Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

2.6.7.10.2 Contd.

Daily Dose (µg/kg/day)	0 (Control 1)		18		70		250		0 (Control 2)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Tongue										
Carcinoma, squamous cell, mal, 1°	0	0	1	0	0	0	0	0	0	0
Urinary bladder										
Papilloma, transitional cell, be, 1°	0	0	1	0	0	0	0	0	0	0
Uterus with cervix	NA		NA		NA		NA		NA	
Granular cell tumor, bn, 1°		1		1		0		0		0
Leiomyoma, bn, 1°		0		0		1		1		0

mc = multicentric mal = malignant undiff = undifferentiated bn = benign 1° = primary cell = cellular BA = bronchiolar alveolar
* – p < 0.05 ** – p < 0.01 Compared to Control 1 | 2 (or Control 2 vs. Control 1); Dunnett's t-test (Welch's t-test if not homogenous); Survival Log-Rank Test; Tumor Analysis Cochran-Armitage trend then Fisher's exact test or survival-adjusted using prevalence methods described by Peto, et al (reference in report).
Multicentric tumors and secondary tumors not included in organ summaries.

Reproductive and Developmental Toxicity

2.6.7.11 - Fertility and Early Embryonic Development to Implantation

Report Title: Subcutaneous Fertility and General Reproduction Toxicity Study of AC2993 in Mice
Species/Strain: Mice/CRL:CD-1 (ICR) BR **Study No.:** REST01001
Initial Age: M-68 days F-68 days **Location in CTD:** Section 4.2.3.5.1
Date of First Dose: 06MAR2001 **Method of Administration:** Subcutaneous injection. BID **GLP Compliance:** GLP
Vehicle/Formulation: AC-2993-F12/AC-2993-F7 **Duration of Dosing:** M-28 days prior to mating through cohabitation; F-15 days prior to mating through DG 7
Special Features: None
Design Similar to ICH 4.1.1: Yes
No Observed Adverse Effect Level for Fertility and Development: **F_0 Males:** 760 µg/kg/day **F_0 Females:** 760 µg/kg/day **F_1 Litters:** 760 µg/kg/day

Daily Dose (µg/kg/day)	0 (Control)	6	68	760
MALES				
Number of Males	25	25	25	25
Toxicokinetics [a] AUC_{0-12h} (pg•h/mL)	N/A	3288	37,264	416,480
No. Died or Sacrificed Moribund	0	1	0	0
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Body Weight DS28 (g)	38.0	39.4*	38.8	39.5**
Food Consumption (%)	-	-	-	-
Organ Weights				
Prostate/Body Weight (x1000)	110	90	97	82**
Mean No. Days Prior to Mating	3.0	3.0	2.3	3.3
Males that Mated (%)	100.0	100.0	100.0	100.0
Fertility Index [b] (%)	96.0	95.8	92.0	96.0
Sperm Count (motile and static)	477.7	448.0	441.1	375.8
Motility (%)	90.0	89.6	89.6	91.1

BID = Dose divided and administered twice daily N/A = not assayed or measured No.= number DG = Presumed Day of Gestation (starting on Day 0) DS = Day of Study
- No noteworthy findings
* p < 0.05 ** p < 0.01 One-way ANOVA with Dunnett's t-test
[a] REST03391R1, Section 4.2.3.7.7.1. All values were obtained from non-pregnant animals.
[b] Number of pregnancies/Number of mice mated x 100; including 1 pregnancy for each male that impregnated more than one female.

2.6.7.11 Contd.

Daily Dose (µg/kg/day)	0 (Control)	6	68	760
FEMALES				
Number of Females	25	25	25	25
Toxicokinetics [a] AUC_{0-12h} (pg•h/mL)	N/A	3288	37264	416480
No. Died or Sacrificed Moribund	0	0	0	0
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Premating Body Weight (g)	28.7	29.8*	31.0**	30.0**
DG 7 Body Weight (g)	34.0	35.0	35.7*	35.4*
Premating Food Consumption	-	-	-	-
Gestation Food Consumption	-	-	-	-
Mean No. Estrous Cycles/14 days	3.4	3.4	3.0	3.0
Mean No. Days Prior to Mating	3.0	3.0	2.3	3.3
Females Sperm Positive (%)	100	100	100	100
Pregnant Females [c] (%)	96.0	96.0	92.0	96.0
No. Aborted or with Total Resorption of Litter	0	0	0	0
Mean No. Corpora Lutea	14.1	14.7	14.2	13.8
Mean No. Implantations	13.0	13.8	13.3	12.6
Mean % Preimplantation Loss	7.4	23.7	5.7	6.2
Mean No. Viable Conceptuses	12.7	12.9	12.7	12.0
Mean No. Nonviable Conceptuses	0.3	0.9	0.6	0.7
Mean % Postimplantation Loss	2.2	6.5	4.6	5.2

BID = Dose divided and administered twice daily N/A = not assayed or measured No.= number DG = Presumed Day of Gestation (starting on Day 0) DS = Day of Study
- No noteworthy findings.
* p < 0.05 ** p < 0.01 One-way ANOVA with Dunnett's t-test
[a] REST03391R1, Section 4.2.3.7.7.1. All values were obtained from non-pregnant animals.
[c] Number of pregnancies/Number of mice in cohabitation x 100.

2.6.7.12 Reproductive and Developmental Toxicity - Effects on Embryo/Fetal Development

2.6.7.12.1 Embryo-Fetal Development in Mice

Report Title: Developmental Toxicity Study of Subcutaneously Administered AC2993 in Mice
Species/Strain: Mice/CRL:CD-1 (ICR) BR **Day of Mating:** DG 0 **Study No.:** REST99060R1
Initial Age: 65 Days **Day of Caesarian Section:** DG 18 **Location in CTD:** Section 4.2.3.5.2
Date of First Dose: 02FEB2000 **Duration of Dosing:** DG 6 – DG 15 **GLP Compliance:** GLP
Vehicle/Formulation: PBO-F11/AC-2993-F4 **Method of Administration:** Subcutaneous injection, daily dose divided BID
Special Features: None
Design Similar to ICH 4.1.3: Yes
No Observed Adverse Effect Level: F_0 Females: 6 µg/kg/day **F_1 Litters:** 6 µg/kg/day

