CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

U.S. FOOD & DRUG ADMINISTRATION

Pharmacology & Toxicology
Center for Drug Evaluation and Research
Division of Metabolism and Endocrinology Products

NDA SECONDARY REVIEW

Date:	September 28, 2017
NDA #	209210
Sponsor:	AstraZeneca
Drug:	Exenatide extended-release, non-aqueous (BYDUREON BCISE)
Primary Reviewer:	Huiqing Hao, Ph.D.
Secondary Reviewer:	Ronald Wange, Ph.D.

AstraZeneca is seeking market approval for exenatide once-weekly suspension (EQWS), with a proposed trade name of BYDUREON BCISE, as a treatment option for Type 2 diabetes mellitus (T2DM). If approved, BYDUREON BCISE would be the third formulation of exenatide marketed by AstraZeneca for T2DM, joining BYETTA (NDA 21-733, twice daily immediate release exenatide) and BYDUREON (NDA 22-200, once weekly extended release exenatide). BYDUREON BCISE contains the same exenatide-containing PLG (50:50 poly (D,L)-lactide-co-glycolide) microspheres that are the active component of BYDUREON. Whereas BYDUREON must be reconstituted with an aqueous vehicle just prior to injection; with BYDUREON BCISE the microspheres are suspended in a nonaqueous, medium-chain triglyceride (MCT) vehicle that is suitable for use with an autoinjector delivery platform, simplifying the process for dose preparation and self-injection by patients.

Exenatide (the active pharmacological ingredient in BYETTA, BYDUREON AND BYDUREON BCISE) is a glucagon-like peptide-1 (GLP-1) receptor agonist that exhibits many of the same glucoregulatory actions as GLP-1, a naturally-occurring incretin hormone. However, unlike GLP-1, exenatide is resistant to degradation by dipeptidyl peptidase-4, and therefore has a longer duration of action. As AstraZeneca has previously characterized the pharmacologic, pharmacokinetic and toxicologic profile of exenatide and exenatide microspheres, the nonclinical development program for EQWS was based primarily on toxicology studies intended to bridge the non-aqueous formulation of EQWS to the nonclinical data used to support the marketing applications for Bydureon and Byetta. In particular, AstraZeneca needed to establish that the MCT vehicle did not exacerbate adverse events attributed to exenatide, and that subcutaneous MCT administration was not itself associated with new toxicity findings. Consequently, this secondary review is also focused on the assessment of whether there are safety concerns related to the MCT vehicle in BYDUREON BCISE.

The principle dataset used in this assessment are the Good Laboratory Practice (GLP) compliant toxicity studies performed by the Applicant with EQWS. These included a single-dose, local tolerability study in rats, and 4-week and 13-week toxicity studies in monkeys. As captured in

Dr. Hao's review, there was no novel toxicity observed in these studies, nor was there evidence of MCT-exacerbation of the effects of exenatide. No systemic adverse findings were observed in these toxicity studies, and the systemic No Adverse Effect Level (NOAEL) was established as 1.1 mg/kg/week in monkeys and 4.4 mg/kg/week in rats, the highest doses tested. Non-adverse, slight decreases in red cell parameters were noted in the EQWS-treated monkeys in the 4- and 13-week studies, consistent with previous observations with exenatide in this species.

The most notable finding in the toxicity studies conducted with EQWS were injection site reactions. In the single-dose rat local tolerance study this manifested as acute (observed on Day 1 and generally resolved by Day 3), very slight to slight edema. This experiment compared equal exenatide doses of the QW (BYDUREON) and EQWS (BYDUREON BCISE) formulations, and found no significant difference between the two formulations, suggesting that MCT does not appreciably affect local tolerability. In the subchronic monkey study, granulomatous inflammation (graded minimal to severe) was observed at the injection sites in monkeys treated with EQWS and in monkeys treated with the PLG microspheres (without exenatide). This is primarily a response to the microspheres (i.e., a foreign body reaction), although the presence of exenatide caused a modest increase in severity of this finding in female monkeys. This pattern is in agreement with the monkey injection site histopathology seen with BYDUREON. MCTs, by themselves, caused only a minimal, transient granulomatous inflammatory response at the injections sites. Together, the rat and monkey data do not indicate an increased risk for injection site reactions arising from the presence of the MCT vehicle.

Two areas of general concern related to the GLP-1 receptor agonist drug class bear comment. These being concerns regarding neoplastic potential in thyroid C-cells and the risk of pancreatic injury. Rodent studies with long-acting GLP-1 receptor agonists (including BYDUREON) have found drug-related C-cell tumors (adenomas and/or carcinomas) at clinically relevant exposures. These findings have resulted in the placement of a boxed warning on these products regarding the risk for thyroid C-cell tumors, and contraindications for use in patients who have a personal or family history of medullary thyroid tumors, and in patients with Multiple Endocrine Neoplasia syndrome type 2 (MEN2). It is considered that BYDUREON BCISE carries a comparable risk for these tumors, and AstraZeneca has proposed the same boxed warning for BYDUREON BCISE that appears on BYDUREON. We agree with this approach.

Clinical post-marketing data have raised concerns about the potential for GLP-1 receptor agonists to cause acute pancreatitis. Consequently, the labels for these products (including BYETTA and BYDUREON) bear a warning regarding the risk for pancreatitis, including fatal and non-fatal hemorrhagic or necrotizing pancreatitis. In the subchronic monkey study, EQWS treatment had no effect on amylase or lipase levels, nor upon pancreas weights or histology. These data are not definitive regarding pancreatitis risk; however, as the vast preponderance of toxicology studies with GLP-1 receptor agonists have failed to identify a risk for pancreatitis. Nonetheless, these data do indicate the absence of any discernable novel MCT-related pancreatitis risk. The proposed label for BYDUREON BCISE does include the class warning regarding the potential risk for pancreatitis.

Lastly, it should be noted that while MCTs are a common excipient in many drug products delivered via myriad routes, and that MCTs are a lipid component (active ingredient) of

parenteral nutrition products approved in the United States and European Union, MCTs are considered to be a novel excipient (in the US) when employed for chronic use via SC injection. I consider that the Applicant has provided adequate data, as captured above, and in Dr. Hao's review to support the safe use of MCT by SC injection in a once weekly formulation, containing 774 mg of MCT.

In conclusion, the toxicologic profile of EQWS appears to be indistinguishable from that of QW exenatide (BYDUREON). I concur with Dr. Hao's conclusion that the MCTs present in BYDUREON BCISE do not constitute a safety risk. Moreover, I agree with her recommendation for approval, and with her proposed labeling for this product.

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/s/

RONALD L WANGE
09/29/2017
Pharm/Tox recommends approval.

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	209210
Supporting document/s:	SDN 1
Applicant's letter date:	12/21/2016
CDER stamp date:	12/21/2016
Product:	Bydureon Bcise (Extended QW suspension injectable of exenatide, or EQWS)
Indication:	To improve glycemic control in type 2 diabetes
Applicant:	AstraZeneca AB
Review Division:	Division of Metabolism and Endocrinology Products
Reviewer:	Huiqing Hao, PhD
Supervisor/Team Leader:	Ronald Wange, PhD
Division Director:	Jean-Marc Guettier
Project Manager:	

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

The proposed product in this NDA is Bydureon Bcise (also referred to as EQWS), which contains the same active ingredient (exenatide) as in the approved products, Byetta and Bydureon. Byetta is an immediate-release formulation for twice daily injection (N21773, approved 04/2005) and Bydureon is an extended-release once weekly injection that is provided as powder to be combined with aqueous vehicle (N22200, approved 01/2012). Bydureon Bcise is an oily extended-release formulation for weekly injection containing the same drug substance, drug load (b) (4), and extended-release microspheres as Bydureon, but with a non-aqueous, medium-chain triglycerides (MCTs) vehicle that is suitable for use in an auto-injector.

Most nonclinical data relevant to the safety and labeling of the current product, EQWS, are available from the development programs for Sponsor's approved products, BYETTA and BYDUREON. To support the current NDA, nonclinical studies were conducted to bridge the current formulation to existing products for determination of the impact of the EQWS formulation on pharmacokinetics and toxicology profile, local tolerance, and to evaluate the toxicity of the new excipient, Medium Chain Triglyceride (MCTs). While there are no approved products in the US with MCT as an excipient, SMOFLIPID™, an MCT-containing lipid suspension for parenteral nutrition was recently approved in the US (7/13/2016).

1.2 Brief Discussion of Nonclinical Findings

- PK/TK profile comparison between EQWS and Bydureon:
  - Following administration of a single subcutaneous dose (2.4 mg/kg) in rats, EQWS produced lower C_{max} (-60%) and lower AUC (-40%) values as compared to Bydureon.
  - Systemic exposures (Cmax and $AUC_{0\text{-}168h}$) in monkeys treated with EQWS were generally dose proportional, reaching steady state in one month (relative to Day 1, AUC increased 9-14 fold by week 4; 15-45 folds by week 13). This profile and AUC values were comparable to Bydureon values reported previously.

- Toxicity profile of EQWS
  - Rats

    A single s.c. dose (2.4 mg/kg) study in rats showed similar effects in EQWS and Bydureon groups, including decreases in body weight and food consumption, and injection site reactions (erythema, edema, foreign body granulomas and histiocytic infiltration). The injection site findings were also observed in the microsphere control group. It is considered that

decreases in body weight and food consumption are pharmacological effects, and injection site findings are microsphere related.

- Monkeys:

  Monkey 4-week and 13-week studies employed EQWS (0.11, 0.44 and 1.1 mg/kg/week s.c.), saline, Miglyol 812 and microsphere control groups. Slightly reduced red blood cell parameters (7-15% reductions from baseline in RBCs, hematocrit and hemoglobin at the high dose) in EQWS groups, and injection site reactions (local erythema, edema, foreign body granuloma, eosinophilic infiltrate, inflammation and/or necrosis) in EQWS and microsphere control groups were observed. The red blood cell findings are typical of GLP-1 receptor agonists, having been seen with exenatide previously, and are not considered to be adverse. The injection site findings are considered to be microsphere related. All of these findings were partially resolved after recovery periods (3 weeks for 4-week study and 13 weeks for 3-month study). The NOAELs for both 4-week and 13-week studies were established as 1.1 mg/kg (the highest dose evaluated), which represents 4.2x and 20.1x the 2 mg/week clinical exenatide exposure, based on AUC (4-w study, 149000 pg.h/mL; 13-w study, 719000 pg.h/mL; humans, 35745 pg.h/mL).

In conclusion, EQWS has similar toxicology profile as Bydureon. There were no MCT-related toxicity findings identified.

- Assessment of the safety of the MCT excipient
  - MCTs are commonly consumed in the diet, as they are present in a variety of foods, and been have been widely used as food supplements. A study comparing PK profiles following oral and s.c. administered Miglyol 812, demonstrated oral relative bioavailability of 1-11% for C6, C8 and C10 free fatty acids. MCTs have also recently been approved for parenteral (intravenous) use in the United States as a source of calories and essential fatty acids for parenteral nutrition when oral or enteral nutrition is not possible, insufficient, or contraindicated
  - In monkey 4-week and 13-week toxicology studies for EQWS, Miglyol 812 control group was included in each of these studies. There were no Miglyol 812 related toxicity findings.
  - MCTs have been used in Europe and the United States in lipid emulsions as parenteral nutrition for patients.

- Container closure system:

  Based on a simulated leachable study, a total of 18 leachables were identified, among which 11 were at estimated human exposure less than (b) (4) mcg/day, 6 were in a range of (b) (4) mcg/day and one was at (b) (4) mcg/day (b) (4) (b) (4). These leachables are of no safety concern based on available guidance

(ICH M7, ICH Q3C) and current practice in CDER as well as detailed deliberations, as captured below in Sections 7.1 and 10.2.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical perspective, this NDA is recommended to be approved.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Nonclinical studies conducted under this NDA have no impact to the existing labeling for Bydureon, and it is recommended that Bcise carry the same labeling in the relevant sections (Sections 8.1, 8.2, 13.1 and 13.3) as that of the PLLR-converted Bydureon label. This is captured below.

The following present reviewer's edits on sponsor proposed labeling.

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use BYDUREON BCISE safely and effectively. See full prescribing information for BYDUREON BCISE.

BYDUREON® BCISE™ (exenatide) extended-release injectable suspension
Initial U.S. Approval:

WARNING: RISK OF THYROID C-CELL TUMORS

See full prescribing information for complete boxed warning.

- **Exenatide extended-release causes thyroid C-cell tumors at clinically relevant exposures in rats. It is unknown whether BYDUREON BCISE causes thyroid C-cell tumors, including medullary thyroid carcinoma (MTC) in humans, as the human relevance of exenatide extended-release-induced rodent thyroid C-cell tumors has not been determined. (5.1, 13.1)**
- **BYDUREON BCISE is contraindicated in patients with a personal or family history of MTC or in patients with Multiple Endocrine Neoplasia syndrome type 2 (MEN 2). Counsel patients regarding the potential risk of MTC and the symptoms of thyroid tumors. (4.1, 5.1)**

---------- WARNINGS AND PRECAUTIONS ----------

- Thyroid C-cell Tumors: (b) (4)

---------- USE IN SPECIFIC POPULATIONS ----------

- Pregnancy: (b) (4) Use during pregnancy only if the potential benefit justifies the potential risk to the fetus. (8.1)

(b) (4)

Reviewer comment: no changes proposed to this section.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Limited data with exenatide, the active ingredient in BYDUREON BCISE, in pregnant women are not sufficient to determine a drug-associated risk for major birth defects or miscarriage. There are risks to the mother and fetus associated with poorly controlled diabetes in pregnancy *[see Clinical Considerations]*. Based on animal reproduction studies, there may be (b) (4) from exposure to BYDUREON BCISE during pregnancy.

Animal reproduction studies identified increased adverse fetal and neonatal outcomes from exposure to exenatide extended-release during pregnancy or from exposure to exenatide during pregnancy and lactation. In rats, exenatide extended-release administered during the period of organogenesis reduced fetal growth and produced skeletal ossification deficits (b) (4) at doses that approximate clinical exposures at the maximum recommended human dose (MRHD) of 2 mg/week. In mice, exenatide administered during (b) (4), caused (b) (4) increased (b) (4) neonatal deaths (b) (4) at doses that approximate clinical exposures at the MRHD [see *Data*]. Based on animal data, advise (b) (4) pregnant women of the potential risk to a fetus.

The estimated background risk of major birth defects is 6-10% in women with pre-gestational diabetes with a HbA1c >7 and has been reported to be as high as 20-25% in women with HbA1c >10. The estimated background risk of miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Clinical Considerations

Disease-associated maternal and/or embryo/fetal risk:
Poorly controlled diabetes in pregnancy increases the maternal risk for diabetic ketoacidosis, preeclampsia, spontaneous abortions, preterm delivery, still birth and delivery complications. Poorly controlled diabetes increases the fetal risk for major birth defects, stillbirth, and macrosomia related morbidity.

Data

Animal Data

Pregnant rats given subcutaneous doses of 0.3, 1, or 3 mg/kg exenatide extended-release every 3 days during organogenesis had systemic exposures 3-, (b) (4)-, and (b) (4)-times human exposure, respectively, at the MRHD of 2 mg/week BYDUREON BCISE based on plasma exenatide exposure (AUC) comparison. Reduced fetal growth at all doses and skeletal ossification deficits at 1 and 3 mg/kg occurred at doses that decreased maternal food intake and body weight gain.
In pregnant mice given 6, 68, 460, or 760 mcg/kg/day exenatide during fetal organogenesis, (b) (4)
(b) (4)

(b) (4)

In pregnant rabbits given 0.2, 2, 22, 156, or 260 mcg/kg/day exenatide during fetal organogenesis, irregular fetal skeletal ossifications were observed at 2 mcg/kg/day, a dose yielding systemic exposure up to (b) (4) times the human exposure from the MRHD of BYDUREON BCISE based on AUC comparison.

In maternal mice given 6, 68, or 760 mcg/kg/day exenatide from gestation day 6 through lactation day 20 (weaning), an increased number of neonatal deaths were observed on postpartum days 2 to 4 in dams given 6 mcg/kg/day, a dose yielding a systemic exposure equivalent to the human exposure from the MRHD of BYDUREON BCISE based on AUC comparison.

Reviewer's Comment: This section has been extensively edited to bring this section of the label into alignment with the proposed PLLR-converted Bydureon label, and to remove redundancy.

8.2 Lactation

Risk Summary

There is no information regarding the presence of exenatide, (b) (4) (b) (4) in human milk, the effects of exenatide on the breastfed infant, or the effects of exenatide on milk production. Exenatide was present in the milk of lactating mice. However, due to species-specific differences in lactation physiology, the clinical relevance of these data is not clear *[see Data]*. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for exenatide and any potential adverse effects on the breastfed child from exenatide or from the underlying maternal condition.

Data

In lactating mice subcutaneously injected twice a day with exenatide, the concentration of exenatide in milk was up to 2.5% of the concentration in maternal plasma.

Reviewer's Comment: This section has been extensively edited to bring this section of the label into alignment with the proposed PLLR-converted Bydureon label, and to remove redundancy.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Thyroid C-cell tumors have been observed in rats and mice with GLP-1 receptor agonists.

A 2 year carcinogenicity study was conducted with exenatide extended-release, the active component of BYDUREON BCISE, in male and female rats at doses of 0.3, 1.0, and 3.0 mg/kg (2-, 10-, and 27-times human systemic exposure at the maximum

recommended human dose (MRHD) of 2 mg/week BYDUREON BCISE based on plasma exenatide AUC, respectively) administered by subcutaneous injection every other week. In this study there was an increased incidence of C-cell adenomas and C-cell carcinomas at all doses. An increase in benign fibromas was seen in the skin subcutis at injection sites of males given 3 mg/kg. No treatment-related injection-site fibrosarcomas were observed at any dose. The human relevance of these findings is currently unknown.

(b) (4)

Carcinogenicity of exenatide extended-release has not been evaluated (b) (4) (b) (4) in mice.

Reviewer's comment: (b) (4) (b) (4)

Exenatide, the active ingredient in BYDUREON BCISE, was not mutagenic or clastogenic, with or without metabolic activation, in the Ames bacterial mutagenicity assay or chromosomal aberration assay in Chinese hamster ovary cells. Exenatide was negative in the in vivo mouse micronucleus assay.

In mouse fertility studies with exenatide, the active ingredient in BYDUREON BCISE, at twice-daily subcutaneous doses of 6, 68, or 760 mcg/kg/day, males were treated for 4 weeks prior to and throughout mating, and females were treated 2 weeks prior to mating and throughout mating until gestation day 7. No adverse effect on fertility was observed at 760 mcg/kg/day, a systemic exposure 163 times the human exposure resulting from the recommended dose of 2 mg/week, based on AUC.

(b) (4)

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number (Optional): 141732-76-5

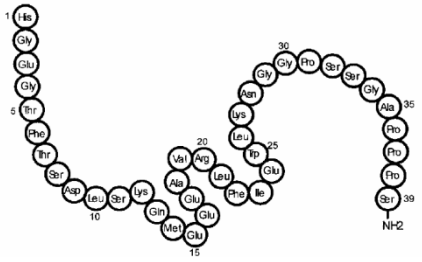
Generic Name: Exenatide once-weekly suspension (EQWS)

Code Name: EQWS, QW RTU, AC2993, AC2993A, AC002993, LY2148568

Chemical Name: L-histidylglycyl-L-glutamylglycyl-L-threonyl-L-phenylalanyl-L-threonyl-Lseryl-L-aspartyl-L-luecyl-L-seryl-L-lysyl-L-glutaminyl-L-methionyl-L-glutamyl-L-lutamyl-Lglutamyl-L-alanyl-L-valyl-L-arginyl-L-leucyl-L-phenylalanyl-L-isoleucyl-L-glutamyl-Ltryptophanyl-L-leucyl-L-lysyl-L-asparaginylglycylglycyl-L-prolyl-L-seryl-L-serylglycyl-Lalanyl-L-prolyl-L-prolyl-L-prolyl-L-serinamide, hydrate (IUPAC)

Molecular Formula/Molecular Weight: $C_{184}H_{282}N_{50}O_{60}S$ / 4186.6 Daltons

Structure or Biochemical Description:



Pharmacologic Class: Glucagon-like peptide-1 (GLP-1) receptor agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

Clinical studies run in the US to support this NDA were performed under IND 107,815.

Exendin-4 analogs

NDA 22200 /IND 67,092 - exenatide, Amylin, once weekly formulation, powder to be dissolved in aqueous vehicle

NDA 21-773 / IND 57,725 - exenatide, BYETTA, twice daily injection, Amylin

NDA 21-919 / IND 57,725 - exenatide, BYETTA, Amylin, monotherapy

(b) (4)

Note: Exenatide is synthetic version of exendin-4, a hormone found in the saliva of the Gila monster that was first isolated by John Eng MD in 1992. Like human GLP-1, exendin-4 binds to the intact human Glucagon-like peptide -1 receptor (GLP-1R), and displays biological effects of regulations on glucose metabolism and insulin secretion.

Medium Chain Triglycerides (MCT)

NDA 207648 – SMOFLIPID is a sterile, homogenous lipid emulsion for intravenous infusion indicated for parenteral nutrition. Per product label, SMOFLIPID contains 20 g of lipid per 100 mL, 6 g of which are MCT. The labeled maximum recommended dose of SMOFLIPID is 2.5 g/kg/day, or 150 mg of lipid per day (for a 60 kg adult), 45 g of which represents MCT. This gives a maximum weekly dose of 315 g of MCT.

2.3 Drug Formulation

The drug product is an oily suspension of exenatide microspheres in medium chain triglycerides (MCTs), packaged in a 2.0 mL USP (b) (4) glass cartridge sealed at one end with an (b) (4) seal/cap (b) (4) and an (b) (4) plunger at the other. The finished drug product is comprised of the suspension filled cartridge assembled into the auto-injector. See the product composition below.

Unit formula for EQWS

Component		%w/w (of microspheres)	Nominal quantity per 0.85 mL dose delivered (mg)	Function
Exenatide microsphere	Exenatide	(b) (4)	2.0	API
	Sucrose		0.8	(b) (4)
	5050 DL (b) (4) polymer		37.2	
MCTs (b) (4) (b) (4)			774.4	Vehicle

API, active pharmaceutical ingredient; MCTs, medium chain triglycerides

The submission did not include MCTs composition information. As per discussion with CMC reviewer, Dr. Suong T Tran, that the MCT composition is controlled by USP-NF specification, which specify the percentages of carbon chains as following:

(b) (4)

The applicant also states the only difference between their MCTs specification and the USP-NF specifications is the applicant's tighter limits (b) (4) (b) (4). Thus the quality is better than the compendial requirement.

The exenatide controlled-release microspheres are the same as those used in the approved product BYDUREON® (NDA022-200).

2.4 Comments on Novel Excipients

Medium chain triglyceride, a vehicle, is contained in the current product but not in BYDUREON®. The safety of MCT as an excipient was established in the toxicology studies reviewed below in Section 6. In addition, it is noted that patients receiving parenteral (intravenous) nutrition with SMOFLIPIDS™ can receive up to (b) (4) g of MCT/week, which compared to the 0.77 g/week of MCT that patients treated with Bydureon Bcise will receive is a significantly higher dosage.

2.5 Comments on Impurities/Degradants of Concern

There are no impurities or degradants of concern.

The sponsor provided product impurity specifications along with estimated safety margins (based on mg/m^2) based on NOAELs obtained in toxicology studies. Some of these toxicology studies were conducted with Bydureon and some were with Bydureon Bcise. This reviewer agrees that the proposed impurity specifications are of no safety concern.

Table 6 Qualification of Product-Related Impurities

Toxicology study	Human safety margin at NOAEL[a]	Lot number	Total impurities, %	Specified largest individual impurity, %	Sum of product-related impurities, %
					(b) (4)
BYDUREON® Studies					
4 month rat	22 x	278-2565A			
		278-2785A			
		278-3465A			
3 month monkey	11 x	278-1414A			
9 month monkey	11 x	278-2565A			
		278-2695A			
		278-2785A			
		278-3465A			
		278-0096A			
		278-0166A			
		278-0326A			
		278-0396A			
		278-0516A			
		278-0586A			
		278-1076A			
		278-1216A			
8 week rat	22 x	07-017-112			
		05-016-051B			
Carcinogenicity	7 x[b]	05-016-051			

		05-016-051B			
		05-016-052			
		05-016-053			
EQWS Studies					
1 month monkey (+ MCT)	11 x	07-017-112			
3 month monkey (+ MCT)	11 x	07-017-112			
Proposed specification limits, %					
Margin of safety					
					(b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

In adults with type 2 diabetes mellitus, EQWS will be administered subcutaneously at 2 mg per week. This dose level is the same as for Bydureon (label, 09/24/2015).

2.7 Regulatory Background

This NDA is a 505b(1) application. The proposed product, EQWS, is a formulation modification of the Sponsor's approved product, BYDUREON (NDA 222000, 01/27/2012). See Introduction Section for more details.

