CENTER FOR DRUG EVALUATION AND RESEARCH

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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**022-200**
SUPPORTING DOCUMENT NUMBER:	**043**
DATE RECEIVED BY CENTER:	**28 July 2011**
PRODUCT:	**Exenatide extended-release (BYDUREON)**
INTENDED CLINICAL POPULATION:	**Type 2 Diabetes**
SPONSOR:	**Amylin Pharmaceuticals, Inc.**
DOCUMENTS REVIEWED:	**Complete Response: resubmission**
REVIEW DIVISION:	**Division of Metabolism and Endocrinology Drug Products (HFD-510)**
PHARM/TOX REVIEWER:	**B. Timothy Hummer, Ph.D., DABT**
PHARM/TOX SUPERVISOR:	**Karen Davis-Bruno, Ph.D.**
DIVISION DIRECTOR:	**Mary Parks, M.D.**
PROJECT MANAGER:	**Pooja Dharia, Pharm.D.**
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EXECUTIVE SUMMARY

I. RECOMMENDATIONS

A. Recommendation on approvability

The initial marketing application for Bydureon was received by the Agency on 05 May 2009 (Supporting Document #001). The application was not approved and a complete response letter was sent to the sponsor on 12 March 2010. The primary deficiencies noted in the complete response letter included product quality issues and the requirement for a Risk Evaluation and Mitigation Strategy (REMS). There were no nonclinical deficiencies noted in the complete response letter. The sponsor then resubmitted the marketing application for Bydureon as a complete response on 22 April 2010. On 18 October 2010, the sponsor was issued a second complete response decision due to concerns for the potential of QT prolongation in humans based on clinical data. The sponsor resubmitted their marketing application on 28 July 2011 to address the QT prolongation concerns. In this submission, the sponsor has also included a nonclinical rat embryo-fetal development study that was conducted at the request of the European Medicines Agency (EMA). This study was not required for the complete response and the data in the study do not raise new safety concerns for embryo-fetal development. However, because the information from the rat study will be included in the label, the study data are discussed in this review.

After review of the initial NDA submission, Pharmacology/Toxicology recommended that Bydureon not be approved based on the finding of drug-related thyroid C-cell tumors in rats after treatment for 2 years at clinically relevant exposures. New information regarding C-cell effects was not included in this submission currently under review or the previous complete response. In the absence of data to determine the human relevance of the rodent tumor signal, the Pharmacology/Toxicology recommendation continues to be a complete response. However, this recommendation is based on the nonclinical data that indentifies potential clinical risk in the absence of considering clinical benefit. It is recognized that when clinical benefit is considered, Bydureon may be found to have a clinical benefit that outweighs the potential risk of C-cell proliferation in humans. In this case it is recommended that the sponsor further evaluate the potential risk of C-cell proliferation to humans in nonclinical post-marketing requirement (PMR) studies.

Please refer to the initial pharmacology/toxicology review dated 22 February 2010 for a detailed review of the nonclinical data submitted for this marketing application.

B. Recommendation for nonclinical studies

The following nonclinical studies are recommended as post-marketing requirements.

1. Cellular hyperplasia is a physiological process in which cells proliferate in response to a specific stimulus. Because the cells in hyperplastic tissue are typically normal in both appearance and organization, hyperplasia is generally thought to be reversible once the stimulus is removed. However, continued proliferation increases the chance of DNA mutations that can allow for the progression of hyperplasia to neoplasia. Although it is assumed that GLP-1 agonist-induced C-cell proliferation is reversible once treatment is discontinued, it is uncertain whether short-term exposure to exenatide extended-release increases the lifetime risk of C-cell tumors even after treatment is discontinued.

To address the question of reversibility of C-cell hyperplasia, the sponsor should conduct a 2-year mouse study consisting of a 6-month treatment period with 3 doses of exenatide extended-release yielding multiples of human exposures of 10-, 30-, and 100X, followed by a 1.5 year recovery period. Animals should be assessed for C-cell hyperplasia/neoplasia at 6-months and 2 years. Additionally, thyroids collected at the 6 month time point should be evaluated for GLP-1 receptor expression using a quantitative technique to determine whether there is a correlation between the level of GLP-1 receptor expression and the degree of C-cell proliferation.

2. It has been speculated that the sensitivity of GLP-1-induced C-cell hyperplasia is dependent on GLP-1 receptor density, with C-cells having higher expression levels of GLP-1 receptor being more susceptible to the proliferative effects of GLP-1 agonists. Limited published reports indicate that human C-cells have a lower expression of GLP-1 receptor than rodents, thereby making humans less susceptible to GLP-1 agonist-induced C-cell proliferation. However, this hypothesis is based on a limited number of human thyroid samples. To compliment the available information on human expression, C-cells from additional human thyroid samples should be assessed for GLP-1 receptor expression. These data should also be compared with the expression levels of GLP-1 receptor in mice after 6 months of treatment, which will be measured in the study for PMR #1.

GLP-1 receptor expression levels should be measured on C-cells from human thyroid biopsy samples with the following histopathology findings:

1. Normal tissue
2. Non-neoplastic C-cell hyperplasia
3. Neoplastic C-Cell hyperplasia (microcarcinoma)
4. C-cell carcinoma

3. It is currently believed that GLP-1 agonist-induced C-cell proliferation is dependent on the GLP-1 receptor. However, this hypothesis should be verified in vivo.

A comparison of C-cell hyperplasia should be made between wild-type and GLP-1 receptor knock-out mice after treatment with exenatide extended-release or vehicle for 3 months. To better ascertain the growth promoting pathways that are involved in the hyperplastic process, gene expression analysis should be conducted on C-cells that have been isolated through laser capture microdissection, dependent upon feasibility, for each of the animals. The gene expression analysis should include a number of genes involved in growth promoting, growth inhibitory, and apoptotic pathways.

C. Recommendations on labeling

The relevant nonclinical sections of the label are shown below. Much of text had already been agreed upon by the Division and Sponsor during previous review cycles. The inclusion of rat teratology data to Sections 8.1 and 13.3 is the only new nonclinical information that has been added since the last version of the draft label. No changes are recommended from what the sponsor has submitted in this latest complete response.

WARNING: RISK OF THYROID C-CELL TUMORS

See full prescribing information for complete boxed warning.

- **Exenatide extended-release caused thyroid C-cell tumors at clinically relevant exposures in rats. It is unknown whether BYDUREON causes thyroid C-cell tumors, including medullary thyroid carcinoma (MTC), in humans, as human relevance has not been determined by clinical or nonclinical studies (5.1).**
- **BYDUREON is contraindicated in patients with a personal or family history of MTC or in patients with Multiple Endocrine Neoplasia syndrome type 2 (MEN 2) (5.1).**

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

- Thyroid C-cell tumors in animals: Human relevance unknown. Counsel patients regarding the risk of medullary thyroid carcinoma and the symptoms of thyroid tumors (5.1).

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

- Pregnancy: (b) (4) To report drug exposure during pregnancy call 1-800-633-9081 (8.1).

- Nursing Mothers: Caution should be exercised when BYDUREON is administered to a nursing woman (8.3).

FULL PRESCRIBING INFORMATION

WARNING: RISK OF THYROID C-CELL TUMORS

(b) (4)

5 WARNINGS AND PRECAUTIONS

5.1 Risk of Thyroid C-cell Tumors

(b) (4)

BYDUREON. Such monitoring may increase the risk of unnecessary procedures, due to the low specificity of serum calcitonin testing for MTC and a high background incidence of thyroid disease. If serum calcitonin is measured and found to be elevated, the patient should be referred to an endocrinologist for further evaluation [see *Patient Counseling Information (17)*].

8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies of BYDUREON use in pregnant women. In rats, exenatide extended-release administered during the major period of organogenesis reduced fetal growth and produced skeletal ossification deficits in association with maternal effects; exenatide extended-release was not teratogenic in rats. In animal developmental studies, exenatide, the active ingredient of BYDUREON, caused cleft palate, irregular skeletal ossification and an increased number of neonatal deaths. BYDUREON should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Fetuses from pregnant rats given SC doses of exenatide extended-release at 0.3, 1 or 3 mg/kg on gestation days 6, 9, 12 and 15 demonstrated reduced fetal growth at all doses and produced skeletal ossification deficits at 1 and 3 mg/kg in association with maternal effects (decreased food intake and decreased body weight gain). There was no evidence of malformations. Doses of 0.3, 1 and 3 mg/kg correspond to systemic exposures of 3, 7 and 17-times, respectively, the human exposure resulting from the recommended dose of 2 mg/week, based on AUC [see *Nonclinical Toxicology (13.3)*].

Female mice given SC doses of exenatide, the active ingredient of BYDUREON, at 6, 68, or 760 mcg/kg/day beginning 2 weeks prior to and throughout mating until gestation day 7, had no adverse fetal effects. At the maximal dose, 760 mcg/kg/day, systemic exposures were up to 148 times the human exposure resulting from the recommended dose of 2 mg/week, based on AUC [see *Nonclinical Toxicology (13.3)*].

In developmental toxicity studies, pregnant animals received exenatide, the active ingredient of BYDUREON, subcutaneously during organogenesis. Specifically, fetuses from pregnant rabbits given SC doses of exenatide at 0.2, 2, 22, 156, or 260 mcg/kg/day from gestation day 6 through 18 experienced irregular skeletal ossifications from exposures 4 times the human exposure resulting from the recommended dose of 2 mg/week, based on AUC. Fetuses from pregnant mice given SC doses of exenatide at 6, 68, 460, or 760 mcg/kg/day from gestation day 6 through 15 demonstrated reduced fetal and neonatal growth, cleft palate and skeletal effects at systemic exposure that is equivalent to the human exposure resulting from the recommended dose of 2 mg/week, based on AUC [see *Nonclinical Toxicology (13.3)*].

Lactating mice given SC doses of exenatide, the active ingredient of BYDUREON, at 6, 68, or 760 mcg/kg/day from gestation day 6 through lactation day 20 (weaning), experienced an increased number of neonatal deaths. Deaths were observed on postpartum days 2-4 in dams given 6 mcg/kg/day, a systemic exposure that is equivalent to the human exposure resulting from the recommended dose of 2 mg/week, based on AUC [see *Nonclinical Toxicology (13.3)*].

Pregnancy Registry

Amylin Pharmaceuticals, Inc. maintains a Pregnancy Registry to monitor pregnancy outcomes of women exposed to exenatide during pregnancy. Physicians are encouraged to register patients by calling (800) 633-9081.

8.3 Nursing Mothers

Exenatide is present in the milk of lactating mice at concentrations less than or equal to 2.5% of the concentration in maternal plasma following subcutaneous dosing. It is not known whether exenatide is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for tumorigenicity shown for exenatide extended-release in animal studies, a decision should be made whether to discontinue nursing or to discontinue BYDUREON, taking into account the importance of the drug to the mother.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

A 104-week carcinogenicity study was conducted with exenatide extended-release in male and female rats at doses of 0.3, 1.0 and 3.0 mg/kg (2, 9, and 26-times human systemic exposure based on AUC, respectively) administered by SC injection every other week. A statistically significant increase in thyroid C-cell tumor incidence was observed in both males and females. The incidence of C-cell adenomas was statistically significantly increased at all doses (27% to 31%) in females and at 1.0 and 3.0 mg/kg (46% and 47%, respectively) in males compared with the control group (13% for males and 7% for females). A statistically significantly higher incidence of C-cell carcinomas occurred in the high dose group females (6%), while numerically higher incidences of 3%, 7%, and 4% (non-statistically significant versus controls) were noted in the low, mid, and high dose group males compared with the control group (0% for both males and females). 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.

A 104-week carcinogenicity study was conducted with exenatide, the active ingredient in BYDUREON, in male and female rats at doses of 18, 70, or 250 mcg/kg/day (3, 6, and 27 times human systemic exposure based on AUC, respectively) administered by once daily bolus SC injection. Benign thyroid C-cell adenomas were observed in female rats at all exenatide doses. The

incidences in female rats were 8% and 5% in the two control groups and 14%, 11%, and 23% in the low, medium, and high dose groups.

In a 104-week carcinogenicity study with exenatide, the active ingredient in BYDUREON, in male and female mice at doses of 18, 70, or 250 mcg/kg/day administered by once daily bolus SC injection, no evidence of tumors was observed at doses up to 250 mcg/kg/day, a systemic exposure up to 16 times the human exposure resulting from the recommended dose of 2 mg/week, based on AUC. The carcinogenicity of exenatide extended-release has not been evaluated in mice.

BYDUREON and exenatide, the active ingredient in BYDUREON, were 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, at twice-daily SC 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 148 times the human exposure resulting from the recommended dose of 2 mg/week, based on AUC.

13.3 Reproductive and Developmental Toxicology

A rat embryo-fetal developmental toxicity study was conducted with exenatide extended-release. A complete reproductive and developmental toxicity program was conducted with exenatide, the active ingredient in BYDUREON.

Fetuses from pregnant rats given SC doses of exenatide extended-release at 0.3, 1 or 3 mg/kg on gestation days 6, 9, 12 and 15 demonstrated reduced fetal growth at all doses and produced skeletal ossification deficits at 1 and 3 mg/kg in association with maternal effects (decreased food intake and decreased body weight gain). There was no evidence of malformations. Doses of 0.3, 1 and 3 mg/kg correspond to systemic exposures of 3, 7 and 17-times, respectively, the human exposure resulting from the recommended dose of 2 mg/week, based on AUC.

In female mice given twice-daily SC doses of 6, 68, or 760 mcg/kg/day exenatide, the active ingredient in BYDUREON, beginning 2 weeks prior to and throughout mating until gestation day 7, there were no adverse fetal effects at doses up to 760 mcg/kg/day, systemic exposures up to 148 times the human exposure resulting from the maximum recommended dose of 2 mg/day, based on AUC.

In pregnant mice given twice-daily SC doses of 6, 68, 460, or 760 mcg/kg/day exenatide, the active ingredient in BYDUREON, from gestation day 6 through 15 (organogenesis), cleft palate (some with holes) and irregular fetal skeletal ossification of rib and skull bones were observed at 6 mcg/kg/day, a systemic exposure equal to the human exposure resulting from the maximum recommended dose of 2 mg/day, based on AUC.

In pregnant rabbits given twice-daily SC doses of 0.2, 2, 22, 156, or 260 mcg/kg/day exenatide, the active ingredient in BYDUREON, from gestation day 6 through 18 (organogenesis), irregular fetal skeletal ossifications were observed at 2 mcg/kg/day, a systemic exposure 4 times the human exposure resulting from the maximum recommended dose of 2 mg/day, based on AUC.

In pregnant mice given twice-daily SC doses of 6, 68, or 760 mcg/kg/day exenatide, the active ingredient in BYDUREON, from gestation day 6 through lactation day 20 (weaning), an increased number of neonatal deaths was observed on postpartum days 2-4 in dams given 6 mcg/kg/day, a systemic exposure equal to the human exposure resulting from the maximum recommended dose of 2 mg/day, based on AUC.

II. SUMMARY OF NONCLINICAL FINDINGS

A. Brief overview of nonclinical findings

The following summary is taken from the first pharmacology/toxicology review for this NDA, with some modification to the summary for developmental and reproductive toxicity.

Repeat-dose studies were conducted with exenatide extended-release, a sustained release formulation of exenatide, to compliment the existing toxicology program that had been conducted with immediate-release exenatide in support of the marketing approval of Byetta (NDA 21-773). These additional studies include repeat-dose studies in rats (1 and 4 months) and monkeys (3 and 9 months), a rat carcinogenicity study, and several in vitro genetic toxicology studies to qualify manufacturing changes. Subsequent to the first review, a rat embryo-fetal developmental toxicity study was conducted with exenatide extended-release.

Mice treated with immediate-release exenatide twice daily at doses up to 380 µg/kg/dose for 6 months had microscopic findings in the eye (retinal atrophy, corneal mineralization, cataract), parotid salivary gland (basophilia), bone marrow hyperplasia, and injection site reactions (inflammation, hemorrhage, fibrosis, epithelial hyperplasia) [NDA 21-773]. A NOAEL could not be determined because parotid gland hyperplasia and pthisis bulbi (shrinkage and wasting of the eyeball) were observed at the low dose (9 µg/kg BID; ~2X the maximum recommended human dose [MRHD] based on exposure [AUC]).

Sprague-Dawley rats receiving exenatide extended-release every other week by subcutaneous injection at doses up to 9 mg/kg for 4 months resulted in slight decreases in body weight gain at all doses that generally correlated with decreased food consumption, especially for males receiving 3 and 9 mg/kg. Injection-site reactions were the primary treatment-related effect for all groups receiving microspheres. Injection-site findings consisted of swelling/palpable lumps, with severity increasing as the dose of microspheres increased. Histopathology revealed foamy macrophages/fibroblasts, lymphocytic infiltrate, and granulomas of minimal to slight severity at all injection sites. Injection site findings reversed or showed a trend for recovery by the end of the 3-month recovery period. One MD and one HD female had renal tubular adenomas (approximately 10X and 27X MRHD, respectively, Ab negative AUC); the relationship to treatment was uncertain. The NOAEL for this study was 9 mg/kg (~27X MRHD, Ab negative AUC) based on a lack of target organ toxicity.

The subcutaneous injection of exenatide extended-release once weekly to cynomolgus monkeys at doses up to 1.1 mg/kg for 3 and 9 months primarily resulted in injection site reactions. Macroscopic lesions at the injection sites were characterized by red or white discoloration, nodules, edema, abscesses, thickened tissue, and injection site enlargement. Some occurrences of abscesses with drainage were noted for all exenatide-treated groups in the 9-month study. Nodules appeared to increase in severity with increase in exenatide dose (3-month study) or microsphere dose (9-month study). Microscopically, the injection sites for all groups were characterized as having chronic inflammation, abscesses, epidermal hyperplasia, fibrosis, and/or hemorrhage, although these occurred at a lower incidence for the diluent control group. Granulomatous inflammation (minimal to severe), granulomas, and foreign material were noted at the injection site of animals receiving microspheres with or without exenatide. Granulomas were well-circumscribed with minimal fibrosis and consisted of foamy macrophages and multinucleated giant cells that often containing microspheres. The incidence of macroscopic and microscopic lesions was drastically reduced at the end of a 3-month recovery period, suggestive of reversibility. The NOAEL for both studies was 1.1 mg/kg (14-19X MRHD, AUC) on the basis of a lack of target organ toxicity.

Rat and monkey TK data showed that steady-state concentrations were achieved within a month of treatment and clearance of the drug after the final dose occurred within 2 months. Anti-exenatide antibodies were detected in both rats and monkeys after repeated dosing with exenatide extended-release. The presence of antibodies did not appear to have neutralizing activity, although it did have an effect on TK results. In rats, AUC values tended to increase in the presence of antibodies and in monkeys, AUC values tended to increase in the presence of low antibody titers but decreased with higher antibody titers. Because of these effects, mean AUC values for antibody negative animals and humans were used for exposure comparisons when available.

A carcinogenicity study in which Sprague-Dawley rats received exenatide extended-release by subcutaneous injection once every other week showed an increase in thyroid C-cell tumors (adenomas plus carcinomas) at all doses (≥0.3 mg/kg; 1X MRHD, Ab negative AUC) for both males and females (statistically significant for all groups except LD males). The incidence of C-cell adenomas was greater in this study than observed with immediate-release exenatide, which is believed to be due to the difference in PK profiles between the two exenatide formulations. A statistically significant increase in fibromas of the skin was also observed in males treated with the high dose (3 mg/kg; 26X MRHD, Ab negative AUC). Fibromas were relatively acellular and were comprised primarily of bundles of collagen. The fibromas were not specifically noted as being at injection sites, but this was implied in the pathologists report. A non-statistically significant slight increase in renal tubular cell tumors (adenomas plus carcinomas) was observed in HD females (25X MRHD, Ab negative AUC); two tubular cell adenomas were also observed in a 4-month rat study; a relationship to test article remains uncertain. Other expected findings for exenatide (reduced body weight) and PLG microspheres (foreign body granulomas at the injection site) were also observed.

The carcinogenicity of exenatide extended-release was not evaluated in mice. Immediate-release exenatide did not induce tumors in a mouse carcinogenicity study. However, based on mouse carcinogenicity results with other long-acting GLP-1 receptor agonists, this class of compounds also induces C-cell tumors in mice, although generally at higher clinical exposure margins than observed for rats. Therefore, based on the available data, it is assumed that if exenatide extended-release were to be tested in a mouse carcinogenicity study, thyroid C-cell adenomas would be observed; however it would also be expected that there would be a greater clinical exposure margin than observed for rats.

Exenatide and exenatide extended-release were not mutagenic or clastogenic in a battery of genetic toxicology studies.

The effect of immediate-release exenatide on reproduction and embryonic development was previously investigated in support of the marketing application for Byetta (NDA 21-773). Results of a fertility and early embryonic development study in mice showed no exenatide-related adverse effects on estrus cycling, mating and fertility indices, numbers of corpora lutea, implantation, viable embryos, non-viable embryos, pre- or post-implantation viability, or cauda epididymal sperm motility, count, or density. Accordingly, the NOAEL for effects on male and female reproduction was the high dose of 380 µg/kd BID (148X MRHD). Note that all exposure margins presented for the developmental and reproductive toxicity are in relation to clinical exposures (AUC) for Bydureon.