Daily Dose (µg/kg/day)	0 (Control)	6	68	460	760
Number of Females					
Main Study	25	25	25	25	25
Toxicokinetics [d]	0	5	5	5	5
Toxicokinetics [a] **AUC_{0-12h} (pg•h/mL)**	N/A	3288	37,264	252,080	416,480
No. Died or Sacrificed Moribund	0	0	0	0	0
Pregnancy (%)	96	84	92	88	76
Abortions (%)	0	0	4	0	5
Premature Deliveries (%)	0	0	4	5	5
Clinical Observations	-	-	-	-	-
Necropsy Observations					
Ovarian cyst (incidence)	0/25	0/25	0/25	1/25	0/25
Body Weight (g)					
DG 6	29.0	28.5	29.0	28.9	28.8
DG 7	29.4	28.7	28.8	27.7**	27.4**
DG 15	42.1	42.5	43.5	41.2	42.4
Food Consumption DG 6-16 (g)	5.1	4.8	4.6	4.2**	4.4**
Mean No. Corpora Lutea	14.1	13.3	13.6	12.9	14.3
Mean No. Implantations	13.0	12.3	12.7	11.8*	13.2

BID = Dose divided and administered twice daily N/A = not assayed or measured No.= number DG = Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings. * = p < 0.05 ** = p < 0.01 One-way ANOVA with Dunnett's t-test
[a] REST03391R1, Section 4.2.3.7.7.1. All values were obtained from non-pregnant animals.
[b] Number of pregnancies/Number of mice mated.
[c] Based on absence of body weight loss.
[d] REST01002, Section 4.2.2.3..2.1. Results discussed in Section 2.6.4.4 Distribution.

2.6.7.12.1 Contd.

Daily Dose (µg/kg/day)	0 (Control)	6	68	460	760
Number of Females					
Main Study	25	25	25	25	25
Litter Sizes	12.7	11.4	12.2	11.0**	12.3
Live Fetuses (%)	99	100	100	100	99
Mean Resorptions	0.4	0.9	0.4	0.7	0.9
Early Resorptions	0.2	0.8	0.2	0.5	0.5
Late Resorptions	0.1	0.1	0.2	0.2	0.4
Mean Fetal Body Weight (g)					
Male	1.29	1.30	1.24	1.20**	1.13**
Female	1.25	1.24	1.19*	1.14**	1.07**
Percent Male Fetuses	47.7	48.5	52.4	45.7	56.6
Fetal Anomalies:					
Gross External % (litter/fetal incidence):					
Cleft Palate	8.3/1.3	19.0/2.1	9.5/0.8	9.5/1.3	17.6/3.4
Umbilical Hernia	0/0	0/0	0/0	4.8/0.4	0/0
Eyelids Open	4.2/0.3	0/0	0/0	0/0	5.9/0.5
Visceral Anomalies % (litter/fetal):					
Hole in Palate	4.2/0.7	9.5/1.7	0/0	4.8/0.9	0/0
Umbilical Artery Left of Urinary Bladder	20.8/4.1	0/0	33.3/7.4	23.8/4.5	35.3/7.1

BID = Dose divided and administered twice daily N/A = not assayed or measured No.= number DG = Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings
* = p < 0.05 ** = p < 0.01 One-way ANOVA with Dunnett's t-test
[a] REST03391R1, Section 4.2.3.7.7.1. All values were obtained from non-pregnant animals.
[b] Number of pregnancies/Number of mice mated.

2.6.7.12.1 Contd.

Daily Dose (µg/kg/day)	0 (Control)	6	68	460	760
Number of Females					
Main Study	25	25	25	25	25
Skeletal Anomalies % (litter/fetal):					
Skull, frontals contained interfontal	58.3/17.9	71.4/29.0	85.7/25.2	52.4/25.6	76.5/29.4
Palate, incomplete ossification	8.3/1.9	4.8/0.8	9.5/1.5	9.5/1.6	17.6/4.6
Cervical rib at 7th cervical vertebra	54.2/15.4	52.4/16.1	61.9/20.0	33.3/9.9	35.3/9.2
Thoracic vertebral arches fused	0/0	0/0	0/0	4.8/1.6	0/0
Thoracic vertebral centra fused	0/0	0/0	0/0	4.8/0.8	0/0
Ribs, wavy	0/0	0/0	0/0	0/0	11.8**/2.8**
Ribs, fused	0/0	0/0	0/0	4.8/1.6	0/0
Manubrium, fused	4.2/0.6	0/0	0/0	4.8/0.8	0/0
Sternal centra fused or asymmetric	12.5/1.9	14.3/3.2	0/0	9.5/1.6	0/0
Xiphoid, bifid	0/0	0/0	0/0	4.8/0.8	0/0
Notable Ossification Sites (no./fetus/litter)					
Vertebrae, thoracic	13.22	13.41	13.23	13.43*	13.40
Vertebrae, lumbar	5.78	5.58	5.77	5.57*	5.59
Ribs, pairs	13.17	13.37*	13.18	13.39*	13.32
% Affected Fetuses/Litter:	19.0	26.0	24.8	20.6	26.0

BID = Dose divided and administered twice daily N/A = not assayed or measured No.= number DG = Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings
* = $p < 0.05$ ** = $p < 0.01$ One-way ANOVA with Dunnett's t-test
[a] REST03391R1, Section 4.2.3.7.7.1. All values were obtained from non-pregnant animals.
[b] Number of pregnancies/Number of mice mated.

2.6.7.12.2 Embryo-Fetal Development Study in Rabbits

Report Title: Developmental Toxicity Study of Subcutaneously Administered AC2993 in Rabbits
Species/Strain: Rabbits/New Zealand White [Hra:(NZW)SPF] **Study No.:** REST99061R2
Initial Age: 5-6 Months **Day of Mating:** DG 0 **Location in CTD:** Section 4.2.3.5.3
Date of First Dose: 06FEB2000 **Day of Caesarian Section:** DG 29 **GLP Compliance:** GLP
Vehicle/Formulation: PBO-F11/AC-2993-F4 **Duration of Dosing:** DG 6 – DG 18 (TK dosed DG 6- DG 24)
Special Features: None **Method of Administration:** Subcutaneous injection, daily dose divided BID
Design Similar to ICH 4.1.3: Yes
No Observed Adverse Effect Level: F_0 Females: 0.2 µg/kg/day **F_1 Litters:** 0.2 µg/kg/day