Nonclinical requirements were discussed in PreNDA meeting on 02/08/2016 under IND107815 (meeting minutes). There are no outstanding issues in the NDA submission.

A Pediatric Study Plan was submitted to IND 107815 on 29 Apr 2016 (Seq. No. 0100) which included a Pediatric Waiver request for ages 0-9 years. The FDA agreed Initial Pediatric Study Plan. In this NDA, AstraZeneca requests a full waiver of the requirement to submit an assessment of the safety and effectiveness of (exenatide) extended-release injectable suspension for the proposed indication in pediatric patients 0-9 years old (inclusive) as studies are impossible or highly impracticable. Also, the sponsor is requesting deferral of the studies to less than 18 years.

The safety and effectiveness of EQWS have not been established in pediatric patients and currently EQWS is not recommended for use in pediatric patients.

There are no juvenile animal studies required at this time.

3 Studies Submitted

3.1 Studies Reviewed

EQWS	Study No.
General toxicology	
AC2993 QW RTU: A Single Dose Pharmacokinetics and Local Tolerance Study Following Subcutaneous Administration in Rats	REST080666
AC2993 QW RTU: A 4-Week Repeated Dose Toxicity Study Following Weekly Subcutaneous Administration in Cynomolgus Monkeys	REST080663
AC2993 QW RTU: A 3-Month Repeated Dose Toxicity Study Following Subcutaneous Administration in Cynomolgus Monkeys	REST080799
Special Toxicology	
Chemically-induced Pancreatitis in ob/ob Mice after a Single Administration of AC2993	REST090186
Histopathology for Study REST090186	REST090020
Caerulein-induced pancreatitis in ob/ob mice after sub-chronic dosing with AC2993 for four weeks	REST090159

Effect of sub-chronic treatment of exenatide on inflammatory cytokines, gene expression, and pancreatic duct cell proliferation in a mouse model of caerulein-induced acute pancreatitis	REST090159R1
Histopathology for Study REST090159	REST090080
Effect of Exenatide on Basal, CCK-8-stimulated and Caerulein hyperstimulated Pancreatic Enzyme Secretion in Normal Sprague Dawley (SD) Rats.	REST090012
Histopathology of REST090012	REST090021
Effect of Exenatide on Basal and Caerulein-hyperstimulated Pancreatic Enzyme Secretion in Diabetic Fatty Zucker (ZDF) Rats.	REST090182
Histology of REST090182	REST090046
Exenatide Does Not Affect Pancreatic Exocrine Secretion in a Sodium Taurocholate Model of Acute Pancreatitis	REST100052
Histology of REST100052	REST100085
Potential Effects of Twice-daily (BID) Subcutaneous Injection of Exenatide (AC2993) for 3 Months on Pancreatic Exocrine Structure and Function in Obese Diabetic (ZDF) Rats	REST100021
MCTs: A Single Dose Pharmacokinetic Study of Tricaprylin (C8) and Miglyol 812 in Rats	8342572

3.2 Studies Not Reviewed (vehicle selection study)

Assessment of novel vehicle tolerability in RccHan:WIST Rats	3113KR
Characterization of different mouse models of caerulein-induced acute pancreatitis	REST080861
Histopathology for “Characterization of different mouse models of caerulein-induced acute pancreatitis (REST080861”	REST090022
Characterization of dose response of caerulein-induced acute pancreatitis in diabetic ob/ob and normal mice	REST090183
Sub-chronic effects of AC3174 on glucose homeostasis and pancreatic function in HF-STZ diabetic mice	REST080862

3.3 Previous Reviews Referenced

Nonclinical reviews for NDA 21773 and NDA 22200

4 Pharmacology

Pharmacology studies were conducted in support of the predecessor products, Byetta and Bydureon. No pharmacology studies were conducted with the medium-chain triglyceride component of Bydureon Bcise.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The proposed product, EQWS is in an oily suspension, which is a formulation modification from the approved aqueous suspension, Bydureon. A rat study was

conducted to compare the PK profiles between these two formulations. Following a single subcutaneous dose (2.4 mg/kg), EQWS and the QW formulations gave qualitatively similar plasma concentration profiles, with an early peak at ~3-4 days, followed by a later peak at ~23-28 days. However the EQWS samples had a markedly diminished initial peak (~5-fold lower than QW), and did exhibit an overall lower C_{max} (-60%) and AUC (-38%), compared to QW. See studies REST080666 and REST080666r1 reviewed in Single Dose Toxicity Section.

5.2 Toxicokinetics

Toxicokinetics data are presented in the toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Study title: AC2993 QW RTU: A Single Dose Pharmacokinetics and Local Tolerance Study Following Subcutaneous Administration in Rats

Study no.:	REST080666/REST080666r1
Study report location:	ECTD 4.2.2.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/08/2008
GLP compliance:	Yes, a GLP compliance statement is included in the report
QA statement:	Yes
Drug, lot #, and % purity:	AC2993 microsphere powder, Lot No. 07-017-112, 97% purity. This test article was reconstituted with appropriate diluent as a sterile microsphere suspension (0.9% saline for QW aqueous formulation, Miglyol 812 for QW RTU formulation and QW RTU "aged" formulation that was prepared three weeks before use)

Key Study Findings

This study compared toxicity profile between the new formulation (AC2993 QW RTU) and the marketed formulation (AC2993 QW). Following a single subcutaneous administration in rats, AC2993 QW RTU, prepared freshly (2.4 mg/kg) and three weeks before use (at 4.4 mg/kg), exhibited similar toxicity findings as that observed after a single dose of AC2993 QW (2.4 mg/kg), including transient body weight losses (in the first 5 days) or reduced body weight gain and decreased food consumption, injection site findings of erythema, edema, foreign body granulomas and histiocytic infiltrations.

Local histopathologic findings were partially recovered after three weeks of recovery period.

Compared to exenatide QW formulation, treatment with exenatide QW RTU had reduced initial exposure levels (lower initial peak), lower C_{max} (-60%) and lower AUC (-38%) values.

Methods

Doses:	2.4 mg/kg for AC2993 QW and AC2993 QW RTU; 4.4 mg/kg for AC2993 QW RTU "aged" (prepared 3 weeks before use)
Route of administration:	Subcutaneous
Dose volume:	1.6 mL/kg
Formulation/Vehicle:	Saline, Miglyol 812, and microsphere control
Species/Strain:	CD® [Crl:CD® (SD)] rats
Number/Sex/Group:	6/sex/group
Age:	11 weeks old
Weight:	Males, 379-435 g; females, 248-282 g
Satellite groups:	6/sex/group for three-week recovery study
Unique study design:	No data were collected for clinical pathology and toxicokinetics; Sacrifices were performed on Day 22 for main groups and Day 43 for recovery groups.

Group Assignments

Group Number	Treatment	Dose Level (mg/kg AC2993)	Dose Volume (mL/kg)	Number of Animals[a]	
				Male	Female
1	NaCl	0	1.6	12	12
2	Miglyol 812 Control	0	1.6	12	12
3	Microsphere Control[b]	0	1.6	12	12
4	AC2993 QW	2.4	1.6	12	12
5	AC2993 QW RTU	2.4	1.6	12	12
6	AC2993 QW RTU "aged"	4.4	1.6	12	12

[a]Six animals/sex/group (main study animals) were necropsied on Day 22 and the remaining six animals/sex/group (recovery animals) were necropsied on Day 43.
[b]Group 3 animals were administered approximately 82 mg/kg microspheres (vs. 47 mg/kg microspheres for Groups 4 and 5).

Deviation from study protocol:	No major protocol deviations occurred that affect result interpretation

Observations and Results

Mortality

No treatment related deaths

Clinical Signs

No treatment related findings

Dermal Irritation Scores

Injection site erythema and edema were observed in most groups except saline control, with higher incidence/severity observed in AC2993 QW and AC2993 QW RTU and

AC2993 QW RTU "aged" groups, among which these were no differences. These findings were mostly disappeared after three weeks of recovery period.

Dermal irritation incidence (severity)

Treatment		Erythema		Edema	
		Days 1-21	Days 28-42	Days 1-21	Days 28-42
Saline	M F				
Miglyol 812 control	M F			3 (1) 3 (1)	
Microsphere control	M F	1 (2)		4 (1)	
AC2993 QW	M F	2 (1)		11 (1.4) 11 (1.3)	
AC2993 QW RTU	M F	1 (1) 1 (1)		8 (1.1) 5 (1.2)	1 (1)
AC2993 QW RTU "aged"	M F			7 (1.3) 12 (1.3)	2 (1)

Days 1-21, N=12/sex/group; Days 28-42, recovery phase, n=6/sex/group
Severity was in a scale of 4 as 1=very slight erythema or edema, 2= well-defined erythema or slight edema, 3=moderate to severe erythema or moderate edema, 4=severe erythema or edema
Blanks indicate zero incidences.

Body Weights

A transient body weight loss (up to 6% at Day 5 versus Day 1) and decreased body weight gain within 21 days were noted in the three groups given various AC2993 test article formulations. During recovery phase, these groups gained more weights than saline and other controls. By end of the recovery period (Day 42), only AC2993 QW RTU (4.4 mg/kg rather than 2.4 mg/kg) male group had body weights lower than control.

Treatment		Day 1	Day 5	Day 15	Day 21	Day 42
Saline	M F	386 257	397 268	428 283	441 290	497 312
Miglyol 812 control	M F	385 254	388 254	422 275	440 284	495 309
Microsphere control	M F	389 257	399 266	433 279	450 292	500 312
AC2993 QW	M F	373 247	358 232	395 261	414 271	471 309
AC2993 QW RTU	M F	378 249	356 238	397 270	410 270	467 298
AC2993 QW RTU "aged"	M F	381 254	361 254	388 265	402 272	452 296

Food Consumption

Reduced food consumption was noted in AC2993 treatment groups, more prominently during week 1 (42-52% less than saline control).

Gross Pathology
No Miglyol 812-, microsphere- or test article-related findings at terminal or recovery necropsies.
Organ Weights
No remarkable findings
Histopathology
Adequate Battery: Yes. With existing data for AC2993 QW, the tissues examined in the current study were limited to injection sites, tissue masses with regional lymph node and tissues with gross lesions for all groups. Recovery animals were not examined unless reversibility of microscopic findings needs to be evaluated.

Peer Review: None

Histological Findings

Foreign body granulomas in injection sites were observed in microsphere control and all microsphere containing articles (QW, QW RTU, and QW RTU "aged"), more prominently for groups given microspheres and QW RTU formulation and between those two there was no significant difference in terms of incidence and severity. The granulomas were comprised of small to moderate numbers of epitheloid macrophages and multinucleate giant cells surrounding variable numbers of sharp bordered empty spaces (not likely to be microspheres) that occasionally contained test article (AC2993 active plus microspheres).

Also, histiocytic infiltration, with moderate amount of foamy cytoplasm in the histiocytes was observed in the injection sites for groups given Miglyol 812 and AC2993 containing articles (QW, QW RTU, and QW RTU "aged"). The highest incidence/severity was seen in AC2993 QW group.

The above findings were partially resolved at Day 43 sacrifice.

As presented below, AC2993 QW RTU and its "aged" formulation were not more toxic than AC2993 QW and/or microsphere alone, and therefore, there is no safety concern for formulation change from AC2993 QW to AC2993 QW RTU.

Injection site finding incidence (severity)

Treatment		Foreign body granuloma		Histiocytic infiltration	
		Terminal	Recovery	Terminal	Recovery
Saline	M F	- -	- -	- -	- -
Miglyol 812 control	M F	- -	- -	1 (1)	1 (1) 2 (1)
Microsphere control	M F	5 (1.6) 3 (2)	2 (1) 2 (1.5)	- -	- -

AC2993 QW	M F	2 (1) 2 (1.5)	2 (1) 1 (1)	3 (2.3) 3 (1.3)	3 (2.3) 3 (1.3)
AC2993 QW RTU	M F	4 (1.5) 6 (2.6)	3 (1) 1 (1)	1 (1) 1 (1)	1 (1) -
AC2993 QW RTU "aged"	M F	5 (2.6) 4 (2)	- 1 (1)	3 (1.7) -	- 1(1)

Severity was in a scale of 4 as 1=minimal, 2=mild, 3=moderate and 4=severe
N=6/sex/group at terminal and recovery sacrifices
"-" indicates no incidence

Pharmacokinetic data

Compared to Exenatide QW (2.4 mg/kg), the new formulation, Exenatide QW RTU (2.4 mg/kg) has an ~5-fold lower initial exposure peak, lower C_{max} (-60%) and lower AUC (-38%) values. The "aged" Exenatide QW RTU, which was prepared three weeks before use, presented similar PK profile as freshly prepared formulation.

As QW RTU associated reduction in initial exposure peak, lower C_{max} and lower AUC value are of no safety concerns, nonclinical data that were generated to support aqueous formulation is acceptable to be used to support the NDA for QW RTU.

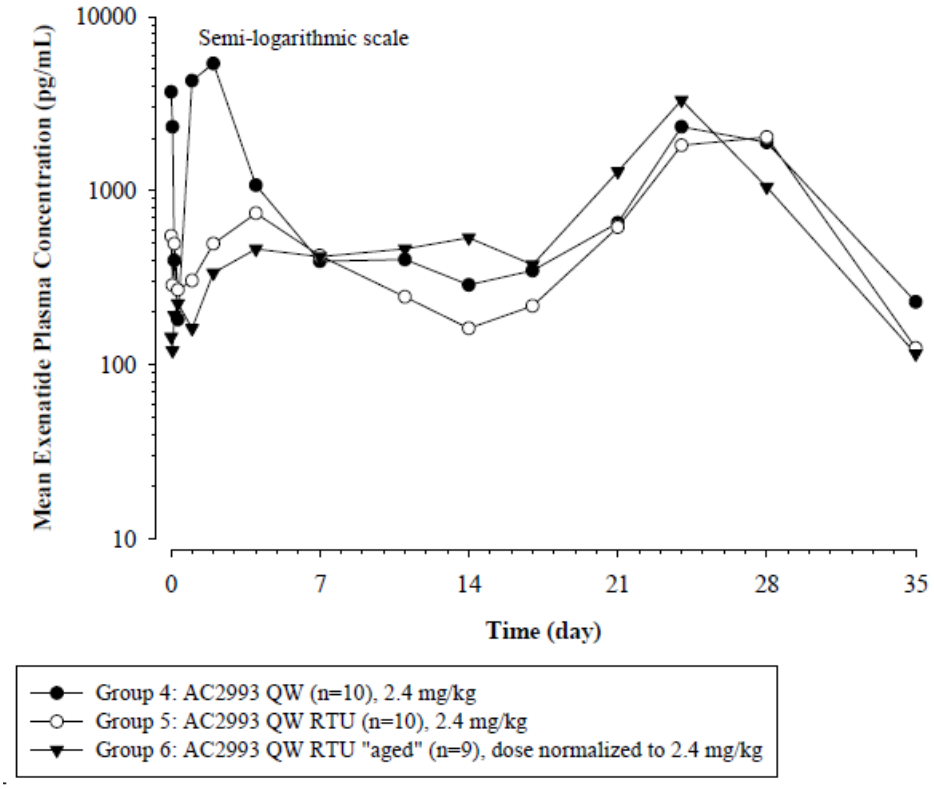
Lower systemic exposure may affect drug efficacy. However, this issue is addressed in human PK and efficacy studies, and rat data is not as informative.

PK data (male and female combined)

	$T_{last,}$ h	C_{max}, ng/mL	$AUC_{0-t,}$ ng.h/mL
2.4 mg/kg Ex. QW	857	6.3	41.4
2.4 mg/kg Ex. QW RTU	826	2.5	25.8
4.4 mg/kg Ex. QW RTU "aged"	840	7.7	63.7
4.4 mg/kg Ex. QW RTU "aged" normalized to 2.4 mg/kg	840	4.2	34.7

AUC_{0-t}, t refers to T_{last} which is the time of the last quantifiable sample

Figure 2: Mean Plasma Concentration of Exenatide in Recovery (Male and Female Combined) Sprague-Dawley Rats Negative for Antibodies to Exenatide Following a Single Subcutaneous Administration of AC2993 QW or AC2993 QW RTU Formulation ([redacted]); REST080666)



Dosing Solution Analysis

The analytical results indicate that the dosing formulations were within ±7% from the nominal concentrations. No test article was detected in any of the control samples.

6.2 Repeat-Dose Toxicity

Study title: AC AC2993 QW RTU A 4-Week Repeated Dose Toxicity Study Following Weekly Subcutaneous Administration in Cynomolgus Monkeys

Study no.	REST080663/ REST080669
Study report location:	ECTD 4.2.3.2
Conducting laboratory and location:	[redacted]
Date of study initiation:	09/24/2008

GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	AC2993, Lot 07-17-112, purity 97%; Miglyol 812, Lot Nos.080628 and 070606, no purity information was provided.

Key Study Findings

This 4-week monkey s.c. study included three control groups (saline, Miglyol 812 and microspheres) and three AC2993 QW RTU groups (0.11, 0.44 and 1.1 mg/kg). Slightly reduced red blood cell parameters (up to -18% of hemoglobin) in AC3993 QW RTU groups, and injection site reactions (erythema, edema, foreign body granulomas, eosinophilic infiltrate and inflammation) in AC2993 QW RTU groups and microsphere control were observed. There were no significant differences in injection site findings between AC3993 QW RTU and microsphere control groups. All these findings were partially resolved after three weeks of recovery period. The NOAEL was considered to be 1.1 mg/kg which represents 4.2x clinical exposure at MRHD of 2 mg/week (AUC_{0-168} 149000 versus 35745 pg.h/mL)

Systemic exposures (Cmax and AUC_{0-168h}) were dose proportional in general. Compared to first dose, 4^{th} dose was associated with longer Tmax (20-144 hours versus 1 hour) and higher AUC values (9-14 fold). Anti-drug antibodies were detected in 13% of treatment monkeys, which did not appear to affect systemic exposure in this relative short study.

Methods

Doses:	0.11, 0.44, 1.1 mg/kg/dose
Frequency of dosing:	Once per week
Route of administration:	Subcutaneous
Dose volume:	0.16 mL/kg (0.11 mL/kg for 0.11 mg/kg/dose)
Formulation/Vehicle:	0.9% NaCl; Miglyol 812 (diluent); AC2993-Placebo PBO F-19 (control microspheres)
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	3/sex/dose
Age:	2.6-3.6 years old
Weight:	Males, 2.2-2.8 kg; females, 2.1-2.7 kg
Satellite groups:	2/sex/dose for recovery study
Unique study design:	Animals were sacrificed on Day 29 for main study and Day 52 for recovery study.

4. STUDY DESIGN

Group	Treatment	Dose Level (mg/kg/dose AC2993)	Dose Volume (mL/kg)	Dose Conc. (mg/mL exenatide)	Dose Regimen	Number of Animals* M	F
Main Study Groups							
1	NaCl	0	0.16	0	4 wk, q 1 wk	5	5
2	Diluent Control	0	0.16	0	4 wk, q 1 wk	5	5
3	Control Microspheres**	0	0.16	0	4 wk, q 1 wk	5	5
4	AC2993 QW RTU	0.11	0.11	1.0	4 wk, q 1 wk	5	5
5	AC2993 QW RTU	0.44	0.16	2.75	4 wk, q 1 wk	5	5
6	AC2993 QW RTU	1.1	0.16	6.92	4 wk, q 1 wk	5	5

*3 animals per sex per group will be necropsied one week after the last dose (a Day 29 necropsy), while 2 animals per sex per group will be sacrificed 3 weeks after the last dose (Day 50 necropsy).

** Group 3 animals will be administered the same amount of microspheres as the high dose (Group 6) animals.

Deviation from study protocol:	No protocol deviations occurred that affect result interpretation.

Observations and Results

Mortality

None

Clinical Signs

No remarkable findings

Dermal Irritation Scores

Injection site erythema and edema were observed during the study and recovery period (Days 36-50), with edema to be more prevalent than erythema. These findings appeared to be microsphere related. The incidence and severity of the findings, especially edema, were closely associated with exposure to microspheres rather than other components (AC2993, Miglyol 812, saline). See the table below.

Dermal irritation incidence (severity)

Dose, mg/kg			Erythema		Edema	
			Days 1-29	Days 36-50	Days 1-29	Days 36-50
0 (Saline)		M F	2 (1) -	- -	- 1 (1)	- -
0 (Miglyol 812)		M F	1 (1) -	- -	- -	- -
0 (Microsphere)		M F	1 (2) -	- 2 (2)	5 (1.2) 5 (1.1)	2 (1) 2 (1.6)
AC2993 QW RTU	0.11	M F	2 (1) -	- 1 (2)	5 (1) 3 (1)	1 (1) 1 (1.1)
	0.44	M F	- -	1 (1) -	4 (1.1) 5 (1)	2(1.1) 2 (1)
	1.1	M F	- -	- -	5 (1.2) 5 (1.3)	2 (1) 2 (1.3)

Days 1-29, N=5/sex/group; Days 36-50, n=2/sex/group
Severity was in a scale of 4 as 1=very slight erythema or edema, 2= well-defined erythema or slight edema, 3=moderate to severe erythema or moderate edema, 4=severe erythema or edema
"-" indicates no incidence

Body Weights
No remarkable findings

Food Consumption
No remarkable findings

Ophthalmoscopy
Not examined

ECG
Not remarkable findings

Hematology
Slightly (up to -18% hemoglobin) decreased red blood cell parameters (erythrocytes, hemoglobin, and hematocrit) and increased reticulocytes were observed in AC2993 QW RTU groups. These changes were partially resolved after four weeks of recovery period, and were not considered to be adverse. See the table below.

dose, mg/kg		RBC, 10^6/microL		Hb, g/dL		Reticulocytes, 10^3/microL	
		terminal	recovery	terminal	recovery	terminal	recovery
0 (Saline)	M F	5.18 5.24	5.62 5.80	12.9 13.5	14.4 14.6	68.9 66.7	29.4 49.3
0 (Miglyol 812)	M F	5.16 5.12	5.61 5.38	12.8 12.6	13.6 13.8	66.7 84.6	43.1 36.9
0 (Microsphere)	M F	5.13 4.98	5.61 5.53	12.8 12.4	13.8 13.8	86.3 71.2	51.4 52.0

AC2993 QW RTU	0.11	M F	4.72 4.66	5.65 5.23	11.5 11.7	13.5 13.5	176.1 114.7	34.2 36.1
	0.44	M F	4.96 4.50	5.49 5.27	12.3 11.3	13.4 13.6	128.8 137.1	27.1 59.0
	1.1	M F	4.78 4.60	4.94 5.50	11.2 11.1	12.3 13.3	141.9 150.1	31.6 56.8

Clinical Chemistry
No remarkable findings (including no effect on lipase or amylase)

Urinalysis
No remarkable findings

Gross Pathology
Injection site swellings/thickenings were observed in animals given microspheres or microsphere containing test articles, but not saline or diluent (Miglyol 812). The incidence and severity of the gross lesions appeared to be associated with the dose of microspheres (the dose in microsphere control matched with that of 1.1 mg/kg AC2993 QW RTU). There were no treatment related findings in thyroid and pancreas.

Incidence (severity) of injection site swelling/thickening

Dose, mg/kg	0 (NaCl)	0 (Diluent)	0 (microspheres)	0.11	0.44	1.1
	Main study (n=12 sites/sex/dose)					
M	0	0	11 (2.4)	3 (2)	12 (2)	12 (2.7)
F	0	0	11 (3.1)	10 (2)	8 (2)	12 (2)
	Recovery study (n=8 sites/sex/dose)					
M	0	0	5 (2.2)	0	3 (2)	3 (3)
F	0	0	3 (2)	3 (2)	8 (2.5)	8 (2.5)

Severity was in a scale of 4 presented as 1= minimal; 2=mild; 3=moderate; 4= severe

Organ Weights
No remarkable findings (including no changes in thyroid weight and pancreas weight)

Histopathology
Adequate Battery: A comprehensive battery of tissues from all animals was microscopically examined (summary table and individual data were included). The study protocol, however, provided that only the following tissues were examined microscopically: injection sites, gross lesions, tissue masses with regional lymph node.

Peer Review: None

Histological Findings

Foreign body granuloma in the subcutis of injection sites were observed in groups given diluent control (Miglyol), microsphere control, and AC2993 QW RTU. Incidence/severity appeared to be lowest with Miglyol group and highest with high dose AC2993 QW RTU

group which was slightly more severe than microsphere control group. The granuloma was characterized by foamy, slightly basophilic macrophages and multinucleated cells centered around variably sized spaces that are clear or contain refractile light yellow material, accompanied by minimal to mild eosinophilic infiltrate and inflammation (perivascular lymphocytic infiltrate in some animals). Following three weeks of recovery period, this finding was slightly lessened. See the table below for details.