Pregnant rats treated with exenatide extended-release on gestation days (GD) 6, 9, 12, and 15 showed an initial body weight loss between GD 6 and 9 and decreased body weight gain from GD 6 until the end of the study. There was a

slight, non-statistically significant increase in early resorptions at all dose levels compared with the control value. A statistically significant decrease in fetal body weight was observed at all dose levels. There were no definitive treatment-related fetal malformations observed. Irregular skeletal ossification, particularly for cervical centrum #1 and 27 presacral vertebrae, was observed at ≥1.0 mg/kg (≥7X MRHD). The NOAEL values for maternal toxicity and for delayed embryonic development was less than 0.3 mg/kg (<3X clinical exposure). There were no definitive treatment-related developmental malformations; accordingly, the NOAEL for teratogenicity was 3.0 mg/kg (17X MRHD). Doses of 0.3, 1, and 3 mg/kg correspond to systemic exposures of 3, 7 and 17-times, respectively, the clinical exposure at the MRHD of 2 mg/week.

In a mouse embryonic development study, maternal body weight gain and food consumption were slightly decreased at ≥230 µg/kg/dose, particularly at the beginning of the dosing period. Some abortions and premature deliveries were observed at ≥34 µg/kg/dose. The number of implantations, litter sizes, and live fetuses were significantly decreased for dams receiving ≥230 µg/kg/dose relative to control. Fetal body weights were decreased at ≥230 µg/kg/dose for males and ≥68 µg/kg/dose for females. Skeletal variations associated with delayed fetal growth included changes in the number of rib pairs or vertebral ossification sites and wavy ribs at ≥230 µg/kg/dose. Rare occurrences of fetuses with multiple abnormalities including cleft palate with or without hole were observed for most dose groups, including control. The maternal NOAEL was 3 µg/kg BID (1X MRHD) based on the observed abortions and the developmental NOAEL was 3 µg/kg BID on the basis of decreased fetal body weights, cleft palate, and wavy ribs.

Two rabbit embryonic development studies were conducted with immediate-release exenatide doses ranging from 0.1 to 130 µg/kg twice daily. Apparent maternal toxicity characterized by profound weight loss and reduced food and water consumption was observed at doses ≥11 µg/kg/dose. Clinical indicators of starvation (β-hydroxybuterate and potassium) were also noted. Morphological markers of fetal growth retardation were observed that included umbilical hernias and skeletal variations of angulated hyoid, altered number of rib pair or vertebral bodies, and fused sternabrae at ≥11 µg/kg/dose. Fetal incidence of small gall bladder was significantly increased at 11, 78, and 130 µg/kg/dose. In the second study, skeletal variations were observed at ≥1 µg/kg/dose, but were also present at a similar incidence in an untreated, pair-fed group, suggesting these effects were a consequence of compromised maternal health. However, an increase in umbilical hernias was not observed in the pair-fed groups.

For the first rabbit study, the maternal NOAEL was determined to be the low dose of 0.1 µg/kg BID (0.1X MRHD) based on dose-related decrease in weight gain during the treatment period. The developmental NOAEL was also 0.1 µg/kg BID (0.1X MRHD) based on the developmental retardation. For the second study, which was conducted to better define the NOAEL, the NOAEL for

developmental toxicity was the low dose of 1 µg/kg BID (4.5X MRHD). TK data showed that the potential for exenatide to cross the placental barrier is very low in both mice and rabbits. Therefore the fetal findings observed in both species may have been a consequence of a reduction in the maternal nutritional state during gestation or maternal toxicity.

The effects of exenatide on gestation, parturition, lactation, and maternal behavior were evaluated in mice from implantation through lactation and weaning. The effects on development and fertility of the offspring were also evaluated. The F_0 maternal NOAEL was less than 3 µg/kg BID (<1X MRHD) due to mortality at ≥3 µg/kg/dose. The NOAEL for F_1 fetal viability and growth was 3 µg/kg BID (1X MRHD) because of reduced preweaning pup body weights at 34 µg/kg BID (19X MRHD) and 380 µg/kg BID (198X MRHD) and increased perinatal mortality and reduced body weight gains postweaning at 380 µg/kg BID. F_0 maternal administration of exenatide at doses as high as 380 µg/kg BID did not affect, learning, memory, day of preputial separation or day of vaginal patency, mating or fertility, or cesarean-sectioning parameters of the F_1 generation mice. There were no treatment-related effects on corpora lutea, implantations, litter sizes, or resorptions in cesarean-sectioned pregnant F_1 females or on the incidence of fetal alterations in F_2 generation mice.

An assessment of the local tolerance of exenatide extended-release at the injection sites was integrated into the repeat-dose toxicology studies. Injection site reactions were generally characterized by swelling, inflammation, and other findings typical of foreign body reactions in both rats and monkeys, with increased incidence and severity with increasing dose of microspheres. In monkeys administered exenatide extended-release for 9 months, swelling with open drainage and/or abscesses was observed in all groups receiving exenatide extended-release. Microscopically, granulomatous inflammation with foreign body giant cells and fibrosis were observed in rats and monkeys receiving microspheres, with or without exenatide.

B. Pharmacologic activity

Exenatide binds to and activates the GLP-1 receptor, a G protein-coupled receptor. Through its activation of the GLP-1 receptor, exenatide mimics many of the glucoregulatory activities of endogenous GLP-1 including stimulation of glucose-mediated insulin secretion and synthesis of pro-insulin, increased insulin sensitivity, suppression of glucagon release, increased pancreatic β-cell mass, slowing of nutrient absorption via inhibition of gastric emptying, and suppression of food intake. Pharmacology studies in rodent models of diabetes and obesity demonstrated that the administration of exenatide resulted in the lowering of serum glucose and HbA1c values in conjunction with decreased food intake and body weight. Like GLP-1, the glucose lowering effect of exenatide has been shown to be dependent on glucose concentrations in the plasma.

C. Nonclinical safety issues relevant to clinical use

In a rat carcinogenicity study conducted with exenatide extended-release, a statistically significant increase in thyroid C-cell tumors (adenomas plus carcinomas) relative to control groups was observed at clinically relevant exposures. Similar findings have been observed for other compounds in this therapeutic class. Currently it is not known whether thyroid C-cell tumors induced by GLP-1 receptor agonists are relevant to human risk. Therefore, until data to the contrary are available, it should be assumed that exenatide extended-release has the potential to induce thyroid C-cell hyperplasia and tumors in humans. Fibromas of the skin were observed in male rats at exposures that are approximately 26-fold higher than at the maximum clinical dose of 2 mg/week.

Injection site findings typical of foreign body reactions were observed in rats and monkeys receiving PLG microspheres, with or without exenatide. Effects at the injection sites were generally mild to moderate and were found to be reversible as the microspheres degraded. Based on these findings, patients could experience some discomfort at the injection sites (e.g., inflammation and granulomas).

Byetta, which contains the same active ingredient as Bydureon, has been placed in the pregnancy category C category based on findings of fetal cleft palate, irregular fetal skeletal ossification of rib and skull bones, and increased neonatal deaths in mice at clinically relevant exposures. Irregular fetal skeletal ossifications were also observed in rabbits at exposures that are approximately 4.5 times the human exposure at the maximum recommended dose. Fetal umbilical hernias were observed at 79-times clinical exposure. Fetal effects generally occurred at doses that caused meaningful decreases in maternal body weight gain compared with controls. As with Byetta, exenatide extended-release should only be used during pregnancy if the potential benefit justifies the potential risk to the fetus.

Through post-marketing adverse event reporting, a possible signal for drug-induced pancreatitis, including life-threatening necrotizing/hemorrhagic pancreatitis, has been rarely observed in patients taking Byetta. A possible increase in the incidence of pancreatitis has also been noted in patients taking other drugs working through the GLP-1 pathway, including other GLP-1 receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors. To further investigate the potential role of diabetes in the development of drug-induced pancreatitis, sponsors of approved GLP-1 receptor agonists, including Amylin, have been asked to conduct toxicology studies in rodent models of diabetes and pancreatitis to assess for drug-induced acinar cell proliferation and pancreatic toxicity. Based on the totality of the data, nonclinical toxicology studies conducted with drugs from these therapeutic classes have failed to induce drug-related pancreatic toxicity or pancreatitis in normal animals or in disease models of diabetes or pancreatitis. If this class of drugs does increase a patient's risk for pancreatitis, it may be human specific or involve other risk factors that have not been studied in

nonclinical studies, such as concomitant medications. Currently, it is not felt that additional nonclinical studies will enhance our understanding of the potential risk of pancreatitis for patients taking a drug in this therapeutic class.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 022-200
Review number: 3
Supporting document number/date: 43 / 28 July 2011
Type of submission: NDA resubmission, Complete Response
Information to sponsor: No
Sponsor and/or agent: Amylin Pharmaceuticals, Inc.
Manufacturer for drug substance: exenatide extended-release [redacted] different suppliers:

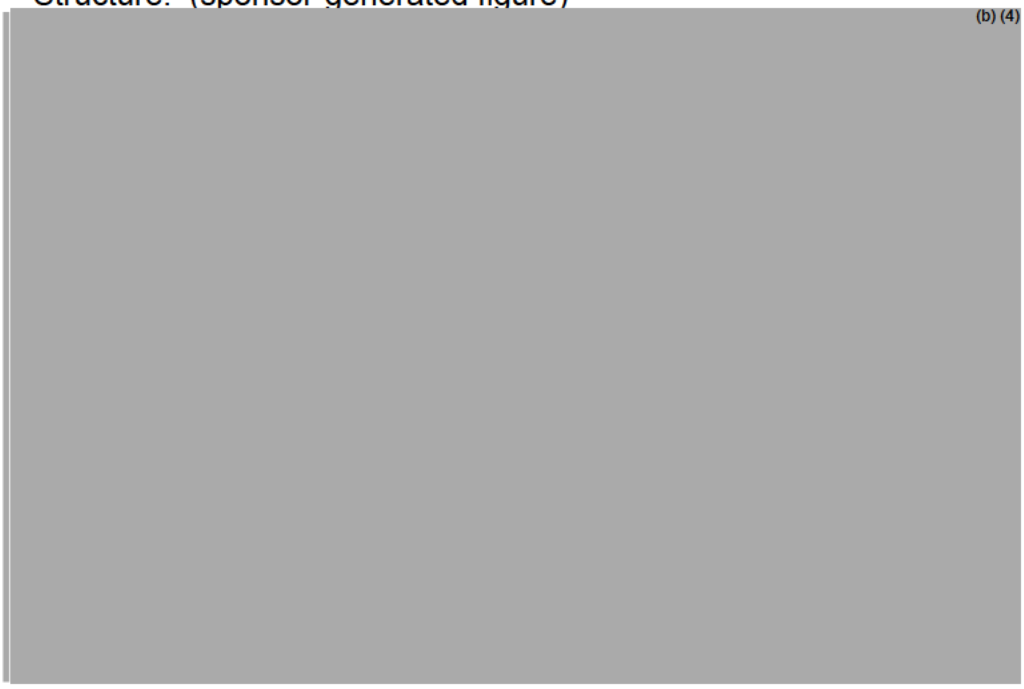
2. Mallinckrodt Inc., St. Louis, MO
3. Lonza, SA, Braine-l'Alleud, Belgium

Reviewer name: B. Timothy Hummer, Ph.D., DABT
Division name: Division of Metabolism and Endocrinology Products
HFD #: 510
Review completion date: 13 December 2011

Drug:

Trade name: BYDUREON
Generic names: exenatide extended-release, exenatide QW, exenatide once weekly, exenatide LAR, exendin-4
Code names: AC2993-F17
Chemical name: L-histidylglycyl-L-glutamylglycyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-aspartyl-L-leucyl-L-seryl-L-lysyl-L-glutaminyl-L-methionyl-L-glutamyl-L-glutamyl-L-glutamyl-Lalanyl-L-valyl-L-arginyl-L-leucyl-L-phenylalanyl-L-isoleucyl-L-glutamyl-L-tryptophanyl-Lleucyl-L-lysyl-L-asparaginylglycylglycyl-L-prolyl-L-seryl-L-serylglycyl-L-alanyl-L-prolyl-L-prolyl-L-prolyl-L-serinamide
CAS registry number: 141732-76-5
Molecular formula: $C_{184}H_{282}N_{50}O_{60}S$
Molecular weight: 4186.6 Daltons

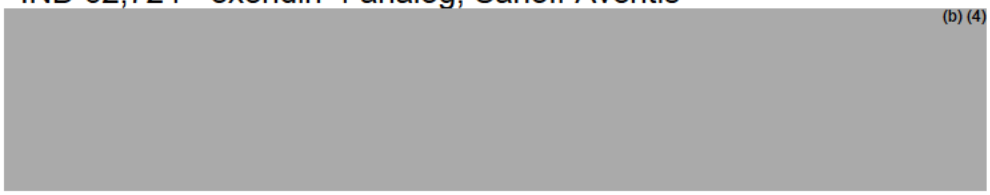
Structure: (sponsor-generated figure)



Relevant INDs/NDAs/DMFs:

Exendin-4 analogs

IND 67,092 - exenatide, Amylin, once weekly formulation
NDA 21-773 / IND 57,725 - exenatide, BYETTA, Amylin
NDA 21-919 / IND 57,725 - exenatide, BYETTA, Amylin, monotherapy
IND 62,724 - exendin-4 analog, Sanofi-Aventis



GLP-1 analogs

NDA 22-341 / IND 61,040 - GLP-1 analog, liraglutide, Novo Nordisk

Drug class: Glucagon-like peptide-1 (GLP-1) receptor agonist

Intended clinical population: Type 2 diabetes

Route of administration: Subcutaneous injection, once weekly

Disclaimer: Some tables, figures, and/or text were taken from the Sponsor's submission, where indicated. Also, some text, tables, and/or figures were taken or modified from Dr. John Colerangle's pharmacology/toxicology review of NDA 21-773 (Byetta), where indicated.

Studies reviewed within this submission:
- An Embryo-Fetal Development and Toxicokinetic Study of Subcutaneously Administered Exenatide (LY2148568) LAR in Sprague-Dawley Rats (REST110051)

Studies not reviewed within this submission: None

2.6.2 PHARMACOLOGY

The reader is referred to Dr. John Colerangle's review of NDA 21-773 for summaries of the primary, secondary, and safety pharmacology studies conducted with immediate-release exenatide. The reader is also referred to the initial pharmacology/toxicology review for NDA 22-200 dated 22 February 2010.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

The reader is referred to Dr. John Colerangle's review of NDA 21-773 for summaries of ADME studies conducted with immediate-release exenatide. The reader is also referred to the initial pharmacology/toxicology review for NDA 22-200, dated 22 February 2010, for a review of ADME studies conducted with exenatide extended-release.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

A complete toxicology program was conducted to support the marketing approval of immediate-release exenatide (Byetta). Summaries of these studies can be found in the pharmacology/toxicology review for NDA 21-773. The sponsor conducted additional toxicology studies with exenatide extended-release to supplement the Byetta toxicology program. These include repeat-dose toxicology studies up to 4 months in rats, a 3-month and 9-month toxicology study in monkeys, a carcinogenicity study in rats, a rat teratology study, and several in vitro genetic toxicology studies to qualify manufacturing changes for the drug substance. All nonclinical studies conducted with exenatide extended-release were previously reviewed, except for the rat teratology study reviewed here. Please refer to the initial pharmacology/toxicology review dated 22 February 2010 for a review of these studies.

A list of toxicology studies conducted with immediate-release exenatide and exenatide extended-release is shown in the sponsor-generated table below.

Table 1: Summary of Exenatide Once Weekly Nonclinical Program

Type of Study	Study	Species	Duration of Dosing	Dosing Regimen	Route	GLP Status
Exenatide (BYETTA)						
General Toxicology	Acute	Mouse	Single dose	QD	IV	Non-GLP
		Rat	Single dose	QD	SC	GLP
		Monkey	Single dose	QD	SC	GLP
	Repeated Dose	Mouse	3-Month	QD	SC	GLP
			3-Month	BID	SC	GLP
			6-Month	BID	SC	GLP
		Rat	14-Day	QD	IV	Non-GLP
			28-Day	QD	SC	GLP
			3-Month	QD	SC	GLP
		Monkey	5-Day	QD	SC	GLP
			28-Day	QD	SC	GLP
			3-Month	BID	SC	GLP
			9-Month	BID	SC	GLP
Genotoxicity	Ames (3 studies)	N/A	N/A	N/A	N/A	GLP
	Chromosomal Aberration (3 studies)	CHO	N/A	N/A	N/A	GLP
	Micronucleus	Mouse	Single Dose	QD	SC	GLP
Carcinogenicity		Mouse	2-year	QD	SC	GLP
		Rat	2-year	QD	SC	GLP
DART	Fertility	Mouse	As per ICH	BID	SC	GLP
	Embryofetal	Mouse (2 studies)	As per ICH	BID	SC	GLP
		Rabbit (2 studies)	As per ICH	BID	SC	GLP
	Peri/PostNatal	Mouse	As per ICH	BID	SC	GLP
Special Toxicology	Impurities & degradation products (3 studies)	Mouse	28-Day	BID	SC	GLP
Exenatide QW						
General Toxicology	Repeated Dose	Mouse [1]	28-Day	BID	SC	GLP
		Rat	8-Week	Every 2 weeks	SC	GLP
			8-Week	Every 2 weeks	SC	GLP
			4-Month	Every 2 weeks	SC	GLP
		Monkey	3-Month	QW	SC	GLP
			9-Month	QW	SC	GLP
Genotoxicity	Ames (3 studies) [1]	N/A	N/A	N/A	N/A	GLP
	Chromosomal Aberration (3 studies) [1]	CHO	N/A	N/A	N/A	GLP
Carcinogenicity		Rat	2-year	Every 2 weeks	SC	GLP
DART	Embryofetal	Rat	As per ICH	Every 72 hours	SC	GLP

BID = twice daily; CHO = Chinese Hamster Ovary Cells; DART = developmental and reproductive toxicology; GLP = Good Laboratory Practice; SC = subcutaneous; IV = intravenous; QD = once daily; QW = once weekly.

[1] A 28-day mouse study and in vitro genotoxicity studies (Ames and chromosomal aberration) were conducted with exenatide (drug substance qualification studies)

2.6.6.6 Developmental and Reproductive Toxicology

Embryo-Fetal Development

Study title: An Embryo-Fetal Development and Toxicokinetic Study of Subcutaneously Administered Exenatide (LY2148568) LAR in Sprague-Dawley Rats

Key study findings:

- There were no mortalities or adverse clinical signs.
- Mean maternal body weights decreased during the first two days after the first dose on GD 6, after which time body weights slowly increased, although, initial body weight loss after dosing was observed at the low dose after each dose administration. Mean final gross maternal body weight, net maternal weight (minus gravid uterus), and body weight gain from Gestational Day (GD) 6 through 20 were statistically significantly lower than control at all dose levels. Effects on maternal body weight occurred in a dose-related manner and correlated with decreased food consumption, especially between GD 6 and GD 9. Decreases in food consumption partially recovered after GD 9.
- A slight non-statistically significant increase in early resorptions was observed, resulting in a slightly lower percentage of viable fetuses for treated dams.
- A statistically significant decrease in fetal body weight was observed at all dose levels.
- There was a trend for a greater male (55%) to female (45%) ratio at the high dose, although the gender ratio was within the historical control data.
- No definitive treatment-related fetal malformations were observed. Although one HD fetus had cleft palate, it is difficult to assign a treatment relationship based on a single occurrence and the incidence falls within the lab's historical range. Situs inversus occurred in two HD fetuses, which is an incidence that is slightly higher than the historical control range (0.5% vs. 0.4%). This finding may have been treatment related, although it is the most common visceral malformation for control animals observed in this lab. There were no treatment-related external or visceral fetal variations. The external and visceral variations noted are common background findings in this laboratory.
- There was a slight imbalance in the number of treated fetuses that had variations in ossification of vertebrae, sternebrae, pubis, and/or sternum compared with control; however the number of fetuses was rather low and did not always occur in a dose-related manner. The most probable treatment-related skeletal variations included a decrease in the number of fetuses with ossified cervical centrum #1 (statistically significant at high dose) and a small increase in fetuses with 27 presacral vertebrae in the mid- and high-dose groups.
- The NOAEL for maternal toxicity was considered to be less than 0.3 mg/kg because of the effects on maternal body weight at all dose levels. The NOAEL for effects on embryonic development was also considered to be less than 0.3 mg/kg because of statistically significant decreases in fetal body weight at all

doses, a potential increase in early resorptions, and skeletal development delays at 1.0 and 3.0 mg/kg. The effects on fetal body weight and skeletal development were likely secondary to maternal toxicity. There were no definitive treatment-related developmental malformations; accordingly, the NOAEL for teratogenicity was 3.0 mg/kg.