Daily Dose (µg/kg/day)	0 (Control)	0.2	22	156	260
Number of Females					
Main Study	20	20	20	20	20
Toxicokinetics (fetal)	5	5	5	5	5
Toxicokinetics [b] AUC_{0-12h} (pg•h/mL)	**N/A**	228	214,883	1,486,667 [c]	3,610,750
No. Died or Sacrificed Moribund	0	1	1	0	0
Pregnancy (%)	95	100	95	100	90
Abortions (%)	0	0	0	5	0
Premature Deliveries (%)	0	0	5	0	0
Clinical Observations (incidence)					
Scant Feces	1/20	3/20	10/20*	16/20**	20/20**
No Feces	0/20	0/20	2/20	3/20	4/20
Necropsy Observations	-	-	-	-	-
Body Weight (kg)					
DG 6	3.55	3.56	3.58	3.56	3.52
DG 7	3.61	3.61	3.45	3.38**	3.34**
DG 18	3.80	3.76	3.60	3.42**	3.34**
DG 29	3.98	3.93	3.89	3.79	3.76

BID = Dose divided and administered twice daily N/A = Not assayed or measured No.= Number DG = Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings. C = cervical T = thoracic Lu = Lumbar Cau = Caudal
* = $p < 0.05$ ** = $p < 0.01$
[a] REST01003, Section 4.2.2.3.4.1. Results reviewed in Section 2.6.4.4 Distribution.
[b] REST03391R1, Section 4.2.3.7.7.1.
[c] Value excludes Day 12.

2.6.7.12.2 Contd.

Daily Dose (µg/kg/day)	0 (Control)	0.2	22	156	260
Food Consumption/Day (g)					
DG 6-9	166.5	165.1	50.6**	20.4**	14.8**
DG 15-19	162.8	150.8	125.2**	97.1**	83.3**
DG 19-29	132.2	131.2	146.0	151.4*	157.3**
Mean No. Corpora Lutea	10.4	10.1	9.2	10.5	10.5
Mean No. Implantations	8.8	8.8	7.6	8.3	9.1
Mean Litter Sizes	8.8	8.6	7.4	7.5	8.0
Mean Live Fetuses/Litter	8.8	8.5	7.4	7.5	8.0
Mean Resorptions	0.0	0.2	0.2	0.8**	1.1**
Early Resorptions	0.0	0.0	0.1	0.3	0.7**
Late Resorptions	0.0	0.2	0.1	0.6**	0.4
Live Fetal Body Weight/Litter (g)					
Male	43.59	44.18	43.86	41.17	40.84
Female	43.17	41.19	42.75	40.73	41.38
Percent Male Fetuses	53.4	40.0	47.2	49.7	43.9
Fetal Anomalies:					
Total Affected Fetuses (%):	7.2	7.4	17.5*	23.9**	23.6**
Gross External % (litter/fetal incidence):					
Umbilical Hernia	0.0/0.0	0.0/0.0	11.8/1.6	10.5/5.6*	33.3**/11.8**

BID – Dose divided and administered twice daily N/A – Not assayed or measured No. – Number DG – Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings. C = cervical T = thoracic Lu = Lumbar Cau = Caudal
* – p < 0.05 ** – p < 0.01

Daily Dose (µg/kg/day)	0 (Control)	0.2	22	156	260
Visceral Anomalies % (litter/fetal):					
Eye-Circumcorneal Hemorrhage	0.0/0.0	0.0/0.0	5.9/2.4**	0.0/0.0	0.0/0.0
Lung-Interm. Lobe Absent	10.5/3.0	15.8/1.8	5.9/1.6	5.3/0.7	16.7/2.1
Kidney-Dilation of Pelvis	0.0/0.0	0.0/0.0	0.0/0.0	5.3/1.4	0.0/0.0
Intestine-Protrude through umbilical opening	0.0/0.0	0.0/0.0	11.8/1.6	10.5/5.6*	33.3**/11.8**
Gallbladder-Absent	0.0/0.0	0.0/0.0	17.6**/2.4**	0.0/0.0	5.6/0.7
Gallbladder-Small	0.0/0.0	10.5/1.2	17.6/5.6**	15.8/3.5*	11.1/2.8**
Skeletal Anomalies % (litter/fetal):					
Skull-Irregular ossification (all)	21.0/2.4	5.3/0.6	5.9/0.8	15.8/2.8	16.7/2.1
Hyoid: Ala, angulated	10.5/1.2	10.5/1.8	35.3/5.6**	21.0/4.9**	44.4/7.6**
C. Vertebrae-centrum misaligned	0.0/0.0	0.0/0.0	5.9/0.8	0.0/0.0	0.0/0.0
C. Vertebrae-C. rib at C7	0.0/0.0	5.3/0.6	0.0/0.0	0.0/0.0	0.0/0.0
T. Vertebrae-Hemivertebrae	0.0/0.0	0.0/0.0	0.0/0.0	15.8/2.1	5.6/0.7
T. Vertebrae-Arch small	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.6/0.7
T. Vertebrae-Centrum, bifid	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.6/0.7
T. Vertebrae-Centra fused	0.0/0.0	0.0/0.0	0.0/0.0	5.3/0.7	0.0/0.0
T. Vertebrae-Centrum, unilateral ossification	0.0/0.0	0.0/0.0	0.0/0.0	5.3/0.7	0.0/0.0
Cau. Vertebrae-Misaligned	0.0/0.0	10.5/1.2	0.0/0.0	5.3/0.7	5.6/0.7
Cau. Vertebrae-Fused	0.0/0.0	0.0/0.0	0.0/0.0	5.3/0.7	5.6/0.7

BID – Dose divided and administered twice daily N/A – Not assayed or measured No. – Number DG – Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings. C = cervical T = thoracic Lu = Lumbar Cau = Caudal
* p < 0.05 ** p < 0.01

Daily Dose (µg/kg/day)	0 (Control)	0.2	22	156	260
Skeletal Anomalies (CONTINUED) % (litter/fetal):					
Ribs-Split	0.0/0.0	5.3/0.6	0.0/0.0	10.5/1.4	0.0/0.0
Ribs-Fused	0.0/0.0	0.0/0.0	0.0/0.0	21.0**/2.8**	5.6/0.7
Ribs-Two segments	0.0/0.0	0.0/0.0	0.0/0.0	5.3/0.7	0.0/0.0
Ribs-Incomplete ossification	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.3/0.7
Ribs-Thickened	0.0/0.0	0.0/0.0	0.0/0.0	5.3/0.7	0.0/0.0
Ribs-Proximate	5.3/0.6	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Ribs-Broad	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.6/0.7
Sternal Centra-Incomplete ossification	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.6/0.7
Sternal Centra-Fused	0.0/0.0	0.0/0.0	0.0/0.0	21.0**/3.5**	22.2**/2.8**
Sternal Centra-Asymmetric	0.0/0.0	0.0/0.0	0.0/0.0	5.3/0.7	5.6/0.7
Scapula-Ala, wavy	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.6/0.7
Pelvis-Pubis not ossified	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.6/0.7
Notable Ossification Sites (no./fetus/litter)					
Vertebrae, thoracic	12.51	12.55	12.80**	12.84**	12.90**
Vertebrae, lumbar	6.48	6.43	6.19**	6.16**	6.09**
Ribs, pairs	12.47	12.49	12.73*	13.10**	12.84**
Total Affected Fetuses (%):	7.2	7.4	17.5*	23.9**	23.6**