Dose, mg/kg		0 (NaCl)	0 (Diluent)	0 (micro-spheres)	0.11	0.44	1.1
		Terminal n=12 injection sites/sex/group (3 animal/sex/group)					
Foreign body granuloma	M	-	10 (1.5)	12 (1.8)	12 (1.6)	12 (2.3)	12 (2.2)
	F	-	10 (1.3)	11 (2.2)	12 (2.4)	11 (2.4)	10 (2.6)
Hemorrhage	M	-	-	1 (1)	-	-	1 (1)
	F	-	-	-	-	-	-
Eosinophilic infiltrate	M	-	6 (1.2)	6 (1.2)	8 (1)	7 (1)	6 (1)
	F	-	3 (1)	6 (1)	8 (1.1)	5 (1)	4 (1)
Inflammation	M	-	-	1 (1)	2 (1)	2 (1)	-
	F	1 (3)	3 (1)	1 (1)	2 (1)	2 (1)	2 (1)
Granulomatous inflammation	M	-	-	-	-	-	-
	F	-	-	-	-	-	1 (4)
		Recovery n=8 injection sites/sex/group (2 animal/sex/group)					
Foreign body granuloma	M	-	-	8 (1.9)	5 (1.8)	8 (2)	8 (1.9)
	F	-	1 (1)	8 (1.9)	8 (1.9)	8 (2.5)	8 (2.2)
Eosinophilic infiltrate	M	-	-	4 (1)	2 (1)	-	-
	F	-	-	1 (1)	-	4 (2)	-
Histiocytic infiltration	M	-	-	-	-	-	-
	F	-	2 (1)	-	-	-	-
Inflammation	M	-	-	1 (1)	2 (1)	2 (1)	-
	F	1 (3)	3 (1)	1 (1)	2 (1)	2 (1)	2 (1)

Severity was in a scale of 4 presented as 1= minimal; 2=mild; 3=moderate; 4= severe
"-" indicates no incidence

With regard to unsolved issues of thyroid tumor and pancreatitis, this study showed no histopathological findings in thyroid and pancreas.

Special Evaluation
None

Toxicokinetics
Systemic exposures (Cmax and AUC) were increased with increasing dose, and also increased over time (C_{max}, 2-4x; AUC, 9-14x following 4th dose compared to 1st dose). T_{max} was significantly longer over time (1 hour following the 1st dose; 18-102 hours following the 4th dose on Day 22). There were no apparent gender effects on TK profile. See the table below.

Table 3. Mean Exposure Parameters in Cynomolgus Monkeys Treated by Weekly Subcutaneous Administration of AC2993 QW RTU for 4 Weeks ((b) (4); REST080663)

Group	Study day	AUC_{0-168h} (pg•h/mL)		C_{max} Day 1 (pg/mL)		C_{max} Overall (pg/mL)		C_{ave} (pg/mL)		T_{max} (h)		T_{last} (h)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.11 mg/kg/dose	Day 1 (N = 10)	1370	1630	139	56.0	139	56.0	7.8	9.23	1.00	0	53	79.5
	Day 22 (N = 10)	19200	13300	336	244	336	244	114	79.4	18.1	52.7	168	0
	Day 22[a] (N = 9)	18700	14000	334	259	334	259	111	83.6	20.0	55.5	168	0
0.44 mg/kg/dose	Day 1 (N = 10)	7170	4360	291	150	291	150	42.1	26.1	1.40	0.843	152	51.2
	Day 22 (N = 10)	61400	19800	819	310	819	310	365	118	101	86.0	168	0
1.1 mg/kg/dose	Day 1 (N = 10)	16600	4090	446	171	446	171	98.7	42.3	1.60	0.966	168	0
	Day 22 (N = 10)	138000	54900	1680	503	1680	503	821	327	102	85.5	168	0
	Day 22[a] (N = 7)	149000	60100	1680	540	1680	540	885	358	144	62.4	168	0

[a]Antibody negative.

Of the 30 monkeys treated with AC2993, 4 monkeys (LD, 1; MD, 0; HD, 3) were antibody positive by day 22 of the study, with titers ranging from 25 to 625. The presence of antibodies does not appear to have impacted the toxicokinetics of AC2993 in this relatively short study.

Dosing Solution Analysis

The actual dosing formulations were within 8% from the nominal concentrations, with the exception for the 0.44 mg/kg/dose group which within 15% from the nominal concentration.

Study title: AC2993 QW RTU: A 3-Month Repeated Dose Toxicity Study Following Subcutaneous Administration in Cynomolgus Monkeys

Study no.	REST080799/ REST080793
Study report location:	ECTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/29/2008
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	AC2993, Lot 07-17-112, purity 97%; Miglyol 812, Lot No 070606, no purity information was provided; Microsphere control (AC2993 placebo, or Formulation PBO-F19) lot No. S428-2717BA, no purity information was provided.

Key Study Findings

This 13-week monkey s.c. study included three control groups (saline, Miglyol and microspheres) and three AC2993 QW RTU groups (0.11, 0.44 and 1.1 mg/kg). Systemic effects were limited to slightly reduced red blood cell parameters (up to -18% of hemoglobin) and reactive increased reticulocytes in AC2993 QW RTU groups. Injection site reactions (erythema, edema, granulomatous inflammation and necrosis) were observed in AC2993 QW RTU groups and microsphere control group. The injection site findings were attributed to presence of microspheres, as that the incidence and severity were primarily associated with microsphere dosage, while the test drug presented no clear contribution. The NOAEL is 1.1 mg/kg in this study which represents 20.1x clinical exposure at MRHD of 2 mg/week (AUC_{0-168} value 719000 versus 35745 pg.h/mL).

Systemic exposures (C_{max} and AUC_{0-168}) were generally dose proportional and accumulated over the repeat treatment (15-45x AUC at Week 13 compared to Day 1). T_{max} at Week 13 is significantly longer than that observed at Day 1 (6-56 hours versus 1 hour) and the associated mechanism is unclear.

Anti-drug antibodies were detected in 66% of treatment monkeys. The presence of antibodies was associated with higher systemic exposure. The neutralization activities of the antibodies were not analyzed. Systemic exposure data from antibody negative animals are to be used for comparisons in safety evaluation.

Methods

Doses:	0.11, 0.44, 1.1 mg/kg/dose
Frequency of dosing:	Once per week
Route of administration:	Subcutaneous
Dose volume:	0.16 mL/kg (0.11 mL/kg for 0.11 mg/kg/dose)
Formulation/Vehicle:	0.9% NaCl; Miglyol 812 (diluent); AC2993-Placebo PBO F-19 (control microspheres)
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	4/sex/dose
Age:	2-4 years old
Weight:	2-3 kg
Satellite groups:	2/sex/dose for recovery study
Unique study design:	Animals were sacrificed on Day 92 for main study and Day 183 for recovery study.

4. STUDY DESIGN

Group	Treatment	Dose Level (mg/kg/dose AC2993)	Dose Volume (mL/kg)	Dose Conc. (mg/mL AC2993)	Dose Regimen	Number of Animals* M	F
1	NaCl	0	0.16	0	13 wk, q 1 wk	6	6
2	Diluent Control	0	0.16	0	13 wk, q 1 wk	6	6
3	Control Microspheres**	0	0.16	0	13 wk, q 1 wk	6	6
4	AC2993 QW RTU	0.11	0.11	1.0	13 wk, q 1 wk	6	6
5	AC2993 QW RTU	0.44	0.16	2.75	13 wk, q 1 wk	6	6
6	AC2993 QW RTU	1.1	0.16	6.92	13 wk, q 1 wk	6	6

*4 animals per sex per group will be necropsied one week after the last dose (a Day 92 necropsy), while 2 animals per sex per group will be sacrificed 13 weeks after the last dose (Day 183 necropsy).

** Group 3 animals will be administered the same amount of microspheres as the high dose (Group 6) animals.

Deviation from study protocol:	No protocol deviations occurred that affect result interpretation.

Observations and Results

Mortality

No mortalities occurred in treatment groups

Clinical Signs

No remarkable findings

Dermal Irritation Scores

Increased incidences of erythema and edema in microsphere control and AC2993 QW RTU groups were observed. Edema was more prevalent than erythema. The dermal irritation findings appeared to be attributable to the microspheres. Of the groups

administered AC2993 QW RTU, the 0.11 mg/kg/dose group was least affected, and the incidence/severity were similar between microsphere control and high dose AC2993 QW RTU (1.1 mg/kg/dose) group. Irritation noted in the Miglyol 812 control was similar to saline group, being rare and sporadic/transient in occurrence, which is considered to be procedure related. The dermal irritation findings were partially recovered after 3 months of recovery period.

Dermal irritation incidence (severity)

dose, mg/kg			Erythema		Edema	
			Days 1-89	Days 92-176	Days 1-89	Days 92-176
0 (Saline)		M F	- 3 (1)	- -	1 (1) -	- -
0 (Miglyol 812)		M F	2 (1) 2 (1)	- -	2 (1) 2 (1.1)	- 1 (1)
0 (Microsphere)		M F	1 (1) 2 (1.5)	- -	6 (1.7) 6 (1.6)	2 (1.8) 2 (1.3)
AC2993 QW RTU	0.11	M F	2 (1) 2 (1)	- -	6 (1.1) 5 (1.0)	1 (1) 1 (1)
	0.44	M F	1 (1) 1 (1.2)	- -	6 (1.1) 6 (1.2)	2 (1.2) 2 (1.1)
	1.1	M F	2 (1.8) 4 (1)	1 (1.5) 1 (1)	6 (1.7) 6 (1.6)	2 (1.4) 2 (1.2)

Days 1-89, N=6/sex/group; Days 92-176, n=2/sex/group
Severity was in a scale of 4 as 1=very slight erythema or edema, 2= well-defined erythema or slight edema, 3=moderate to severe erythema or moderate edema, 4=severe erythema or edema; severity average is weighted by occurrence and number of animal affected.
"-" indicates no incidence

Body Weights
No microsphere-, diluent-, or test article-related effects noted

Food Consumption
No microsphere-, diluent-, or test article-related effects noted

Ophthalmoscopy
No microsphere-, diluent-, or test article-related effects noted

ECG
No microsphere-, diluent-, or test article-related effects noted

Hematology
Slightly decreased red blood cell parameters (up to -18% hemoglobin, in a similarly or less degree for reduced erythrocyte counts and hematocrit value) and increased reticulocytes in AC2993 QW RTU groups were observed. Recovery data obtained after 13 weeks of recovery period showed partial reversibility of this finding. See the table below.

dose, mg/kg			RBC, 10^6/microL		Hb, g/dL		Reticulocytes, 10^3/microL	
			terminal	recovery	terminal	recovery	terminal	recovery
0 (Saline)		M	5.55	5.69	13.18	13.30	66.40	64.10
		F	5.31	5.55	12.85	13.15	61.62	67.10
0 (Miglyol 812)		M	5.50	5.91	13.04	13.55	61.78	34.95
		F	5.21	5.47	12.55	13.25	67.25	54.45
0 (Microsphere)		M	5.52	6.01	12.83	14.40	61.80	56.00
		F	5.20	5.50	12.23	12.30	60.22	52.55
AC2993 QW RTU	0.11	M	5.11	5.87	11.78	13.45	98.27	57.20
		F	4.90	5.44	11.95	12.80	84.92	47.30
	0.44	M	5.03	5.91	11.65	12.50	88.70	49.20
		F	4.72	5.45	11.58	13.20	95.07	73.55
	1.1	M	4.80	5.41	11.22	12.55	99.45	36.65
		F	4.75	5.15	11.23	12.50	106.83	75.85

Clinical Chemistry
No remarkable findings (including no effect on lipase or amylase)

Urinalysis
No remarkable findings

Gross Pathology
Injection site swelling/thickening was noted in microsphere control and AC2993 QW RTU groups. The incidence and severity were related to microsphere dose (most prominently in microsphere control and 1.1 mg/kg/dose of AC2993 QW RTU where highest dose of microspheres were used). No diluent- (Miglyol 812), or test article-related macroscopic findings. See the table below for incidence and severity. There were no similar findings in the recovery animals after 13 weeks of recovery period.

Test Article-Related Macroscopic Findings
Terminal
Male

Dose Level (mg/kg/dose)	0 (NaCl)	0 (Diluent)	0 (Microsphere Control)	0.11	0.44	1.1
Number Examined	4	4	4	4	4	4
Injection sites combined [Total of 13 sites]						
swollen/thickened	0	0	25	5	5	23
- mild	0	0	14	3	5	13
- moderate	0	0	11	2	0	10

Test Article-Related Macroscopic Findings Terminal Female						
Dose Level (mg/kg/dose)	**0 (NaCl)**	**0 (Diluent)**	**0 (Microsphere Control)**	**0.11**	**0.44**	**1.1**
Number Examined	**4**	**4**	**4**	**4**	**4**	**4**
Injection sites combined [Total of 13 sites]						
swollen/thickened	0	0	32	0	18	23
- mild	0	0	26	0	13	11
- moderate	0	0	6	0	5	9
- severe	0	0	0	0	0	3

Organ Weights
No treatment related findings (including no changes in pancreas weight and thyroid weight)

Histopathology
Adequate Battery: A comprehensive battery of tissues from all animals was microscopically examined.

Peer Review: None

Histological Findings

Histopathological findings were limited to injection site granulomatous inflammation and accompanied necrosis in some cases, mainly in microsphere control and AC2993 QW RTU groups. The granulomatous inflammation was generally consistent with a foreign body reaction, characterized by discrete, variably-sized focal to multifocal aggregates of macrophages ad multinucleated giant cells with rare lymphocytes and eosinophils that often appeared to be encompassed by a thin band of fibrous tissues. The macrophages and multinucleated giant cells typically contained single large cytoplasmic vacuoles that were either clear or contained pale granular eosinophilic material that likely represented phagocytized test article material. The granulomatous inflammation occurred at a higher incidence and severity in recent injection sites relative to injection sites used earlier in the study. This suggested ongoing resolution of the inflammation over the course of the study in all dose groups. Following 13 weeks of recovery, all microscopic findings at the injection sites were of minimal severity, indicating near complete resolution of these findings over the recovery period. See the table below for details.

With regarding to unsolved issues of thyroid tumors and pancreatitis, there were remarkable findings in thyroid and pancreas.

Test Article-Related Microscopic Findings Terminal Male						
Dose Level (mg/kg/dose)	**0 (NaCl)**	**0 (Diluent)**	**0 (Microsphere Control)**	**0.11**	**0.44**	**1.1**
Number Examined	**4**	**4**	**4**	**4**	**4**	**4**
Injection sites combined [Total of 13 sites]						
Inflammation, granulomatous	1	11	37	28	35	38
- minimal	1	11	11	14	16	8
- mild	0	0	9	13	14	9
- moderate	0	0	16	1	5	20
- severe	0	0	1	0	0	1
Inflammation, mixed	0	0	0	4	0	0
- minimal	0	0	0	2	0	0
- mild	0	0	0	1	0	0
- moderate	0	0	0	1	0	0
Infiltration, lymphocytic	0	0	2	5	0	2
- minimal	0	0	2	2	0	2
- mild	0	0	0	3	0	0
Necrosis	0	0	20	1	8	25
- minimal	0	0	3	0	3	4
- mild	0	0	8	1	5	11
- moderate	0	0	9	0	0	10

Test Article-Related Microscopic Findings Terminal Female						
Dose Level (mg/kg/dose)	**0 (NaCl)**	**0 (Diluent)**	**0 (Microsphere Control)**	**0.11**	**0.44**	**1.1**
Number Examined	**4**	**4**	**4**	**4**	**4**	**4**
Injection sites combined [Total of 13 sites]						
Inflammation, granulomatous	0	10	38	22	37	33
- minimal	0	10	11	6	12	5
- mild	0	0	8	13	12	6
- moderate	0	0	19	3	13	15
- severe	0	0	0	0	0	7
Inflammation, mixed	0	1	0	2	0	1
- minimal	0	1	0	2	0	0
- mild	0	0	0	0	0	1
Infiltration, lymphocytic	0	0	0	11	17	13
- minimal	0	0	0	10	17	8
- mild	0	0	0	1	0	5
Necrosis	0	0	21	2	12	23
- minimal	0	0	7	2	2	3
- mild	0	0	7	0	6	6
- moderate	0	0	7	0	4	14

Toxicokinetics

Systemic exposures (C_{max} and AUC_{0-168}) were generally dose proportional (form antibody negative animals), which accumulated over the repeat treatment (15-45x AUC at Week 13 compared to Day 1). This range of AUC fold increase at week 13 is similar to that observed in Week 4 of the 4-week monkey study (reviewed earlier), suggesting that a steady state was reached in 4 weeks.

Similar to the 4-week study, this study also showed longer T_{max} following Week 13 dosing than Day 1 dosing (1 versus 6-56 hours). The cause of this finding is unclear especially in light of common practice of injection site rotation in the monkeys. There were no gender effects on TK profile.

The following table presents male and female combined TK data.

Table 4. Mean Exposure Parameters in Cynomolgus Monkeys Treated by Weekly Subcutaneous Administration of AC2993 QW RTU for 13 Weeks ((b) (4) REST080799)

Group	Study week	$AUC_{0\text{-}168h}$ (pg•h/mL)		C_{max} Day 1 (pg/mL)		C_{max} Overall (pg/mL)		C_{ave} (pg/mL)		T_{max} (h)		T_{last} (h)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.11 mg/kg/dose	Week 1 (N = 12)	5350	3810	250	56.6	250	56.6	33.2	22.9	1.00	0	126	57.4
	Week 13 (N = 12)	107000	47500	1580	1320	1680	1260	640	283	18.1	19.1	168	0
	Week 13* (N = 4)	69000	47600	1000	805	1090	780	411	284	56.7	96.4	168	0
0.44 mg/kg/dose	Week 1 (N = 12)	19000	8220	760	229	760	229	113	49.0	1.00	0	168	0
	Week 13 (N = 12)	291000	201000	4150	3530	4480	3460	1850	1280	42.8	65.1	156	41.6
	Week 13* (N = 5)	197000	18500	3310	1660	3340	1620	1170	110	34.4	74.7	168	0
1.1 mg/kg/dose	Week 1 (N = 12)	22400	9200	1880	1650	1680	1260	640	283	1.17	0.577	168	0
	Week 13 (N = 12)	1020000	1200000	10700	8050	13800	11900	6090	7160	26.1	50.5	168	0
	Week 13* (N = 2)	719000	NC	7310	NC	7310	NC	4280	NC	6.00	NC	168	NC

*Antibody negative animals only.

Of the 36 monkeys treated with AC2993, 24 (LD, 8; MD, 7; HD, 9) were antibody positive by week 13 of the study, with titers ranging from 25 to 390,625. The neutralizing activities of the antibodies were not analyzed. At the group mean level, the presence of antibodies was associated with increased $AUC_{0\text{-}168h}$ values, reflecting that, in the majority of animals, ADA caused markedly increased exposures. However, in a subset of animals ADA caused decreased, or loss of, exposure.

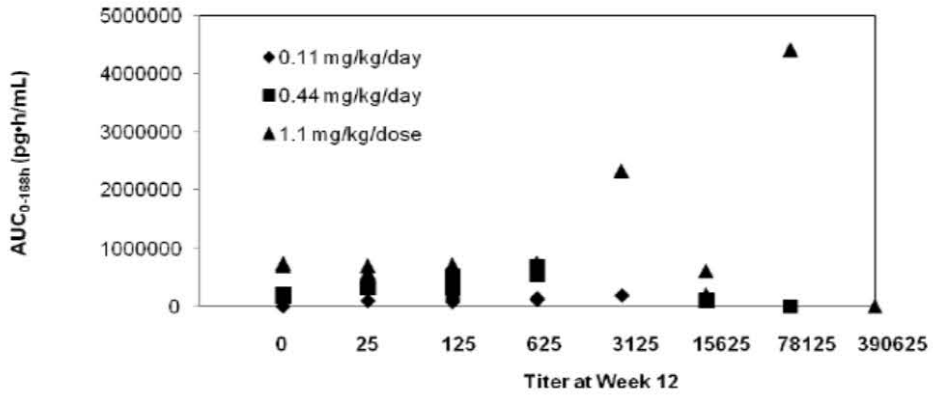


Figure 9. Plasma $AUC_{0\text{-}168h}$ vs. AC2993 Antibody Titer

Appendix B (continued). Toxicokinetic Parameters for AC2993 in Individual Male and Female Monkeys after Weekly Subcutaneous Injections of AC2993 QW RTU

Week	Dose (mg/kg/dose)	Sex	Animal #	$AUC_{0\text{-}168h}$ (hr•pg/mL)	C_{max} Day 1 (pg/mL)	C_{max} Overall (pg/mL)	C_{ave} (pg/mL)	T_{max} (hr)	T_{last} (hr)
13	0.11	M	119	93100	700	1060	554	168	168
			120[b]	76500	1170	1170	456	1.00	168
			121	0	0	0	0	NC	NC
			155	136000	1380	1390	812	24.0	168
			156[c]	107000	1630	1630	635	1.00	168
			157[b]	167000	2820	2820	993	3.00	168
			Mean	96600	1280	1340	575	39.4	168
			SD	57200	946	913	341	72.5	0
			Mean*	66600	775	894	396	84.5	168
			SD*	58000	815	825	346	118	0
		F	122[d]	187000	2120	2120	1110	3.00	168
			123[b]	119000	615	1080	706	48.0	168
			124[a]	95200	5020	5020	567	1.00	168
			158[c]	115000	616	906	683	24.0	168
			159	76100	1680	1680	453	1.00	168
			160[c]	119000	1250	1250	707	3.00	168
			Mean	118000	1880	2010	705	13.3	168
			SD	37500	1650	1540	223	19.1	0
			Mean*	76000	1680	1680	453	1.00	168
			SD*	NA	NA	NA	NA	NA	NA

NA = not applicable; N<3.
NC = not calculated; all plasma concentrations were below limit of quantitation.

*Excluding antibody positive animals.
[a]Antibody titer = 25.
[b]Antibody titer = 125.
[c]Antibody titer = 625.
[d]Antibody titer = 3125.

Appendix B (continued). Toxicokinetic Parameters for AC2993 in Individual Male and Female Monkeys after Weekly Subcutaneous Injections of AC2993 QW RTU

Week	Dose (mg/kg/dose)	Sex	Animal #	$AUC_{0\text{-}168h}$ (hr•pg/mL)	C_{max} Day 1 (pg/mL)	C_{max} Overall (pg/mL)	C_{ave} (pg/mL)	T_{max} (hr)	T_{last} (hr)
13	0.44	M	125	215000	1580	1760	1280	168	168
			126	180000	3190	3190	1070	1.00	168
			127[a]	320000	3650	5830	3360	96.0	168
			161	211000	5940	5940	1260	1.00	168
			162[b]	320000	3790	3790	1910	1.00	168
			163[e]	107000	607	1350	634	48.0	168
			Mean	225000	3130	3640	1580	52.5	168
			SD	83000	1860	1950	961	68.1	0
			Mean*	202000	3570	3630	1200	56.7	168
			SD*	19400	2210	2120	115	96.4	0
		F	128[c]	689000	10600	10600	4100	3.00	168
			129	175000	3560	3560	1040	1.00	168
			130	204000	2270	2270	1210	1.00	168
			164[b]	518000	3240	4120	3090	168	168
			165[f]	1320	72.2	86.2	14.0	24.0	24.0
			166[c]	554000	11300	11300	3300	1.00	168
			Mean	357000	5170	5310	2130	33.0	144
			SD	268000	4630	4570	1590	66.8	58.8
			Mean*	189000	2910	2910	1130	1.00	168
			SD*	NA	NA	NA	NA	NA	NA

NA = not applicable; N<3.
*Excluding antibody positive animals.
[a]Antibody titer = 25.
[b]Antibody titer = 125.
[c]Antibody titer = 625.
[e]Antibody titer = 15625.
[f]Antibody titer = 78125.

Appendix B (continued). Toxicokinetic Parameters for AC2993 in Individual Male and Female M Weekly Subcutaneous Injections of AC2993 QW RTU

Week	Dose (mg/kg/dose)	Sex	Animal #	$AUC_{0\text{-}168h}$ (hr•pg/mL)	C_{max} Day 1 (pg/mL)	C_{max} Overall (pg/mL)	C_{ave} (pg/mL)	T_{max} (hr)	T_{last} (hr)
13	1.1	M	131	700000	8640	8640	4170	6.00	168
			132[a]	700000	25500	25500	4170	1.00	168
			133[c]	748000	13300	13300	4450	1.00	168
			167[b]	715000	8710	8710	4250	1.00	168
			168[d]	2320000	14500	18600	13800	168	168
			169[g]	0	0	0	0	NC	NC
			Mean	864000	11700	12500	5140	35.4	168
			SD	769000	8420	8860	4580	74.2	0
			Mean*	700000	8640	8640	4170	6.00	168
			SD*	NA	NA	NA	NA	NA	NA
		F	134[a]	585000	25100	25100	3480	1.00	168
			135	738000	5990	5990	4390	6.00	168
			136[e]	206000	1300	1930	1230	48.0	168
			170[f]	4410000	8730	41300	26300	48.0	168
			171[a]	550000	5210	5210	3280	1.00	168
			172[e]	609000	11300	11300	3620	6.00	168
			Mean	1180000	9610	15100	7040	18.3	168
			SD	1590000	8320	15200	9470	23.1	0
			Mean*	738000	5990	5990	4390	6	168
			SD*	NA	NA	NA	NA	NA	NA

NA = not applicable; N<3.
NC = not calculated; all plasma concentrations were below limit of quantitation.