- Exposure ($AUC_{0\text{-}72h}$) approximately doubled between GD 6 and GD 15 for all dose levels. Mean C_{max} values increased by approximately twofold at the mid- and high-dose levels between GD 6 and 15. C_{max} at the low dose only increased by 1.3 fold. Exposure increased with dose in a slightly less than dose proportional manner.

Study no.: (b) (4)-353252 (REST110051)
Conducting laboratory and location: (b) (4)
Date of study initiation: 30 November 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Exenatide (LY2148568) LAR, Lot #0083, 97.3% pure

Methods

Species/strain: Rat/Sprague-Dawley
Study Design:

				Number of Females	
Group Number	Treatment	Dose Level (mg/kg)	Dose Volume (mL/kg)	Number of EFD Females[a]	Number of TK Females[b]
1	Vehicle	0	3.0	25	4
2	Exenatide	0.3	0.3	25	8
3	Exenatide	1.0	1.0	25	8
4	Exenatide	3.0	3.0	25	8

[a] = Embryo-Fetal Development Phase ((b) (4)353252)
[b] = Toxicokinetic Phase ((b) (4)-353252T)

Route and regimen: Subcutaneous, dosed every 3 days from GD 6 through GD 15; c-sections conducted on GD 20
Formulation: Exenatide/microsphere powder was resuspended in an appropriate volume of aqueous diluent supplied by the sponsor
Parameters and endpoints evaluated: Standard Segment II endpoints

Results

Mortality (dams):
There were no unscheduled deaths.

Clinical signs (dams):

Dose (mg/kg)	0	0.3	1.0	3.0
Decreased defecation	0/0	0/0	3/3	6/6
Hair loss - ventral abdominal area	0/0	28/3	34/3	21/5
Hair loss - ventral thoracic area	0/0	14/3	1/1	1/1

Number of observations for abnormal sign/number of animals

Body weight (dams):

Dose (mg/kg)	0	0.3	1.0	3.0
Weight (g) - GD 6	273	278	278	276
Weight (g) - GD 20	408	377**	363**	338**
Weight gain (g)	135	99	85	62
Diff from control (g)		-36	-50	-73
% diff from control		↓27%	↓37%	↓54%
Gravid uterine weight (g)	87.8	78.6**	79.9*	73.1**
Net final body weight (g)	320.0	298.4**	283.3**	265.0**
Diff from control (g)		-21.6	-36.7	-55.0
% diff from control		↓7%	↓11%	↓17%

*p<0.05; **p<0.01 (Dunnett's test); GD = gestation day.

Summary of Maternal Body Weight

Dose (mg/kg):	0[a]	0.3	1.0	3.0
Mean Body Weight (g)				
Gestation Day 6	273	278 (1.8%)	278 (1.8%)	276 (1.1%)
Gestation Day 9	287	263 (-8.4%)**	249 (-13.2%)**	242 (-15.7%)**
Gestation Day 12	308	287 (-6.8%)**	275 (-10.7%)**	261 (-15.3%)**
Gestation Day 15	332	308 (-7.2%)**	297 (-10.5%)**	282 (-15.1%)**
Gestation Day 20	408	377 (-7.6%)**	363 (-11.0%)**	338 (-17.2%)**

(sponsor-generated table)

Food consumption (dams):

Dose (mg/kg)	0	0.3	1.0	3.0
GD 6 - 9 (g/animal/day)	22	11**	8**	6**
GD 9 - 12 (g/animal/day)	24	18**	18**	14**
GD 12 - 15 (g/animal/day)	29	23**	22**	21**
GD 15 - 18 (g/animal/day)	25	19**	17**	15**

**p<0.01 (Dunnett's test); GD = gestation day.

Macroscopic findings (maternal): No treatment-related maternal macroscopic findings were noted.

Toxicokinetics: (sponsor-generated table)

	Administered Dose (mg/kg)		
Parameter	**0.3**	**1**	**3**
LY2148568			
Day 6			
C_{max} (pg/mL)	858	1130	3073
$AUC_{0\text{-}72h}$ (pg*Hours/mL)	24047	64184	124238
Day 15			
C_{max} (pg/mL)	1089	2570	5827
$AUC_{0\text{-}72h}$ (pg*Hours/mL)	43468	121305	284698
C_{avg} (pg/mL)	604	1685	3954

Abbreviations: $AUC_{0\text{-}72ht}$ = area under the curve from time 0 to 72 hours, C_{max} = maximum plasma concentration, C_{avg} = average plasma concentration during the dosing period.

Terminal and necroscopic evaluations:C-section data:

Summary of Fetal Data at Scheduled Necropsy

GROUP	SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1 TOTAL	174	178	352	0	9	0	9	361	380	19	NA	24
MEAN	7.3	7.4	14.7	0.0	0.4	0.0	0.4	15.0	15.8	0.8	3.9	
S.D.	2.47	2.28	2.10	0.00	0.65	0.00	0.65	2.14	1.93	1.28	0.23	
S.E.	0.50	0.47	0.43	0.00	0.13	0.00	0.13	0.44	0.39	0.26	0.05	
2 TOTAL	172	162	334	0	17	0	17	351	366	15	NA	24
MEAN	7.2	6.8	13.9	0.0	0.7	0.0	0.7	14.6	15.3	0.6	3.6*	
S.D.	2.33	2.36	1.64	0.00	0.81	0.00	0.81	1.61	2.05	0.97	0.22	
S.E.	0.48	0.48	0.33	0.00	0.16	0.00	0.16	0.33	0.42	0.20	0.05	
3 TOTAL	187	176	363	0	17	1	18	381	416	35	NA	25
MEAN	7.5	7.0	14.5	0.0	0.7	0.0	0.7	15.2	16.6	1.4	3.5**	
S.D.	1.66	1.97	1.50	0.00	0.75	0.20	0.74	1.54	1.98	2.04	0.33	
S.E.	0.33	0.39	0.30	0.00	0.15	0.04	0.15	0.31	0.40	0.41	0.07	
4 TOTAL	186	161	347	0	20	0	20	367	396	29	NA	25
MEAN	7.4	6.4	13.9	0.0	0.8	0.0	0.8	14.7	15.8	1.2	3.4**	
S.D.	1.87	2.66	2.30	0.00	0.82	0.00	0.82	2.19	1.84	2.08	0.19	
S.E.	0.37	0.53	0.46	0.00	0.16	0.00	0.16	0.44	0.37	0.42	0.04	

* = Significantly different from the control group at 0.05
** = Significantly different from the control group at 0.01
NA = NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA,
FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

1- 0 MG/KG 2- 0.3 MG/KG 3- 1.0 MG/KG 4- 3.0 MG/KG

sponsor-generated table modified by reviewer

Summary of Fetal Data at Scheduled Necropsy [% per Litter]

GROUP:	0 MG/KG	0.3 MG/KG	1.0 MG/KG	3.0 MG/KG
CORPORA LUTEA				
MEAN	15.8	15.3	16.6	15.8
S.D.	1.93	2.05	1.98	1.84
S.E.	0.39	0.42	0.40	0.37
N	24	24	25	25
IMPLANTATION SITES				
MEAN	15.0	14.6	15.2	14.7
S.D.	2.14	1.61	1.54	2.19
S.E.	0.44	0.33	0.31	0.44
N	24	24	25	25
VIABLE FETUSES (%)				
MEAN	97.6	95.2	95.4	94.5
S.D.	4.06	5.48	4.67	5.61
S.E.	0.83	1.12	0.93	1.12
N	24	24	25	25
DEAD FETUSES (%)				
MEAN	0.0	0.0	0.0	0.0
S.D.	0.00	0.00	0.00	0.00
S.E.	0.00	0.00	0.00	0.00
N	24	24	25	25
EARLY RESORPTIONS (%)				
MEAN	2.4	4.8	4.4	5.6
S.D.	4.06	5.48	4.75	5.62
S.E.	0.83	1.12	0.95	1.12
N	24	24	25	25

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
CORPORA LUTEA AND IMPLANTATION SITES COMPARED USING DUNNETT'S TEST
MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group

sponsor-generated table modified by reviewer

Summary of Fetal Data at Scheduled Necropsy [% per Litter]

GROUP:	0 MG/KG	0.3 MG/KG	1.0 MG/KG	3.0 MG/KG
LATE RESORPTIONS (%)				
MEAN	0.0	0.0	0.3	0.0
S.D.	0.00	0.00	1.26	0.00
S.E.	0.00	0.00	0.25	0.00
N	24	24	25	25
TOTAL RESORPTIONS (%)				
MEAN	2.4	4.8	4.7	5.6
S.D.	4.06	5.48	4.68	5.62
S.E.	0.83	1.12	0.94	1.12
N	24	24	25	25
PRE-IMPLANTATION LOSS (%)				
MEAN	4.9	3.7	7.7	6.9
S.D.	7.94	5.49	9.90	11.73
S.E.	1.62	1.12	1.98	2.35
N	24	24	25	25
POST-IMPLANTATION LOSS (%)				
MEAN	2.4	4.8	4.7	5.6
S.D.	4.06	5.48	4.68	5.62
S.E.	0.83	1.12	0.94	1.12
N	24	24	25	25
MALES (%)				
MEAN	49.2	51.6	51.8	54.7
S.D.	14.86	16.12	11.83	15.53
S.E.	3.03	3.29	2.37	3.11
N	24	24	25	25
FEMALES (%)				
MEAN	50.8	48.4	48.2	45.3
S.D.	14.86	16.12	11.83	15.53
S.E.	3.03	3.29	2.37	3.11
N	24	24	25	25
MALE FETAL WEIGHTS (g)				
MEAN	4.0	3.7**	3.6**	3.5**
% DIFFERENCE		-7.5	-10.0	-12.5
S.D.	0.25	0.26	0.32	0.21
S.E.	0.05	0.05	0.06	0.04
N	24	24	25	25
FEMALE FETAL WEIGHTS (g)				
MEAN	3.7	3.5**	3.5**	3.4**
% DIFFERENCE		-5.4	-5.4	-8.1
S.D.	0.24	0.21	0.34	0.21
S.E.	0.05	0.04	0.07	0.04
N	24	24	25	25
COMBINED FETAL WEIGHTS (g)				
MEAN	3.9	3.6**	3.5**	3.4**
% DIFFERENCE		-7.7	-10.3	-12.8
S.D.	0.23	0.22	0.33	0.19
S.E.	0.05	0.05	0.07	0.04
N	24	24	25	25

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
FETAL WEIGHTS COMPARED USING DUNNETT'S TEST
MODIFIED STATISTICS USED.
* INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
** = Significantly different from the control group at 0.01

sponsor-generated table modified by reviewer

Offspring Evaluations:

Summary of Fetuses and Litters with Malformations [absolute number]

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	352	334	363	347	24	24	25	25
CLEFT PALATE	0	0	0	1	0	0	0	1
NUMBER EXAMINED VISCERALLY	352	334	363	347	24	24	25	25
SITUS INVERSUS	0	0	0	2	0	0	0	2
NUMBER EXAMINED SKELETALLY	352	334	363	347	24	24	25	25
TOTAL NUMBER WITH MALFORMATIONS								
EXTERNAL :	0	1	0	1	0	1	0	1
SOFT TISSUE :	0	0	1	2	0	0	1	2
SKELETAL :	0	2	0	0	0	2	0	0
COMBINED :	0	2	1	3	0	2	1	3

1- 0 MG/KG 2- 0.3 MG/KG 3- 1.0 MG/KG 4- 3.0 MG/KG

sponsor-generated table modified by reviewer

Summary of Litter Proportions of Malformations % per Litter

DOSE GROUP:		1	2	3	4
NUMBER OF LITTERS EXAMINED VISCERALLY		24	24	25	25
CLEFT PALATE	MEAN	0.0	0.0	0.0	0.3
	S.D.	0.00	0.00	0.00	1.43
	S.E.	0.00	0.00	0.00	0.29
SITUS INVERSUS	MEAN	0.0	0.0	0.0	0.5
	S.D.	0.00	0.00	0.00	1.74
	S.E.	0.00	0.00	0.00	0.35

1- 0 MG/KG 2- 0.3 MG/KG 3- 1.0 MG/KG 4- 3.0 MG/KG
MODIFIED STATISTICS USED.
None significantly different from control group using Dunn's test

sponsor-generated table modified by reviewer

Summary of Fetuses and Litters with Variations [absolute number]

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	352	334	363	347	24	24	25	25
NUMBER WITH FINDINGS	0	0	0	0	0	0	0	0
NUMBER EXAMINED VISCERALLY	352	334	363	347	24	24	25	25
RENAL PAPILLA(E) NOT DEVELOPED AND/OR DISTENDED URETER(S)	0	2	1	3	0	2	1	2
MAJOR BLOOD VESSEL VARIATION	0	1	1	1	0	1	1	1
NUMBER EXAMINED SKELETALLY	352	334	363	347	24	24	25	25
14TH RUDIMENTARY RIB(S)	20	32	18	17	11	14	9	8
CERVICAL CENTRUM #1 OSSIFIED	58	72	29	21	21	21	11	11
27 PRESACRAL VERTEBRAE	0	0	2	4	0	0	2	3
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	20	11	18	29	9	8	11	9
25 PRESACRAL VERTEBRAE	0	0	1	1	0	0	1	1
STERNEBRA(E) #1,#2,#3 AND/OR #4 UNOSSIFIED	0	1	4	2	0	1	3	2
REDUCED OSSIFICATION OF THE VERTEBRAL ARCHES	0	1	5	2	0	1	3	1
PUBIS UNOSSIFIED	0	2	1	1	0	2	1	1
ENTIRE STERNUM UNOSSIFIED	0	0	1	2	0	0	1	2

1- 0 MG/KG 2- 0.3 MG/KG 3- 1.0 MG/KG 4- 3.0 MG/KG

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Summary of Litter Proportions of Variations % per Litter

DOSE GROUP:		1	2	3	4
NUMBER OF LITTERS EXAMINED VISCERALLY		24	24	25	25
RENAL PAPILLA(E) NOT DEVELOPED AND/OR DISTENDED URETER(S)	MEAN	0.0	0.6	0.3	0.9
	S.D.	0.00	1.95	1.43	3.34
	S.E.	0.00	0.40	0.29	0.67
MAJOR BLOOD VESSEL VARIATION	MEAN	0.0	0.3	0.3	0.3
	S.D.	0.00	1.57	1.33	1.33
	S.E.	0.00	0.32	0.27	0.27
NUMBER OF LITTERS EXAMINED SKELETALLY		24	24	25	25
14TH RUDIMENTARY RIB(S)	MEAN	6.1	9.6	4.7	5.1
	S.D.	8.59	12.36	9.09	8.86
	S.E.	1.75	2.52	1.82	1.77
CERVICAL CENTRUM #1 OSSIFIED	MEAN	17.6	22.5	8.0	6.8*
	S.D.	15.83	20.55	10.60	11.21
	S.E.	3.23	4.19	2.12	2.24
27 PRESACRAL VERTEBRAE	MEAN	0.0	0.0	0.5	1.3
	S.D.	0.00	0.00	1.63	3.83
	S.E.	0.00	0.00	0.33	0.77
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	MEAN	5.4	3.2	5.0	8.6
	S.D.	9.02	5.45	7.92	16.28
	S.E.	1.84	1.11	1.58	3.26
25 PRESACRAL VERTEBRAE	MEAN	0.0	0.0	0.3	0.3
	S.D.	0.00	0.00	1.25	1.54
	S.E.	0.00	0.00	0.25	0.31
STERNEBRA(E) #1,#2,#3 AND/OR #4 UNOSSIFIED	MEAN	0.0	0.3	1.1	0.6
	S.D.	0.00	1.46	3.13	2.15
	S.E.	0.00	0.30	0.63	0.43
REDUCED OSSIFICATION OF THE VERTEBRAL ARCHES	MEAN	0.0	0.3	1.2	0.6
	S.D.	0.00	1.36	3.84	2.86
	S.E.	0.00	0.28	0.77	0.57
PUBIS UNOSSIFIED	MEAN	0.0	0.6	0.2	0.3
	S.D.	0.00	1.95	1.18	1.43
	S.E.	0.00	0.40	0.24	0.29
ENTIRE STERNUM UNOSSIFIED	MEAN	0.0	0.0	0.3	0.5
	S.D.	0.00	0.00	1.33	1.86
	S.E.	0.00	0.00	0.27	0.37
14TH FULL RIB(S)	MEAN	0.0	0.3	0.2	0.0
	S.D.	0.00	1.57	1.18	0.00
	S.E.	0.00	0.32	0.24	0.00

1- 0 MG/KG 2- 0.3 MG/KG 3- 1.0 MG/KG 4- 3.0 MG/KG
MODIFIED STATISTICS USED.
None significantly different from control group using Dunn's test

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Summary of Litter Proportions of Variations % per Litter

DOSE GROUP:		1	2	3	4
NUMBER OF LITTERS EXAMINED		24	24	25	25
TOTAL VARIATIONS					
PERCENT PER LITTER WITH EXTERNAL VARIATIONS	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00
	S.E.	0.00	0.00	0.00	0.00
PERCENT PER LITTER WITH SOFT TISSUE VARIATIONS	MEAN	0.0	0.9	0.8	1.2
	S.D.	0.00	2.43	2.27	3.52
	S.E.	0.00	0.50	0.45	0.70
PERCENT PER LITTER WITH SKELETAL VARIATIONS	MEAN	29.7	36.9	21.9	24.4
	S.D.	18.92	20.51	16.98	20.37
	S.E.	3.86	4.19	3.40	4.07
TOTAL PERCENT PER LITTER WITH VARIATIONS	MEAN	29.7	37.4	22.7	25.0
	S.D.	18.92	20.60	16.46	20.32
	S.E.	3.86	4.21	3.29	4.06

1- 0 MG/KG 2- 0.3 MG/KG 3- 1.0 MG/KG 4- 3.0 MG/KG
MODIFIED STATISTICS USED.
None significantly different from control group using Dunn's test

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Historical Control Data ((b) (4) : 4/21/1998 - 8/27/2010)

Mean of Study Means

Endpoint	Total	Mean	S.D.	SEM	Median	Min	Max	25th Quartile	75th Quartile
NO. OF DATASETS	**168**								
No. of Animals Examined at Laparohysterectomy	4273								
Mean Gravid Uterine Weight (g)		85.4	3.70	0.29	85.7	72.2	95.4	82.8	88.1
Mean No. Viable Fetuses/Dam		15.1	0.69	0.05	15.1	12.2	17.1	14.7	15.5
Total No. Viable Fetuses	60839								
Viable Fetuses (%/Litter)		95.1	1.57	0.12	95.4	90.1	98.0	94.0	96.2
Mean No. Postimplantation Loss/Dam		0.7	0.22	0.02	0.7	0.3	1.4	0.6	0.9
Total No. of Postimplantation Losses	3025								
Postimplantation Loss (%/Litter)		4.9	1.57	0.12	4.6	2.0	9.9	3.8	6.0
Early Resorptions (%/Litter)		4.8	1.59	0.12	4.5	1.5	9.9	3.6	5.8
Late Resorptions (%/Litter)		0.1	0.19	0.01	0.0	0.0	0.8	0.0	0.2
Dead Fetuses (%/Litter)		0.0	0.05	0.00	0.0	0.0	0.5	0.0	0.0
Mean No. Implantations/Dam		15.9	0.68	0.05	15.9	13.0	17.6	15.4	16.3
Mean No. Corpora Lutea/Dam		17.2	0.83	0.06	17.2	14.5	19.4	16.6	17.7
Mean No. Preimplantation Loss/Dam		1.4	0.59	0.05	1.3	0.3	3.1	0.9	1.7
Total No. Preimplantation Losses	5427								
Preimplantation Loss (%/Litter)		7.3	2.97	0.23	7.1	1.5	15.7	5.2	9.1
Total No. Male Fetuses	30376								
Total No. Female Fetuses	30463								
% Males/Litter		49.9	2.70	0.21	49.8	43.1	56.7	47.9	51.8
% Females/Litter		50.1	2.70	0.21	50.2	43.3	56.9	48.2	52.1
Mean Fetal Body Weight (g)		3.7	0.11	0.01	3.6	3.4	3.9	3.6	3.7
Mean Male Body Weight (g)		3.8	0.12	0.01	3.7	3.5	4.0	3.7	3.8
Mean Female Body Weight (g)		3.6	0.11	0.01	3.6	3.4	3.8	3.5	3.6

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		Mean of Study Means
NO. OF DATASETS	**165**	
Total No. of Fetuses/Litters Examined Externally	59744	3942
Total No. of Fetuses/Litters Examined Viscerally	59550	3942
Total No. of Fetuses/Litters Examined Skeletally	59537	3941