BID = Dose divided and administered twice daily N/A = Not assayed or measured No. = Number DG = Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings. C = cervical T = thoracic Lu = Lumbar Cau = Caudal
* = p < 0.05 ** = p < 0.01

2.6.7.12.3 Embryo-Fetal Development Study in Rabbits

Report Title: A Comparative Evaluation of the Effects on Normal Development and Growth of the Embryo and Fetus in Rabbits of Subcutaneously Administered AC2993 at Dosages That Cause Depression in Feed Consumption and Matched Pair-Fed Animals.

Species/Strain: Rabbits/New Zealand White [Hra:(NZW)SPF] **Day of Mating:** DG 0 **Study No.:** REST02022

Initial Age: 6 Months **Day of Caesarian Section:** DG 29 **Location in CTD:** Section 4.2.3.5.4

Date of First Dose: 31MAR2002 **Duration of Dosing:** DG 6 – DG 18 **GLP Compliance:** GLP

Vehicle/Formulation: AC-2993-F12/AC-2993-F6, AC-2663-F7 **Method of Administration:** Subcutaneous injection, daily dose divided BID

Special Features: Study designed to assess the contribution made by reduced feed consumption to developmental effects observed in REST99061R2, Section 4.2.3.5.3, with pair-fed treated groups for relevant doses of exenatide. Includes measurements of biochemical endpoints indicative of fasting state and quantitative water consumption.

Design Similar to ICH 4.1.3: Based on 4.1.3 with addition of pair-fed groups

Daily Dose (µg/kg/day)	0 (Control)	2	22	260	0 (PF=2)	0 (PF=22)	0 (PF=260)
Number of Females	20	20	20	20	20	20	20
No. Died or Sacrificed Moribund	0	0	0	1	0	0	0
Toxicokinetics [a] AUC $_{0\text{-}12h}$ (pg•h/mL)	N/A	12,164	214,883	3,610,750	N/A	N/A	N/A
Clinical Observations (incidence)							
Scant Feces	1/20	20/20**	19/20**	20/20**	6/20	19/20**	20/20**
No Feces	0/20	0/20	9/20*	13/20**	0/20	2/20	1/20
No Urine	0/20	0/20	6/20**	4/20*	0/20	0/20	0/20
Necropsy Observations	-	-	-	-	-	-	-
Pregnancy (%)	95	100	95	95	95	90	100
Abortions (%)	0	0	10	5	0	0	0
Premature Deliveries (%)	0	5	0	0	0	0	0

BID = Dose divided and administered twice daily N/A = Not assayed or measured No.= Number DG = Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings PF = Pair-fed to match respective exenatide-treated group
C = cervical T = thoracic Lu = Lumbar Cau = Caudal S = Sacral BHBA = β-hydroxybutyric acid
* = p < 0.05 ** = p < 0.01
[a] Values from REST03391R1, Section 4.2.3.7.7.1.

Daily Dose (µg/kg/day)	0 (Control)	2	22	260	0 (PF=2)	0 (PF=22)	0 (PF=260)
Number of Females	20	20	20	20	20	20	20
Body Weight (kg)							
DG 6	3.43	3.52	3.54	3.48	3.68*	3.68*	3.69*
DG 7	3.45	3.44	3.41	3.27	3.65	3.62	3.57
DG 18	3.64	3.54	3.43	3.27**	3.71	3.65	3.61
DG 29	3.84	3.76	3.77	3.68	3.92	3.91	3.85
Food Consumption/Day (g)							
DG 6-9	171.6	108.0**	54.4**	19.8**	107.4**	53.3**	18.7**
DG 15-19	177.5	143.1**	123.9**	90.9**	142.3**	117.9**	85.2**
DG 19-29	153.2	161.8	169.0**	171.4**	147.0	157.4	168.5
Water Consumption/Day (g)							
DG 6-9	290.6	240.6*	149.7**	52.5**	285.0	310.4	283.7
DG 15-19	404.5	373.1	330.8	243.8**	340.9	367.0	365.8
DG 19-29	357.8	377.6	408.8	424.5	326.2	361.1	387.4

BID = Dose divided and administered twice daily N/A = Not assayed or measured No.= Number DG = Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings PF = Pair-fed to match respective exenatide-treated group
C = cervical T = thoracic Lu = Lumbar Cau = Caudal S = Sacral BHBA = β-hydroxybutyric acid
* = p < 0.05 ** = p < 0.01

2.6.7.13.3 Contd.

Daily Dose (µg/kg/day)	0 (Control)	2	22	260	0 (PF=2)	0 (PF=22)	0 (PF=260)
Number of Females	20	20	20	20	20	20	20
Hematology							
Lymphocytes (10^3/mm^3) DG 9	7.8	5.8**	4.8**	4.9**	7.0	7.9	8.1
Serum Chemistry							
Glucose DG 9 (mg/dL)	121	124	121	123	133**	133**	134**
Glucose DG 18 (mg/dL)	126	131	128	126	124	123	123
Glucose DG 29 (mg/dL)	122	124	124	127	111	115	113
Lactate DG 9 (mg/dL)	23.68	21.25	20.78	24.29	23.54	24.58	29.86
Lactate DG 18 (mg/dL)	26.13	25.78	23.95	26.38	38.61	41.00*	51.68**
Lactate DG 29 (mg/dL)	20.98	38.32	24.42	20.96	38.94	31.90	30.70
BHBA DG 9 (mg/dL)	0.91	1.14	2.67**	2.82**	0.77	0.85	2.33**
BHBA DG 18 (mg/dL)	1.03	1.58	1.95	1.72	1.10	1.16	1.00
BHBA DG 29 (mg/dL)	4.07	3.63	2.08*	1.94**	2.98	2.57	2.22*
Potassium DG 9 (mmol/L)	4.7	4.3**	4.0**	3.8**	4.8	4.6	4.4*
Potassium DG 18 (mmol/L)	4.8	4.2**	4.3**	4.3*	4.8	4.9	5.0
Potassium DG 29 (mmol/L)	4.3	4.3	4.4	4.4	4.6	4.5	4.6
Total protein DG 9 (g/dL)	5.7	5.8	5.9	5.9	5.6	5.6	5.8
Total protein DG 18 (g/dL)	5.7	5.7	5.6	5.2**	5.5*	5.4**	5.1**
Total protein DG 29 (g/dL)	4.7	4.7	5.0	4.9	4.6	4.6	4.8
Albumin DG 9 (g/dL)	4.3	4.4	4.3	4.3	4.3	4.3	4.3
Albumin DG 18 (g/dL)	4.2	4.3	4.2	3.8**	4.1	4.1	3.8**
Albumin DG 29 (g/dL)	3.3	3.4	3.5	3.5	3.3	3.4	3.5