*Excluding antibody positive animals.
[a]Antibody titer = 25.
[b]Antibody titer = 125.
[c]Antibody titer = 625.
[d]Antibody titer = 3125.
[e]Antibody titer = 15625.
[f]Antibody titer = 78125.
[g]Antibody titer = 390625.

Analysis of dosing formulations:
The test article dosing formulations were within 6% for their nominal concentration, with the exception of the Group 5 formulation (0.44 mg/kg, 2.75 mg/mL) at Week 13 where a concentration of 118.9% of nominal was measured.

7 Genetic Toxicology

Genetic toxicology studies were conducted in support of the predecessor products Byetta and Bydureon. No genotoxicity studies were conducted with the medium-chain triglyceride component of Bydureon Bcise, and the Applicant cites scientific literature demonstrating that neither caprylic acid, nor tricaprylin were mutagenic in bacterial reverse mutation assays[1]. QSAR and an Ames study were conducted to qualify two leachables that were identified by the Applicant, and are reviewed below.

[1] Traul, KA, et al., "Review of the toxicologic properties of medium-chain triglycerides," *Food Chem. Toxicol.* 2000, 38(1):79-98.

7.1 Impurity Qualification

Impurity Qualification Genotoxicity study (submission dated 8/14/2017)

Two leachables observed in the simulation study, (b) (4) (b) (4) contain structural allerts baed on QSAR (DEREK and Leadscope analyses). The sponsor reported the followng Ames test for (b) (4), which was used as a proxy for this class of leachables. This study report was not submitted until requested by the Division.

Study Title: Ames Reverse-Mutation Study In Salmonella Typhimurium and Escherichia Coli

Key findings: BMT-173773-01 was negative in this Ames assay as that no increased revertant colony number was observed in any of the tester starins at any test concentration, both with and without the metabolic activation system.

Study No. DE13308

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

Date of study initiation: 12/17/2014
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: (b) (4) Lot A027C-08, purity 94%
(b) (4)

Methods

Strains/species/cell line:
TA98, TA100, TA1535, TA1537 and E coli WP2 uvrA
Unique study design: (b) (4) was dissolved and diluted in dimethyl formamide
Basis of dose selection: 5000 mcg/plate, ±S9
Vehicle controls: Dimethylformamide
Positive controls: see below

Positive controls

Chemical	Final concentration, mcg/plate	Strain(s)	S-9
2-nitroflurene (2NF)	1.0	TA98	-
Sodium azide (NaN_3)	0.5	TA100, TA1535	-
9-aminoacridine hemihydrate (9AC)	50.0	TA1537	-
4-nitroquinoline N-oxide (NQO)	0.5	WP2 uvrA	-
Benzo[α]pyrene (B[α]P)	5.0	TA98, TA1537, TA100	+
2-animoanthracene (2AA)	5.0	TA1535	+
	20.0	WP2 uvrA	+

Stock solutions were formulated in water for NaN3, and in DMSO for all other positive control articles

Incubation and sampling times: Pre-incubation method was used. Sampling time was at 64-68 hours.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
All these studies were considered valid based on the following:

- Triplicate cultures were used for all culture conditions
- Revertant colonies were counted electronically with a Sorcerer Colony Counter, and manually for occasions at the discretion of study director.
- At least 5 different concentrations were accountable for revertant colonies.
- Results of positive and negative control were in expected range
- Criteria for positive results for most studies: dose-related increase in revertant colony numbers to at least 2x of vehicle control levels with bacterial strains TA98, TA100, and WP2 uvrA (3x for TA1535 and TA1537) either in the presence or absence of S9 mix.

Study outcome:

- Toxicity (absence of background lawn or diminution in mean revertant colony counts) was observed in the absence of S9 in strain TA1537 at ≥625 mcg/plate and in TA100 at ≥313 mcg/plate; in the presence of S9 in strains TA100 at ≥1250 mcg/plate.
- Precipitate was observed in most strains at concentrations≥ 2500 mcg/plate without S9, and 5000 mcg/plate with S9, except the culture of WP2 uvrA no precipitate was observed up to 5000 mcg/plate.
- There were no meaningful increases in revertant colonies in any test conditions in this study.

8 Carcinogenicity

Carcinogenicity studies were conducted in support of the predecessor products Byetta and Bydureon. No carcinogenicity studies were conducted with the medium-chain triglyceride component of Bydureon Bcise.

9 Reproductive and Developmental Toxicology

Developmental and Reproductive Toxicology (DART) studies were conducted in support of the predecessor products Byetta and Bydureon. No DART studies were conducted with the medium-chain triglyceride component of Bydureon Bcise.

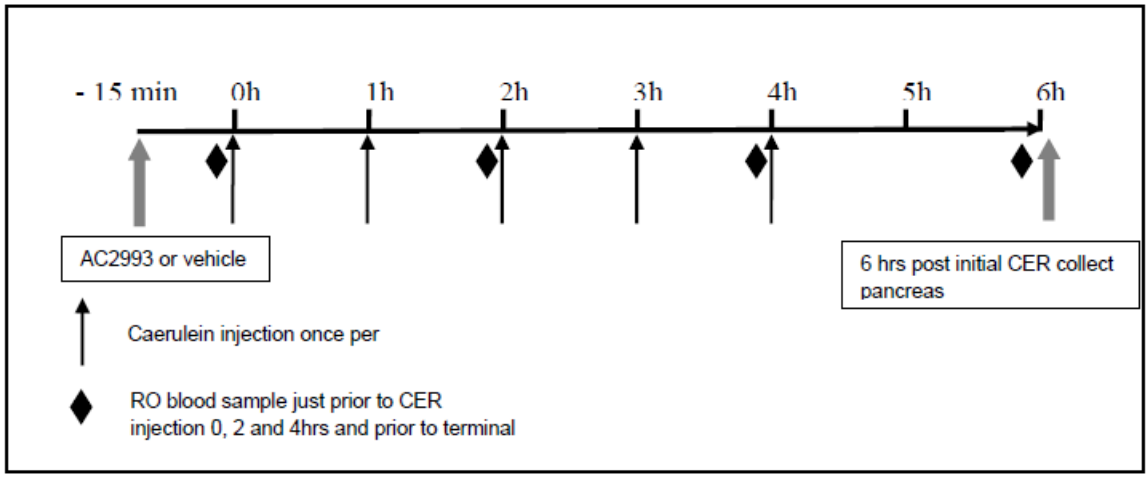
10 Special Toxicology Studies

10.1 Pancreatic Toxicity

Title: Chemically-induced Pancreatitis in ob/ob Mice after a Single Administration of AC2993

Study no.: REST090186/ REST090020

Study design (*NB – Non-GLP)*: 12-week old male B6.V-lepob/j (ob/ob) mice received a single 5 nmol/kg dose of exenatide (AC2993) or vehicle by SC injection. Beginning 15 min post exenatide dosing, mice received five intraperitoneal (IP) hourly injections of 10 µg/kg caerulein. Blood samples were collected at baseline and at several points throughout the study and glucose, amylase and lipase levels measured. After the last caerulein injection, pancreata were collected for weighing and histological examination.



Results:

Figure 1: Amylase

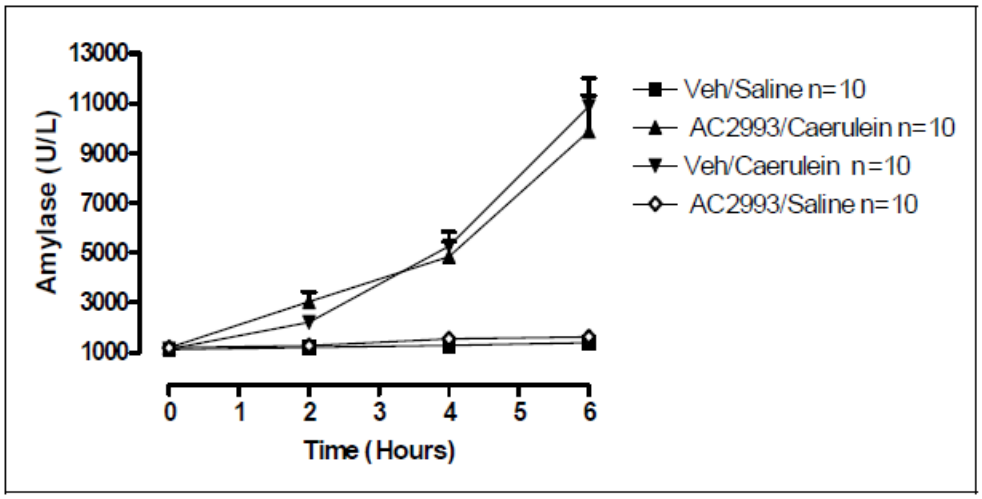


Figure 2: Lipase

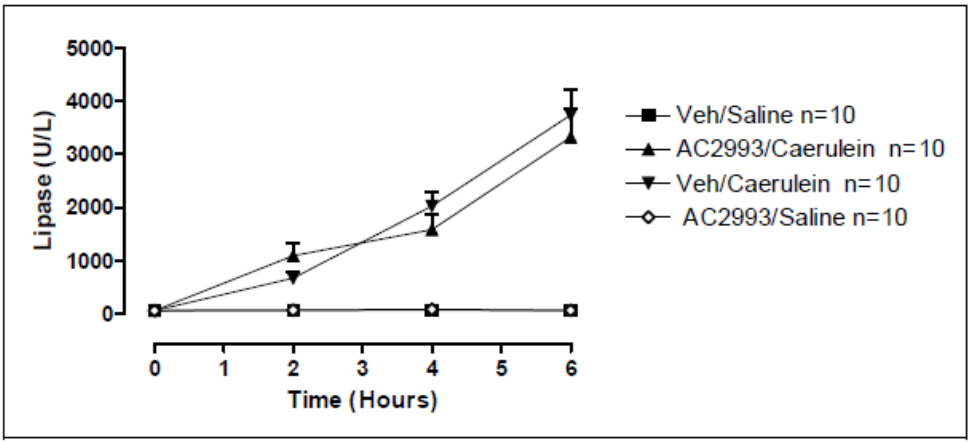


Figure 3: Glucose

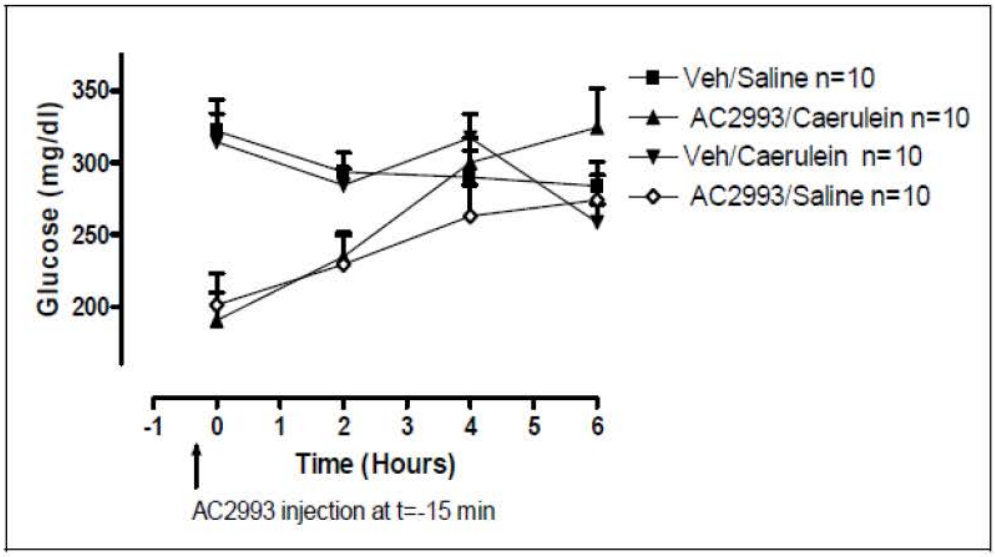
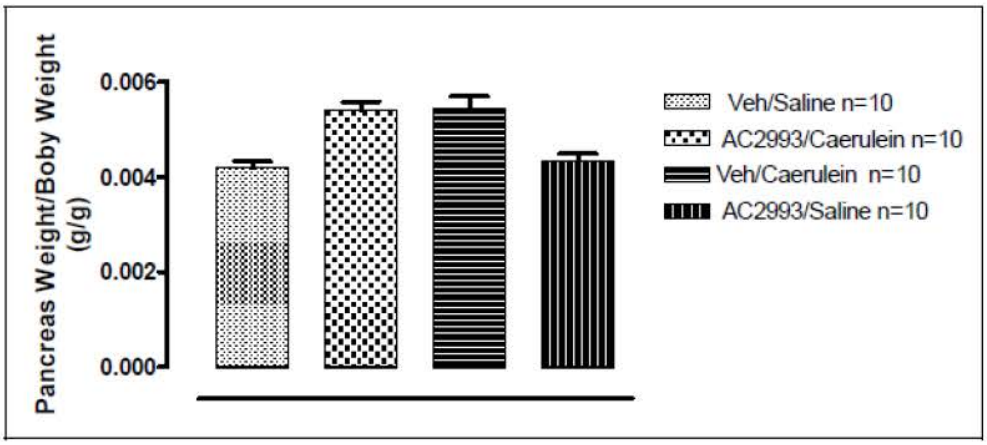


Figure 4: Pancreas Weight/Body Weight



Group	1											2										
Strain	ob/ob											ob/ob										
Treatment	Vehicle 10% DMSO											AC2993 5 nmol/kg										
Caerulein Insult	Vehicle Saline											Caerulein 10 µg/kg										
Animal Number	301	302	303	304	305	306	307	308	309	310	INC	311	312	313	314	315	316	317	318	319	320	INC
Pancreas	N		N	N		N	N	N	N		7											0
Lymphocytic Infiltrate											0											0
Exocrine Acute Inflammation											0	1>	1>	1>	2>	1>	1>	1>	1>	1>	1>	10
Acinar Cell Vacuolation											0	2]	1>	1>	2>	2>	2>	2]	2]	2]	2>	10
Acute Necrotizing Inflammation, Fat					2)						1	3)	1)	1)		2>						4
Acinar Single Cell Necrosis											0	1>		1>	1>	1>	1>	1>	1>	1>		8
Atrophy, Lobule		2									1										1>	1
Ductal Necrosis					2)						1											0
Islet Vascular Ectasia										3)	1											0
Vascular Mixed Inflammation											0				2)							1
Chronic Inflammation, Fat											0											0

= With focal mild acute vasculitis

Group	3											4									
Strain	ob/ob											ob/ob									
Treatment	Vehicle 10% DMSO											AC2993 5 nmol/kg									
Caerulein Insult	Caerulein 10 µg/kg											Vehicle Saline									
Animal Number	321	322	323	324	325	326	327	328	329	330	INC	331	332	333	334	335	336	337	338	339	340	INC
Pancreas											0	N	N	N		N		N	N	N		7
Lymphocytic Infiltrate	1>										1											0
Exocrine Acute Inflammation	1>	1>	1>	1>	1>	1>	1>	1>	1>	1>	10											0
Acinar Cell Vacuolation	1>	1]	1>	2>	2>	2>	2>	1>	1>	1>	10											0
Acute Necrotizing Inflammation, Fat					2)	1)	1)	1)	1)	2)	6						1)					1
Acinar Single Cell Necrosis		1>		1>	1>	1>	1>	1>	1>	1>	8											0
Atrophy, Lobule	1)	2>									2											0
Ductal Necrosis											0											0
Islet Vascular Ectasia											0											0
Vascular Mixed Inflammation								2)			1				2)							1
Chronic Inflammation, Fat											0										2)	1

= With focal mild acute vasculitis

Abbreviations and Glossary

N	Normal
INC	Incidence
)	Focal
>	Multifocal
]	Diffuse
1	Minimal
2	Mild
3	Moderate
4	Marked

Conclusion: Caerulein caused increases in amylase, lipase and pancreas weight. Exenatide had no effect on these parameters, but did decrease blood glucose. Exenatide had no discernable effect on pancreas histology, either on its own, or in combination with caerulein.

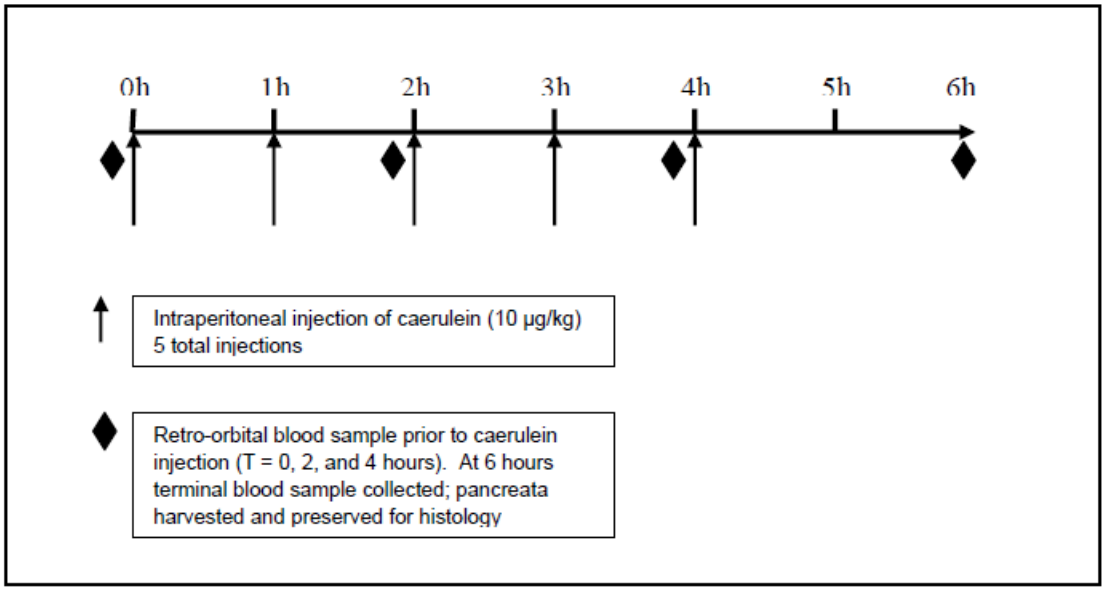
Title: Caerulein-induced pancreatitis in ob/ob mice after sub-chronic dosing with AC2993 for four weeks

Study no.: REST090159/REST090159R1

Study design (*NB – Non-GLP)*: 7-week old male B6.V-lepob/j (ob/ob) mice received AC2993 (5 or 30 µg/kg/day) or vehicle by SC minipump infusion for 4 weeks. WT (lean) mice received vehicle by SC minipump infusion. After 4 weeks of drug treatment, pancreatitis was induced by administered of caerulein as five intraperitoneal hourly injections at 10 µg/kg (5 ml/kg). Blood samples were collected for measurement of glucose, amylase and lipase levels. Cytokine levels (IL-1β, IL-2, IL-6, IFN-γ, MCP-1, TNF-α) were also measured 6 hours after initiation of caerulein treatment. The levels of mRNA for genes known to be associated with pancreatitis (Reg3b, Egr1, Icam1, IL6,

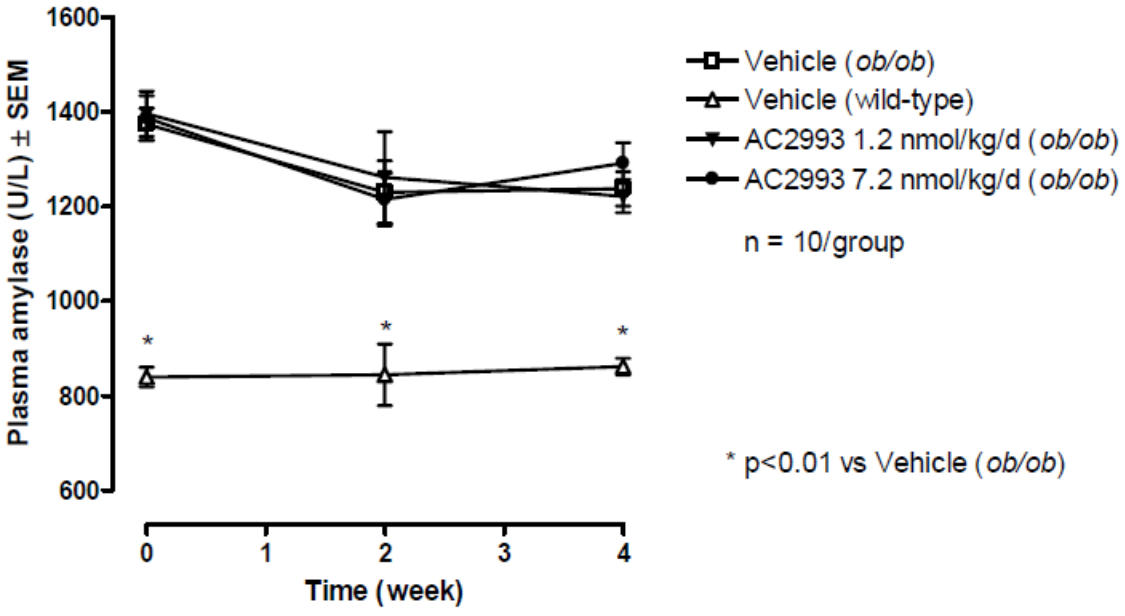
Ccl2 [encoding MCP-1], Nfkb1, Mpo and Vamp8). After the last caerulein injection, pancreata were collected for weighing and histological examination. Pancreatic ductal cell proliferation was assessed by co-staining with Ki-67 (proliferation marker) and pan-cytokeratin (pancreatic duct marker).

Figure 1: Caerulein injection schedule.