MALFORMATIONS (% Per Litter)	**Mean**	**S.D.**	**SEM**	**Median**	**Min**	**Max**	**25th Quartile**	**75th Quartile**
TOTAL EXTERNAL MALFORMATIONS	0.1	0.21	0.02	0.0	0.0	1.3	0.0	0.2
TOTAL VISCERAL MALFORMATIONS	0.1	0.17	0.01	0.0	0.0	0.9	0.0	0.0
TOTAL SKELETAL MALFORMATIONS	0.1	0.23	0.02	0.0	0.0	1.1	0.0	0.3
TOTAL MALFORMATIONS	0.3	0.35	0.02	0.2	0.0	1.6	0.0	0.5
EXTERNAL								
Cleft Palate	0.0	0.04	0.00	0.0	0.0	0.3	0.0	0.0
VISCERAL								
Situs Inversus	0.0	0.08	0.01	0.0	0.0	0.4	0.0	0.0

VARIATIONS (% Per Litter)	**Mean**	**S.D.**	**SEM**	**Median**	**Min**	**Max**	**25th Quartile**	**75th Quartile**
TOTAL EXTERNAL VARIATIONS	0.0	0.02	0.00	0.0	0.0	0.3	0.0	0.0
TOTAL VISCERAL VARIATIONS	0.5	0.74	0.06	0.3	0.0	4.0	0.0	0.6
TOTAL SKELETAL VARIATIONS	33.7	6.68	0.52	33.6	18.0	50.4	29.7	37.3
TOTAL VARIATIONS	33.9	6.80	0.38	33.7	17.1	51.1	29.7	37.8
VISCERAL								
Major Blood Vessel Variation	0.1	0.16	0.01	0.0	0.0	0.8	0.0	0.0
Renal Papilla(e) not Developed and/or Distended Ureter(s)	0.3	0.64	0.05	0.0	0.0	4.0	0.0	0.3

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VARIATIONS (% Per Litter)	Mean	S.D.	SEM	Median	Min	Max	25th Quartile	75th Quartile
SKELETAL								
14th Rudimentary Rib(s)	7.0	3.15	0.25	6.4	0.0	18.9	4.8	8.7
25 Presacral Vertebrae	0.1	0.30	0.02	0.0	0.0	2.0	0.0	0.0
27 Presacral Vertebrae	0.2	0.27	0.02	0.0	0.0	1.8	0.0	0.3
Cervical Centrum #1 Ossified	20.4	5.80	0.45	19.7	6.6	35.8	16.5	24.6
Entire Sternum Unossified	0.0	0.07	0.01	0.0	0.0	0.4	0.0	0.0
Pubis Unossified	0.1	0.22	0.02	0.0	0.0	2.3	0.0	0.0
Reduced Ossification of the Vertebral Arches	0.1	0.19	0.02	0.0	0.0	1.1	0.0	0.2
Sternebra(e) #1, #2, #3 and/or #4 Unossified	0.2	0.26	0.02	0.0	0.0	1.3	0.0	0.3
Sternebra(e) #5 and/or #6 Unossified	6.4	5.87	0.46	4.1	0.0	26.1	2.5	8.8

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Summary Incidence Malformations and Variations
(Total Number Fetuses/Litters Affected)
Ranked Uppermost to Nethermost

NO. OF DATASETS	**165**	
Total No. of Fetuses/Litters Examined Externally	59744	3942
Total No. of Fetuses/Litters Examined Viscerally	59550	3942
Total No. of Fetuses/Litters Examined Skeletally	59537	3941

	Number	
MALFORMATIONS	**Fetuses**	**Litters**
EXTERNAL		
Microphthalmia and/or Anophthalmia	18	18
Mandibular Micrognathia	7	7
Omphalocele	7	7
Fetal Anasarca	7	5
Umbilical Herniation of the Intestine	6	6
Anal Atresia	5	5
Filamentous Tail	5	5
Cleft Palate	4	4
Exencephaly with or without Open Eyelid(s)	3	3
Tail- Short	3	3
Aglossia	2	2
Carpal and/or Tarsal Flexure	2	2
Cyclopia	2	2
Mandibular Agnathia	2	2
Open Eyelid(s)	2	2
Apodia	1	1
Astomia	1	1
Cleft Face	1	1
Cleft Lip	1	1
Craniorachischisis	1	1
Ectromelia	1	1
Gastroschisis	1	1
Hydrocephaly with or without Dome Head	1	1
Maxillary Agnathia	1	1
Meningoencephalocele	1	1
Micromelia	1	1
Proboscis-Like Nose	1	1
Spina Bifida	1	1

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Summary Incidence Malformations and Variations
(Total Number Fetuses/Litters Affected)
Ranked Uppermost to Nethermost

NO. OF DATASETS	**165**	
Total No. of Fetuses/Litters Examined Externally	59744	3942
Total No. of Fetuses/Litters Examined Viscerally	59550	3942
Total No. of Fetuses/Litters Examined Skeletally	59537	3941

	Number	
MALFORMATIONS	**Fetuses**	**Litters**
VISCERAL		
Situs Inversus	14	14
Hydrocephaly	9	9
Lung(s)- Lobular Dysgenesis	7	6
Retroesophageal Aortic Arch	5	5
Kidney(s) and/or Ureter(s) Absent	4	4
Interrupted Aortic Arch	2	2
Thyroid Gland(s)- Absent	2	2
Transposition of the Great Vessels	2	2
Aorta- Narrowed	1	1
Epididymis- Absent	1	1
Heart and/or Great Vessel Anomaly	1	1
Kidney(s)- Absent	1	1
Lung(s)- Lobular Agenesis	1	1
Testis- Absent	1	1
Vessel(s)- Malpositioned	1	1

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Summary Incidence Malformations and Variations
(Total Number Fetuses/Litters Affected)
Ranked Uppermost to Nethermost

NO. OF DATASETS	**165**	
Total No. of Fetuses/Litters Examined Externally	59744	3942
Total No. of Fetuses/Litters Examined Viscerally	59550	3942
Total No. of Fetuses/Litters Examined Skeletally	59537	3941

VARIATIONS	**Number Fetuses**	**Litters**
VISCERAL		
Renal Papilla(e) not Developed and/or Distended Ureter(s)	156	97
Major Blood Vessel Variation	47	45
Liver- Accessory Lobule(s)	16	10
Hemorrhagic Ring Around the Iris	12	12
Spleen- Pale	9	9
Spleen- Accessory	8	5
Spleen Small	6	6
Adrenal Gland(s)- Enlarged	5	1
Liver- Pale	4	4
Hemorrhagic Iris	2	2
Kidney(s)- Small	2	2
Testis Small	2	2
Retrocaval Ureter(s)	2	1
Adrenal Gland(s)- Accessory	1	1
Adrenal Gland(s)- Pale	1	1
Diaphragm- Thin	1	1
Heart Small	1	1
Liver- Swollen	1	1

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Summary Incidence Malformations and Variations
(Total Number Fetuses/Litters Affected)
Ranked Uppermost to Nethermost

NO. OF DATASETS	165	
Total No. of Fetuses/Litters Examined Externally	59744	3942
Total No. of Fetuses/Litters Examined Viscerally	59550	3942
Total No. of Fetuses/Litters Examined Skeletally	59537	3941

VARIATIONS	Number Fetuses	Litters
SKELETAL		
Cervical Centrum #1 Ossified	11969	3004
14th Rudimentary Rib(s)	4154	1710
Sternebra(e) #5 and/or #6 Unossified	3908	1415
Hyoid Unossified	767	492
7th Cervical Rib(s)	492	372
Reduced Ossification of the 13th Rib(s)	366	249
Sternebra(e)- Malaligned (Slight or Moderate)	229	204
Sternebra(e) #1, #2, #3 and/or #4 Unossified	114	106
Bent Rib(s)	99	77
27 Presacral Vertebrae	89	71
Reduced Ossification of the Vertebral Arches	67	62
25 Presacral Vertebrae	62	37
Reduced Ossification of the Skull	52	45
Pubis Unossified	42	36
7th Sternebra	24	10
Reduced Ossification of the Rib(s)	20	17
Entire Sternum Unossified	9	9
Skull Bone(s)- Accessory	8	8
Unco-Ossified Vertebral Centra	6	6
Vertebral Centra Unossified	5	5
Ischium Unossified	4	3
Extra Site of Ossification Anterior to Sternebra #1	3	3
Sternebrae with Thread-Like Attachment	3	2
Reduced Ossification of the Limb Bone(s)	2	2
Extra Site of Ossification Anterior to Cervical Centrum #2	1	1

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INTEGRATED SUMMARY

Embryo-Fetal Toxicity

The effect of exenatide on embryonic development was evaluated in mice and rabbits during the nonclinical program for immediate-release exenatide (Byetta; NDA 21-773). The results of these studies led to Byetta being labeled as Pregnancy Category C. Additionally, at the request of the EMA, Amylin conducted a Segment II teratology study in rats with exenatide extended-release to determine whether continuous steady-state exposure of exenatide results in a similar developmental safety profile compared with immediate-release exenatide. Although inclusion of this study report in the submission was not considered essential for the sponsor's complete response, the sponsor plans on including the information in the label. A comparison of noteworthy teratology study findings is summarized in the table below.

In pregnant mice treated with immediate-release exenatide, maternal body weight gain was statistically significantly decreased at ≥460 µg/kg/day between GD 6 and 9; no noteworthy decrease in body weight gain was noted for any exenatide-treated group by the end of the study. A slight increase in abortions and/or early deliveries was observed at ≥68 µg/kg/day. An increase in post-implantation loss was observed at 6, 460, and 760 µg/kg/day but not at 68 µg/kg/day. Considering a lack of dose response and a lack of a statistically significant increase from control, it is uncertain whether this effect was truly treatment related.

Fetal body weights were statistically significantly decreased for both genders at ≥460 µg/kg/day and for females at 68 µg/kg/day (≥19X clinical exposure). Effects on vertebrae and rib ossification were observed in exenatide-treated groups, but these effects did not occur in a dose-related manner. A statistically significant increase in wavy ribs was observed at 760 µg/kg/day (~200X clinical exposure). Additionally, a slight increase in cleft palate was observed at 760 µg/kg/day compared with control animals (7 for HD vs. 4 for control). The percentage of fetuses with cleft palate at the high-dose was 3.4%, which exceeds the historical control range for the laboratory that conducted the study (0%-1.2%); however, the control and low-dose groups also slightly exceeded the historical control range, with percentages of 1.3% and 2.1%, respectively, while the cleft palate incidence for the two mid-dose groups were within the historical control values. Overall, because the incidence of cleft palate was high for the control group, it is difficult to determine a treatment relationship for this finding. However, because the incidence of cleft palate at 760 µg/kg/day was greater than the concurrent control and the historical control range, an increase in the incidence of cleft palate may have been treatment related at the high dose. Therefore, from a conservative standpoint, the NOAEL for cleft palate is considered to be 460 µg/kg/day (~100X clinical exposure). However, I do not agree that there is a potential treatment-related increase in fetuses with multiple malformations as noted in the review for NDA 21-773. The NOAEL for maternal toxicity is less

than 6 µg/kg/day because of treatment-related decreases in food consumption and possible treatment-induced abortions/early deliveries. The NOAEL for fetal toxicity is considered to be 6 µg/kg/day (1.3X clinical exposure) due to effects on fetal body weight at ≥68 µg/kg/day. The NOAEL for wavy ribs (developmental delay) and possibly treatment-related cleft palate is 460 µg/kg/day (~100X clinical exposure).

Two rabbit teratology studies were conducted. The second study was conducted to better define the NOAEL for maternal and fetal effects and to help determine the contribution of decreased food consumption on post-implantation loss and fetal growth retardation. In pregnant rabbits treated with immediate-release exenatide, maternal body weight loss was observed at ≥2 µg/kg/day between GD6 and 9, and a meaningful decrease in body weight gain from GD6 to the end of the study was observed at ≥0.2 µg/kg/day. A statistically significant increase in post-implantation loss was observed at exposures ≥156 µg/kg/day (≥544X clinical exposure); a numerical increase in post-implantation loss was also observed at 22 µg/kg/d (79X clinical exposure). In fetuses, a statistically significant increase in umbilical hernias was observed at ≥156 µg/kg/day and slight numerical increase was also observed at 22 µg/kg/day. Statistically significant irregular skeletal ossifications occurred at ≥2 µg/kg/day (≥4.5X clinical exposure). A slight decrease in fetal body weight was also observed at ≥22 µg/kg/day.

When pair-fed controls were assessed, skeletal ossification irregularities were also observed for all groups indicating that the skeletal effects were due to decreased maternal nutrition. Fetal body weights were also decreased for the group that was feed matched with the 260 µg/kg/day group, but not the 22 µg/kg/day group. The treatment related effects on post-implantation loss and fetal umbilical hernias did not appear to be affected by a decrease in maternal food consumption suggesting a direct exenatide effect on these parameters. The NOAEL for developmental effects not associated with diminished maternal nutrition was determined to be 2 µg/kg/day (~4.5X clinical exposure).

Pregnant rats treated with exenatide extended-release showed an initial body weight loss between GD 6 and 9. After GD 9, mean maternal body weights rebounded slightly, but mean body weight gain from GD 6 until the end of the study was still statistically significantly lower than the control group. There was a slight, non-statistically significant increase in early resorptions at all dose levels compared with the control value. A statistically significant decrease in fetal body weight was observed at all dose levels. There were no definitive treatment-related fetal malformations observed; the single incidence of cleft palate fell within the lab's historical control range and situs inversus occurred in two high-dose fetuses, which is an incidence that is only slightly higher than the historical control range (0.5% vs. 0.4%). There was a slight imbalance in the number of treated fetuses that had variations in ossification of vertebrae, sternebrae, pubis, and/or sternum compared with control; however the number of fetuses was

rather low and did not always occur in a dose-related manner. The most probable treatment-related skeletal variations included a decrease in the number of fetuses with ossified cervical centrum #1 (statistically significant at high dose) and a small increase in fetuses with 27 presacral vertebrae at ≥1.0 mg/kg (≥7X clinical exposure).

The NOAEL for maternal toxicity was considered to be less than 0.3 mg/kg because of the effects on maternal body weight at all dose levels. The NOAEL for effects on embryonic development was also considered to be less than 0.3 mg/kg (<3X clinical exposure) because of statistically significant decreases in fetal body weight at all doses, as well as a potential increase in early resorptions and skeletal development delays at 1.0 and 3.0 mg/kg. The effects on fetal body weight and skeletal development were likely secondary to maternal toxicity. There were no definitive treatment-related developmental malformations; accordingly, the NOAEL for teratogenicity was 3.0 mg/kg (17X clinical exposure).

Summary of Developmental Findings

Species	Rat				Mouse†					Rabbit††					
Test Article	Exenatide extended-release				Exenatide					Exenatide					
Dose Level (µg/kg)	0	300	1000	3000	0	6	68	460	760	0	0.2	2	22	156	260
$AUC_{0\text{-}24h}$ (ng·h/mL)	NA	14.49	40.44	94.90	NA	6.97	102.8	504.2	1079.9	NA	0.456	24.33	429.8	2973.3	7221.5
Clinical Exposure Margin †††	NA	3X	7X	17X	NA	1.3X	19X	92X	198X	NA	0.1X	4.5X	79X	544X	1322X
Findings - Maternal															
Found dead	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/20 0/20	1/20 NA	NA 0/20	1/20 0/20	0/20 NA	0/20 1/20
Abortions	0/25	0/25	0/25	0/25	0/25	0/25	1/25	0/25	1/25	0/20 0/20	0/20 NA	NA 0/20	0/20 2/20	1/20 NA	0/20 1/20
Premature delivery	0/25	0/25	0/25	0/25	0/25	0/25	1/25	1/25	1/25	0/20 0/20	0/20 NA	NA 1/20	1/20 0/20	0/20 NA	0/20 0/20
Body weight gain vs. con. (D6 to D9)	-	↓200% **	↓300% **	↓327% **	-	↓12%	↓12%	↓53% **	↓76% **	- -	NC NA	NA ↓240% **	↓267% ** ↓520% **	↓400% ** NA	↓422% ** ↓780% **
Body weight gain vs. con. (D6 to end of study)	-	↓40% **	↓51% **	↓63% **	-	NC	NC	NC	NC	- -	↓12% * NA	NA ↓41%	↓35% * ↓63%*	↓42% ** NA	↓42% ** ↓66%*
Early resorptions	9	17	17	20	6	16	4	10	9	0 6	0 NA	NA 2	2 14	5 NA	13** 11
Late resorptions	0	0	1	0	3	3	5	5	6	0 2	4 NA	NA 3	2 6	11* NA	7 6
Postimplantation loss/litter	2.4%	4.8%	4.7%	5.6%	3.4%	7.7%	3.7%	6.3%	6.8%	0% 5.0%	3.1% NA	NA 2.7%	5.9% 11.4%	10.1%* NA	12.7%** 11.4%
Dams/Does with any resorption	7	12	14	15	7	12	9	11	8	0 6	3 NA	NA 4	3 9	7** NA	11** 11**
Findings - Fetal incidence															
Fetal body weight (mean)	-	↓8% **	↓10% **	↓13% **	-	NC	↓4%	↓8%**	↓13%**	- -	NC NA	NA NC	NC ↓8%	NC NA	NC ↓8%

Species	Rat				Mouse[†]					Rabbit[††]					
Test Article	Exenatide extended-release				Exenatide					Exenatide					
Dose Level (µg/kg)	0	300	1000	3000	0	6	68	460	760	0	0.2	2	22	156	260
Cleft palate[#]	0	0	0	1 (0.3%)	4 (1.3%)	5 (2.1%)	2 (0.8%)	3 (1.3%)	7 (3.4%)	0 0	0 NA	NA 0	0 1 (0.7%)	0 NA	0 0
Situs inversus	0	0	0	2 (0.6%)	0	0	0	0	0	0 0	0 NA	NA 0	0 0	0 NA	0 0
Umbilical hernia	0	0	0	0	0	0	0	1	0	0 0	0 NA	NA 0	2 (1.6%) 1 (0.7%)	8* (5.6%) NA	17** (11.8%) 8 (6.2%)
Gallbladder - small	0	0	0	0	0	0	0	0	0	0 1 (0.6%)	2 (1.2%) NA	NA 0	7** (5.6%) 0	5** (3.5%) NA	4* (2.8%) 0
Skeletal															
Hyoid - ala, angulated	0	0	0	0	0	0	0	0	0	2 (1.2%) 0	3 (1.8%) NA	NA 5 (3.5%)	7* (5.6%) 3 (2.2%)	7* (4.9%) NA	11** (7.6%) 2 (1.5%)
Sternal centra: fused	0	0	0	0	2	3	0	1	0	0 1 (0.6%)	0 NA	NA 2 (1.4%)	0 8 (5.8%)	5** (3.5%) NA	4** (2.8%) 3 (2.3%)
Ossification sites -vertebrae, thoracic	NR	NR	NR	NR	13.22	13.41	13.23	13.43*	13.40	12.51 12.55	12.55 NA	NA 12.80 **	12.80** 12.71	12.84** NA	12.90** 12.85**
Ossification sites -vertebrae, lumbar	NR	NR	NR	NR	5.78	5.58	5.77	5.57*	5.59	6.48 6.44	6.43 NA	NA 6.20*	6.19** 6.27	6.16** NA	6.09** 6.14**

Species	Rat				Mouse†					Rabbit††					
Test Article	Exenatide extended-release				Exenatide					Exenatide					
Dose Level (µg/kg)	0	300	1000	3000	0	6	68	460	760	0	0.2	2	22	156	260
Ossification sites -ribs (pairs)	NR	NR	NR	NR	13.17	13.37*	13.18	13.39*	13.32	12.47 12.49	12.49 NA	NA 12.73*	12.73* 12.65	13.10** NA	12.84** 12.78**
-Wavy ribs (rat) or irregular shaped ribs (rabbit)	0	0	0	0	0	0	0	0	3** (2.8%)	0 0	0 NA	NA 0	0 1 (0.7%)	0 NA	0 1 (0.8%)
Cervical centrum #1 ossified	58 (17.6%)	72 (22.5%)	29 (8.0%)	21* (6.8%)	NR	NR	NR	NR	NR	NR NR	NR NA	NA NR	NR NR	NR NA	NR NR
Reduced ossification of the vertebral arches	0	1 (0.3%)	5 (1.2%)	2 (0.6%)	NR	NR	NR	NR	NR	NR NR	NR NA	NA NR	NR NR	NR NA	NR NR
Entire sternum unossified	0	0	1 (0.3%)	2 (0.6%)	NR	NR	NR	NR	NR	NR NR	NR NA	NA NR	NR NR	NR NA	NR NR

MRHD = maximum recommended human dose; NA = not applicable; NC = no meaningful change from control; NOAEL = no observed adverse effect level; NR = specific endpoint not reported.

*p≤0.05; **p≤0.01

†Mouse TK data was extrapolated from 91 day toxicity study conducted with nonpregnant mice.

††Two rabbit studies were conducted, REST99061R2 and REST02022, each with slightly different dose levels. For the rabbit data rows, the top row within a data cell is from REST99061R2 and the bottom row is from REST02022. AUC was not calculated for these rabbit studies; TK data are from other studies obtained from the review of NDA 21-773.