BID = Dose divided and administered twice daily N/A = Not assayed or measured No.= Number DG = Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings PF = Pair-fed to match respective exenatide-treated group
C = cervical T = thoracic Lu = Lumbar Cau = Caudal S = Sacral BHBA = β-hydroxybutyric acid
* = p < 0.05 ** = p < 0.01

Daily Dose (µg/kg/day)	0 (Control)	2	22	260	0 (PF=2)	0 (PF=22)	0 (PF=260)
Number of Females	20	20	20	20	20	20	20
Mean No. Corpora Lutea	10.6	10.3	11.0	10.6	10.3	10.2	10.0
Mean No. Implantations	8.7	7.7	9.2	8.6	9.7	9.4	9.3
Mean Litter Sizes	8.3	7.5	8.0	7.6	9.1	8.6	8.8
Mean Live Fetuses/Litter	8.3	7.5	8.0	7.6	9.0	8.6	8.8
Mean Resorptions	0.4	0.3	1.2	1.0	0.6	0.8	0.5
Early Resorptions	0.3	0.1	0.8	0.6	0.4	0.3	0.2
Late Resorptions	0.1	0.2	0.4	0.4	0.2	0.6	0.2
Mean Live Fetal Body Weight/Litter(g)							
Male	44.30	45.53	40.72	41.22	44.96	44.55	42.92
Female	43.65	43.29	40.24	39.43	44.86	42.72	41.43
Mean Percent Male Fetuses	48.6	46.6	45.2	53.3	55.4	50.6	47.8
Fetal Anomalies:							
Gross External % (litter/fetal):							
Umbilical Hernia	0.0/0.0	0.0/0.0	5.9/0.7	29.4/6.2	0.0/0.0	0.0/0.0	0.0/0.0
Visceral Anomalies % (litter/fetal):							
Eyes-Circumcorneal Hemorrhage	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	5.3/0.6	5.6/0.6	0.0/0.0
Eyes-Microphthalmia	5.3/0.6	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Heart-Septal Defect	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Vessels-Positional changes (all)	10.5/1.9	0.0/0.0	11.8/1.5	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Lung-Interm. Lobe Absent	5.3/0.6	5.3/0.7	23.5/2.9	5.9/0.8	5.3/0.6	11.1/1.3	5.0/0.6
Lung-Large	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Kidney-Absent	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Kidney-Dilation of Pelvis	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Intestine-Protrude, umbilical	0.0/0.0	0.0/0.0	5.9/0.7	29.4/6.2	0.0/0.0	0.0/0.0	0.0/0.0
Gallbladder-Absent	5.3/0.6	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Gallbladder-Small	5.3/0.6	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Adrenal-Misplaced	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.0/0.6

BID = Dose divided and administered twice daily N/A = Not assayed or measured No.= Number DG = Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings PF = Pair-fed to match respective exenatide-treated group
* = p < 0.05 ** = p < 0.01

2.6.7.13.3 Contd.

Daily Dose (µg/kg/day)	0 (Control)	2	22	260	0 (PF=2)	0 (PF=22)	0 (PF=260)
Number of Females	20	20	20	20	20	20	20
Skeletal Anomalies % (litter/fetal):							
Skull-Irregular ossification (all)	0.0/0.0	5.3/1.4	5.9/0.7	5.9/0.8	5.3/0.6	0.0/0.0	10.0/1.1
Hyoid: Ala, angulated	0.0/0.0	21.0/3.5	17.6/2.2	11.8/1.5	5.3/0.6	5.6/1.3	10.0/1.7
C. Vertebrae-C6 present	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
C. Vertebrae-C. rib at C7	0.0/0.0	5.3/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
C. Vertebrae-Centra fused	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.0/0.6
T. Vertebrae-Hemivertebrae	0.0/0.0	5.3/0.7	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
T. Vertebrae-Arch fused	5.3/0.6	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
T.Vertebrae-Centrum not ossified	5.3/0.6	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
T. Vertebrae-Extra ossification	0.0/0.0	0.0/0.0	0.0/0.0	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
T. Vertebrae-Centrum, bifid	0.0/0.0	0.0/0.0	5.9/0.7	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
T. Vertebrae-Centrum fused	0.0/0.0	0.0/0.0	5.9/0.7	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
T. Vertebrae-Unilat. ossification	0.0/0.0	0.0/0.0	11.8/1.4	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
T. Vertebrae-Arch small	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Lu. Vertebrae-Arch large	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
S. Vertebrae-Fused	0.0/0.0	0.0/0.0	5.9/1.4	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
S. Vertebrae-Arch large	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Cau. Vertebrae-Misaligned	0.0/0.0	5.3/0.7	5.9/0.7	0.0/0.0	5.3/0.6	16.7/2.6**	0.0/0.0
Cau. Vertebrae-Fused	0.0/0.0	0.0/0.0	5.9/1.4	0.0/0.0	0.0/0.0	0.0/0.0	5.0/0.6
Cau. Vertebrae-Cau2 present	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Cau. Vertebrae-Small	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Cau. Vertebrae-Cau9 present	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0

BID = Dose divided and administered twice daily N/A = Not assayed or measured No.= Number DG = Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings PF = Pair-fed to match respective exenatide-treated group
C = cervical T = thoracic Lu = Lumbar Cau = Caudal S = Sacral BHBA = β-hydroxybutyric acid
* = p < 0.05 ** = p < 0.01