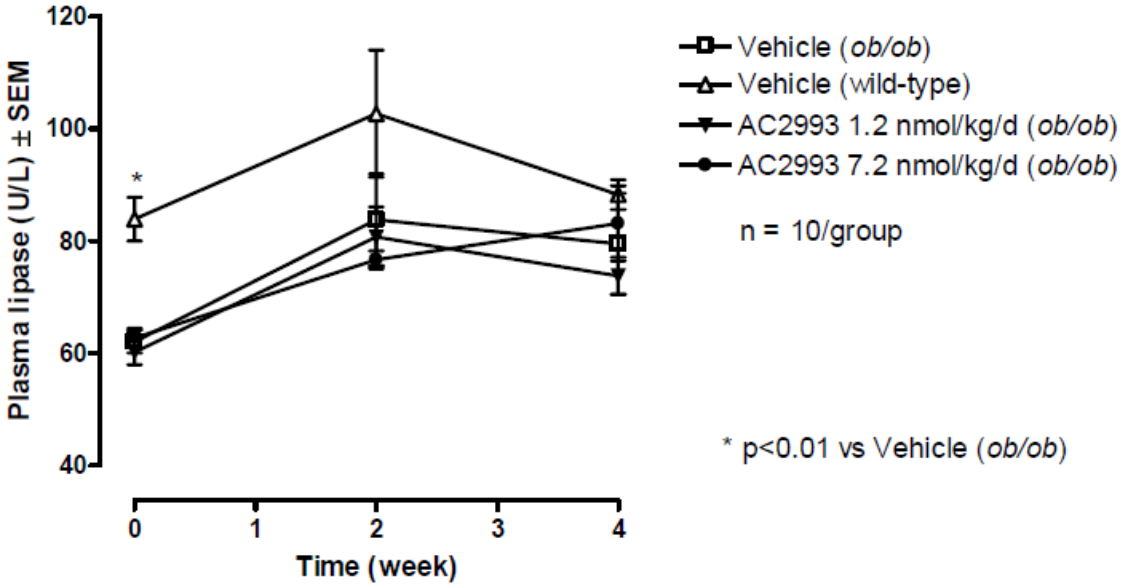


Results:

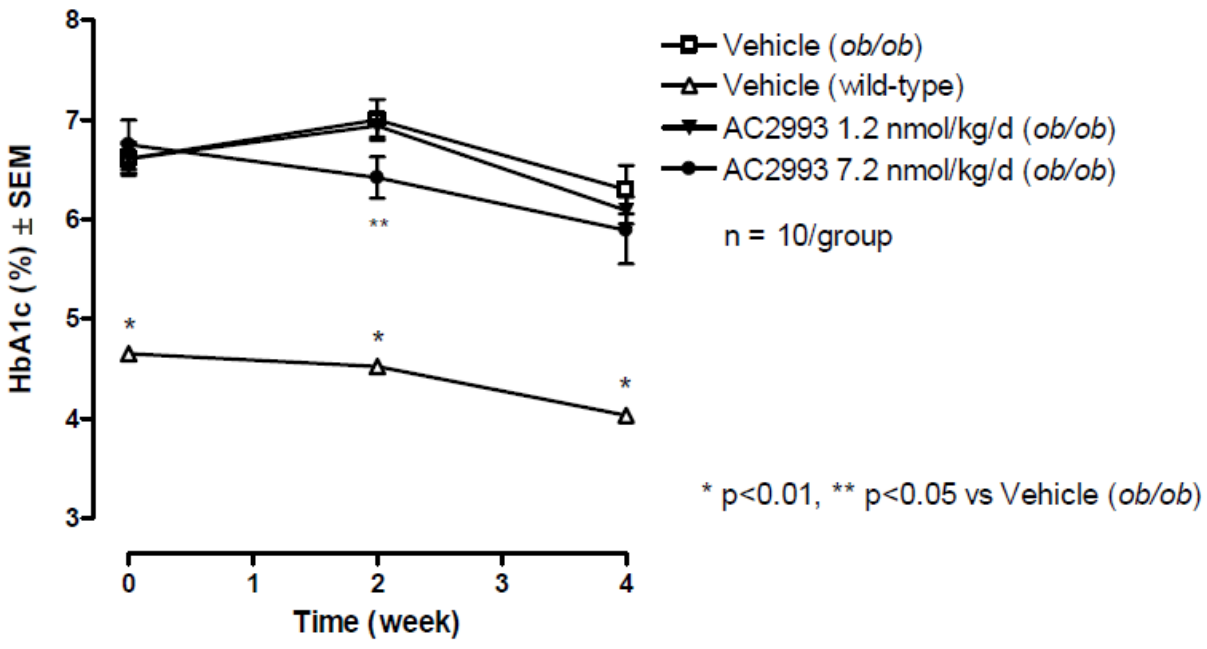
Effects of AC2993 on plasma amylase over 4 weeks



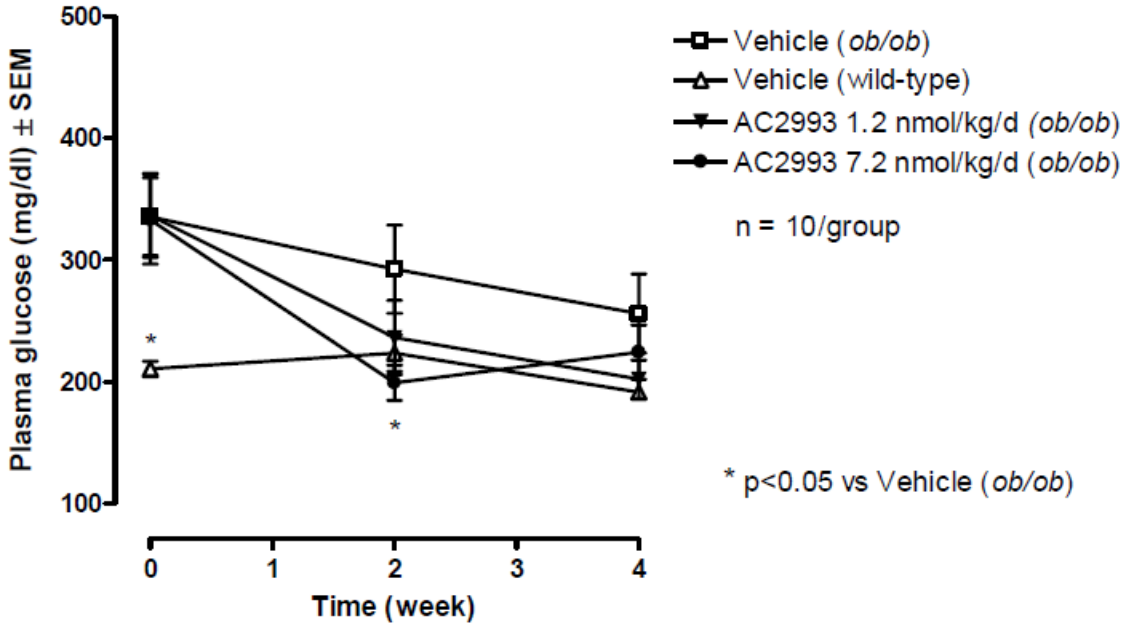
Effects of AC2993 on plasma lipase over 4 weeks



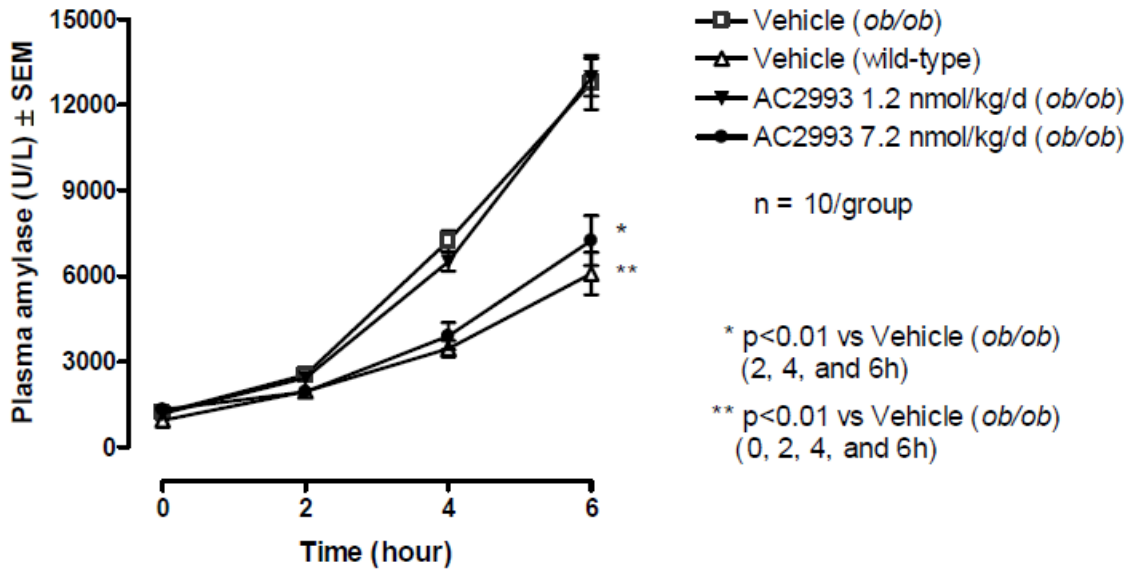
Effects of AC2993 on plasma HbA1c over 4 weeks



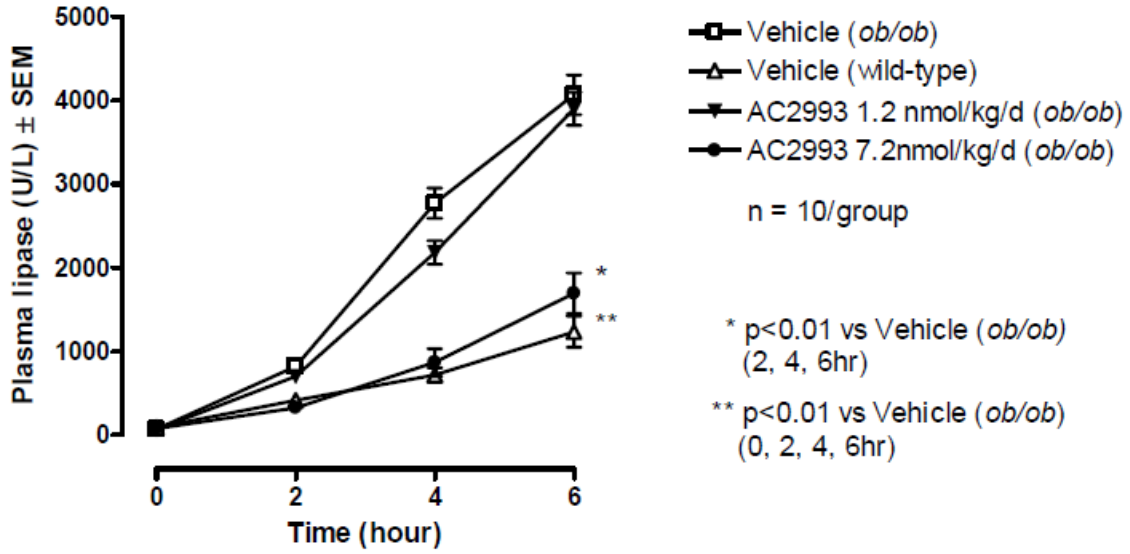
Effects of AC2993 on plasma glucose over 4 weeks



Effects of caerulein on plasma amylase over 4 weeks



Effects of caerulein on plasma lipase over 4 weeks



Effects of caerulein on plasma glucose over 4 weeks

Figure 10: Plasma glucose in response to caerulein.

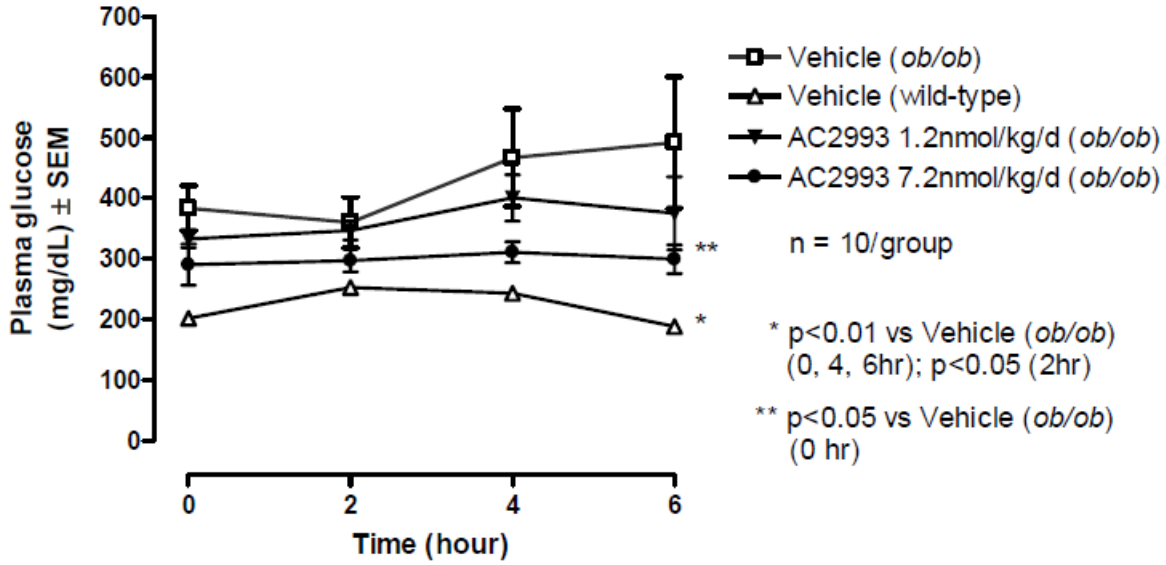


Figure 12: Pancreatic weight normalized to body weight.

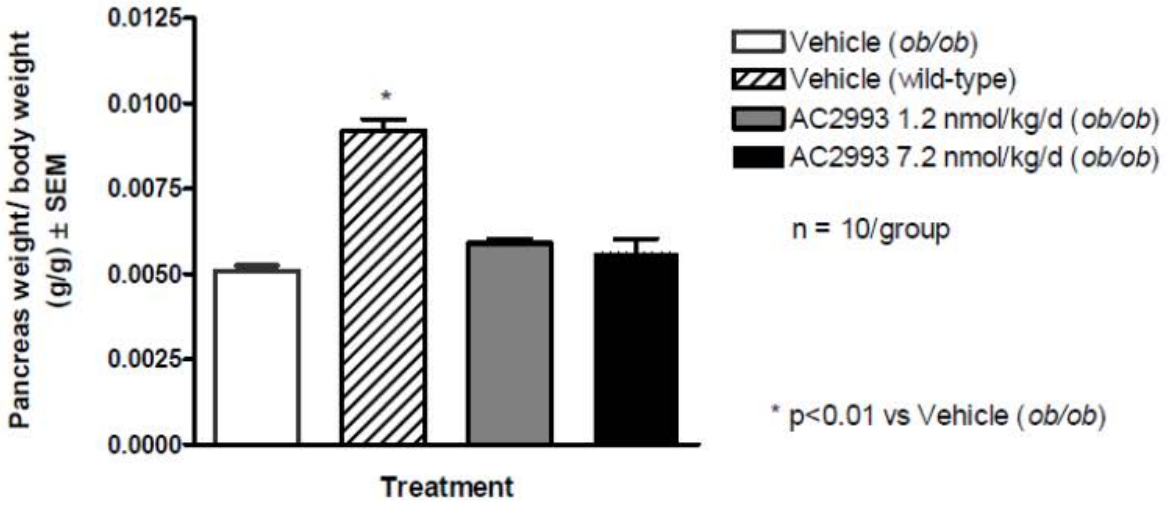
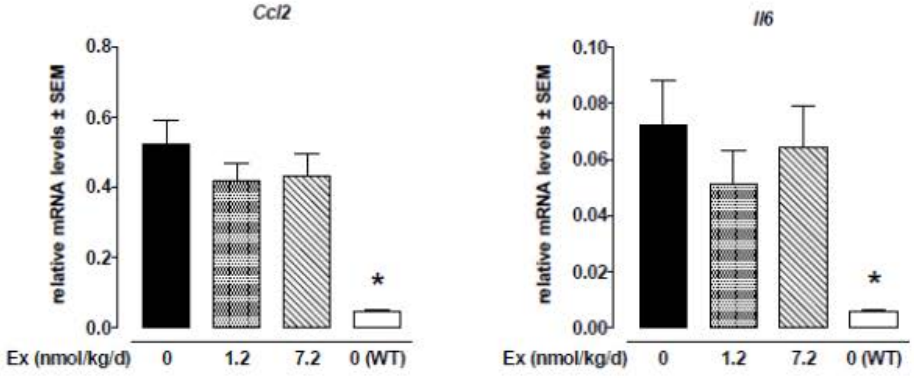


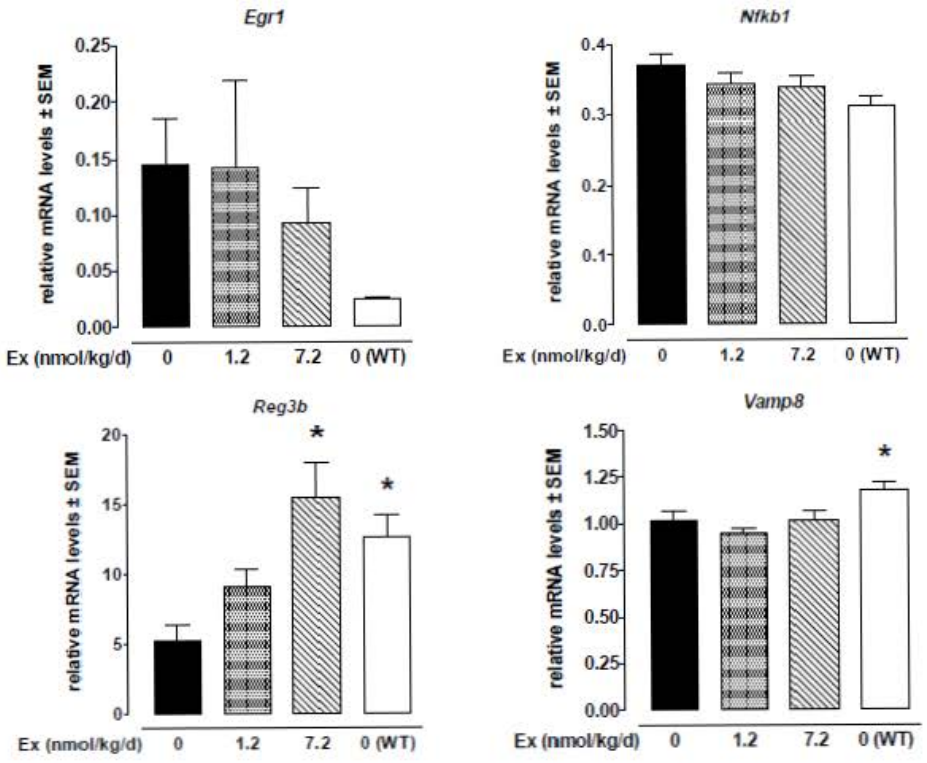
Table 1: Effects of 4-week pretreatment with exenatide on plasma cytokines at 6h post CRN-induced acute pancreatitis in diabetic *ob/ob* mice.

Group	Exenatide Dose (nmol/kg/d)	CRN insult	IL-1β (pg/ml)	IL-2 (pg/ml)	IL-6 (pg/ml)	IFN-γ (pg/ml)	MCP-1 (pg/ml)	TNF-α (pg/ml)
4-week continuous infusion								
WT	0	Yes	9.6 ± 0.4	6.6 ± 0.1 [a]	23.8 ± 6.7 [a]	8.1 ± 0.2	42.1 ± 5.1 [a]	6.2 ± 0.2 [a]
ob/ob	0	Yes	11.9 ± 1.0	8.3 ± 0.7	5910 ± 1642	8.8 ± 0.6	779 ± 205	12 ± 1.5
ob/ob	1.2	Yes	9.7 ± 0.6	7.2 ± 0.2	3607 ± 489	7.5 ± 0.4	421 ± 63	8.1 ± 0.4 [a]
ob/ob	7.2	Yes	12.1 ± 2.1	7.3 ± 0.3	5877 ± 1507	7.4 ± 0.3	541 ± 143	10.1 ± 1.3

n=8-10; [a] p<0.05 vs. vehicle (*ob/ob*).
Log_{10}-transformed data were used for ANOVA analysis followed with Bonferroni's test.

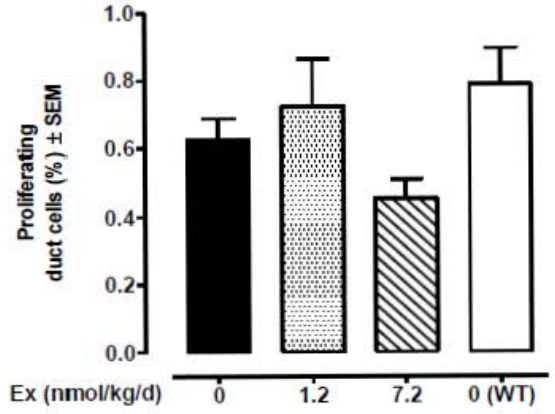
Figure 2: Effects of 4-week pretreatment with exenatide on gene expression at 6h post CRN-induced acute pancreatitis in diabetic *ob/ob* mice.





n=8-10; * p<0.05 vs. vehicle (*ob/ob*).

Figure 3: Effects of 4-week pretreatment with exenatide on duct cell proliferation at 6h post CRN-induced acute pancreatitis in diabetic *ob/ob* mice.



Group	1											2										
Strain	ob/ob											ob/ob										
Treatment	Vehicle											AC2993										
Dose	0 nmol/kg/d											1.2 nmol/kg/d										
Animal Number	101	102	103	104	105	106	107	108	109	110	INC	111	112	113	114	115	116	117	118	119	120	INC
Pancreas											0											0
Acute Inflammation, Exocrine	2	2	2	2	2	2	2	2	2	2	10	2	2	2	2	2	2	2	2	2	2	10
Vacuolation, Acinar Cell	2>	2>	2>	2>	2>	2>	2>	2>	2>	2>	10	2>	2>	2>	2>	2>	2>	2>	2>	2>	2>	10
Single Cell Necrosis, Acinar Cell	1>	1>	1>	1>	1>	1>	1>	1>	1>	1>	10	1>	1>	1>	1>	1>	1>	1>	1>	1>	1>	10
Acute Inflammation, Fat	2>	2>	2>	2>	2>	3)	3)	3>		4)	9		4)	3>	3>	1)			3>	2>	1>	7
Granulomatous Inflammation, Fat											0						2)	3)				2
Necrosis, Fat	2>	2>			2)		2>	2>		3)	6		3)	2>	2>	1)			2>	1)	1>	7
Single Cell Degeneration, Islet	1>	1>	1>	1>	1>	1>	1>	1>	1>	1>	10	1>	1>	1>	1>	1>	1>	1>	1>	1>	1>	10
Vascular Ectasia, Islet											0	3)				2)	2)			3>		4
Acute Inflammation, Vascular											0				2)						2)	2
Ductal Proliferation											0							3)		2)		2
Chronic Inflammation, Periductal											0							3)[A]				1
Atrophy, Lobule						2				2	2		2		2							2

A=With macrophage hemosiderin pigmentation

Group	3											4										
Strain	ob/ob											WT Lean Control										
Treatment	AC2993											Vehicle										
Dose	7.2 nmol/kg/d											0 nmol/kg/d										
Animal Number	121	122	123	124	125	126	127	128	129	130	INC	131	132	133	134	135	136	137	138	139	140	INC
Pancreas											0											0
Acute Inflammation, Exocrine	1>	2>	1>	2>	2>	1>	2>	2>	2>	2>	10	2>	1>	1>	1>	1>	1>	1>	1>	1>	1>	10
Vacuolation, Acinar Cell	1>	1>	1>	1>	1>	1>	2>	1>	1>	1>	10											0
Single Cell Necrosis, Acinar Cell					1>						1	1>	1>	1>	1>	1>	1>	1>	1>	1>	1>	10
Acute Inflammation, Fat		2>	2>	4>			3>	3)	3>	2)	7					2>[B]				2>[B]		2
Granulomatous Inflammation, Fat											0											0
Necrosis, Fat				2)		1)	2>	2)	1>	1)	6											0
Single Cell Degeneration, Islet	1>										1											0
Vascular Ectasia, Islet								3>		2)	2											0
Acute Inflammation, Vascular											0											0
Ductal Proliferation			2)								1			2)								1
Chronic Inflammation, Periductal			2)				1>				2			2)								1
Atrophy, Lobule									2		1											0

B=Involves peripancreatic fat

Appendix 1
Abbreviations and Glossary

N	Normal
INC	Incidence
)	Focal
>	Multifocal
1	Minimal
2	Mild
3	Moderate
4	Marked

Conclusion: Exenatide had no effect on baseline (the 4 weeks prior to caerulein treatment) amylase or lipase levels, nor did it potentiate the effect of caerulein on these parameters. Indeed, HD (but not LD) exenatide markedly blunted the caerulein-induced increases in pancreatic enzymes in the ob/ob mice; reducing the enzymes to levels comparable to those seen in the WT mice. Exenatide reduced blood glucose and HbA1c. Exenatide had no effect on pancreas weight. Exenatide did not potentate the

effects of caerulein on cytokine levels or mRNA levels; indeed, exenatide appeared to moderate the caerulein-induced reduction in Reg3b mRNA expression. Exenatide had no effect on the proliferation index of pancreatic ductal cells.

The histopathology findings are somewhat more complex to interpret. For multiple presumably caerulein-induced findings, HD exenatide was protective, while LD exenatide had no effect. The findings that showed improvement at HD were:

acute inflammation, exocrine (↓ severity)
vacuolation, acinar cell (↓ severity)
single cell necrosis, acinar cell (↓ incidence)
acute inflammation, fat (↓ incidence)
single cell degeneration, islet (↓ incidence)

However, other findings showed an increased incidence/severity, particularly at LD:

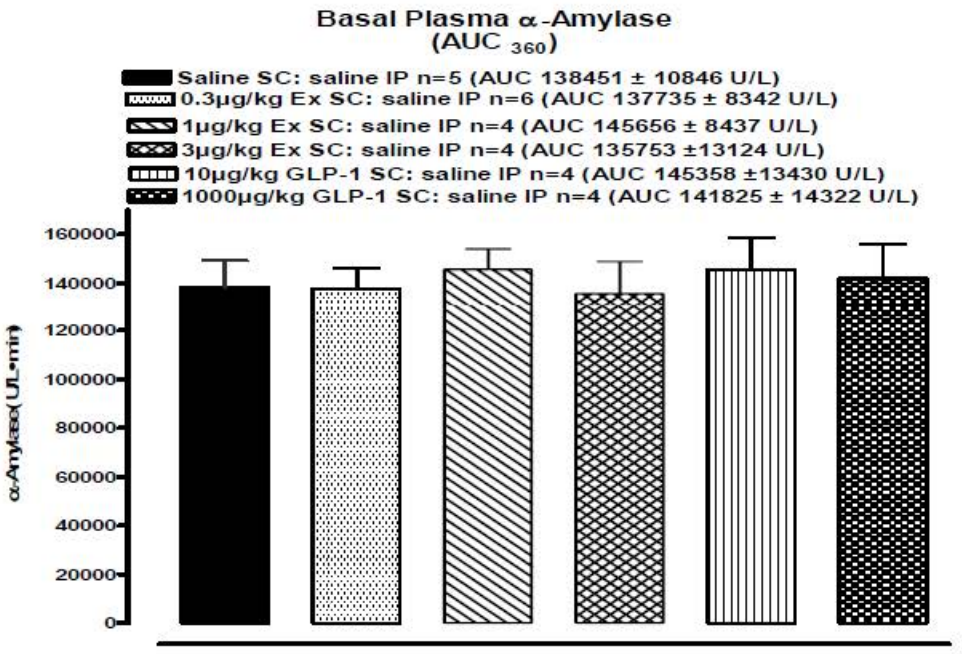
granulomatous inflammation, fat
vascular ectasia, islet
acute inflammation, vascular

Title: Effect of Exenatide on Basal, CCK-8-stimulated and Caeruleinhyperstimulated Pancreatic Enzyme Secretion in Normal Sprague Dawley (SD) Rats.