†††Exposure margins were calculated by dividing the mean nonclinical $AUC_{0\text{-}24h}$ value by the mean clinical $AUC_{0\text{-}24h}$ value for Bydureon. Note that the presence or absence of antibodies was not factored into the AUC values for the DART studies. Mean clinical daily exposure for exenatide extended-release (Bydureon) is 5.461 ng·h/mL, which is based on an $AUC_{0\text{-}168h}$ of 38.23 ng·h/mL for antibody-negative patients at Week 30 in Study 2993LAR-105.

#Historical control data for cleft palate at the laboratory conducting the mouse study is 0-4 (0-1.2%) fetuses with cleft palate per study.

Conclusions:

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 (especially in rabbits). These effects generally occurred in the presence of maternal toxicity (decreased body weight gain and decreased food consumption), with effects on maternal body weight being greatest in rats and rabbits. 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 the consequence of reduced maternal nutrition due to decreased food consumption and decreased weight gain. The only developmental structural malformations that were attributed to or possibly attributed to exenatide were umbilical hernias in rabbits (statistically significantly increased at 544X clinical exposure) and a slight numerical increase in the incidence of cleft palate at the highest dose tested in the mouse teratogenicity study (~200X clinical exposure).

It is difficult to determine whether the difference in exposure profiles between exenatide extended-release and immediate-release exenatide (i.e., steady state vs. pulsatile) has a meaningful effect on maternal toxicity and fetal development because studies were not conducted in the same species with both exenatide formulations. Rats are more sensitive to exenatide-induced body weight and food consumption effects than mice, so it would not be unexpected that maternal toxicity and delayed fetal growth would occur at lower exposures in rats than in mice, regardless of the formulation. Therefore, it cannot be determined whether the effects observed in rats at lower clinical exposure margins are due to a difference in exposure profile between the two exenatide formulations or a difference in sensitivity to exenatide between these two rodent species. Overall, the continuous exposure of exenatide in rats did not result in malformations or a fetal toxicity profile that is significantly different from the immediate-release formulation when tested in mice and rabbits. Therefore, the overall embryo-fetal toxicity profile appears similar between exenatide extended-release (Bydureon) and immediate-release exenatide (Byetta).

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/s/

BRIAN T HUMMER
12/14/2011

KAREN L DAVIS BRUNO
12/16/2011

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**022-200**
SUPPORTING DOCUMENT NUMBER:	**022**
DATE RECEIVED BY CENTER:	**22 April 2010**
PRODUCT:	**Exenatide Once Weekly (BYDUREON)**
INTENDED CLINICAL POPULATION:	**Type 2 Diabetes**
SPONSOR:	**Amylin Pharmaceuticals, Inc.**
DOCUMENTS REVIEWED:	**Complete Response: resubmission**
REVIEW DIVISION:	**Division of Metabolism and Endocrinology Drug Products (HFD-510)**
PHARM/TOX REVIEWER:	**B. Timothy Hummer, Ph.D., DABT**
PHARM/TOX SUPERVISOR:	**Karen Davis-Bruno, Ph.D.**
DIVISION DIRECTOR:	**Mary Parks, M.D.**
PROJECT MANAGER:	**John Bishai, Ph.D.**
Date of review submission to Document Archiving, Reporting, & Regulatory Tracking System (DARRTS):	**08 October 2010**

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The initial marketing application for Bydureon was received by the Agency on 05 May 2009 (Supporting Document #001). The application was not approved and a complete response letter was sent to the sponsor on 12 March 2010. The primary deficiencies noted in the complete response letter included product quality issues and the requirement for a Risk Evaluation and Mitigation Strategy (REMS). There were no nonclinical deficiencies noted in the complete response letter. The sponsor has resubmitted the marketing application for Bydureon as a complete response. This review addresses the nonclinical aspect of the resubmission of the marketing application for Bydureon.

After review of the initial NDA submission, it was the nonclinical reviewer's recommendation that Bydureon not be approved based on the finding of drug-related thyroid c-cell tumors in rats after treatment for 2 years at clinically relevant exposures. New nonclinical data were not included in the complete response currently under review. The deficiencies noted in the complete response letter for the original application noted clinical safety issues and not nonclinical issues per se. There continues to be insufficient data to determine the human relevance of drug induced thyroid c-cell tumors in rats. Therefore because new information has not been provided in this application, the Pharmacology/ Toxicology recommendation continues to be a complete response. Nonclinical post-marketing requirements (PMRs) aimed at providing additional information to address species specificity of the thyroid c-cell tumors were included in the pharmacology/toxicology reviews of the original submission.

Please refer to the initial pharmacology/toxicology review dated 22 February 2010 and supervisor's memo dated 01 March 2010 for detailed recommendations, nonclinical PMRs, and labeling.

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/s/

BRIAN T HUMMER
10/08/2010
Recommendation: Complete Response

KAREN L DAVIS BRUNO
10/08/2010

DEPARTMENT OF HEALTH & HUMAN SERVICES
Food and Drug Administration

Memorandum

Date: February 22, 2010
From: Karen Davis-Bruno PhD; Pharmacology Supervisor; DMEP
Subject: Supervisory Memo
To: NDA 22-200 Bydureon (exenatide QW)/Amylin
Re: B.Timothy Hummer PhD. Pharmacology/Toxicology Review NDA 22-200

Endogenous GLP-1 is degraded in minutes by dipeptidyl peptidase IV resulting in a half-life that limits clinical use. GLP-1 analogues are modified or formulated to create a prolonged half-life and favorable clinical dosing interval. However data from GLP-1 analogues under review have demonstrated that those with longer half-lives as well as sustained-release of short-acting analogues (NDA 22-341 A. Parola review pg. 466) are associated with rodent pre-neoplastic lesions and neoplasia. These rodent findings may be related to persistent GLP-1 receptor activation.

Bydureon is a synthetic peptide, GLP-1 receptor agonist with an extended duration of action; given once weekly compared to exenatide. Exenatide is GLP-1(7-36)amide (Byetta NDA 21-773) which is administered BID. Exenatide mimics several glucoregulatory actions of the endogenous incretin; GLP-1 including: glucose-dependent enhancement of insulin synthesis and secretion, inhibition of glucagon secretion and slowing of gastric emptying. Bydureon is a sustained release formulation of exenatide developed using PLG microspheres (50:50 poly (D,L)-lactide-co-glycolide). The PLG microspheres are likely contributors to the injection site findings in the toxicology studies noted in Dr. Hummer's review. Generally speaking injection site findings in animals are not necessarily of clinical significance because in humans injection site irritation becomes limiting to product use well before any tissue damage is seen.

The nonclinical development program for Bydureon (exenatide QW) is based on bridging new information with this once weekly formulation to the safety profile of exenatide immediate release characterized for Byetta (NDA 21-773). The bridging studies provided in NDA 22-200 include repeat dose studies with exenatide QW in rats (4-month duration) and monkeys (3, 9 months), a rat 2-year carcinogenicity and in vitro genotoxicity studies to qualify manufacturing changes and to establish the similarity in toxicity profiles of exenatide QW and immediate release exenatide (Byetta) formulations. Amylin previously conducted a rat and mouse carcinogenicity study with immediate release exenatide to support the market approval of Byetta. The primary concern is that Bydureon was associated with increased thyroid C-cell tumors in rats compared to Byetta.

Pharmacokinetics & Thyroid C-cell Tumors

Recent data from other GLP-1 receptor agonists with longer half-lives than endogenous GLP-1 as well as sustained release formulations of short acting GLP-1 analogues (SC infusion of exenatide immediate release in mice NDA 22-341 pg 466) suggest that persistent receptor activation may be associated with thyroid C-cell hyperproliferation in rodents.

Thyroid C-cell hyperproliferative changes (hyperplasia, adenoma, carcinoma) are rare

findings (<1%) in mouse carcinogenicity studies. Diffuse and focal hyperplastic responses as well as adenomas are common findings in aging rats, however malignant C-cell carcinoma is a rare finding (<1%) in a rat carcinogenicity study.

Statistically significant increases in rat thyroid C-cell adenomas appear in males at 1 mg/kg and in females at 0.3 mg/kg suggesting a NOAEL <0.3 mg/kg; which is less than clinical exposure based on AUC comparisons across species. The combined incidence (adenomas + carcinomas) appears to be driven primarily by the adenoma incidence. Therefore the NOAEL for tumorigenicity (benign + malignant) with Bydureon is less than clinical exposures based on AUC (<0.3 mg/kg). The malignant carcinomas appear in males at 1 mg/kg (8-times clinical exposure) and exceed the the historical control range in females at 3 mg/kg however the incidence is not statistically significantly increased for males or females. Fibromas (benign) do not appear in females but are statistically significantly increased at 3 mg/kg in males at exposures 26-times higher than clinical exposure. The fibromas are likely a result of the continued presence of microspheres over the lifetime of the rodent. The clinical significance of this finding is questionable because the concentrations of drug needed to elicit this finding was 26-times higher than the maximum clinical exposure. As stated earlier clinical use would likely be discontinued due to irritation reactions well before any tissue damage resulted with the product.

Summary of Tumor Incidence from 2-Year Carcinogenicity Study in Male Rats Treated With Bydureon

Dose (mg/kg)	Diluent Control	Micro-sphere Control	0.3	1.0	3.0	Histor-ical Control
Exposure Margin¤			2X	10X	26X	
Thyroid, c-cell hyperplasia	15/70 (21%)	10/70 (14%)	23/70 (33%)	19/70 (27%)	23/70 (33%)	NP
c-cell adenoma	9/70 (14%) **p<0.001†**	9/70 (14%) **p<0.001†**	**20/70** (29%) p=0.038	**32/70** (46%) **p<0.001***	**33/70** (47%) **p<0.001***	8.8% (1.9-15.4%)
c-cell carcinoma	0/70 (0%) p=0.164	1/70 (1.4%) p=0.237	2/70 (2.9%) p=0.268	**5/70** (7.1%) **p=0.036***	**3/70** (4.3%) p=0.133	0.6% (0-1.7%)
c-cell adenoma + carcinoma	9/70 (13%) **p<0.001†**	10/70 (14%) **p<0.001†**	**22/70** (31%) p=0.019	**34/70** (49%) **p<0.001***	**35/70** (50%) **p<0.001***	NP
Skin, subcutis, Fibroma	0/70 (0%) **p=0.004†**	3/70 (4.3%) p=0.034	4/70 (5.7%) p=0.069	2/70 (2.9%) p=0.273	**8/70** (11%) **p=0.004***	2.2% (0-5%)

Historical control data from 11 studies; NP = not provided.
*Statistically significant by pair-wise analysis compared with diluent control.
†Statistically significant for dose response.
¤Based on mean AUC values for antibody negative animals on Day 183 compared with mean AUC values for antibody negative humans.

Summary of Tumor Incidence from 2-Year Carcinogenicity Study in Females Treated with Bydureon

Dose (mg/kg)	Diluent Control	Micro-sphere Control	0.3	1.0	3.0	Histor-ical Control
Exposure Margin¤			1X	8X	25X	
Thyroid, c-cell hyperplasia	13/70 (19%)	12/70 (17%)	31/70 (44%)	29/70 (41%)	40/70 (57%)	NP
c-cell adenoma	5/70 (7.1%) p=0.024	9/70 (13%) p=0.072	**22/70** (31%) **p<0.001***	**19/70** (27%) **p=0.003***	**21/70** (30%) **p<0.001***	8.1% (2.0-11.4%)
c-cell carcinoma	0/70 (0%) **p=0.014+**	1/70 (1.4%) p=0.042	1/70 (1.4%) p=0.533	1/70 (1.4%) p=0.517	**4/70** (5.7%) p=0.064	0.6% (0-4.0%)
c-cell adenoma + carcinoma	5/70 (7%) **p=0.003+**	10/70 (14%) p=0.016	23/70 (33%) **p<0.001***	20/70 (29%) **p=0.002***	25/70 (36%) **p<0.001***	NP

Historical control data from 11 studies; NP = not provided.
*Statistically significant by pair-wise analysis compared with diluent control.
+Statistically significant for dose response.
¤Based on mean AUC values for antibody negative animals on Day 183 compared with mean AUC values for antibody negative humans.

The tumor incidence with Bydureon is distinctly different than that observed with immediate release exenatide (Byetta). Statistically significant increases in drug-related tumors were not observed in either the rat or mouse with Byetta. However an increased incidence of thyroid C-cell adenomas was observed in all Byetta treated females relative to controls. The incidence in high dose females was 23% relative to concurrent controls (5%. 8%) and exceeds the historical control mean (5%) and range (0-10%) suggesting that the thyroid C-cell adenomas at 95-times higher exposure than that achieved at the highest clinical dose, were drug-related. Dose selection for the Byetta carcinogenicity study was a single daily dose that differs from the clinical twice daily (BID) dosing. The lowest total daily doses tested in the carcinogenicity studies exceed the maximum human daily exposure by at least 5-times.

Thyroid C-cell Tumors in 2-year Rodent Carcinogenicity Studies with Byetta Treatment					
Rat Dose mcg/kg/d	0	0	18	70	250
Human Exposure multiple[a]			12X	28X	95X
Female C-cell adenoma (common)[b]	(5/65)8%	(3/65)5%	(9/65)14%	(7/65)11%	(15/65)23%
Male C-cell adenoma (common)[b]	(8/65) 12%	(10/65) 15%	(10/65) 15%	(15/65) 23%	(10/65) 15%

Mouse Dose mcg/kg/d	0	0	18	70	250
Human Exposure multiple[a]			5X	21X	74X
No dose related or statistically signficant mouse tumors					

a= based on AUC relative to 10 mcg/d MRHD
b= tumor considered common or rare based on incidence in historical control groups of >1% or <1% respectively Historical control range for C-cell adenoma reported as 0-10%

Previous experience with GLP-1 agonists also includes the recent market approval of Victoza (NDA 22-341) where 2-year rat and mouse carcinogenicity studies were performed. Dose and treatment duration dependent, statistically significant thyroid C-cell tumors (benign + malignant) were observed in rats treated at exposures less than clinical exposure based on AUC (0.075 mg/kg). As with Bydureon the total tumor incidence appears to be driven primarily by the adenoma (benign) incidence. Trend analysis in contrast to pairwise statistical signficance, reveals an increase in tumorigenicity in both male and female rats given at less than clinical exposure to Victoza. Thyroid C-cell carcinomas were statistically significantly increased in high dose male rats at 8-times clinical exposure based on AUC comparisons. Statistically significant increases in adenomas occurred at ≥1 mg/kg Victoza in mice at exposures 10-times clinical exposure. C-cell tumors (benign or malignant) are rarely seen in mice in contrast to rats and therefore their appearance is noteworthy. Overall the tumor findings with Victoza are remarkably similar to those seen with Bydureon.

Table 1. C-cell tumors in the 2-year rodent carcinogenicity studies with Victoza

RATS	**Males**				**Females**			
Dose (mg/kg/day)	**0**	**0.075**	**0.25**	**0.75**	**0**	**0.075**	**0.25**	**0.75**
Human exposure multiple[a]	**-**	**0.5**	**2.2**	**7.6**	**-**	**0.5**	**2.2**	**7.6**
N	50	49	50	50	50	49	49	50
C-cell adenoma (common)[b]	6 (12%)	8 (16%)	**21 (42%)**	**23 (46%)**	5 (10%)	**13 (27%)**	**16 (33%)**	**28 (56%)**
C-cell carcinoma (rare)[b]	1 (2%)	**4 (8%)**	**3 (6%)**	**7 (14%)**	0	0	**2 (4%)**	**3 (6%)**

MICE	**Males**					**Females**				
Dose (mg/kg/day)	**0**	**0.03**	**0.2**	**1**	**3**	**0**	**0.03**	**0.2**	**1**	**3**
Human exposure multiple[a]	**-**	**0.2**	**1.8**	**10.0**	**45.0**	**-**	**0.2**	**1.8**	**10.0**	**45.0**
N	79	66	65	67	79	75	66	67	66	76
C-cell adenoma (rare)[b]	0	0	0	**9 (13%)**	**15 (19%)**	0	0	0	**4 (6%)**	**15 (20%)**
C-cell carcinoma (rare)[b]	0	0	0	0	0	0	0	0	0	**2 (3%)**

Bolded data reflect treatment-related increases
a=based on area under the time-concentration curve relative to the 1.8 mg dose
b=tumor considered common or rare based on incidence in historical control groups of >1% or <1%, respectively

In a similar manner to Victoza, the occurrence of thyroid C-cell tumors in both sexes with Bydureon treatment of the rat in 2-year carcinogenicity studies at or below relevant human therapeutic exposures raises the possibility that patients may be at increased risk for this tumor. Studies submitted with the Victoza application explored the mechanism of tumor formation and its human relevance focused on Victoza binding to GLP-1 receptors on thyroid C-cells and increased synthesis and secretion of calcitonin from these cells. As a result of this activation the applicant proposed that C-cells underwent proliferation and with persistent activation, progressed to adenomas and carcinomas in rodents but this was not operational in primates or in humans. The review of this mechanistic data by the Agency as well as outside experts in an Advisory Committee disagreed with the sponsor, concluding that the mechanism of tumor formation was not established nor did they convincingly demonstrate that the tumor findings were irrelevant to humans. Since at least some humans express GLP-1 receptors in the thyroid, while it is unclear if primates do, there is a concern for human relevance of the demonstrated rodent thyroid C-cell tumors.

Important distinctions occur with veterinary histopathology relative to clinical pathology. A major difference is that for clinical pathology the biopsied tissues are extensively sectioned in order to determine a definitive diagnosis. This is not the case in the histopathology of a

standard tissue battery during nonclinical drug development where minimal numbers of tissue sections are typically taken from a tissue of interest or identified rodent tumor. Furthermore, the criteria for differentiating benign from malignant are far from clear. The morphology is quite similar. The distinction relates to a judgment by the reviewing pathologist on the aggressive/invasive behavior of the carcinomas. C-cell adenomas are usually well circumscribed, nodular proliferations that do not penetrate the capsule surrounding the thyroid. Carcinomas do penetrate the capsule and may require evaluation of multiple sections to see it and they metastasize. Often necrosis or an increased mitotic rate and/or fibrodysplasia occurs with carcinomas. While malignant carcinomas might be of greater clinical concern than benign adenomas, the distinction between these tumor types in animal thyroid histopathology from carcinogenicity studies is not as decisive.

An association between C-cell focal hyperplasia/neoplasias and persistent GLP-1 receptor activation can be inferred based on the increased incidence of these findings with administration of long-acting GLP-1 agonists, or shorter-acting analogues when administered continuously compared to GLP-1. In rat carcinogenicity studies there is an increased tumor signal for Bydureon (exenatide QW) compared to Byetta. However the plasma exposures (AUC) suggest reduced exposure with Bydureon compared to Byetta (see sponsor Table 6 below). This indirectly supports the importance of exposure pattern (pulsatile vs. continuous) rather than absolute exposure as being important in comparing the tumorigenicity signals across products. This effect might be of greater magnitude given that anti-Bydureon antibodies in rat tended to increase exposure (AUC) to drug.

Table 6: Comparison of Representative Derived Daily AUC in Repeated Dose Toxicology and Carcinogenicity Studies With BYETTA and Exenatide QW

					Derived Daily AUC (pg·h/mL) [1]	
Study Type	**Species**	**Duration**	**Dose [2]**	**Comment**	**BYETTA**	**Exenatide QW**
Repeated Dose	Rats	2 Months	9000 [3]	NOAEL	n/a	192,269 [4]
		3 Months	250	NOAEL	201,764	n/a
		4 Months	9000 [3]	NOAEL	n/a	146,914[5]
	Monkeys	3 Months	1100	NOAEL	n/a	104,777
		9 Months	150	NOAEL	1,000,708	n/a
		9 Months	1100	NOAEL	n/a	77,949
Carcinogenicity	Rats	2 Years	18 [6]	Low	20,188	n/a
			70 [6]	Mid	45,619	n/a
			250 [6]	High	201,764	n/a
			300 [3] [7]	Low	n/a	9483
			1000 [3] [7]	Mid	n/a	50,869
			3000 [3] [7]	High	n/a	140,546

AUC = area under the plasma concentration-time curve; n/a = 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 exenatide QW studies antibody-negative animals at end of study). Daily AUC estimates for BYETTA were determined by doubling the exposure following a single dose, and daily AUC estimates for exenatide QW were determined by dividing the exposure following a single dose by the dosing interval (days).

[2] Doses expressed in mcg/kg/day for BYETTA and mcg/kg/week for exenatide QW, unless otherwise specified.

[3] Dose administered once every other week.

[4] In this 2-month study (REST060307), the daily AUC value obtained at a dose of 3 mg/kg, the highest dose tested in the ongoing rat carcinogenicity with exenatide QW, was 74,676 pg·h/mL.

[5] In this 4-month study (REST050369), the daily AUC value obtained at a dose of 3 mg/kg, the highest dose tested in the ongoing rat carcinogenicity with exenatide QW, was 142,488 pg·h/mL.

[6] Data is from the 3 month rat study conducted at the same doses.

[7] Data is from TK animals within the study at the 6 month time point.