Daily Dose (µg/kg/day)	0 (Control)	2	22	260	0 (PF=2)	0 (PF=22)	0 (PF=260)
Number of Females	20	20	20	20	20	20	20
Skeletal Anomalies (CONTINUED) % (litter/fetal):							
Ribs-Thickened	5.3/0.6	0.0/0.0	5.9/0.7	0.0/0.0	5.3/0.6	0.0/0.0	0.0/0.0
Ribs-Split	0.0/0.0	0.0/0.0	5.9/0.7	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
Ribs-Fused	5.3/0.6	0.0/0.0	11.8/1.4	5.9/1.5	0.0/0.0	0.0/0.0	5.0/0.6
Ribs-Short	0.0/0.0	0.0/0.0	0.0/0.0	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
Ribs-Irregularly shaped	0.0/0.0	0.0/0.0	5.9/0.7	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
Ribs-Broad	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Ribs-Thin	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Ribs-Small	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.3/0.6	0.0/0.0	0.0/0.0
Manubrium-Fused	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Sternal Centra-Incomplete ossification	0.0/0.0	0.0/0.0	11.8/1.4	11.8/1.5	5.3/1.2	0.0/0.0	0.0/0.0
Sternal Centra-Fused	5.3/0.6	10.5/1.4	23.5/5.8	5.9/2.3	21.0/2.3	16.7/1.9	15.0/3.4
Sternal Centra-Asymmetric	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Sternal Centra-Irregular shape	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Scapula-Irregular shape	0.0/0.0	0.0/0.0	5.9/0.7	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Pelvis-Pubis not ossified	0.0/0.0	0.0/0.0	0.0/0.0	5.9/0.8	0.0/0.0	0.0/0.0	0.0/0.0
Notable Ossification Sites (no./fetus/litter)							
Vertebrae, thoracic	12.55	12.80**	12.71	12.85**	12.74*	12.74*	12.82**
Vertebrae, lumbar	6.44	6.20*	6.27	6.14**	6.26*	6.24*	6.17**
Ribs, pairs	12.49	12.73*	12.65	12.78**	12.67*	12.67*	12.73**
Total Affected Fetuses (%):	7.0	9.2	19.0**	13.1*	7.6	7.7	10.3

BID = Dose divided and administered twice daily N/A = Not assayed or measured No.= Number DG = Presumed Day of Gestation (starting on Day 0)
- = No noteworthy findings PF = Pair-fed to match respective exenatide-treated group
C = cervical T = thoracic Lu = Lumbar Cau = Caudal S = Sacral BHBA = β-hydroxybutyric acid
* = p < 0.05 ** = p < 0.01

2.6.7.14 Effects on Pre- and Postnatal Development and Maternal Function

Report Title:	Developmental and Perinatal Reproduction Toxicity Study of AC2993 in Mice, Including a Postnatal Behavioral/Function Evaluation				
Species/Strain:	Mice/CRL:CD-1 (ICR) BR	**Day of Mating:**	DG 0	**Study No.:**	REST00150R1
Initial Age:	66 Days	**Day of Birth:**	DL 1	**Location in CTD:**	Section 4.2.3.5.6
Date of First Dose:	26JUL2000	**Litters Culled/Not Culled:**	No litters culled	**GLP Compliance:**	GLP
Vehicle/Formulation:	PBO-F11/AC-2993-F4	**Method of Administration:**	Subcutaneous injection, daily dose divided BID		
Special Features:	None.	**Duration of Dosing:**	DG 6 – DL 20 (DL 22 if no parturition)		

Design Similar to ICH 4.1.2: Yes

No Observed Adverse Effect Level: F_0 Females: <6 µg/kg/day **F_1 Males:** 6 µg/kg/day **F_1 Females:** 6 µg/kg/day

Daily Dose (µg/kg/day)	0 (Control)	6	68	760
F_0 Females				
Number of Females	25	25	25	25
Toxicokinetics (milk) [a]	3	3	3	3
Toxicokinetics				
AUC_{0-12h} (pg•h/mL) [b]	N/A	3288	37,264	416,480
No. Died or Sacrificed Moribund	0	1	1	1
Pregnancy (%)	87.5	96.4	89.3	75.0
Dams with Stillborns Pups (%)	0.0	4.2	0.0	23.5**
Dams with Liveborn Pups (%)	100.0	100.0	100.0	100.0
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Body Weight (g)				
End Gestation DG 18	55.6	53.5	53.1	50.6**
End Lactation DL 21	40.8	42.6	43.9	43.3
Body Weight Gain DL 1-21	5.4	7.4	9.0**	8.3*
Food Consumption (g)				
End Gestation (DG 15-18)	8.7	8.1	7.7**	7.7*
End Lactation (DL 10-14)	20.3	19.7	18.5	17.1**
Mean Gestation (days)	19.4	19.4	19.2	19.5

BID = Dose divided and administered twice daily N/A = not assayed or measured No.= Number DG = Presumed Day of Gestation (starting on Day 0) DS = Day of Study
DL = Day of Lactation - = No noteworthy findings. * = p < 0.05 ** = p < 0.01 One-way ANOVA with Dunnett's t-test
[a] REST01076R1, Section 4.2.2.5.5. Results discussed in Excretion, Section 2.6.4.6.
[b] REST03391R1, Section 4.2.3.7.7.1. All values were obtained from non-pregnant animals.

Daily Dose (µg/kg/day)	0 (Control)	6	68	760
F_1 Litters (Preweaning)				
Mean Litter Sizes	12.2	11.7	11.3	10.8
Live Fetuses (%)	100.0	99.6	100.0	92.3**
Stillborn Fetuses (%)	0.0	0.4	0.0	6.0**
Postnatal Survival DL 1-4 (%) [c]	98.8	96.4	98.8	91.7
Postnatal Survival DL 4-21 (%) [d]	96.8	98.5	92.3	100.0
Mean Live Pups/Litter DL4	12.0	11.2	11.2	9.1**
Mean Pup Weight/Litter DL21 (g)	8.9	9.0	8.4	7.8
Mean Percent Male Fetuses (DL1)	47.6	48.7	48.3	54.7
Pup Clinical Signs	-	-	10 found dead	7 stillborn
Pup Necropsy Observations	-	-	-	-

BID = Dose divided and administered twice daily N/A = not assayed or measured No.= Number DG = Presumed Day of Gestation (starting on Day 0) DS = Day of Study
DL = Day of Lactation - = No noteworthy findings * = p < 0.05 ** = p < 0.01 One-way ANOVA with Dunnett's t-test
[c] Live pups on DL 4/Live pups on DL 1.
[d] Live pups on DL 21/Live pups on DL 4.