Study no.: REST090012/REST090021

Study design (*NB – non-GLP)*: Fasted, male, anesthetized SD rats were injected subcutaneously with saline or exenatide (0.3, 1 or 3µg/kg) 15 minutes before saline (100µl), CCK-8 (10µg/kg) or caerulein (10µg/kg) IP administration. In other groups of animals GLP-1 (10, 1000µg/kg) SC injections were administered two minutes prior to saline or caerulein IP injections. Plasma α-amylase and lipase concentrations were measured for up to 6 hours after IP injections (t= -15, 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min). Pancreata were collected, weighed and analyzed histologically.

Results:


Basal Plasma α-Amylase
(AUC 360)
Saline SC: saline IP n=5 (AUC 138451 ± 10846 U/L)
0.3µg/kg Ex SC: saline IP n=6 (AUC 137735 ± 8342 U/L)
1µg/kg Ex SC: saline IP n=4 (AUC 145656 ± 8437 U/L)
3µg/kg Ex SC: saline IP n=4 (AUC 135753 ±13124 U/L)
10µg/kg GLP-1 SC: saline IP n=4 (AUC 145358 ±13430 U/L)
1000µg/kg GLP-1 SC: saline IP n=4 (AUC 141825 ± 14322 U/L)
α-Amylase(U/L*min)
160000
140000
120000
100000
80000
60000
40000
20000
0

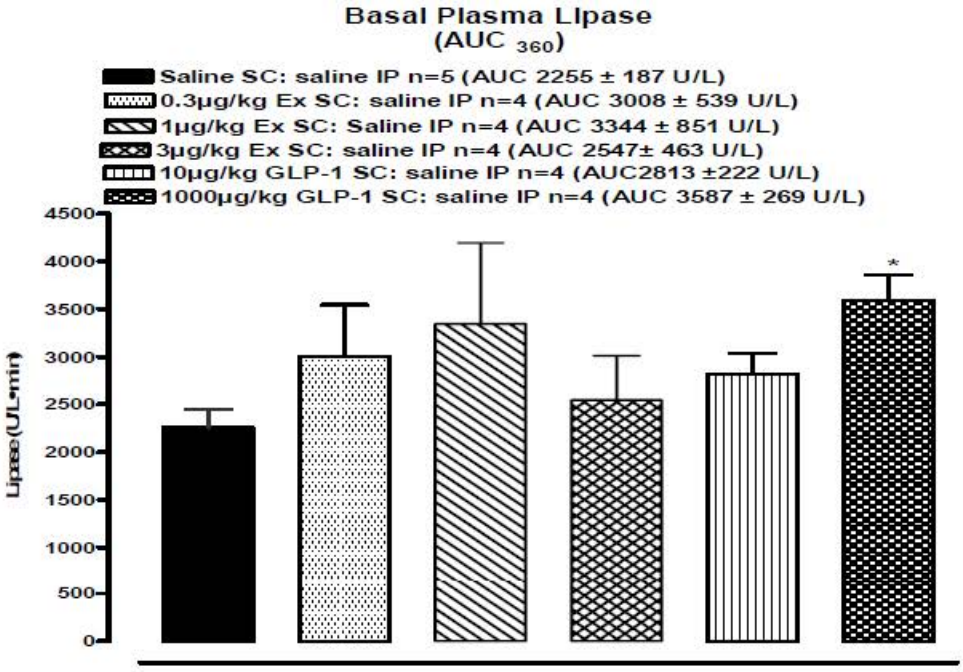

Basal Plasma Lipase
(AUC 360)
Saline SC: saline IP n=5 (AUC 2255 ± 187 U/L)
0.3µg/kg Ex SC: saline IP n=4 (AUC 3008 ± 539 U/L)
1µg/kg Ex SC: Saline IP n=4 (AUC 3344 ± 851 U/L)
3µg/kg Ex SC: saline IP n=4 (AUC 2547± 463 U/L)
10µg/kg GLP-1 SC: saline IP n=4 (AUC2813 ±222 U/L)
1000µg/kg GLP-1 SC: saline IP n=4 (AUC 3587 ± 269 U/L)
Lipase(U/L*min)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
*

Figure 3: Pancreas weight 6h after IP injections with no stimulation.

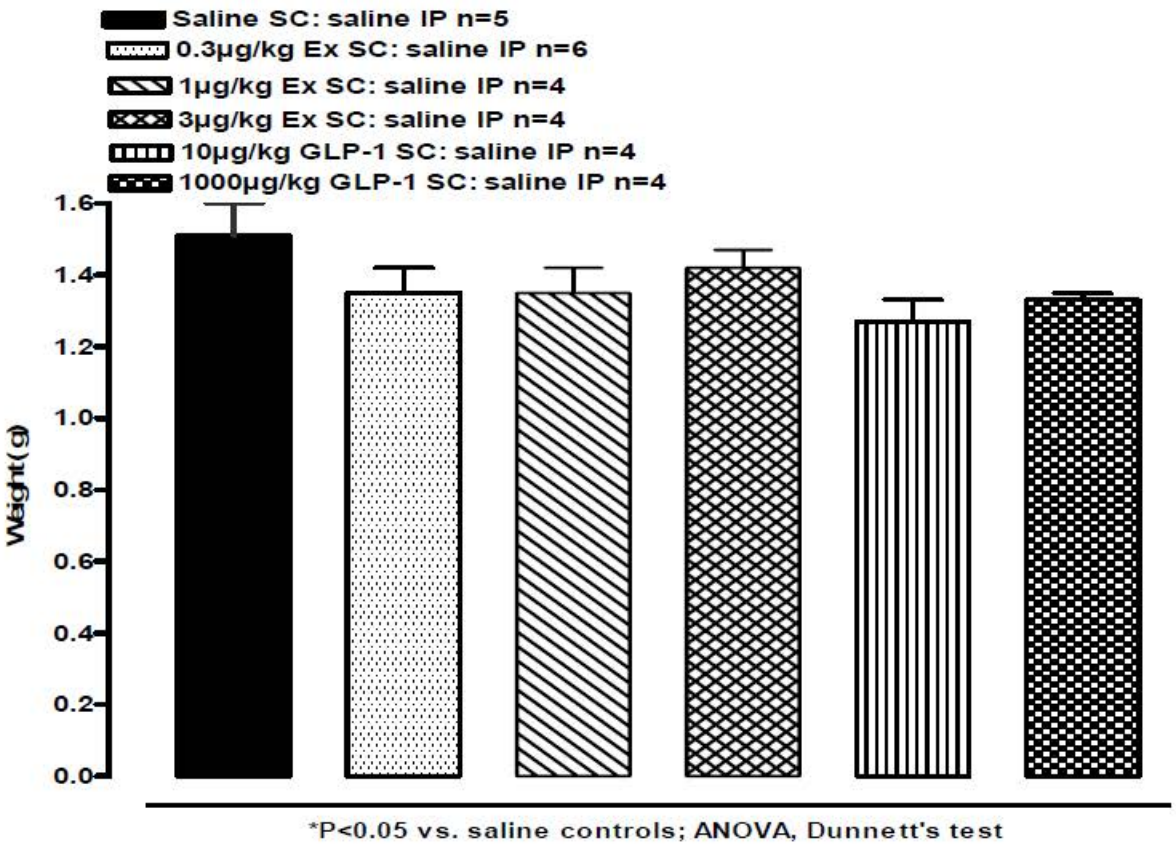
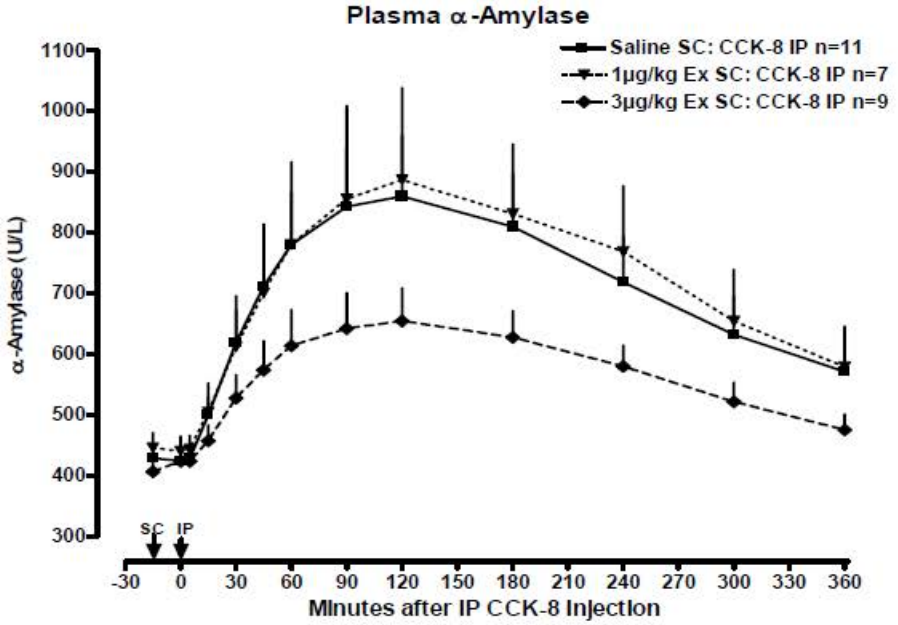


Figure 4: Plasma α-amylase and lipase concentrations over 6 hours after CCK-8 stimulation.



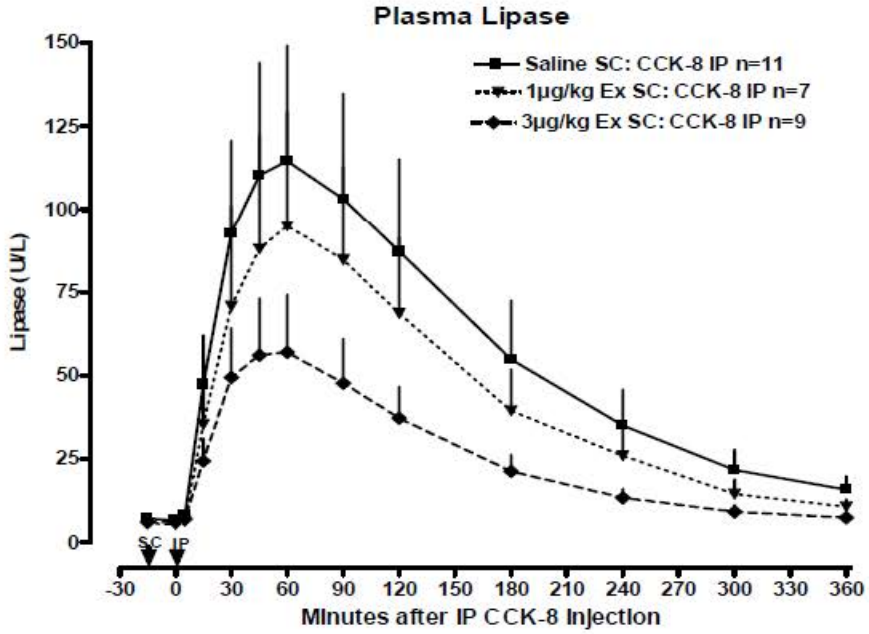


Figure 6: Pancreas Weight 6h after CCK-8 stimulation.

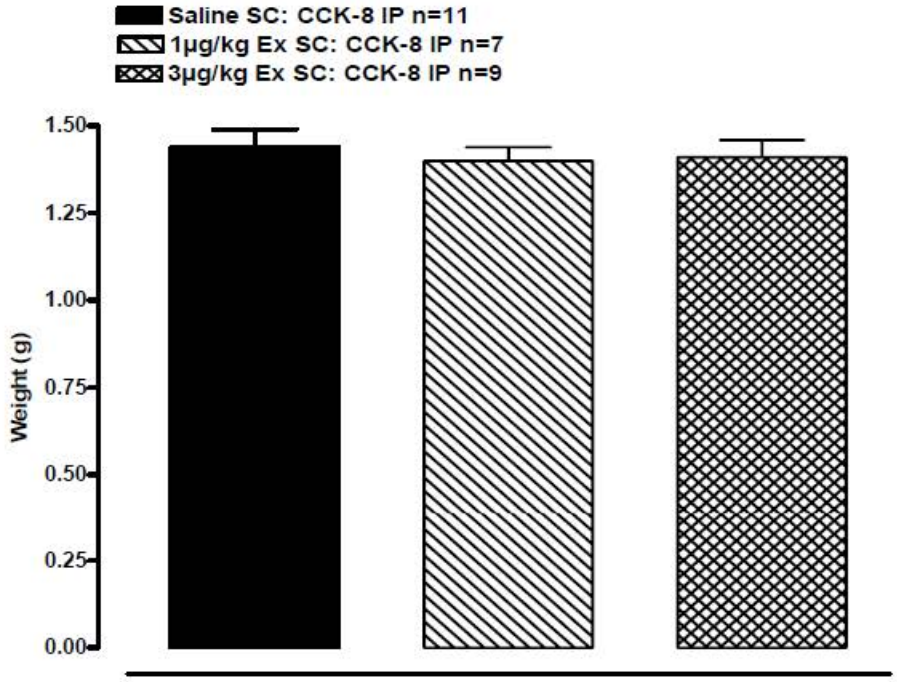
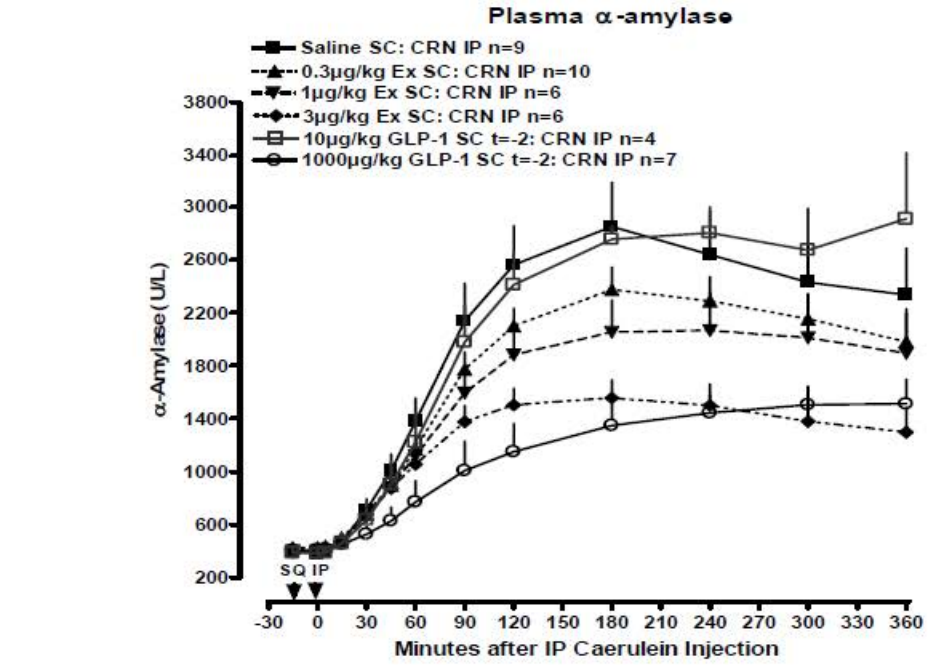


Figure 7: Plasma α-amylase and lipase concentrations over 6 hours after caerulein-hyperstimulation.



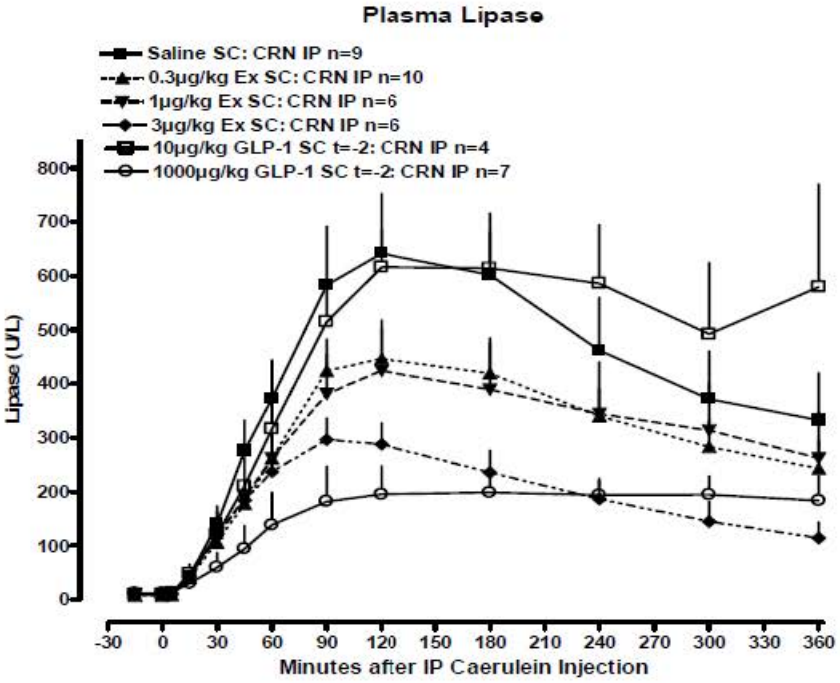
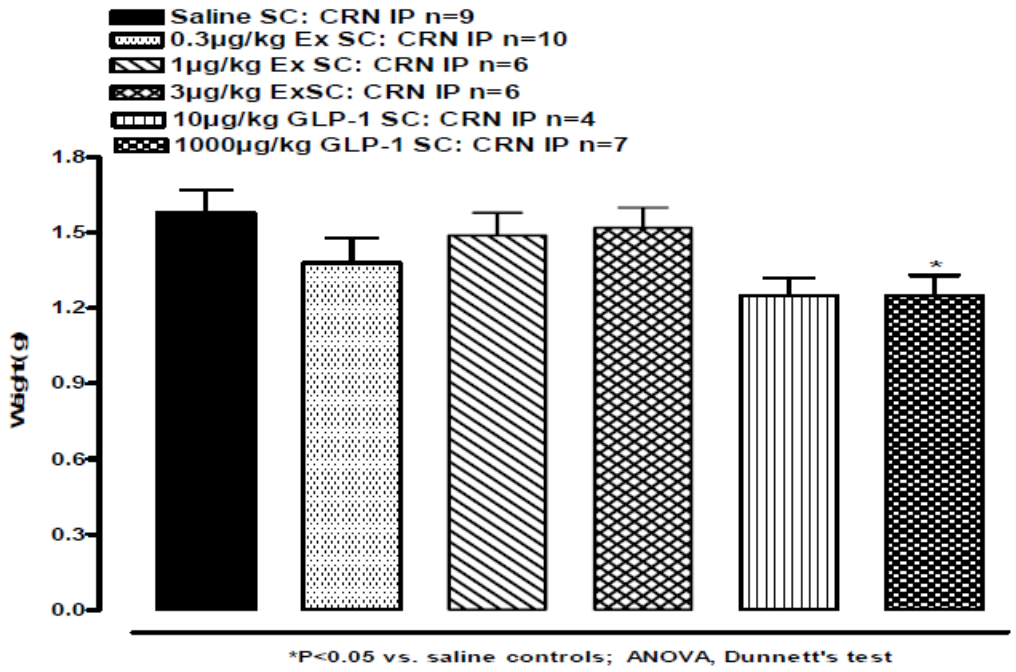


Figure 9: Pancreas weight 6h after caerulein-hyperstimulation.



	Group 1: Basal																						
Subcutaneous injection at t=-15	Saline					3 µg/kg Exendin				1 µg/kg Exendin				0.3 µg/kg Exendin					1 mg/kg GLP-1			
Intraperitoneal injection at t=0	Saline					Saline				Saline				Saline					Saline			
Animal Number	9	10	39	40	49	17	18	41	42	27	28	56	57	82	83	85	131	132	190	191	192	193
Pancreas		N	N	N		N	N		N		N	N	N	N	N	N	N			N	N	N
Hyperplasia, Exocrine Acinar Cell					1)													1)				
Periductal Lymphocytic Inflammation								1)														
Chronic Inflammation	1>									1)												
Lobular Atrophy	1																					
Ductular Obstruction/Proliferation																			1)			

	Group 2: CCK-8																								
Subcutaneous injection at t=-15	Saline											3 µg/kg Exendin								1 µg/kg Exendin					
Intraperitoneal injection at t=0	3.5 µg CCK-8											3.5 µg CCK-8								3.5 µg CCK-8					
Animal Number	13	14	43	44	45	50	130	150	151	152	153	23	24	25	26	46	47	48	161	162	51	52	53	54	55	166	167
Pancreas		N		N	N	N		N	N	N	N	N	N			N	N	N	N	N		N	N	N	N		N
Lobular Atrophy	1						1							1	1											1	
Hyperplasia, Ductal Epithelium																					3)						
Thrombus, Arteriole			1)																								

	Group 3: Caerulein																														
Subcutaneous injection at t=-15	Saline									3 µg/kg Exendin						1 µg/kg Exendin						0.3 µg/kg Exendin								1 mg/kg GLP-1						
Intraperitoneal injection at t=0	3.5 µg Caerulein									3.5 µg Caerulein						3.5 µg Caerulein						3.5 µg Caerulein								3.5 µg Caerulein						
Animal Number	62	63	101	102	104	105	123	124	125	116	117	118	119	120	122	111	112	113	114	115	121	64	65	66	67	86	87	89	106	107	108	195	196	197	198	199	200	201
Pancreas					N	N		N				N	N			N			N					N			N	N						N		N	N
Acinar Cell Vacuolation			1>														1>	1>			1>	1>			1>	1>						1>	1>			1>		
Acinar Single Cell Necrosis	1]	1>	2>	1>			1>		1]	1>	1>			1>	1>					1>					1>				1>	1>	1>	1>	1>	1>		1>		
Periductal Lymphocytic Infiltration																	1																					
Chronic Inflammation				2)																																		
Lobular Atrophy																				1			1															

Conclusion: Exenatide had no significant effect on basal (unstimulated) amylase and lipase activity. After stimulation with CCK-8 at 10 µg/kg, exenatide at 3µg/kg non-significantly reduced both plasma amylase and lipase activity. Exenatide at 3µg/kg significantly reduced plasma amylase and lipase increases after IP caerulein at 10 µg/kg. Exenatide had no effect on basal, CCK-8-stimulated or caerulein-stimulated pancreas weight. No exenatide-related histopathological findings were observed in the

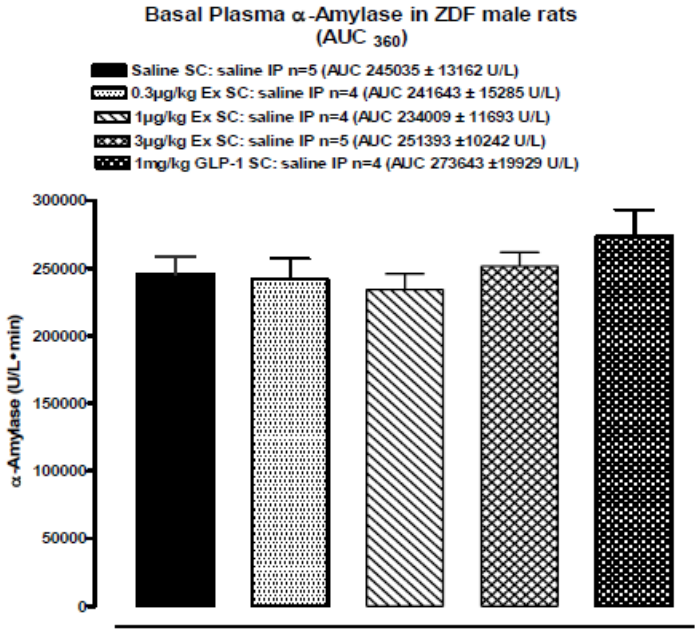
pancreas after injection of exenatide up to 3µg/kg followed by IP administration of saline, CCK-8 at 3.5 µg/kg, or 3.5µg of caerulein.

Title: Effect of Exenatide on Basal and Caerulein-hyperstimulated Pancreatic Enzyme Secretion in Diabetic Fatty Zucker (ZDF) Rats.

Study no.: REST090182/REST090046

Study design (*NB – non-GLP)*: Fasted, male, anesthetized type 2 diabetic Fatty Zucker rats were injected subcutaneously with saline or exenatide (0.3, 1 or 3µg/kg) 15 minutes before saline (100µl) or caerulein (10µg/kg) IP administration. In other groups of animals GLP-1 (10, 1000µg/kg) SC injections were administered two minutes prior to saline or caerulein IP injections. Plasma α-amylase and lipase concentrations were measured for up to 6 hours after IP injections (t= -15, 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min). Pancreata were collected, weighed and analyzed histologically.