Rat and monkey toxicokinetic data showed that steady-state concentrations were achieved within a month of Bydureon treatment and clearance of the drug post-dose occurred within 2 months. Anti-drug antibodies (ADA) were observed in both rat and monkeys without a neutralizing effect, although there was an affect on drug exposure. In rats, exposure (AUC) increased in the presence of ADA and in monkeys AUC increased in the presence of low antibody titers but decreased with higher antibody titers. This was the rationale for using ADA negative exposure values for the calculation of exposure margins in the toxicity studies

in Dr. Hummer's review. This may reflect the perturbation of the renal clearance of exenatide in the presence of ADA. Mechanistic studies performed with Byetta under NDA 21-773 suggest that reabsorption and proteolytic degradation of exenatide occurs in the renal tubule after filtration. This process might be influenced by the presence of large molecular weight antibodies. A 4.3% increase (4/70 vs. 0/70 controls) in renal tubular cell tumors (adenomas + carcinomas) was observed in high dose females (25-times higher than human exposure) from the 2-year Bydureon carcinogenicity study which were not statistically significant. Renal tubular cell adenomas were observed in a 4-month rat toxicity study.

Victoza and Bydureon are non-genotoxic carcinogens and therefore it is possible that early preneoplastic events induced by these agents could be reversible. Further studies may address this (see PMR #3). Studies up to 26 weeks in rats given Victoza did not show preneoplastic (thyroid C-cell focal hyperplasia; FCCH) effects. However, thyroid C-cell hyperplasia was noted in mouse studies of 4-13-weeks duration. The reversibility of the hyperplasia was partially reversed after a 15-week recovery period. Whether any residual thyroid effects would have progressed to adenoma or carcinoma are unknown. Bydureon 2-year rat carcinogenicity study results indicate the presence of treatment related C-cell hyperplasia. However studies of shorter duration treatment did not demonstrate a thyroid preneoplastic response. Six-month treatment of mice with exenatide BID doesn't result in FCCH and we have rat data with exenatide QW every other week at up to 9 mg/kg/dose without FCCH. New data that can be obtained as a PMR may provide some mouse preneoplastic data with exenatide QW and confirm the mechanistic data provided in Victoza NDA 22-341 where SC infused exenatide (NovoNordisk Byetta) resulted in focal C-cell hyperplasia in mice. In contrast, thyroid C-cell hyperplasia was not noted with Byetta. Minimal information is available regarding GLP-1 receptor expression and tissue localization in humans. There is a publication[1] that assesses binding affinity and density in thyroid across species:

	GLP-1 R	Rat	Mouse	Human
Thyroid	Incidence	12/12(100%)	3/5 (60%)	1/18 (6%)
	Density (dpm/mg)	2289 ± 282	1982 ± 470	1193
Lung	Incidence	3/3 (100%)	6/6 (100%)	11/28 (39%)
	Density (dpm/mg)	3477 ± 1539	1677 ± 439	636 ± 164

This same publication states that the highest GLP-1 receptor expression is present in insulinomas, gastrinomas and pheochromocytomas. Human carcinomas were found to express virtually no GLP-1 receptors, although studies by others indicate functional GLP-1 receptors in human pancreatic adenocarcinoma cell lines. The down-regulation of GLP-1 receptors in islets in chronic pancreatitis suggests that the impaired glucose tolerance in these patients may be due not only to a loss of islets but also to a reduced responsivenes of β-cells to GLP-1. There is a remarkable difference between human and rodents in GLP-1 receptor expression in thyroid tissue based on this limited data. This suggests that not all human thyroids have detectable GLP-1 receptors. The authors note that receptors could not be assigned with certainty to either the follicular epithelium or the medullary C-cells in the rodent or human tissues used.

Recommendation:
Dr. Hummer's Pharmacology/Toxicology review of the available nonclinical data recommends not approving this marketing application. I agree with his recommendation

[1] J Nuclear Med 48(5):736-743; 2007 Korner, Stockli, Waser, Reubi; GLP-1 Receptor Expression in Tumors and Human Normal Tissues: Potential for in vivo Targeting

based on the drug-related carcinogenicity signal observed in the rat life-time bioassay at relevant therapeutic exposures. By inference these rodent tumorigenic findings are potentially clinically relevant as there is an absence of mechanistic data to suggest otherwise. Suggestions for further studies have been offered in Dr. Hummer's review and are discussed as potential PMRs in this memo. This recommendation is based on the nonclinical data which is useful in identifying risk without assessing clinical benefit, both of which are important in the regulatory decision making process.

The rodent carcinogenicity data raise concern for long-term human safety rather than providing reassurance that no clinical risk exists. Nonclinical post-marketing studies (PMR) may provide some additional background to inform prescribing physicians in labeling, although they may not provide any true assessment of long-term human outcome. These recommended studies include:

1. A 2-year mouse study with 3 doses of exenatide QW to yield multiple of human exposure of 10-, 30-, 100X and assess thyroid C-cell focal hyperplasia; a preneoplastic lesion in mice. Interim assessments after 13-weeks, and 26-weeks treatment with termination of treatment after 26 weeks and a follow-up of a subgroup after 1.5 years treatment free for focal thyroid C-cell hyperplasia and tumors at the end of the study.

The first PMR study recommended is designed to determine if Bydureon increases C-cell tumor incidence in mice treated long enough (based on the composite liraglutide data) to induce focal C-cell hyperplasia, but terminating treatment before tumors would be anticipated to develop. This proposal would inform us if limited exposure to Bydureon increases the lifetime risk of C-cell tumor induction even after treatment has been discontinued as well as informing us about whether another rodent species shows a demonstration of preneoplastic thyroid signals with Bydureon. The duration of treatment in this study is based on established C-cell hyperplasia (considered pre-neoplastic) after 9-weeks of liraglutide treatment and with continued lifetime exposure in the two year mouse carcinogenicity study, malignant tumors were not detected until lifetime exposure to drug.

2. A nonclinical study to determine whether thyroid C-cell hyperplasia is dependent on cell signaling mediated through GLP-1 receptor activation and/or RET oncogene signaling activation. The study should be conducted under steady state exenatide concentrations to determine whether the incidence of thyroid C-cell hyperplasia is a result of continuous cell signaling. Autoradiographic/immunohistochemical staining in thyroid tissue sections can be used to determine GLP-1 receptor localization in mice with and without focal C-cell hyperplasia. RET activation and downstream signaling assessments in normal C-cells and focal hyperplastic C-cells from mouse thyroid tissue sections are recommended.

The second PMR study recommended is intended to explore potential mechanisms of receptor mediated thyroid carcinoma formation across species. Activating mutations in rearranged during transfection (RET) proto-oncogene is responsible for most familial forms of medullary thyroid carcinoma (MTC)in humans. Somatic RET mutations have been observed as well in many sporadic MTC cases when thyroid tissue samples are examined. Results from this study might impact recommendations for further clinical monitoring. Limited published literature indicates that only 60% of mice thyroids express GLP-1 receptors. This proposed study will determine if GLP-1 receptor expression occurs in normal, preneoplastic (focal C-cell hyeprplastic) or neoplastic C-cells. If the C-cell tumors are linked to GLP-1 receptors, then GLP-1 knockout mice would not be anticipated to develop focal C-cell hyperplasia.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22200	ORIG-1	AMYLIN PHARMACEUTICALS INC	Bydureon (exenatide LAR)

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/s/

KAREN L DAVIS BRUNO
03/01/2010

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**022-200**
SUPPORTING DOCUMENT NUMBER:	**001**
DATE RECEIVED BY CENTER:	**05 May 2009**
PRODUCT:	**Exenatide Once Weekly (BYDUREON)**
INTENDED CLINICAL POPULATION:	**Type 2 Diabetes**
SPONSOR:	**Amylin Pharmaceuticals, Inc.**
DOCUMENTS REVIEWED:	**Electronic CTD**
REVIEW DIVISION:	**Division of Metabolism and Endocrinology Drug Products (HFD-510)**
PHARM/TOX REVIEWER:	**B. Timothy Hummer, Ph.D., DABT**
PHARM/TOX SUPERVISOR:	**Karen Davis-Bruno, Ph.D.**
DIVISION DIRECTOR:	**Mary Parks, M.D.**
PROJECT MANAGER:	**John Bishai, Ph.D.**
Date of review submission to Document Archiving, Reporting, & Regulatory Tracking System (DARRTS):	**22 February 2010**

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Not approvable (CR)

On the basis of the nonclinical data, it is recommended that this application not be approved. In a 2-year carcinogenicity study, rats developed thyroid c-cell tumors at clinically relevant exposures. There is currently insufficient information to conclude that GLP-1 receptor agonist-induced c-cell tumors are not relevant to human risk. Due to a lack of data, human risk cannot be dismissed or quantified, and therefore, additional nonclinical data are required to adequately assess the human relevance of this nonclinical finding.

Although a tumorigenicity signal exists in rodents for this product, as well as for other compounds in this therapeutic class, the ultimate decision for marketing approval will be at the discretion of the medical review team, as the risk to benefit ratio for this therapeutic product will be considered to a greater extent than for the nonclinical review. It is recognized that there may be mitigating factors that could allow this product to be approved when considering the information from all review disciplines. Some of these factors include:

- Clinical benefit: Once weekly administration compared with once or twice daily administration of currently approved GLP-1 receptor agonists should enhance patient compliance, thereby increasing the likelihood of adequate, long-term glucose control.
- Labeling: A boxed warning on the product label would provide a strong message to health care providers and consumers about the potential risk for thyroid c-cell tumors.
- Post-marketing requirements: Information from nonclinical studies investigating the mechanism of c-cell tumor formation in rodents and the relevance to humans could alleviate concerns about the risk for drug-induced c-cell tumors in humans within a few years. Additionally, a human cancer registry for patients taking Bydureon would allow the Agency to further assess human risk by collecting additional clinical data.
- Regulatory precedence: Another GLP-1 receptor agonist that shows a similar risk for thyroid c-cell tumors as Bydureon was recently granted marketing approval.

B. Recommendation for nonclinical studies

The primary concern regarding the rodent carcinogenicity data is our current lack of understanding regarding human relevance. Therefore, nonclinical studies with an objective to determine a mechanism of action for the development of thyroid c-cell tumors would be most beneficial. By understanding the mechanism(s) for c-cell hyperplasia and progression to adenomas and carcinomas in rats and mice, one would then be able to apply that knowledge to determine whether human c-cells respond in a similar manner. Although it would be ideal to receive this information before marketing approval, a clinical risk to benefit evaluation of this product may warrant such studies to be conducted as a post-marketing requirement.

Specific study recommendations will be communicated to the sponsor through a letter describing the need for a complete response or a letter listing specific nonclinical post marketing requirements. However such studies could include, but are not limited to, those that investigate:

a. Whether the development of c-cell hyperplasia is dependent on thyroid GLP-1 receptor activation? This question could be investigated through the use of a GLP-1 receptor knockout mouse model.

b. If hyperplasia and tumor development is dependent on the GLP-1 receptor on c-cells, how does the presence of GLP-1 receptors on c-cells compare across species? Does receptor density or extent of receptor activation play a role in the degree of the hyperplastic response? Is the hyperplastic response dependent on exenatide-induced alterations of growth regulatory gene expression and do those alterations also occur in human c-cells?

C. Recommendations on labeling

Because c-cell tumors occurred in rats at clinically relevant exposures and the relevance to human risk is currently unknown, if Bydureon is granted marketing approval, a boxed warning is being recommended. Inclusion of a boxed warning is consistent with how the c-cell tumor risk for Victoza is being communicated to physicians and patients.

WARNING: RISK OF THYROID C-CELL TUMORS

See full prescribing information for complete boxed warning.

- **Exenatide QW causes thyroid C-cell tumors at clinically relevant exposures in rats. It is unknown whether BYDUREON causes thyroid C-cell tumors, including medullary thyroid carcinoma (MTC), in humans, as human relevance has not been determined by clinical or nonclinical studies (5.1).**
- **BYDUREON is contraindicated in patients with a personal or family history of MTC or in patients with Multiple Endocrine Neoplasia syndrome type 2 (MEN 2) (5.1).**

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

- Thyroid C-cell tumors in animals: Human relevance unknown. Counsel patients regarding the risk of medullary thyroid carcinoma and the symptoms of thyroid tumors (5.1).

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

- Pregnancy: Based on animal data, BYDUREON may cause fetal harm. BYDUREON should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. To report drug exposure during pregnancy call 1-800-XXX-XXXX (8.1).
- Nursing Mothers: Caution should be exercised when BYDUREON is administered to a nursing woman (8.3).

5 WARNINGS AND PRECAUTIONS

5.1 Risk of Thyroid C-cell Tumors

(b) (4)

[redacted]

[redacted]

8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies of BYDUREON use in pregnant women. (b) (4) [redacted]

Female mice given SC doses of exenatide, the active ingredient of BYDUREON, at 6, 68, or 760 mcg/kg/day beginning 2 weeks prior to and throughout mating until gestation day 7, had no adverse fetal effects. At the maximal dose, 760 mcg/kg/day, systemic exposures were up to 148 times the human exposure resulting from the recommended dose of 2 mg/week, based on AUC [see *Nonclinical Toxicology (13.3)*].

In developmental toxicity studies, pregnant animals received exenatide, the active ingredient of BYDUREON, subcutaneously during organogenesis. Specifically, fetuses from pregnant rabbits given SC doses of exenatide at 0.2, 2, 22, 156, or 260 mcg/kg/day from gestation day 6 through 18 experienced irregular skeletal ossifications from exposures (b) (4) times the human exposure resulting from the recommended dose of 2 mg/week, based on AUC. Fetuses from pregnant mice given SC doses of exenatide at 6, 68, 460, or 760 mcg/kg/day from gestation day 6 through 15 demonstrated reduced fetal and neonatal growth, cleft palate, and skeletal effects at a systemic exposure that is equivalent to human exposure resulting from the recommended dose of 2 mg/week, based on AUC [see *Nonclinical Toxicology (13.3)*].

Lactating mice given SC doses of exenatide, the active ingredient of BYDUREON, at 6, 68, or 760 mcg/kg/day from gestation day 6 through lactation day 20 (weaning), experienced an increased number of neonatal deaths. Deaths were observed on postpartum days 2-4 in dams given 6 mcg/kg/day, a systemic exposure that is equivalent to human exposure resulting from the recommended dose of 2 mg/week, based on AUC [see *Nonclinical Toxicology (13.3)*].

Pregnancy Registry

Amylin Pharmaceuticals, Inc. maintains a Pregnancy Registry to monitor pregnancy outcomes of women exposed to exenatide during pregnancy. Physicians are encouraged to register patients by calling (800) 633-9081.

8.3 Nursing Mothers

(b) (4)

[redacted]

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

A 104-week carcinogenicity study was conducted with exenatide QW in male and female rats at doses of 0.3, 1.0, and 3.0 mg/kg (2, 9, and 26-times human systemic exposure based on AUC, respectively) administered by SC injection every other week. A statistically significant increase in thyroid C-cell tumor incidence was observed in both males and females. The incidence of C-cell adenomas was significantly increased at all doses (27% to 31%) in females and at 1.0 and 3.0 mg/kg (46% and 47%, respectively) in males compared with the control group (13% for males and 7% for females). A numerically higher incidence of C-cell carcinomas occurred in the high dose group females (6%), and numerically higher incidences of 3%, 7%, and 4% (non-statistically significant versus controls) were noted in the low, mid, and high dose group males compared with the control group (0% for both males and females). An increase in benign

fibromas was seen in the skin subcutis at injection sites of males given 3 mg/kg. The human relevance of these findings is currently unknown.

A 104-week carcinogenicity study was conducted with exenatide, the active ingredient in BYDUREON, in male and female rats at doses of 18, 70, or 250 mcg/kg/day administered by once daily bolus SC injection. Benign thyroid C-cell adenomas were observed in female rats at all exenatide doses. The incidences in female rats were 8% and 5% in the two control groups and 14%, 11%, and 23% in the low-, medium-, and high-dose groups with systemic exposures of 3, 6, and 27 times, respectively, the human exposure resulting from the recommended dose of 2 mg/week, based on plasma area under the curve (AUC).

In a 104-week carcinogenicity study with exenatide, the active ingredient in BYDUREON, in male and female mice at doses of 18, 70, or 250 mcg/kg/day administered by once daily bolus SC injection, no evidence of tumors was observed at doses up to 250 mcg/kg/day, a systemic exposure up to 16 times the human exposure resulting from the recommended dose of 2 mg/week, based on AUC. The carcinogenicity of BYDUREON has not been evaluated in mice.

Exenatide QW and exenatide were 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, at twice-daily SC 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 148 times the human exposure resulting from the recommended dose of 2 mg/week, based on AUC.

13.3 Reproductive and Developmental Toxicology

(b) (4)

A complete reproductive and developmental toxicity program was conducted with exenatide, the active ingredient in BYDUREON.

In female mice given twice-daily SC doses of 6, 68, or 760 mcg/kg/day exenatide, the active ingredient in BYDUREON, beginning 2 weeks prior to and throughout mating until gestation day 7, there were no adverse fetal effects at doses up to 760 mcg/kg/day, systemic exposures up to 148 times the human exposure resulting from the maximum recommended dose of 2 mg/day, based on AUC.

In pregnant mice given twice-daily SC doses of 6, 68, 460, or 760 mcg/kg/day exenatide, the active ingredient in BYDUREON, from gestation day 6 through 15 (organogenesis), cleft palate (some with holes) and irregular fetal skeletal ossification of rib and skull

bones were observed at 6 mcg/kg/day, a systemic exposure equal to the human exposure resulting from the maximum recommended dose of 2 mg/day, based on AUC.

In pregnant rabbits given twice-daily SC doses of 0.2, 2, 22, 156, or 260 mcg/kg/day exenatide, the active ingredient in BYDUREON, from gestation day 6 through 18 (organogenesis), irregular fetal skeletal ossifications were observed at 2 mcg/kg/day, a systemic exposure (b) (4) times the human exposure resulting from the maximum recommended dose of 2 mg/day, based on AUC.

In pregnant mice given twice-daily SC doses of 6, 68, or 760 mcg/kg/day exenatide, the active ingredient in BYDUREON, from gestation day 6 through lactation day 20 (weaning), an increased number of neonatal deaths was observed on postpartum days 2-4 in dams given 6 mcg/kg/day, a systemic exposure equal to the human exposure resulting from the maximum recommended dose of 2 mg/day, based on AUC.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Repeat-dose studies were conducted with exenatide QW, a sustained release formulation of exenatide, to compliment the existing toxicology program that had been conducted with immediate release exenatide in support of the marketing approval of Byetta (NDA 21-773). These additional studies include repeat-dose studies in rats (1 and 4 months) and monkeys (3 and 9 months), a rat carcinogenicity study, and several in vitro genetic toxicology studies to qualify manufacturing changes.

Based on the review of NDA 21-773, mice treated with immediate release exenatide twice daily at doses up to 380 µg/kg/dose for 6 months had microscopic findings in the eye (retinal atrophy, corneal mineralization, cataract), parotid salivary gland (basophilia), bone marrow hyperplasia, and injection site reactions (inflammation, hemorrhage, fibrosis, epithelial hyperplasia). A NOAEL could not be determined because parotid gland hyperplasia and pthisis bulbi (shrinkage and wasting of the eyeball) were observed at the low dose (9 µg/kg BID; ~2X the maximum recommended human dose [MRHD] based on exposure [AUC]).

Sprague-Dawley rats receiving exenatide QW every other week by subcutaneous injection at doses up to 9 mg/kg for 4 months resulted in slight decreases in body weight gain that generally correlated with decreased food consumption were observed at all doses, but particularly for males receiving 3 and 9 mg/kg. Injection-site reactions were the primary treatment-related effect for all groups receiving microspheres. Injection-site findings consisted of swelling/palpable lumps, with severity increasing as the dose of microspheres increased. Histopathology revealed foamy macrophages/fibroblasts, lymphocytic infiltrate, and granulomas of minimal to slight severity at all injection sites. Injection site findings reversed or showed a trend for recovery by the end of the 3-month recovery period. One MD and one HD female had renal tubular adenomas (approximately 10X and 27X MRHD, respectively, Ab negative AUC); the relationship to treatment was uncertain. The NOAEL for this study was placed at 9 mg/kg (~27X MRHD, Ab negative AUC) based on a lack of target organ toxicity.

The subcutaneous injection of exenatide QW once weekly to cynomolgus monkeys at doses up to 1.1 mg/kg for 3 and 9 months primarily resulted in injection site reactions. Macroscopic lesions at the injection sites were characterized by red or white discoloration, nodules, edema, abscesses, thickened tissue, and injection site enlargement. Some occurrences of abscesses with drainage were noted for all exenatide-treated groups in the 9-month study. Nodules appeared to increase in severity with increase in exenatide dose (3-month study) or microsphere dose (9-month study). Microscopically, the injection sites for all groups were characterized as having chronic inflammation, abscesses, epidermal hyperplasia, fibrosis, and/or hemorrhage, although these occurred at a lower incidence for the diluent control group. Granulomatous inflammation (minimal to severe), granulomas, and foreign material were noted at the injection site of animals receiving microspheres with or without exenatide. Granulomas were well-circumscribed with minimal fibrosis and consisted of foamy macrophages and multinucleated giant cells that often containing microspheres. The incidence of macroscopic and microscopic lesions was drastically reduced at the end of a 3-month recovery period, suggestive of reversibility. The NOAEL for both studies was 1.1 mg/kg (14-19X MRHD, AUC) on the basis of a lack of target organ toxicity.