Daily Dose (µg/kg/day)	0 (Control)	6	68	760
F_1 Males (Post-Weaning)				
Number Evaluated	25	25	25	25
Mortality	2	1	1	1
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Mean Body Weight (g)				
DW1	10.5	10.9	9.5	9.0*
Precohabitation (DW96-101)	37.1	37.3	37.3	34.3**
Weight Gain (DW1-101)	26.3	26.4	27.8	25.4
Reproductive Organ Weights	-	-	-	-
Mean Age of Preputial Separation (day)	29.8	29.9	30.5	30.5
Motor Activity	-	-	-	-
Learning and Memory	-	-	-	-
Mean Days to Mating	2.4	2.2	2.3	2.1
Males Mated (%)	100.0	100.0	100.0	100.0
Fertile Males (%)	78.3	87.5	95.8	91.7

BID = Dose divided and administered twice daily N/A = not assayed or measured No.= Number DG = Presumed Day of Gestation (starting on Day 0) DS = Day of Study
DL = Day of Lactation - = No noteworthy findings * = p < 0.05 ** = p < 0.01 One-way ANOVA with Dunnett's t-test

2.6.7.14 Contd.

Daily Dose (µg/kg/day)	0 (Control)	6	68	760
F_1 Females (Post-Weaning)				
Number Evaluated	25	25	25	25
Mortality	1	0	0	1
Clinical Observations	1 found dead	-	-	1 moribund
Necropsy Observations	-	-	-	-
Mean Body Weight (g)				
DW1	10.0	10.1	9.0	8.5*
Precohabitation (DW 96-101)	29.8	30.2	29.2	27.9**
Body Weight Gain (DW 1-101)	19.5	20.1	20.2	19.6
Gestation (DG18)	65.2	64.1	60.8*	61.7*
Body Weight Gain (DG0-18)	35.5	34.4	32.1	34.0
Age of Vaginal Patency (days)	31.7	31.7	31.9	32.1
Motor Activity	-	-	-	-
Learning and Memory	-	-	-	-
Mean Days to Mating	2.3	2.2	2.3	2.1
Females Presumed Mated (%)	100.0	100.0	100.0	100.0
Pregnant Females (%)	79.2	88.0	96.0	91.7
Mean Number Corpora Lutea	15.3	16.0	15.1	15.4
Mean Number Implantations	14.4	14.7	14.1	14.3
Preimplantation Loss	1.0	1.0	1.4	1.0

BID = Dose divided and administered twice daily N/A = not assayed or measured No.= Number DG = Presumed Day of Gestation (starting on Day 0) DS = Day of Study
DL = Day of Lactation - = No noteworthy findings * = $p < 0.05$ ** = $p < 0.01$ One-way ANOVA with Dunnett's t-test

Daily Dose (µg/kg/day)	0 (Control)	6	68	760
F_2 Litters				
Mean Live Fetuses/Litter	13.0	13.4	12.3	13.2
Mean Number Resorptions	1.3	1.2	1.6	1.1
Mean Dead Fetuses/Litter	0.0	0.0	0.2	0.0
Mean Postimplantation Loss	0.3	0.2	0.1	0.1
Mean Fetal Body Weight (g)	1.36	1.32	1.34	1.38
Mean Percent Males/Litter	50.2	50.3	51.9	52.8
Fetal Anomalies (litter/fetal)				
Head-Exencephaly	0.0/0.0	0.0/0.0	4.3/0.4	0.0/0.0
Eye-Lid opened	0.0/0.0	0.0/0.0	4.3/0.4	0.0/0.0
Snout-Cleft	0.0/0.0	0.0/0.0	4.3/0.4	0.0/0.0
Palate-Cleft	0.0/0.0	4.5/0.3	0.0/0.0	0.0/0.0
Tail-Tip forked	0.0/0.0	0.0/0.0	0.0/0.0	4.5/0.4
Hindlimb-Flexed downward	0.0/0.0	0.0/0.0	0.0/0.0	4.5/0.4

BID = Dose divided and administered twice daily N/A = not assayed or measured No.= Number DG = Presumed Day of Gestation (starting on Day 0) DS = Day of Study
DL = Day of Lactation - = No noteworthy findings * = $p < 0.05$ ** = $p < 0.01$ One-way ANOVA with Dunnett's t-test

2.6.7.15 Impurities (Degradation Products)

Report Title:	28-Day Toxicity Evaluation of Heat-Degraded AC2993 in CD-1 Mice Administered Subcutaneously Twice Daily				
Species/Strain:	Mice/ Crl:CD-1	**Duration of Dosing:**	28 days	**Study No.:**	REST01029
Initial Age:	6 weeks	**Duration of Postdose:**	0 days	**Location in CTD:**	Section 4.2.3.7.6.1
Date of First Dose:	07Jun2001	**Method of Administration:**	Subcutaneous, BID	**GLP Compliance:**	GLP
Vehicle/Formulation:	AC-2993-F12 /AC-2993-F6, Heat Degraded AC-2993-F6 (50°C for 10 Days)				
Special Features:	General toxicity evaluation of heat degraded exenatide drug product				
No Observed Adverse Effect Level:	N/A				

Daily Dose (µg/kg/day)	0 (Control)		760 (94.0% SCX-HPLC purity)		Heat Degraded 760 (76.7% SCX-HPLC purity)	
Number of Animals	M	F	M	F	M	F
Main study and TK	10	10	12	12	12	12
Clinical pathology only	10	10	10	10	10	10
Antibody assay only	[a]	[a]	10	10	10	10
Toxicokinetics [a]						
AUC_{0-4hr} (pg•hr/mL):	N/A		301,298		356,018	
Noteworthy Findings						
Died or Sacrificed Moribund:	0	0	0	0	0	0
Mean Body Weight (g):						
Week 4	36.54	28.06	38.06	28.99	37.20	30.16*
Mean Food Consumption (g):						
Week 1	6.1	5.4	5.0**	4.8*	5.5	5.1
Week 4	6.9	6.3	6.4	6.1	6.3	6.1
Clinical Observations (incidence):						
Hair discolored, yellow	0	0	3	0	2	0
Unkempt appearance	1	0	3	0	4	0