Results:



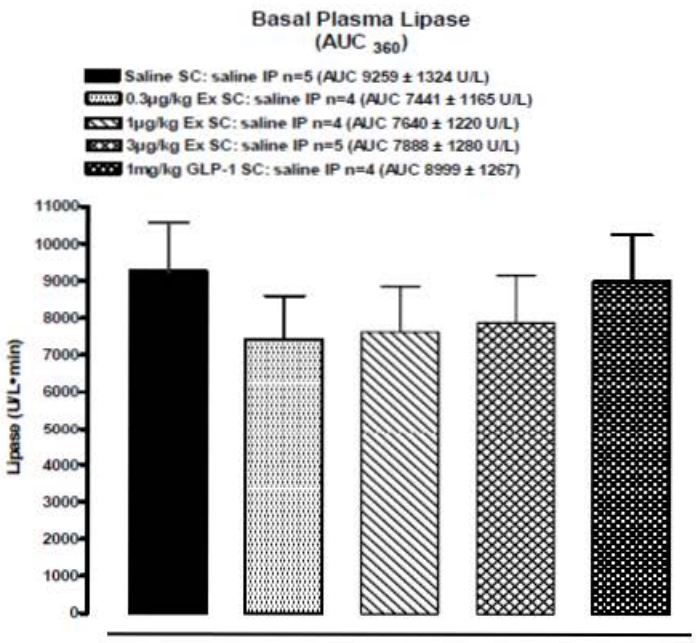


Figure 3: Pancreas weight 6h after IP injections with no stimulation.

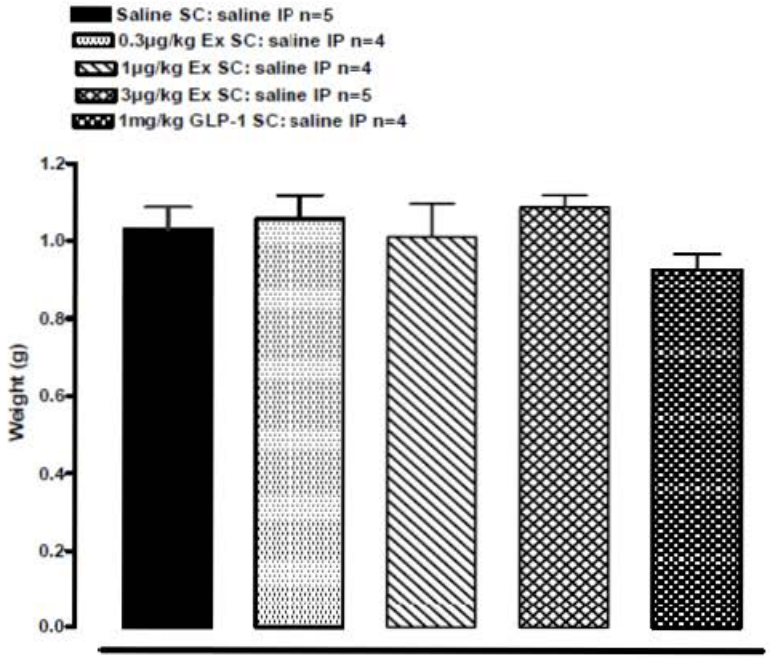


Figure 5: Trapezoidal area under the concentration time curve for 360 minutes following caerulein-hyperstimulation.

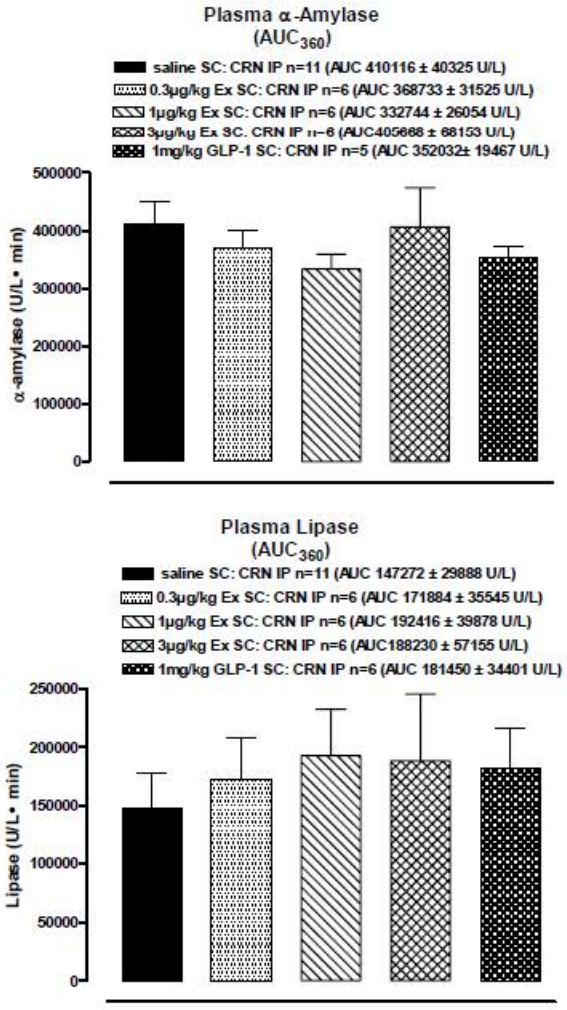
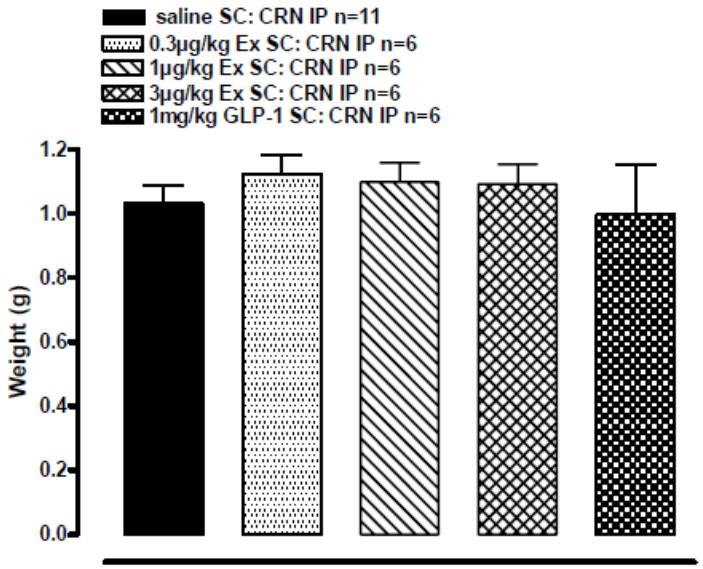


Figure 6: Pancreas Weight 6h after caerulein-hyperstimulation.



	Group 1: Basal																				
Subcutaneous injection at t=-15	Saline					0.3 µg/kg Exendin				1 µg/kg Exendin				3 µg/kg Exendin					1 mg/kg GLP-1			
Intraperitoneal injection at t=0	Saline					Saline				Saline				Saline					Saline			
Animal Number	1	2	3	4	5	62	63	64	65	41	42	43	44	21	22	23	24	25	81	82	83	84
Pancreas																						
Exocrine Necrotizing Inflammation	1>																					
Periductal Mononuclear Cell Infiltration	1>			1)	1)			1)	1>	1)					2)	1>	1)		2)	1)		
Macrophage Pigmentation					1)			1)								1>	1>				1)	
Perivascular Mixed Inflammation															1)							
Increased Islet Cell Apoptosis														1>	1>							
Islet Cell Hypertrophy					1>		1>						1>	1>			1>		1>			
Increased Islet Cell Vacuolation		1>		1>	1>		1>						1>		1>	1>	1>	1>	1>	1>		1>
Increased Islet Stroma	2>	2>	1>	2>	2>	2>	2>	2>	2>	1>	2>	2>	2>	1>	1>	1>	1>	2>	1>	2>	2>	2>
Periductal Fibrosis												1>		1>							1)	
Ductal Cell Proliferation					1)							1>									1)	

	Group 2: Caerulein																														
Subcutaneous injection at t=-15	Saline											0.3 µg/kg Exendin						1 µg/kg Exendin						3 µg/kg Exendin						1 mg/kg GLP-1					
Intraperitoneal injection at t=0	3.5 µg Caerulein											3.5 µg Caerulein						3.5 µg Caerulein						3.5 µg Caerulein						3.5 µg Caerulein					
Animal Number	11	12	13	14	15	16	17	18	19	20	101	72	73	74	75	76	77	51	52	55	56	57	58	35	36	37	38	39	40	91	92	93	94	95	96
Pancreas									N[B]																										
Periductal Mononuclear Cell Infiltration			1)		1>					1)			1>	1>	1>			1)		1)		1>		1)				1>			1)				
Macrophage Pigmentation				1)		1>				1)		1>	1>	1>	1>			1)	1>			1>	1>												
Periductal Fibrosis				1)				2)		2)								2)				1)			2)										
Peri-islet Chronic Inflammation				2)																															
Granulomatous Inflammation, Peripancreatic Fat	2)[A]																																		
Islet Cell Hypertrophy		1>						1>			1>				1>			1>						1>							1>				1>
Increased Islet Cell Vacuolation	1>		1>		1>	1>	1>	1>		1>	1>	1>	1>	1>	1>	1>	1>		1>	1>		1>	1>	1>	1>	1>	1>	1>	1>	1>		1>	1>	1>
Increased Islet Stroma	2>	2>	2>	2>	1>	2>	1>	2>		2>	2>	2>	2>	2>	2>	2>	2>	2>	2>	2>	2>	2>	2>	2>	2>	1>	2>	2>	2>	2>	2>	2>	2>	2>	2>
Islet Vascular Ectasia	2>																										1)				1)			1>	
Islet Cell Vacuolar Degeneration		2)																																	

A=With mineralization B=Few lobules present

Conclusion: Exenatide pre-treatment had no effect on basal (unstimulated) or caerulein-induced amylase and lipase activity; no effect on pancreas weight after caerulein insult; and no effect on exenatide-related histopathological findings in the pancreas either without or with caerulein hyperstimulation.

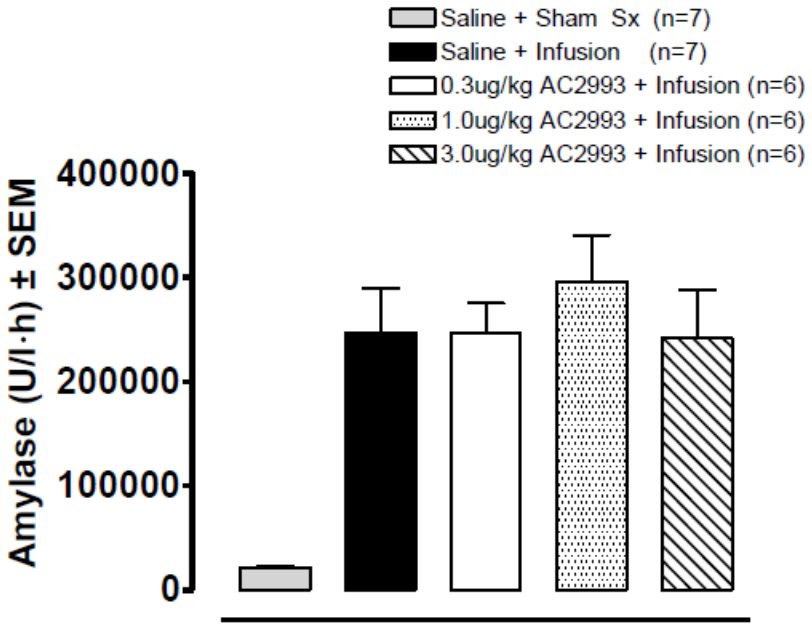
Title: Exenatide Does Not Affect Pancreatic Exocrine Secretion in a Sodium Taurocholate Model of Acute Pancreatitis

Study no.: REST100052/REST100085

Study design (*NB—non-GLP*): The purpose of this study was to characterize the effect of exenatide on pancreatic enzyme secretion in the sodium taurocholate (ST)-induced model of severe necrotizing acute pancreatitis (AP) in male Wistar rats. Fasted rats were administered saline or exenatide at 0.3, 1 or 3 µg/kg subcutaneously 15 min prior to ST-infusion surgery. Transduodenal cannulation of the biliopancreatic duct was followed by retrograde infusion of 5% ST at a rate of 0.05 mL/min and a total volume of 1mL/kg body weight. Sham surgery consisted of laparotomy, duodenal incision and closure. Exenatide was administered 12 h following recovery and continued as twice daily injections through 48 hours. At 3, 6, 24 and 48 hours following ST infusion, plasma was collected for amylase and lipase. Pancreata were weighed and processed for histology.

Results:

Figure 2: Area under the curve ($AUC_{0\text{-}48\,h}$) for amylase and lipase plasma concentrations



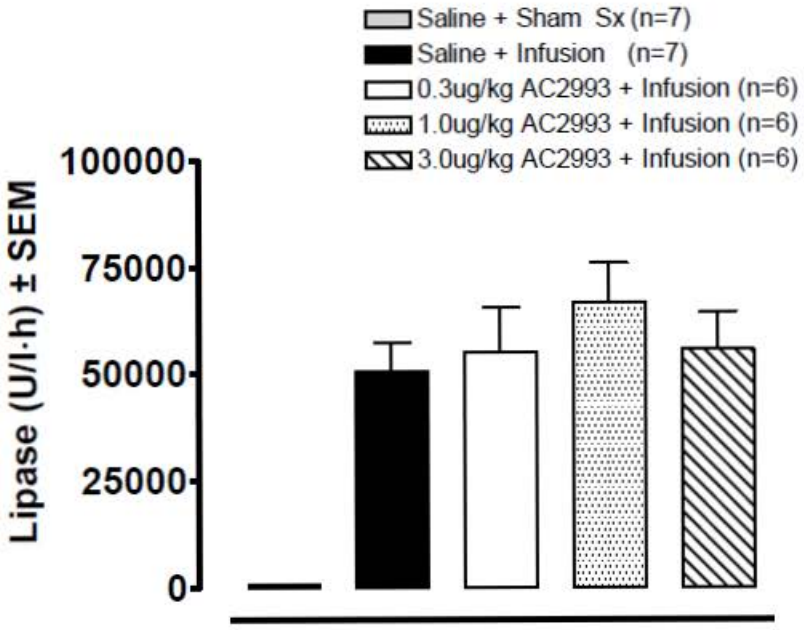


Figure 3: Wet pancreatic weight at 48 hours

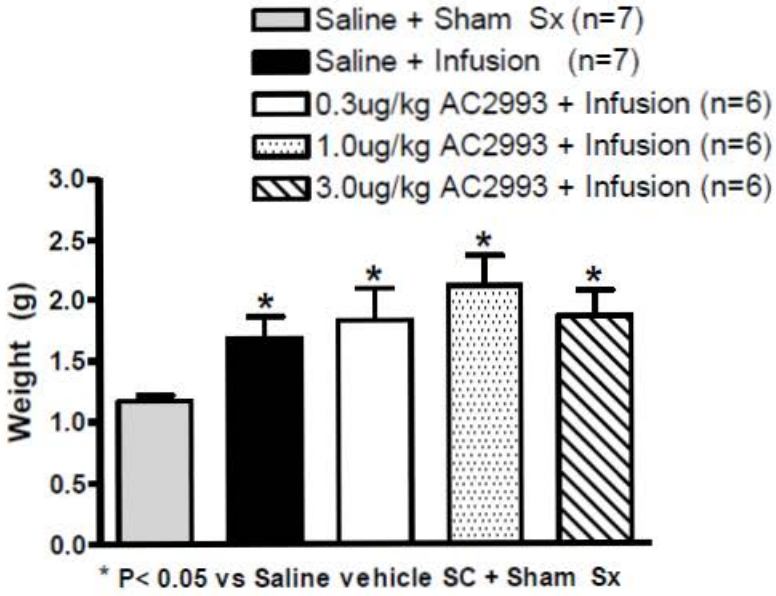


Table 1: Effects of twice daily injections of exenatide on percentage incidence and mean severity of morphological changes in pancreas of Wistar rats at 48 h post ST-induced pancreatitis

			Exenatide dose (nmol/kg)							
	Sham/Vehicle (*n*=7)		Vehicle (*n*=7)		0.072 (*n*=6)		0.24 (*n*=6)		0.72 (*n*=6)	
Microscopic Finding	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity
Infiltration, mixed-cell, interstitium	71	1.6	100	2.7	100	2.7	100	3.0	100	2.5
Vacuolation, acinar cell,	43	1.7	100	2.9	100	2.7	100	2.7	100	2.5
Edema, interstitium	14	1.0	100	3.1	100	3.3	100	3.2	100	2.3
Necrosis, adipose tissue, interstitium	0		100	3.4	100	3.5	100	3.8	100	2.7
Hemorrhage, interstitium	0		29	1.5	50	1.7	33	1.5	33	1.5
Dilation, ducts	0		86	2.3	100	2.2	100	3.0	100	2.8
Necrosis, acinar tissue	0		86	2.5	83	3.0	100	2.7	83	2.6
Necrosis, single-cell, acinar cell	0		57	1.3	83	1.2	33	1.5	50	1.3

Conclusion: Exenatide did not affect activity of amylase and lipase or pancreatic weight in the sodium taurocholate (ST)-induced severe acute pancreatitis model. Sponsor concludes that exenatide at 3µg/kg (0.72 nmol/kg) decreased the incidence and severity of major degenerative and inflammatory changes (mixed-cell infiltration, degeneration of acinar cells, edema, and necrosis of adipose tissue) in the pancreas induced by ST insult; however, to this reviewer, the picture is more subtle, with slightly increased severity in some ST-induced findings at 1.0 µg/kg (0.24 nmol/kg) exenatide, and slight improvement at 3 µg/kg. The overall picture rather suggests a neutral effect of exenatide in this model.

Title: Potential Effects of Twice-daily (BID) Subcutaneous Injection of Exenatide (AC2993) for 3 Months on Pancreatic Exocrine Structure and Function in Obese Diabetic (ZDF) Rats

Study no.: REST100021

NB – this GLP study was previously reviewed by Dr. Hummer under NDA 21773 (DARRTS 7-19-2011) for Byetta. This is a brief summary of his review.

Study design: Male Zucker Dibetic Fatty (ZDF) rats were treated with exenatide SC injection at nominal daily doses of 6, 40 & 250 µg/kg (half this amount BID) for 3 months. At various timepoints during the study and at terminal sacrifice, blood was collected for assessment of HbA1c, insulin, glucose, amylase, lipase and plasma drug levels. A focused histopathological assessment was made of the pancreas. In addition to standard H&E staining, tissue samples were analyzed by immunohistochemistry and histomorphometry, as below.

Text Table 3 Study Design

Group Number / Treatment	Nominal Dose Level* (BID SC Injections)		Nominal Dose Conc.	Dose Volume	Number of Animals (Males)		
	(µg/kg/dose)	(µg/kg/day)	(µg/mL)	(mL/kg/dose)	Main[a]	Rec[b]	TK[c]
1/ Vehicle	0	0	0	0.5	15	8	3
2/ AC2993	3	6	10	0.3	15	8	9
3/ AC2993	20	40	40	0.5	15	8	9
4/ AC2993	125	250	250	0.5	15	8	9

BID = twice daily; Conc. = concentration; Rec = recovery; SC = subcutaneous; TK = Toxicokinetics.

* Actual Group 2 dose levels ranged from 3.2 to 5.0 µg/kg/day, while actual Group 3 dose levels ranged from 33.5 to 37.4 µg/kg/day; these were determined based on the measured predose concentration results obtained (see Section 7.1 for more details).

a Surviving animals were euthanized and had a necropsy performed on the day following the last dose (Day 92).

b Surviving animals were euthanized and had a necropsy performed 4 weeks following the last dose (Day 120).

c Toxicokinetic animals were used for toxicokinetic evaluation only and surviving TK animals were euthanized following their last blood collection occasion.

Measured Histomorphometry Parameters	
Parameter	*Direct Measure*
Ki-67 Cell Count	Total Ki-67 positive duct cells (Cytokeratin-20 positive cells when the cell nucleus was present in the section & Ki-67)
Total Duct Cell Count	Total number of duct cells (1000-2000) (Cytokeratin-20 positive cells when the cell nucleus was present in the section)
Derived Histomorphometry Parameters	
Parameter	*Calculation*
Percent Proliferation	Ki-67 Cell Count / Total Duct Cell Count * 100

Measured Histomorphometry Parameters	
Parameter	*Direct Measure*
TUNEL Cell Count	Total TUNEL positive duct cells (Cytokeratin-20 positive cells when the cell nucleus was present in the section & TUNEL)
Total Duct Cell Count	Total number of duct cells (1000-2000) (Cytokeratin-20 positive cells when the cell nucleus was present in the section)
Derived Histomorphometry Parameters	
Parameter	*Calculation*
Percent Apoptosis	TUNEL Cell Count / Total Duct Cell Count * 100

Selected Results:

Percentage differences of Amylase values from Group 1 are presented in the table below:

Group	Dose Level	Day 30	Day 60	Day 90	End of recovery
1 - Control	0 µg/kg/day	-	-	-	-
2 - AC2993	6 µg/kg/day	+25.2%[a]	+29.2%[a]	+20.7%[a]	-2.5%
3 - AC2993	40 µg/kg/day	+20.7%[a]	+32.8%[a]	+30.0%[a]	-0.52%
4 - AC2993	250 µg/kg/day	+24.4%[a]	+32.6%[a]	+21.4%[a]	-3.9%

-: not compared

[a] Statistically significantly different ($p \leq 0.01$) from control group (Group 1) value at same timepoint

Percentage differences of lipase values from Group 1 are presented in the table below:

Group	Dose Level	Day 30	Day 60	Day 90	End of recovery
1 - Control	0 µg/kg/day	-	-	-	-
2 - AC2993	6 µg/kg/day	-0.71%	-7.8%[a]	+2.1%	-6.0%
3 - AC2993	40 µg/kg/day	-2.9%	-11.0%[a]	+0.70%	-10.0%[a]
4 - AC2993	250 µg/kg/day	-2.1%	-13.6%[a]	-7.0%[a]	-13.3%[a]

-: not compared

[a] Statistically significantly different ($p \leq 0.01$ or $p \leq 0.05$) from control group (Group 1) value at same timepoint

Table 11 Summary of Microscopic Gradings by Organ/Group/Sex

Treatment Period

MALE

DOSE GROUP		1	2	3	4
NUMBER OF ANIMALS EXAMINED		16	15	15	15
PANCREAS	EXAMIN:	16	15	15	15
	N.A.D.	11	6	2	2
- Hypertrophy: islet		-	7	12	13
	Grade 1	-	6	11	10
	Grade 2	-	1	1	2
	Grade 3	-	-	-	1
- Hyperplasia acinar cell: focal		1	1	2	1
	Grade 1	-	1	1	1
	Grade 2	1	-	1	-
- Intraluminal concretion: duct		2	3	4	5
	Grade 1	1	2	4	4
	Grade 2	-	1	-	1
	Grade 3	1	-	-	-
- Inflammation: subacute		-	-	-	1
	Grade 2	-	-	-	1

- Inflammation: acute		3	1	-	-
	Grade 1	1	1	-	-
	Grade 2	1	-	-	-
	Grade 3	1	-	-	-

Table 11 Summary of Microscopic Gradings by Organ/Group/Sex

Recovery Period

MALE

DOSE GROUP		1	2	3	4
NUMBER OF ANIMALS EXAMINED		7	8	8	8
PANCREAS	EXAMIN:	7	8	8	8
	N.A.D.	5	3	1	-
- Hypertrophy: islet		-	5	7	7
	Grade 1	-	4	5	7
	Grade 2	-	1	2	-
- Hyperplasia acinar cell: focal		-	-	2	1
	Grade 1	-	-	1	1
	Grade 2	-	-	1	-
- Intraluminal concretion: duct		-	2	1	-
	Grade 1	-	1	1	-
	Grade 2	-	1	-	-
- Regeneration: acinar cell		-	1	-	-
	Grade 2	-	1	-	-
- Inflammation: subacute		-	-	1	-
	Grade 2	-	-	1	-
- Inflammation: acute		1	-	-	1
	Grade 1	1	-	-	1
- Inflammation: vascular/perivascular		1	-	-	-
	Grade 2	1	-	-	-
- Necrosis: acinar cell		1	-	-	-
	Grade 2	1	-	-	-
- Hemorrhage		1	-	-	-
	Grade 2	1	-	-	-

Key study findings:

- Seven control group animals were sacrificed moribund or found dead during the treatment and recovery periods. The deaths were likely a consequence of the progression of type 2 diabetes in these animals. One HD animal was found dead during the recovery period shortly after blood collection. The cause of death could not be determined but may have been associated with the blood collection procedure.
- No adverse clinical signs were noted in the exenatide-treated groups. Clinical signs for the control group were consistent with deterioration of health associated with the progression of type 2 diabetes.
- Mean body weight gains were greater for exenatide-treated animals, which occurred in a dose-related manner. Body weight gains for control animals diminished and became statistically significantly less than treated animals after approximately Week 4 even though control animals consumed significantly more feed. The decreased body weight gain of the control group correlated with declining insulin levels and an overall decline in health due to diabetes progression and it is likely that control animals were not able to fully utilize the glucose they were consuming.
- Exenatide-treated rats had dose-independent higher mean amylase levels at all time points during the treatment period for all dose groups, with Day 90 mean values being 21% to 30% higher than the control value. Amylase values for control animals increased by the end of recovery to equal the levels of the treated rats. The increase in amylase levels did not correlate with microscopic findings or an increase in mean lipase levels.
- Exenatide-treated animals had lower mean values for glucose and HbA1c and higher mean values for insulin, which demonstrates the efficacy of exenatide in this animal model.
- There were no treatment-related effects on pancreas weights or macroscopic findings.
- The primary treatment-related effect noted microscopically was a dose-related increase in minimal to moderate, multifocal islet cell hypertrophy. There was also a slight dose-related increase in the incidence of concretions in the intraluminal duct (2, 3, 4, and 5 animals for control, LD, MD, and HD, respectively). However, the severity of this finding did not increase with increasing dose. One HD animal had slight focal subacute inflammation with multinucleate giant cells and this animal also had multifocal concretions of slight severity with degeneration/regeneration of epithelial cells. There was no apparent effect on focal acinar cell hyperplasia or acute inflammation. For individual animals, there was no definitive correlation between the presence of concretions, acinar cell hyperplasia, inflammation, and/or amylase levels. After recovery, there was still an increase in islet cell hypertrophy and a very slight increase in incidence of focal acinar cell hyperplasia for exenatide-treated animals.
- Histomorphometric assessment through immunohistochemistry techniques found no significant effect of exenatide on duct cell proliferation or apoptosis.