Rat and monkey TK data showed that steady-state concentrations were achieved within a month of treatment and clearance of the drug after the final dose occurred within 2 months. Anti-exenatide antibodies were detected in both rats and monkeys after repeated dosing with exenatide QW. The presence of antibodies did not appear to have neutralizing activity, although it did have an effect on TK results. In rats, AUC values tended to increase in the presence of antibodies and in monkeys, AUC values tended to increase in the presence of low antibody titers but decreased with higher antibody titers. Because of these effects, mean AUC values for antibody negative animals and humans were used for exposure comparisons when available.

A carcinogenicity study in which Sprague-Dawley rats received exenatide QW by subcutaneous injection once every other week showed an increase in thyroid c-cell tumors (adenomas plus carcinomas) at all doses (≥0.3 mg/kg; 1X MRHD, Ab negative AUC) for both males and females (statistically significant for all groups except LD males). The incidence of c-cell adenomas was greater in this study than observed with immediate release exenatide, which is believed to be due to the difference in PK profiles between the two exenatide formulations. A statistical significant increase in fibromas of the skin was also observed in males treated with the high dose (3 mg/kg; 26X MRHD, Ab negative AUC). Fibromas were relatively acellular and were comprised primarily of bundles of collagen. The fibromas were not specifically noted as being at injection sites, but this was implied in the pathologists report. A non-statistically significant slight increase in renal tubular cell tumors (adenomas plus carcinomas) was observed in HD females (25X MRHD, Ab negative AUC); two tubular cell adenomas were also observed in a 4-month rat study; a relationship to test article remains uncertain. Other expected findings for exenatide (reduced body weight) and PLG microspheres (foreign body granulomas at the injection site) were also observed.

The carcinogenicity of exenatide QW was not evaluated in mice. Immediate release exenatide did not induce tumors in a mouse carcinogenicity study. However, based on mouse carcinogenicity results with other long-acting GLP-1 receptor agonists, this class of compounds also induces c-cell tumors in mice, although generally at higher clinical exposure margins than observed for rats. Therefore, based on the available data, it is assumed that if exenatide QW were to be tested in a mouse carcinogenicity study, thyroid c-cell adenomas would be observed; however it would also be expected that there would be a greater clinical exposure margin than observed for rats.

Exenatide and exenatide QW was not mutagenic or clastogenic in a battery of genetic toxicology studies.

The effect of immediate release exenatide on reproduction and embryonic development was previously investigated in support of the marketing application for Byetta (NDA 21-773). Results of a fertility and early embryonic development study in mice showed no exenatide-related adverse effects on estrus cycling, mating and fertility indices, numbers of corpora lutea, implantation, viable embryos, non-viable embryos, pre- or post-implantation viability, or cauda epididymal sperm motility, count, or density. Accordingly, the NOAEL for effects on male and female reproduction was the high dose of 380 µg/kd BID (148X MRHD). Note that all exposure margins presented for the developmental and reproductive toxicity are in relation to clinical exposures (AUC) for Bydureon.

In a mouse embryonic development study, maternal body weight gain and food consumption were slightly decreased at ≥230 µg/kg/dose, which is consistent with the pharmacodynamic activity of exenatide. Some abortions and premature deliveries were observed at ≥34 µg/kg/dose. The number of implantations, litter sizes, and live fetuses were significantly decreased for dams receiving ≥230 µg/kg/dose relative to control. Fetal body weights were decreased at ≥230 µg/kg/dose for males and ≥68 µg/kg/dose for females. Skeletal variations associated with delayed fetal growth included changes in the number of rib pairs or vertebral ossification sites and wavy ribs at ≥230 µg/kg/dose. Rare occurrences of fetuses with multiple abnormalities including cleft palate with or without hole were observed for most dose groups, including control. The maternal NOAEL was 3 µg/kg BID (1X MRHD) based on the observed abortions and the developmental NOAEL was 3 µg/kg BID on the basis of decreased fetal body weights, cleft palate, and wavy ribs.

Two rabbit embryonic development studies were conducted with immediate release exenatide doses ranging from 0.1 to 130 µg/kg twice daily. Apparent maternal toxicity characterized by profound weight loss and reduced food and water consumption was observed at doses ≥11 µg/kg/dose. Clinical indicators of starvation (β-hydroxybuterate and potassium) were also noted. Morphological markers of fetal growth retardation were observed that included umbilical hernias and skeletal variations of angulated hyoid, altered number of rib pair or vertebral bodies, and fused sternabrae at ≥11 µg/kg/dose. Fetal incidence of small gall bladder was significantly increased at 11, 78, and 130 µg/kg/dose. In the second study, skeletal variations were observed at ≥1 µg/kg/dose,

but were also present at a similar incidence in an untreated, pair-fed group, suggesting these effects were a consequence of compromised maternal health. For the first study, the maternal NOAEL was determined to be the low dose of 0.1 μg/kg BID (0.1X MRHD) based on dose-related decrease in weight gain during the treatment period. The developmental NOAEL was also 0.1 μg/kg BID (0.1X MRHD) based on the developmental retardation. For the second study, which was conducted to better define the NOAEL, the NOAEL for developmental toxicity was the low dose of 1 μg/kg BID (4X MRHD). TK data showed that the potential for exenatide to cross the placental barrier is very low in both mice and rabbits. Therefore the fetal findings observed in both species may have been a consequence of a reduction in the maternal nutritional state during gestation or maternal toxicity.

The effects of exenatide on gestation, parturition, lactation, and maternal behavior were evaluated in mice from implantation through lactation and weaning. The effects on development and fertility of the offspring were also evaluated. The F_0 maternal NOAEL was less than 3 μg/kg BID (<1X MRHD) due to mortality at ≥3 μg/kg/dose. The NOAEL for F_1 fetal viability and growth was 3 μg/kg BID (1X MRHD) because of reduced preweaning pup body weights at 34 μg/kg BID (9X MRHD) and 380 μg/kg BID (148X MRHD) and increased perinatal mortality and reduced body weight gains postweaning at 380 μg/kg BID. F_0 maternal administration of exenatide at doses as high as 380 μg/kg BID did not affect, learning, memory, day of preputial separation or day of vaginal patency, mating or fertility, or cesarean-sectioning parameters of the F_1 generation mice. There were no treatment-related effects on corpora lutea, implantations, litter sizes, or resorptions in cesarean-sectioned pregnant F_1 females or on the incidence of fetal alterations in F_2 generation mice.

An assessment of the local tolerance of exenatide QW at the injection sites was integrated into the repeat-dose toxicology studies. Injection site reactions were generally characterized by swelling, inflammation, and other findings typical of foreign body reactions in both rats and monkeys, with increased incidence and severity with increasing dose of microspheres. In monkeys administered exenatide QW for 9 months, swelling with open drainage and/or abscesses were observed in all groups receiving exenatide QW. Microscopically, granulomatous inflammation with foreign body giant cells and fibrosis were observed in rats and monkeys receiving microspheres, with or without exenatide.

B. Pharmacologic activity

Exenatide binds to and activates the GLP-1 receptor, a G protein-coupled receptor. Through its activation of the GLP-1 receptor, exenatide mimics many of the glucoregulatory activities of endogenous GLP-1 including stimulation of glucose-mediated insulin secretion and synthesis of pro-insulin, increased insulin sensitivity, suppression of glucagon release, increased pancreatic β-cell mass, slowing of nutrient absorption via inhibition of gastric emptying, and suppression of food intake. Pharmacology studies in rodent models of diabetes and obesity demonstrated that the administration of exenatide resulted in the lowering of serum glucose and HbA1c values in conjunction with decreased food intake and body weight. Like GLP-1, the glucose

lowering effect of exenatide has been shown to be dependent on glucose concentrations in the plasma.

C. Nonclinical safety issues relevant to clinical use:
In a rat carcinogenicity study conducted with exenatide QW, a statistically significant increase in thyroid c-cell tumors (adenomas plus carcinomas) relative to control groups was observed at clinically relevant exposures. Similar findings have been observed for other compounds in this therapeutic class. Currently it is not known whether thyroid c-cell tumors induced by GLP-1 receptor agonists are relevant to human risk. Therefore, until data to the contrary are available, it should be assumed that exenatide QW has the potential to induce thyroid c-cell hyperplasia and tumors in humans. Fibromas of the skin were observed in male rats at exposures that are approximately 26-fold higher than achieved at the maximum clinical dose of 2 mg/week.

Injection site findings typical of foreign body reactions were observed in rats and monkeys receiving PLG microspheres, with or without exenatide. Effects at the injection sites were generally mild to moderate and were found to be reversible as the microspheres degraded. Based on these findings, patients could experience some discomfort at the injection sites (e.g., inflammation and granulomas).

Byetta, which contains the same active ingredient as Bydureon, has been placed in the pregnancy category C category based on findings of fetal cleft palate, irregular fetal skeletal ossification of rib and skull bones, and increased neonatal deaths in mice at clinically relevant exposures. Irregular fetal skeletal ossifications were also observed in rabbits at exposures that are approximately 4.5 times the human exposure at the maximum recommended dose. Fetal effects generally occurred at doses that caused meaningful decreases in maternal body weight gain compared with controls. As with Byetta, exenatide QW should only be used during pregnancy if the potential benefit justifies the potential risk to the fetus.

Through post-marketing adverse event reporting, a signal for drug-induced pancreatitis, including life-threatening necrotizing/hemorrhagic pancreatitis, has been observed in patients taking Byetta. Pancreatitis signals have also been noted in patients taking other drugs working through the GLP-1 pathway, including other GLP-1 receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors. However, nonclinical toxicology studies conducted with drugs from these therapeutic classes failed to induce drug-related pancreatic toxicity or pancreatitis in animals. It is possible that other factors in addition to drug exposure are required for pancreatitis to develop, such as diabetes or concomitant medications. To further investigate the potential role of diabetes in the development of drug-induced pancreatitis, sponsors of approved GLP-1 receptor agonists have been asked to conduct toxicology studies in a rodent model for diabetes and thoroughly evaluate for acinar cell proliferation and toxicity in the pancreas. Because the sponsor has already been asked to conduct a study in a diabetic rodent model with immediate release exenatide, additional studies with exenatide QW are not warranted at this time.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 022-200
Review number: 1
Supporting document number: 001
Submit date: 04 May 2009
Type of submission: 505(b)(2) NDA referencing NDA 21-773 (Byetta)
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Amylin Pharmaceuticals
Manufacturer for drug substance: exenatide QW [redacted] (b) (4) different suppliers:

[redacted] (b) (4)

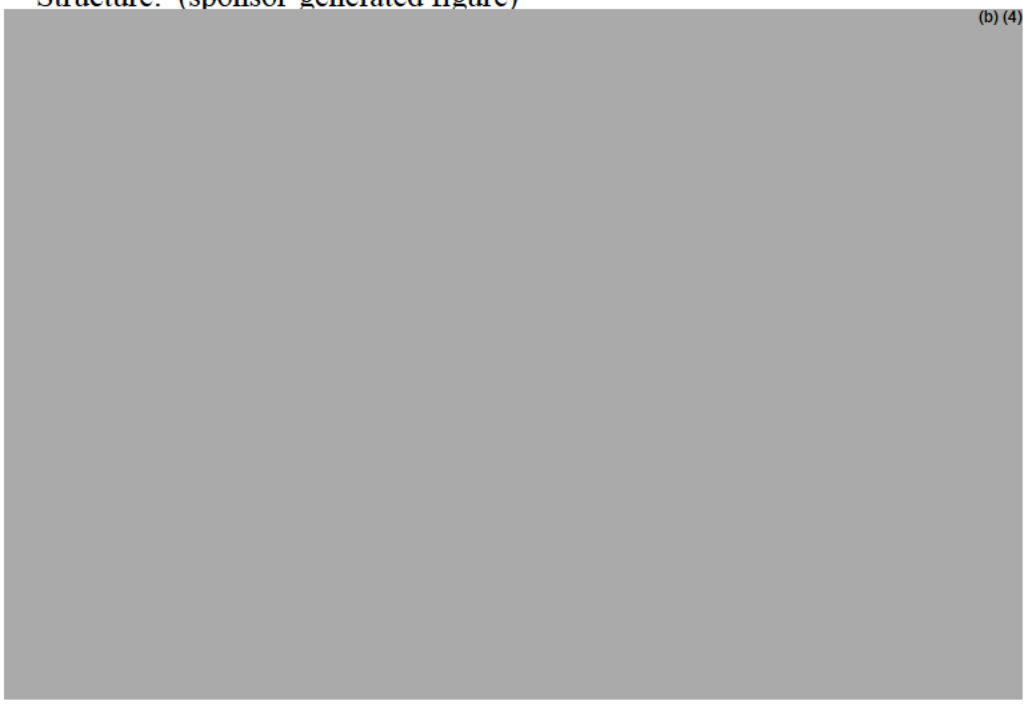
2. Mallinckrodt Inc., St. Louis, MO
3. Lonza, SA, Braine-l'Alleud, Belgium

Reviewer name: B. Timothy Hummer, Ph.D., DABT
Division name: Division of Metabolism and Endocrinology Products
HFD #: 510
Review completion date: 22 February 2010

Drug:

Trade name: BYDUREON
Generic names: exenatide QW, exenatide once weekly, exenatide LAR, exendin-4
Code names: AC2993-F17
Chemical name: L-histidylglycyl-L-glutamylglycyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-aspartyl-L-leucyl-L-seryl-L-lysyl-L-glutaminyl-L-methionyl-L-glutamyl-L-glutamyl-L-glutamyl-Lalanyl-L-valyl-L-arginyl-L-leucyl-L-phenylalanyl-L-isoleucyl-L-glutamyl-L-tryptophanyl-Lleucyl-L-lysyl-L-asparaginylglycylglycyl-L-prolyl-L-seryl-L-serylglycyl-L-alanyl-L-prolyl-L-prolyl-L-prolyl-L-serinamide
CAS registry number: 141732-76-5
Molecular formula: $C_{184}H_{282}N_{50}O_{60}S$
Molecular weight: 4186.6 Daltons

Structure: (sponsor-generated figure)



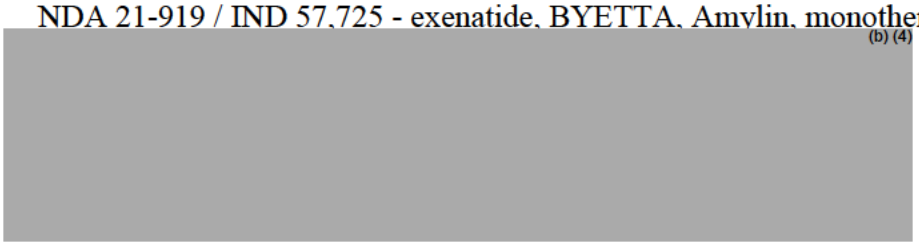
Relevant INDs/NDAs/DMFs:

Exendin-4 analogs

IND 67,092 - exenatide, Amylin, once weekly formulation

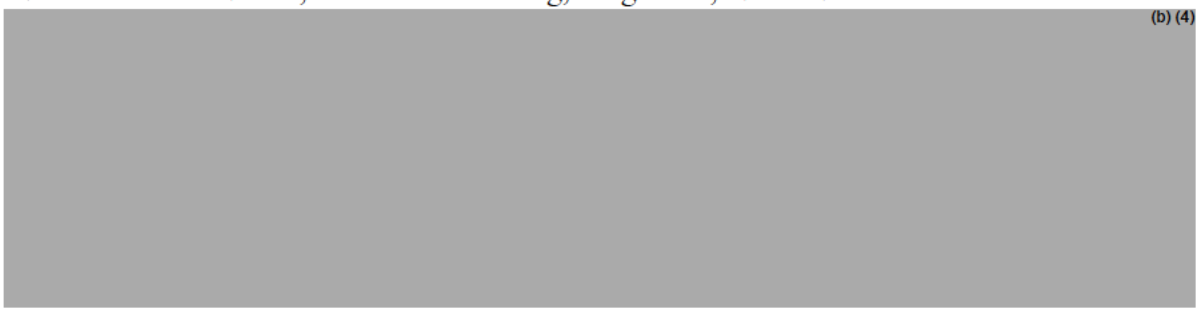
NDA 21-773 / IND 57,725 - exenatide, BYETTA, Amylin

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



GLP-1 analogs

NDA 22-341 / IND 61,040 - GLP-1 analog, liraglutide, Novo Nordisk



Drug class: Glucagon-like peptide-1 (GLP-1) receptor agonist

Intended clinical population: Type 2 diabetes

Clinical formulation: The product will be supplied in a kit consisting of:

- Microsphere/exenatide powder filled into a (b) (4) vial
- Diluent in a pre-filled syringe (b) (4))
- A vial connector
- Injection needles

The exenatide QW dose is prepared by mixing one vial of microspheres with one syringe of diluent. The resulting suspension is then administered by subcutaneous injection using the diluent syringe. Summary tables for the components of exenatide QW (sponsor-generated Tables 1 and 2) and the components of the diluent (sponsor-generated Tables 3 and 4) are presented below.

Table 1: Components of Exenatide QW

Name of Ingredient	Function	Reference to Quality Standard
Exenatide	Active ingredient	In-house
(b) (4)		
a (b) (4)		
b		
c		

Table 2: Unit Formula for Exenatide QW

Name of Ingredient	Quantity (mg/vial)[a]
Exenatide	2.0
(b) (4)	

[a] N/A = Not applicable.

Table 3: Components of Diluent

Name of Ingredient	Function	Reference to Quality Standard
Carboxymethylcellulose Sodium	(b) (4)	USP[a]
Sodium Chloride		USP
Polysorbate 20		NF
Monobasic Sodium Phosphate Monohydrate		USP
Dibasic Sodium Phosphate Heptahydrate		USP
Water for Injection		USP
Components Used in the Process		
(b) (4)		NF[b]
		NF

[a] (b) (4)

[b]

Table 4: Unit Formula of Diluent

Name of Ingredient	Quantity (mg/syringe) [a]
Carboxymethylcellulose Sodium	(b) (4)
Sodium Chloride	
Polysorbate 20	
Monobasic Sodium Phosphate Monohydrate	
Dibasic Sodium Phosphate Heptahydrate	
Water for Injection	
Components Used in the Process	
(b) (4)	N/A[c]

[a] Each syringe contains (b) (4) mL of diluent.
[b] Amount may be adjusted to (b) (4)
[c] N/A = Not applicable.

The diluent is used to suspend the exenatide QW microspheres prior to administration. Clinical and preclinical studies used the microsphere diluent formulation PBO-F27. It was later determined that PBO-F27 was not compatible with the proposed commercial syringe because it is (b) (4) The (b) (4) commercial formulation PBO-F26 was developed to address this issue and is the diluent formulation intended to be used for the marketed product. PBO-F26 is a (b) (4)

Table 5: Diluent Formulation Comparison

Name of Ingredient	Ingredient Concentration (mg/g)	
	PBO-F27 (Vial)	PBO-F26 (Syringe)
Carboxymethylcellulose Sodium		(b) (4)
Sodium Chloride		
Polysorbate 20		
Monobasic Sodium Phosphate Monohydrate		
Dibasic Sodium Phosphate Heptahydrate		

[a] Amount may be adjusted to (b) (4) material.

Route of administration: Subcutaneous, once weekly

Disclaimer: Some tables, figures, and/or text were taken from the Sponsor's submission, where indicated. Also, some text, tables, and/or figures were taken or modified from Dr. John Colerangle's pharmacology/toxicology review of NDA 21-773 (Byetta), where indicated.