Daily Dose (µg/kg/day)	0 (Control)		760 (94.0% SCX-HPLC purity)		Heat Degraded 760 (76.7% SCX-HPLC purity)	
Number of Animals	M	F	M	F	M	F
Main study	10	10	12	12	12	12
Clinical pathology only	10	10	10	10	10	10
Antibody assay only	[b]	[b]	10	10	10	10
Ophthalmology:	-	-	-	-	-	-
Hematology [b]:						
Lymphocytes Pretest (1000/µL)	7.412	7.247	7.412	7.247	7.412	7.247
Lymphocytes Week 4 (1000/µL)	2.542	4.084	3.730	3.254	3.694	2.508*
Clinical Chemistry [b]:	-	-	-	-	-	-
Organ Weights:	-	-	-	-	-	-
Macroscopic Pathology:	-	-	-	-	-	-
Injection site	-	-	-	-	-	-
Microscopic Pathology:	-	-	-	-	-	-
Injection sites	-	-	-	-	-	-
Anti-Exenatide Antibody [c]:						
Positive Tier 1:25	0		0		1	

BID = Dose divided and administered twice daily N/A = not assayed
- = No noteworthy findings or finding not different from controls
* = $p < 0.05$ ** = $p < 0.01$ Dunnett's or Welch's t-test
[a] REST01196, Section 4.2.3.7.6.1.2. Antibody assay for controls obtained from TK plasma specimens.
[b] Pretest values n = 10 from animals not selected for study; Week 4 values include n=5/group.
[c] REST01195, Section 4.2.3.7.6.1.3

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Exenatide, an incretin mimetic acts through multiple mechanisms to potently and immediately promote lowering of plasma glucose levels and to promote long-term actions to significantly lower HbA1c. Exenatide decreases fasting glucose levels in all animal models of type 2 diabetes assessed to date (rat, mouse, and monkey) and exhibits a durable effect to lower HbA1c levels in diabetic rats. Improvements in glycemic control are achieved through both modulation of the rate of glucose appearance in the circulation (slowing of gastric emptying rate, reduced food intake, suppression of glucagon secretion), and modulation of the rate of glucose clearance from the blood (glucose-dependent insulin secretion, improved insulin sensitivity, increased β-cell mass). The results from glucose-lowering studies in several animal species support an efficacious dosage range of 0.01 to 1 µg/kg BID.

Exenatide caused no lethality and minimal toxic responses when administered as a single, IV dose in mice at doses up to 1500 µg/kg, as a SC dose in rats up to 30,000 µg/kg, and as a SC dose in monkeys up to 5000 µg/kg. Exenatide caused minimal toxicity following SC dosing in repeat-dose toxicity studies in mice at ≤760 µg/kg/day for up to 182 days, rats at ≤250 µg/kg/day for up to 91 days, and monkeys at ≤150 µg/kg/day for up to 273 days.

Exenatide-related effects demonstrated most consistently in rats and monkeys (not mice) were decreased food consumption and correlative decrease in body weight/body weight gain. Effects on body weight and food consumption were related to the known pharmacologic effects of exenatide. Treatment in rats at ≥18 µg/kg/day (5X MRHD) and monkeys at ≥13.4 µg/kg/day (131X MRHD) decreased body weight/body weight gain and food consumption. Conversely, exenatide treatment in mice generally tended to mildly elevate body weight and food consumption, but these effects subsided with chronic dosing. The two most notable exenatide-related microscopic pathology changes were basophilic foci in the parotid salivary gland of mice and focal islet cell hypercellularity in the pancreas of monkeys. Basophilic foci in the parotid salivary gland were noted in mice at ≥18 µg/kg/day exenatide (10-12X MRHD) at 91 and 182 days, and at 760 µg/kg/day exenatide (520X MRHD) at 28 days. Reversibility of these lesions was demonstrated in mice treated for 91 days and allowed a 30-day recovery period following completion of exenatide treatment. These lesions were of minimal to moderate severity. Basophilic foci were noted in all exenatide-treated groups of mice at ≥18 µg/kg/day exenatide (5X MRHD) in the 2-year carcinogenicity. However, despite the lesion's relatively common occurrence (~ 45% to 65% across all exenatide-treated groups) there were no exenatide-related increases in salivary gland tumors and no exenatide-related adverse effects on survival. Sponsor stated that the physiologic significance of this lesion remains unclear, but the lack of any adverse or preneoplastic consequence of the lesion suggest that the basophilic foci of the parotid salivary gland is not a toxicologically important effect. Focal, minimal to mild islet cell hypercellularity was noted in the pancreas of monkeys treated at 150 µg/kg/day exenatide (994 to 2007X MRHD) for 91 and 273 days. Islet cell hypercellularity was accompanied by increased staining with Gomori's Aldehyde Fuchsin, suggesting the hypercellularity was an increase in the β-cell population. No islet cell changes were noted in mice or rats. Sponsor stated that exenatide, exendin-4 (naturally occurring form of exenatide), GLP-1, and GLP-1 analogs have been demonstrated to increase β-cell mass both in vitro and in vivo. There were no changes in serum glucose noted in either study and no degenerative microscopic changes. There were no neoplastic changes in the pancreas of mice or rats treated with 250 µg/kg/day exenatide (>90X MRHD) in two-year carcinogenicity studies. Based on the minimal to mild severity and lack of adverse effects, these changes were considered a pharmacologic effect of exenatide, not toxicity. Thus, exenatide was generally well-tolerated in repeat-dose toxicity studies with durations of up to 182 days in mice, 91 days in rats, and 273 days in monkeys. In general, effects on body weight and food consumption were noted in all repeat-dose toxicity studies, a known pharmacologic effect of exenatide. Production of anti-exenatide antibody was limited to monkeys, and may be neutralizing at neutralizing 75 µg/kg BID (994X MRHD, AUC) due to the decreased systemic exposure relative to systemic exposure at 9 µg/kg BID (1360X MRHD, AUC).

Exenatide produced no impairment of fertility, sperm concentration, or sperm motility in male mice, or fertility or estrous cycling in female mice at doses upto 760 µg/kg/d resulting in exposures 260X the clinical exposure at 10 µg BID. Exenatide was not teratogenic in mice at doses up to 6 µg/kg/d resulting in exposures 5X the clinical exposure and in rabbits at doses up to 2 µg/kg/d resulting in exposures 12X the clinical exposure, 10 µg BID. In mice and particularly rabbits, higher exposures resulted in maternal toxicity which precluded the developmental assessment of exenatide.

Exenatide did not show a mutagenic or clastogenic potential with or without metabolic activation in *in vitro* Ames or chromosomal aberration assay in CHO cells or *in vivo* in the mouse micronucleus assay.

Lifetime carcinogenicity evaluations in rats and mice demonstrate increased thyroid C-cell adenomas in female rats at exposures 130X the clinical dose of 20 µg/day. Mice did not demonstrate a tumorigenic potential.

Unresolved toxicology issues: None.

Recommendations: Approval (AP).

Suggested labeling: Please see page 1.

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/s/

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