10.2 Systemic Exposures to Medium-chain Fatty Acids via the Oral and SC Routes

MCTs: A Single Dose Pharmacokinetic Study of Tricaprylin (C8) and Miglyol 812 in Rats (study No. 8342572)

Background:
MCTs (Miglyol 812 contained in Bydureon Bcise) are present in many food products (such as coconut, and palm kernel oils), and are used in Europe as lipid emulsions for parenteral nutrition. At the time of the Pre-NDA meeting (2/8/2016), however, parenteral administration of MCTs had not been previously approved in the United States. Additionally, circulating triglyceride in humans are almost exclusively composed of C16 or C18 fatty acids (*ca.* 95%) with much lower amounts of C14 fatty acids (ca. 5%) and levels of MCTs in human blood are normally below the level of detection. The Division recommended the Sponsor to compare PK profile between oral and subcutaneously administered MCTs (pre-NDA meeting minutes, 2/8/2016). This study was conducted to address this issue. Of note, on July 13 of 2016, FDA approved SMOFLIPID™ as an intravenous nutrition source. Thus, this study is no longer considered to be a critical element of the safety assessment for Miglyol 812.

Methods:
Miglyol 812 is composed of fatty acids mainly C8 (50-80%) and C10 (20-50%), with minimum amounts of C6 (≤2.0%), C12 (≤3.0%) and C14 (≤1.0%). In the current study, PK profiles of C8 and C8 +C10 were studied.

Male rats were assigned to four groups and given single oral (gavage) or SC doses of Miglyol 812 (containing 50-65% C8 and 30-45% C10), or an oral dose of Tricaprylin (C8 only). See the table below. Blood was collected at various time points for quantification of MCTs associated caproic acid (C6), caprylic acid (C8) and capric acid (C10) after the treatments.

Group	Number of Male Animals	Dose Route	Test Article	MCT	Dose Level (g/kg)[a]	Dose Volume (mL/kg)
1	6	Oral	Miglyol 812	C8; C10	10.0	10.5
2	6	Oral	Tricaprylin	C8	9.48	10.0
3	6	SC	Migylol 812	C8; C10	0.08	0.084
4	6	SC	Migylol 812	C8; C10	0.80	0.842

SC Subcutaneous.
a Based on a density of 0.95 g/mL.

Observation and results:

Clinical signs:
Clinical signs were limited to non-formed feces on Day 1 in most animals given test articles orally (10 mg/kg Miglyol 812 and 9.48 mg/kg tricaprylin).

Food consumption:
A transient decrease in food consumption was noted from day 1 to Day 2 in tricaprylin-treated animals only.

Pharmacokinetics:

- Medium chain fatty acids (C8, C6 and C10) were detected following both oral (10 mg/kg) and subcutaneous (0.08 and 0.8 mg/kg) administration of Miglyol 812, as well as orally administered tricaprylin (9.8 mg/kg). Thus, both oral and s.c administered miglyol 812 and tricaprylin were absorbed.
- A ten-fold different s.c. dose of Miglyol (0.08 mg/kg and 0.8 mg/kg) produced similar fatty acid exposures including C10 (17000 versus 17500 ng.h/mL) and C6 (22900 versus 19000 ng.h/mL), and less than dose proportional exposure for C8 fatty acids (18800 versus 47200 ng.h/mL), suggesting an exposure limit with s.c. administration.
- Compared to 0.08 mg/kg s.c. dose, Miglyol 812 oral relative bioavailability was 1%, 7% and 11% based C6, C8 and C10 fatty acid, respectively; compared to 0.8 mg/kg s.c dose, Miglyol oral bioavailability was 102%, 12% and 28%, respectively. As rat s.c. dose of 0.08 mg/kg Miglyol is equivalent to clinical dose of 774 mg Miglyol 814 that is contained in MRHD of EQWS (2 mg), the relative oral bioavailability of 1-11% will be used in safety evaluation.

PK data (group mean values) following oral and s.c dosing of MCTs

Analyte	Test article	Dose, mg/kg	Route	C_{max}, ng/mL	T_{max}, h	$AUC_{0-t,}$ ng.h/mL	Relative bioavailability
C10	Miglyol 812	10	PO	10300	15.4	223000	11%-102%*
		0.08	SC	205	0.5	17000	
		0.8	SC	485	0.75	17500	
C6	Miglyol 812	10	PO	6120	0.625	29500	1%-12%*
		0.08	SC	444	0.708	22900	
		0.8	SC	348	2.95	19000	
C8	Miglyol 812	10	PO	45800	0.625	166000	7%-28%*
		0.08	SC	256	2.54	18800	
		0.8	SC	621	26.5	47200	
	Tricaprylin	9.48	PO	47800	1.17	203000	

* the lower and upper range refer to values generated with 0.08 mg/kg and 0.8 mg/kg Miglyol, respectively.

Daily consumption of MCTs at 20-30 g has been reported in internet. Based on oral bioavailabilities, it is estimated that 77.7 g/week p.o. is expected to produce similar C6 fatty acid exposure as from 2 mg/week clinical dose of EQWS (11.1 g/week for C8, and 7.1 g/week for C10). Therefore, it is likely that the systemic exposures to MCTs related fatty acids from the proposed product are within the range generated by commonly consumed 20-30 g/day MCTs as dietary supplement.

The Sponsor did not measure systemic exposures of MCTs, and the reported AUC of MCTs were actually sum of the concentrations achieved from individual medium chain fatty acids (Caproic, Caprylic and Capric) for which the sponsor clarified in the submission dated 08/04/2017. The systemic exposure to MCTs might be higher when MCTs is given by SC route than oral route because some MCTs might be broken down by lipase in GI tract. The shortage of MCTs exposure data, however does not render a significant safety concern in light of negative systemic toxicity findings in monkey studies with Miglyol 812 and recent FDA approval of SMOFLIPID™ and European practice of using MCTs as IV nutrients.

10.3 Leachables and Extractables

This drug product is presented as an oil-based suspension contained in a glass cartridge within an auto-injector. The packaging system is a 2.0 mL, (b) (4) glass cartridge, with an (b) (4) plunger and an (b) (4) seal/aluminum cap (b) (4). As FDA required, the Sponsor conducted extractable and leachable studies for this product.

- Controlled extractable studies (CES): the sponsor reported a list of extractables that was provided by the (b) (4) supplier, (b) (4) from controlled extraction studies (b) (4) (b) (4) (b) (4) The extractables identified were the following.

(b) (4)

- To assess potential leachable levels at end of shelf life of three years, a simulation study was conducted under following conditions.

(b) (4)

Samples were analyzed by GC-MS and UPLC-PDA-MS. Chromatograms (b) (4) (b) (4) were compared to identify the peaks of interest and the method was then optimized to resolve impurities that may co-elute with the (b) (4) peaks. Based on (b) (4) guidance (b) (4) (b) (4) analytical evaluation threshold (AET) of (b) (4) mcg/mL

was established. The AET was applied in the evaluation of leachables and consequently, only those leachables present at levels above the AET are reported. See the table below.

The extractables identified in the simulation study are a subset of those found in the controlled extraction studies with the exception of (b) (4) (b) (4) which were only found in the simulation study.

The acceptance of extraction and simulation study methods was confirmed by the CMC review team (Dr. Suong T Tran and Dr. Christopher Galliford).

Worst case leachable levels:
The Sponsor proposed product shelf life is 3 years at 2-8^{o}C and in use storage period is 4 weeks at 30^{o}C. It is unlikely that the levels of potential leachables during 3 years storage under long-term storage conditions would significantly exceed a linear extrapolation of the levels after storage for 1 year at 5^{o}C, i.e., the levels of potential leachables after storage at 5^{o}C for 3 years would not be greater than 3 times the level observed after storage at 5^{o}C for 1 year. The worst case leachable levels at end of 3 years of shelf life are estimated to be 3 times that observed after storage at 5^{o}C for 1 year or at 25^{o}C for 1 year, whichever is higher, plus in-use leachables that were observed after storage at 40^{o}C for 60 days.

Estimated maximum human daily dose:
Based on the estimated worst case leachable levels, human maximum daily dose was calculated factoring 0.85 mL per dose and 7 days per dose, for example, maximum human daily dose of (b) (4) is estimated to be (b) (4) (b) (4) The table below presents summary results.

Table 1 Results from the simulation study

(b) (4)

[redacted]

Safety evaluation:
Toxicity concerns for subcutaneously administered compounds include systemic and local effects. Systemic toxicities could be of wide range of potentials, and local toxicities are mainly irritation/ulceration, necrosis and carcinogenicity. Considering the proposed once weekly dosing frequence and injection site reactions are monitorable, carcinogenicity evaluation is more of focus for leachable safety evaluation.

The Sponsor considered all the leachables at worst exposure levels are of no safety concern based on the following:

- Leachables with maximum human exposure equal or below (b) (4) mcg/day present negligible risk to humans (ICH M7, mutagenic impurity of (b) (4) mcg/day is associated with negligible risk)
- (b) (4) mcg/day in humans is (b) (4) times lower than acceptable daily intake (ADI, by oral ingestion) of (b) (4) mcg/day established by WHO, which is adequate to ensure human safety including possible low oral bioavailability with ADI relative to the proposed s.c. administration of this product.
- (b) (4) mcg/day is covered by acceptable level of (b) (4) mcg/day presented in ICH Q3C (chemical- or class-specific acceptable levels).
- The rest of five leachables (b) (4) (b) (4) (b) (4) which have limited toxicology data available, were evaluated against ADIs recommended by Dolan et al (2005) who categorized chemicals that have limited toxicology data into three classes based on their chemical

structures and corresponding ADIs were proposed, as (b) (4) mcg/day for compounds that may be carcinogenic, (b) (4) mcg/day for compounds that may be potent or highly toxic and (b) (4) mcg/day for compounds that are not likely to be potent, highly toxic or carcinogenic. The Sponsor considered all these five leachables qualified. Of note, (b) (4) compounds contain genotoxic structural alerts as assessed by (Q)SAR, the Sponsor conducted an in vitro mutation assay (Ames test) with (b) (4), found negative results (reviewed above under Impurity Qualification Genotoxicity Study, in Section 7.1). Therefore, ADI of (b) (4) mcg/day instead of (b) (4) mcg/day for potential carcinogens was applied for (b) (4).

(b) (4)

A literature search obtained information that is consistent with the sponsor's categorization of these five leachables. (b) (4) has relatively low toxicity, which is primarily irritation to skin, eye, nose and throat (b) (4) (b) (4) may have narcotic action (b) (4) and minor irritation to skin and mucous (b) (4) (b) (4)

(b) (4) Toxicities associated with (b) (4) include anesthetic effects, damaging effects onliver and kidney and in cases of certain compounds, carcinogenicity (b) (4)

Currently, there are no acceptable leachable levels established in CDER's practice. For noncarcinogenic leachables in orally inhaled and nasal drug products, (b) (4) (b) (4) recommends qualification threshold of 5 mcg/day (b) (4) (b) (4) With this standard, most leachables observed in the current submission are acceptable except (b) (4) mcg/day level which exceed the 5 mcg/day threshold. Considering that the 5 mcg/day threshold was set for irritant compounds (b) (4) is expected to be a minimal irritant and is to be administered subcutanously. Thus, in such a case, applying (b) (4) mcg/day threshold as recommended by Dalon et al. appears to be reasonable. (b) (4) at a level of (b) (4) mcg/day is considered to be of no safety concern. As described above, this reviewer considers human exposures to the leachales reported in the simulation study to be acceptable.

11 Integrated Summary and Safety Evaluation

Background:
Exenatide, the active ingredient in Byetta, Bydureon and EQWS, is a 39 amino acid peptide that binds to and activates the GLP-1 receptor. Exenatide mimics many of the glucoregulatory actions of GLP-1, including stimulation of glucose-mediated insulin secretion and synthesis of pro-insulin, increased insulin sensitivity, suppression of glucagon release, increased pancreatic beta-cell mass, slowing of nutrient absorption via inhibition of gastric emptying and suppression of food intake.

The Sponsor previously received marketing approval for Byetta (immediate release formulation) and Bydureon (exenatide QW, aqueous formulation) for treatment of type 2 diabetes. The currently proposed EQWS differs from exenatide QW only in two aspects, inclusion of a new excipient, medium chain triglyceride and using of an auto-injector for administration.

The primary pharmacologic, pharmacokinetic, and toxicology properties of exenatide were well characterized during the development of Byetta (NDA 21773) and exenatide QW (NDA 22200). Readers are referred to reviews by Dr. Colerangle (NDA 21773) and Dr. Hummer (NDA 22200) for further details of nonclinical data.

- General toxicology: exenatide daily dosing exhibited no target organ or dose-limiting toxicity in mice, rats and monkeys in studies up to 6, 3 and 9 months, respectively. Studies of exenatide QW in rats (up to 4 months) and monkeys (up to 9 months) showed similar results.

- Carcinogenicity: Carcinogenicity studies with QW exenatide (active component of Bydureon) showed an increased incidence of thyroid C-cell adenomas in mice

and rats at clinical exposures, and thyroid C-cell carcinomas in rats. The doses used in the exenatide QW rat carcinogenicity study were 2.4-fold (low dose), 23-fold (mid dose), and 74-fold (high dose) the human systemic exposure with EQWS, based on AUC. Bydureon was approved based on clinical benefit/risk ratio, and inclusion of three nonclinical and one clinical Post-Marketing Requirement (PMR) studies (see approval letter for Bydureon).

At present, the thyroid C-cell proliferative lesions in rodent remains an unresolved safety concern, and the potential human risk cannot be excluded. For the current product, the Sponsor proposed to reflect the risk in EQWS label. This reviewer considers this approach acceptable.

Developmental and reproductive toxicology studies: a complete battery of development and reproductive toxicology studies were conducted in mice and rabbits with exenatide. With Bydureon, an additional embryo-fetal developmental study was conducted in rats. The treatment-related fetal effects in mice, rats and rabbits included decreased fetal body weight, irregularities in skeletal ossification, and an increase in post-implantation loss. These effects generally occurred in the presence of maternal toxicity (decreased body weight and decreased food consumption). Additionally, TK data showed that the potential for exenatide to cross the placental barrier was low in mice and rabbits and therefore, the observed developmental findings were likely secondary to maternal toxicity. For details, see the reviews of Dr. Colerangle (NDA 21773) and Dr. Hummer (NDA 22200). Sponsor proposes to use these data to inform DART risk in the label for Bydureon Bcise. This is considered to be reasonable.

Pediatric Study issue:
The safety and effectiveness of EQWS have not been established in pediatric patients and currently EQWS is not recommended for use in pediatric patients.

The Sponsor plans to request a partial waiver of children from birth to less than 10 years as studies are impossible or highly impracticable. The sponsor is requesting deferral of studies of children from age 10 to less than 18 years. The sponsor has submitted iPSP for this plan and the Division agreed with the iPSP.

There are no juvenile animal studies required at this time.

<u>Nonclinical studies conducted for the current NDA</u>

- PK profile comparison between EQWS and Bydureon:
    - Following a single subcutaneous dose (2.4 mg/kg) administration in rats, EQWS produced lower C_{max} (-60%) and lower AUC (-40%) values as compared to Bydureon.

- Systemic exposures (Cmax and $AUC_{0\text{-}168h}$) in monkeys treated with EQWS were generally dose proportional, reaching steady state in one month (relative to Day 1, AUC increased 9-14 fold by week 4; 15-45 folds by week 13). This profile and AUC values were comparable to Bydureon values reported previously (see the table below). Note, Anti-drug antibodies were detected in treatment monkeys (13% and 66% in the 4-week and 3-month studies, respectively). The presence of anti-drug antibodies showed increased mean systemic exposures as a result of mixed alterations in some individuals in the 3-month study, but had no impact on the 4-week study. The neutralizing activities of the antibodies were not analyzed. TK data from antibody negative animals were used when comparisons are made.

Table 5 Comparison of Representative Derived Daily AUC at the NOAEL in Repeat-Dose Toxicology and Carcinogenicity Studies with Exenatide Immediate Release (BYETTA), Exenatide QW (BYDUREON) and EQWS

Study Type	Species	Duration	Derived Daily AUC at NOAEL (pg·h/mL)		
			BYETTA [1]	Exenatide QW [1]	EQWS [1]
Repeated Dose	Mice	6 Months	859,596	-	-
	Rats	28 Days	1,211,247	-	-
		2 Months	-	192,269 [2]	-
		3 Months	201,764	-	-
		4 Months	-	146,914 [3]	-
	Monkeys	1 Month	-	-	21,286
		3 Months	-	104,777	102,714
		9 Months	1,000,708	77,949	-
Carcinogenicity	Mice	2 Years	123,241	-	-
	Rats	2 Years	201,764 [4]	< 9483 [5]	-
DART	Rats	2 Weeks	-	< 43,468 [6]	-

AUC = area under the plasma concentration-time curve; - = not applicable;
NOAEL = no-observable-adverse-effect-level; QW = once weekly.

[1] For each study, the most representative AUC values are presented for that dosing paradigm (i.e., for BYETTA studies, all animals on Day 1 of each study, and for both exenatide QW and EQWS studies antibody-negative animals at end of study).

[2] In this 2 month study, the daily AUC value obtained at a dose of 3 mg/kg, the highest dose tested in the rat carcinogenicity with exenatide QW, was 74,676 pg·h/mL.

[3] In this 4 month study, the daily AUC value obtained at a dose of 3 mg/kg, the highest dose tested in the rat carcinogenicity with exenatide QW, was 142,488 pg·h/mL.

[4] Derived daily AUC at the highest dose tested of 3 mg/kg/day (BYETTA label includes language about all doses tested).

[5] No-Effect-Level not established in this study.

[6] NOAEL not established in this study that was associated with maternal toxicity (pharmacologically mediated weight loss).

Based on the PK data, the introduction of MCTs as the diluent in EQWS does not appear to alter the overall PK characteristics when compared to Bydureon.

- Toxicity profiles of EQWS:
  - Rats: A single s.c.dose (2.4 mg/kg) study in rats showed similar effects in EQWS and Bydureion groups, including decreased body weight and food consumption, and injection site reactions (erythema. edema, foreign body granulomas and histiocytic infiltration). The reduced body weight and food consumption are considered to be pharmacological effects, and injection

site findings are microsphere related. There are no EQWS-related unique toxicities observed as compared to Bydureon treatment group.

- Monkeys: Monkey 4-week and 13-week studies employed EQWS (0.11, 0.44 and 1.1 mg/kg/week s.c.), saline, Miglyol 812 and microsphere control groups. Slightly reduced red blood cell parameters (7-15% reduction from baseline in RBCs, hematocrit and hemoglobin at the high dose) in EQWS groups and injection site reactions (local erythema, edema, foreign body granuloma, eosinophilic infiltrate, inflammation and/or necrosis) in EQWS and microsphere groups were observed. The red blood cell findings were typical of GLP-1 receptor agonists, having been seen with exenatide previously, and are not considered adverse. Of note, for some unclear reasons this was not seen with Bydureon (13 and 39 weeks at 0.11, 0.44 and 1.1 mg/kg, NDA 22200). Injection site findings are considered to be microsphere related. All these findings were partially resolved after recovery periods (3 weeks for 4-week study and 13 weeks for 3-month study). The dose of 1.1 mg/kg was considered to be NOAEL for both the 4-week and 13-week studies, which represents 4.2x and 20.1x MRHD exposure based on AUC comparisons (4-w study, 149000 pg.h/mL; 13-w study, 719000 pg.h/mL; humans, 35745 pg.h/mL).

In conclusion, EQWS has similar toxicology profile as Bydureon.

- Assessment of the safety of MCT excipient:
  - MCTs are commonly consumed in the diet, as they are present in a variety of foods, and been have been widely used as food supplements. A study comparing PK profiles following oral and s.c. administered Miglyol 812, demonstrated oral relative bioavailability of 1-11% for C6, C8 and C10 free fatty acids. MCTs have also recently been approved for parenteral (intravenous) use in the United States as a source of calories and essential fatty acids for parenteral nutrition when oral or enteral nutrition is not possible, insufficient, or contraindicated
  - In monkey 4-week and 13-week toxicology studies for EQWS, Miglyol 812 control group was included in each of these studies. There were no Miglyol 812 related toxicity findings.
  - MCTs have been used in Europe and the United States in lipid emulsions as parenteral nutrition for patients.

- Container closure system:
  Based on a simulated leachable study, total 18 leachables were identified, among which estimated human exposure were less than (b) (4) mcg/day for 11, in a range of (b) (4) mcg/day for 6 and at (b) (4) mcg/day for one (b) (4) These leachables are of no safety concern based on available guidance (ICH M7, ICH Q3C) and current practice in CDER as well as detailed deliberations.

- Product impurity specifications:
  There are no safety concerns for impurity specifications based on toxicology studies where these impurities were contained in the test batches.

- Exenatide treatment-related pancreatitis issue:
  Post-marketing surveillance data for exenatide has suggested a potential link between GLP-1 receptor agonists and the development of acute pancreatitis. Literature reports have provided conflicting and controversial data. There were no pancreatic lesions in histopathology examinations for toxicology studies conducted with Byetta, Bydureon or EQWS. Like Byetta and Bydureon, pancreatitis risk is included in the Sponsor proposed labeling for EQWS.

  The Sponsor submitted several mechanistic studies to address the pancreatitis issue. Pancreatitis was induced by caerulein, CCK-8, or sodium taurocholate in ob/ob mice, normal SD rats, Wistar rats and Diabetic Fatty Zucker (ZDF) rats. Pretreatment with AC2993 (3-30 mcg/kg) did not affect baseline of serum pancreatic enzyme levels (amylase and lipase) or potentiate pancreatitis agents induced elevations of these enzymes, rather in some cases blunted the elevations of the enzymes. There were no AC2993 related findings in pancreas weight or histopathology.

  ZDF rats receiving sc AC2993 at 6-250 mcg/kg for three months showed dose-independent higher mean amylase levels throughout the treatment period, with Day 90 mean values being 21 to 30% higher than control value. The increase in amylase levels did not correlate with microscopic findings or increase in mean lipase levels. There was a slight dose-related increase in the incidence of concretions in the intraluminal duct (2, 3, 4, and 5 animals for control, LD, MD, and HD, respectively). However, the severity of this finding did not increase with increasing dose. There was no definitive correction between the presence of concretions, acinar cell hyperplasia, inflammation, and/or amylase levels. After recovery, there was still an increase in islet cell hypertrophy and a very slight increase in incidence of focal acinar cell hyperplasia for exenatide-treated animals. Histomorphometric assessment through immunohistochemistry techniques found no significant effect of exenatide on duct cell proliferation or apoptosis.

  In the 4-week and 13-week monkey studies with EQWS, there were no changes in serum pancreatic enzymes (amylase and lipase), pancreas weight, gross pathology or histopathology.

  Overall, there was no evidence for exenatide related pancreatitis observed in above animal studies. However, clinical relevance of these animal data is unknown. At this time, no additional animal studies are considered to be more informative to this human risk that has been arisen from clinical surveillance.

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/s/

HUIQING HAO
09/28/2017

RONALD L WANGE
09/28/2017
Recommended for approval. See Secondary Review.