Studies reviewed within this submission:
(Note: the sponsor is relying on many nonclinical studies that were previously reviewed for the approval of Byetta under NDA 21-773. In addition to those studies, the sponsor has conducted the following studies to supplement the previously conducted nonclinical program):

Primary Pharmacology
- Effect of Single Injection of Long-Acting Release Exenatide (Synthetic Exendin-4) in Diabetic Fatty Zucker (ZDF) Rats (Report #REST04093)

ADME
- Effects of Anti-Exenatide Antibodies on the Quantitation of Exenatide in Cynomolgus Monkey Specimens from 3-Month LAR Toxicity Study (in support of study (b) (4) 843-032; Report #REST070069R1)

- Pharmacokinetic Study of Exenatide (AC2993) LAR Formulations in Sprague-Dawley Rats after a Single Subcutaneous Injection (Report #REST070794)

- Pharmacokinetic Study of Exenatide (AC2993) LAR Formulations in Sprague-Dawley Rats after a Single Subcutaneous Injection (Report #REST070881)

- Exenatide LAR (AC2993-F17): Pharmacokinetics and Immunogenicity following Single-Dose Subcutaneous administration in the Cynomolgus Monkey (Report #REST04182R1)

Toxicology
- AC2993: Toxicity Evaluation of AC2993 Made by the (b) (4) Versus a Comparitor Lot from the (b) (4) as a Positive Control when Administered Subcutaneously Twice Daily for 28 Days to CD-1 Mice (Report #REST04568)

- An 8-Week Toxicity Study of Exenatide LAR F-17 Following Bi-weekly Subcutaneous Administration in the Rat with a 12-Week Recovery (Report #REST060307)

- Exenatide LAR: an 8-Week Toxicity Study Following Every Other Week Subcutaneous Administration in Sprague-Dawley Rats with a 12-Week Recovery Period (Report #REST080043)

- Exenatide LAR (F-17): 18-Week Biweekly Subcutaneous Injection [9 Doses] Toxicity and Toxicokinetic Study in Rats with a 3-Month Recovery (Report #REST050369)

- A 3-Month Toxicity Study of Exenatide LAR (AC2993-F17) Following Weekly Subcutaneous Administration in the Cynomolgus Monkey (Report #REST04289R2)

- Exenatide LAR: 39-Week (One Injection/Week) Subcutaneous Injection Toxicity and Toxicokinetic Study in Cynomolgus Monkeys with a 3-Month Recovery (Report #REST050370)

- Exenatide LAR: 104-Week Carcinogenicity Study Following Every Other Week Subcutaneous Administration in Rats (Report #REST060229R1)

Genetic Toxicology

- AC2993 ((b) (4) *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay Treat and Plate Method (Report #REST04571)

- AC2993 ((b) (4) Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells (Report #REST04569)

- Bacterial Reverse Mutation Assay Using the Treat and Plate Method (Report #REST060302)

- Bacterial Reverse Mutation Assay (Report #REST080228)

- *In Vitro* Mammalian Chromosome Aberration Test (Report #REST060306)

- *In Vitro* Mammalian Chromosome Aberration Test (Report #REST080139)

Studies not reviewed within this submission: None

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Exenatide, also known as exendin-4, is a 39 amino acid peptide originally isolated from the salivary secretions of *Heloderma suspectum* (Gila monster). Exenatide has approximately 50% sequence similarity to the endogenously occurring mammalian incretin hormone, glucagon like peptide-1 (7-36) amide (GLP-1). The pharmacodynamic activity of exenatide was investigated during the nonclinical development program for BYETTA (NDA 21-773). The activity of exenatide was examined in vitro and in animal models for short-term and long-term glucoregulatory and anti-diabetic activity. Exenatide binds to and activates the GLP-1 receptor, a G protein-coupled receptor, and mimics many of the glucoregulatory activities of endogenous 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. In subchronic dosing studies in rodent models of diabetes and obesity, administration of exenatide resulted in the lowering of serum glucose and HbA1c values in conjunction with decreased food intake and body weight. To establish that the altered PK profile of exenatide QW compared with Byetta retains adequate pharmacodynamic activity, the anti-diabetic activity of exenatide QW was studied in a rodent model of type 2 diabetes (summarized below). Other than the one pharmacology study conducted with exenatide QW, the sponsor is relying upon the pharmacology information submitted for the NDA approval of Byetta. The reader is referred to Dr. John Colerangle's review of NDA 21-773 for summaries of the primary, secondary, and safety pharmacology studies conducted with exenatide.

2.6.2.2 Primary pharmacodynamics

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

Drug activity related to proposed indication: The sponsor previously received marketing approval for an immediate release formulation of exenatide (Byetta, NDA 21-773) for the treatment of type 2 diabetes mellitus. Exenatide QW contains the same active ingredient as Byetta, but has been combined with polymer microspheres, thereby creating a sustained release formulation. For the Byetta application, the sponsor submitted several pharmacology studies that demonstrated the relevance of the drug activity for the proposed indication. The following summary is adapted from Dr. Colerangle's review of

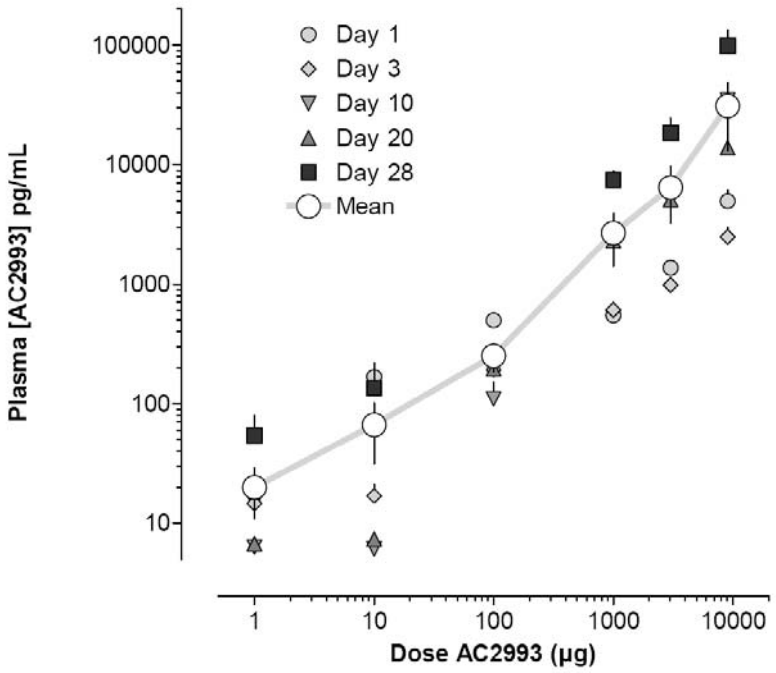
NDA 21-773. In normal and diabetic rats and mice, tissue binding distribution is identical to GLP-1 (including brain lateral septum and basal forebrain, *area postrema* and *medulla oblongata*). Significant binding was observed in pancreas and outer cortex of the kidney. There was wide distribution throughout the pancreas, but focal distribution within the Islets of Langerhans. Potency for stimulating cAMP production in human and rat based receptor systems was similar for exenatide and GLP-1. The EC_{50} values in RINm5f cells (rat insulinoma pancreatic cell line) were 0.31 nM for exenatide and 0.23 nM for GLP-1. The duration of effect on cAMP production was similar (~30 minutes) for both compounds. Exenatide was approximately 1,000 times more potent than GLP-1 in reducing plasma glucose concentration after a single subcutaneous dose to hyperglycemic *db/db* mice. Both compounds induced approximately 30% maximum decrease in glucose in monkeys or mice but the ED_{50} for GLP-1 was approximately 10,000-fold greater than exenatide. The magnitude of glucose lowering effect was related to the pre-existing plasma glucose concentration. This effect has also been well characterized for GLP-1. It is suggested that the glucose lowering ability of GLP-1 is dependent on glucose concentrations in the plasma and thus hypoglycemic effects are not expected to be common events, which has been verified clinically.

Pharmacology studies conducted with exenatide QW

"Effect of Single Injection of Long-Acting Release Exenatide (Synthetic Exendin-4) in Diabetic Fatty Zucker (ZDF) Rats" (Report REST04093). A primary pharmacology study was conducted to evaluate the effects of exenatide-LAR on the progression of glycemic and lipidemic parameters in male prediabetic Zucker diabetic fatty rats. Rats were administered a single subcutaneous injection of long-acting exenatide (0.001, 0.01, 0.1, 1, 3, or 9 mg) or placebo control. Treatment with 1 mg and greater resulted in a dose-dependent reduction in the glycemic indices of HbA1c (≥0.1 mg), fasting glucose, and fasting fructosamine and lipidemic indices of fasting cholesterol and fasting triglyceride for up to 28 days after administration when compared with placebo controls. Mean daily food intake was also significantly decreased with exenatide-LAR doses of 1 mg and higher compared with placebo controls but had no significant effect on body weight gain.

Hyperinsulinemic euglycemic clamp procedures incorporating an intraclamp glucose challenge showed increased β-cell response to the glucose challenge with low doses of exenatide. Dose response analysis of glucose infusion rate showed an increase in insulin sensitivity of up to 2.0-fold *versus* control. Insulin sensitization was apparent and significant with exenatide LAR doses of 1 mg and higher. Exposure data demonstrated that plasma exenatide was sustained for at least 28 days (sponsor-generated Figure 10).

Figure 10: Plasma exenatide concentration (pg/ml).



2.6.2.3 Secondary pharmacodynamics

No secondary pharmacodynamic studies were conducted with exenatide QW.

2.6.2.4 Safety pharmacology

Cardiovascular and neurobehavioral safety pharmacology studies were conducted with exenatide in support of NDA 21-773. Safety pharmacology studies were not conducted with exenatide QW. A summary of safety pharmacology studies conducted with immediate release exenatide is shown in the sponsor-generated table below (from NDA 21-773).

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

IV = intravenous SC = subcutaneous BID = Dose divided and administered twice daily M = Male F = Female.
[a] Qualitative and quantitative electrocardiographic data obtained as part of 273-day repeat-dose toxicity study. General toxicity data summarized in Section 2.6.7.7.9.

2.6.2.5 Pharmacodynamic drug interactions

No drug interaction studies were conducted with exenatide QW.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

See review of NDA 21-773 for pharmacology tabulated summaries of studies conducted with immediate release exenatide.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The ADME characteristics of immediate release exenatide were examined to support the marketing approval of Byetta (NDA 21-773). Exenatide QW, which contains the same active ingredient as Byetta, is formulated with poly(D,L-lactide-co-glycolide) microspheres to prolong absorption. The effect of this slow release formulation on PK parameters in comparison with the immediate release formulation was investigated in three PK studies (shown in the sponsor-generated table below). Distribution, metabolism, and excretion of exenatide are expected to be the same whether administered as an immediate release formulation or a long-acting release formulation; therefore, no additional distribution, metabolism, or excretion studies were conducted with exenatide QW.

Type of Study	Test System Species/Strain/Gender	Method of Administration	Testing Facility	Study Reference
Absorption	Rat / Sprague-Dawley / Male	Subcutaneous injection	(b) (4)	REST070794 (main study) REST070809 (PK analysis)
	Rat / Sprague-Dawley / Male	Subcutaneous injection		REST070881 (main study) REST070887 (PK analysis)
	Monkey / Cynomolgus / Male	Subcutaneous injection		REST04182R1
Distribution	NNS	NA	NA	NA
Metabolism	NNS	NA	NA	NA
Excretion	NNS	NA	NA	NA
Pharmacokinetic Drug Interactions	NNS	NA	NA	NA
Other	NNS	NA	NA	NA

NA = not applicable; NNS = no new study conducted; refer to BYETTA NDA 021-773.

2.6.4.2 Methods of Analysis

An immunoenzymetric assay (IEMA) was developed and validated for the quantitation of the immediate release formulation of exenatide in mouse, rat, monkey, and human biological matrices, which were described in the marketing application for Byetta (NDA 21-773). For the development of the exenatide QW program, the exenatide IEMA was modified and validated at (b) (4) and the sponsor's laboratories to reduce

non-specific background, eliminate the use of (b) (4) and move to a more environmentally friendly and less toxic substrate (b) (4). The modified IEMA was used for the quantification of exenatide QW in pivotal nonclinical and clinical studies for this marketing application.

Two enzyme-linked immunosorbent assays (ELISA) were also developed and validated to detect anti-exenatide antibodies in biological matrices from nonclinical and clinical samples. Details of validation results for precision, accuracy, assay linearity, and reproducibility can be viewed in the application. The lower and upper limits of quantitation for the measurement of exenatide were 10 and 500 pg/mL in mouse plasma, 20 and 400 pg/mL in rat plasma measured at (b) (4). The LLOQ was 10 pg/mL for exenatide in rat and monkey plasma for samples analyzed at the sponsor's laboratory.

A study was conducted in conjunction with a 3-month monkey study (REST04289) to investigate the effects of anti-exenatide antibodies on the quantitation of exenatide in plasma from cynomolgus monkeys (REST070069R1). The results demonstrated that the presence of high titers of anti-drug antibodies in cynomolgus monkey plasma may sometimes interfere with the recovery and quantitation of exenatide in the immunoassay, and may also affect the quantitation of diluted specimens. Dilution of the antibody in the plasma results in an apparent increase in exenatide concentrations, which is non linear. A conclusion was made that anti-drug antibody titers should be considered when evaluating pharmacokinetic or toxicokinetic data.

2.6.4.3 Absorption

The PK characteristics of exenatide QW were investigated in two single-dose studies in rats and a single-dose study in monkeys. Exenatide TK characteristics were also evaluated in repeat-dose toxicology studies (see Section 2.6.6). A summary of absolute bioavailability of exenatide QW and bioavailability relative to the immediate release formulation of exenatide is shown in the sponsor-generated table below.

Rat	
Bioavailability	
Absolute (Exenatide QW)	28%[a]
Relative Exenatide QW to Exenatide Immediate Release	63%[a]
Monkey	
Bioavailability	
Absolute (Exenatide QW)	13%[b]
Relative Exenatide QW to Exenatide Immediate Release	23%[b]

QW = once weekly.
[a] Data mean from 2 rat PK studies (REST070794 and REST070881; Section 2.6.4.3.2).
[b] Data from pilot monkey exposure study (REST04182R1; Section 2.6.4.3.3).

Pharmacokinetic Study of Exenatide (AC2993) LAR Formulations in Sprague-Dawley Rats after a Single Subcutaneous Injection (REST070794).

The pharmacokinetic profile of various exenatide (AC2993) LAR formulations was evaluated in male Sprague-Dawley rats after a single subcutaneous administration of exenatide LAR formulation or immediate release exenatide. The formulations, dose levels, and group sizes are shown in the sponsor-generated table below. Note that AC2993 LAR F17 is the intended clinical formulation for marketing and is the formulation that was used for the pivotal toxicology studies conducted with exenatide QW.

Group Assignments

Group	Formulation	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)	Route	Number of Animals Males
1	AC2993 (control)	0.01	0.4	0.025	SC	15
2	AC2993 LAR IV3	2.4	1.6	1.5	SC	15
3	AC2993 LAR IV4	2.4	1.6	1.5	SC	15
4	AC2993 LAR F17, lot S426-2377CA	2.4	1.6	1.5	SC	15
5	AC2993 LAR F17, lot S426-2507AA	2.4	1.6	1.5	SC	15
6	AC2993 LAR IV2	2.4	1.6	1.5	SC	15
7	AC2993 LAR IV1	2.4	1.6	1.5	SC	15

Mortality, adverse clinical signs, and body weights were assessed throughout the study. Blood samples were collected on Day 1 at 0.25, 1, 4, 8, and 24 hours post-dose for LAR formulations and at 0.033, 0.083, 0.25, 0.75, 1.5, and 3 hours post-dose for immediate release. Samples were also collected at a single time point on several days after dosing up to Day 57. Blood samples were also collected for anti-exenatide antibody analysis.

No mortality or treatment-related clinical findings were observed following a single subcutaneous dose of various exenatide LAR formulations. PK and antigenicity results are shown in the sponsor-generated tables below. The various LAR formulations differed primarily in (b) (4) When the two F-17 lots are considered together, for the 20 antibody negative animals, mean C_{max} (6,116 pg/mL) occurred in the 2nd post-dose peak. Exenatide was measurable in plasma up to approximately 40 days after a single injection of F17 formulations. The mean AUC_{0-t} for the F-17 formulation was 1,003,422 pg·h/mL. Treatment-emergent antibodies to exenatide were observed in approximately 30% of the exenatide LAR-treated animals with titers ranging from 25 to 15,625. These animals were excluded from the pharmacokinetic analysis. Generally, the presence of anti-exenatide antibodies resulted in higher AUC values.

Table 1: Mean Pharmacokinetic Parameters of Exenatide Following a Single Subcutaneous Administration of Various Exenatide LAR Formulations at (b) (4) mg/kg in Male Sprague-Dawley Rats Negative for Antibodies to Exenatide.

Group	Formulation, MW Lot No.		Initial T_{max} (h)	Initial C_{max} (pg/mL)	T_{max} (day)	C_{max} (pg/mL)	T_{last} (day)	AUC_{0-t} (pg·day/mL)	% Relative Bioavailability
2	IV3, (b) (4) kDa	N	11	11	11	11	11	11	11
	Lot 200-00238-01-16	Mean	0.25	5,647	0.19	6,076	33	33,331	59
3	IV4, (b) (4) kDa	N	11	11	11	11	11	11	11
	Lot 200-00238-01-20	Mean	1.09	2,904	1.00	4,321	35	24,360	43
4	F17, (b) (4) kDa	N	10	10	10	10	10	10	10
	Lot S426-2377CA	Mean	1.70	647	2.40	7,546	40	43,571	77
5	F17, (b) (4) kDa	N	10	10	10	10	10	10	10
	Lot S426-2507AA	Mean	2.25	552	7.9	4,686	39	40,047	71
6	IV2, (b) kDa	N	11	11	11	11	11	11	11
	Lot 200-00238-01-12	Mean	0.32	448	1.55	2,298	41	19,532	35
7	IV1, (b) (4) kDa	N	9	9	9	9	9	9	9
	Lot 200-00238-01-08	Mean	0.67	767	8.78	2,865	45	35,259	62

Abbreviations: AUC_{0-t} = Area under the plasma concentration-time curve from time zero through time of last quantifiable sample; C_{max} = Maximum observed plasma concentration over entire duration of study (up to 57 days); Initial C_{max} = Maximum observed plasma concentration in first 8 hours post dose; kDa= kiloDaltons; Initial T_{max} = Time to Initial C_{max}; MW= Molecular Weight; T_{max} = Time to C_{max}; T_{last} = Time of the last quantifiable sample.
Notes: Twenty seven animals had antibody titers ranging from 25 to 15,625 and were excluded from pharmacokinetic analysis; Animal 1090 (group 6, negative) was excluded due to high exenatide concentration (112.5 pg/mL) in predose sample.

Note: F17 is the intended clinical formulation for marketing and was also used for the bridging toxicology program.

Antigenicity after a Single Subcutaneous Injection with Various Exenatide QW Formulations or Immediate Release Exenatide

Group/Formulation		Study day					
		-7	24	36	43	53	57
1/ AC2993 (control)	Incidence of Positive Result (+/N)	0/15					
	Range of Titers	NA					
2/ AC2993 LAR IV3	Incidence of Positive Result (+/N)	0/15	2/15	3/15			
	Range of Titers	NA	25	25-125			
3/ AC2993 LAR IV4	Incidence of Positive Result (+/N)	1/15	2/15		4/15		
	Range of Titers	25	125-625		25-625		
4/ AC2993 LAR F17 S426-2377CA	Incidence of Positive Result (+/N)	0/15	3/15			5/15	
	Range of Titers	NA	25-125			25-3125	
5/ AC2993 LAR F17 S426-2507AA	Incidence of Positive Result (+/N)	0/15	3/15			5/15	
	Range of Titers	NA	125			25-625	
6/ AC2993 LAR IV2	Incidence of Positive Result (+/N)	0/15	3/15				3/15
	Range of Titers	NA	125-3125				625-15,625
7/ AC2993 LAR IV1	Incidence of Positive Result (+/N)	0/15	2/15				6/15
	Range of Titers	NA	25-125				25-125

Pharmacokinetic Study of Exenatide (AC2993) LAR Formulations in Sprague-Dawley Rats after a Single Subcutaneous Injection (REST070881)

The pharmacokinetic profiles of two exenatide (AC2993) LAR F17 batches were investigated in male rats following subcutaneous administration. Two treatment groups of 15 Sprague-Dawley rats were administered a single subcutaneous injection of exenatide LAR from either Lot S426-3206AA or 07-017-112 at a dose level of 2.4 mg/kg and a dose volume of 1.6 mL/kg. The LAR F17 formulations differed in scale and site of manufacture. Lot S426-3206AA was manufactured as a (b) (4) scale at Alkermes and Lot 07-017-112 was made as a (b) (4) scale at Amylin.

Observations for morbidity, mortality, and injury were conducted twice daily for all animals. Observations for clinical signs were conducted weekly. Body weights were measured and recorded on Day 1 prior to dosing. Blood samples for determination of exenatide plasma concentrations were collected from the animals at 0.25, 1, 4, 8, and 24 hours after injection on Day 1 and at a single time point every 2 to 4 days thereafter up to Day 53. Blood samples for anti-exenatide antibody analysis were collected from the animals on Day -7, Day 24, and Day 53. At the end of the study, animals were discarded without further evaluation, although a necropsy examination was performed for one animal that was found dead on Day 24 (treated with Lot 07-017-112). On Day 14 and Day 21, this animal was noted as having brown discolored skin on both forefeet, black material around its nose, and brown material around its mouth. This animal was also noted as having decreased activity and being thin. The cause of death could not be determined. No treatment-related adverse clinical findings were observed in any of the other animals.

A summary of the antigenicity and PK data is shown below in the sponsor-generated tables and figures. Anti-exenatide antibodies were detected in approximately 37% of the animals (11 out of 30). Titers ranged from 25 to 625 with a longer duration of treatment resulting in a higher number of antibody positive animals. PK data from antibody positive animals were excluded from the PK analysis. The plasma concentration versus time profile for exenatide LAR is characterized by an initial peak during the first few hours after injection, a second peak during the first few days after injection, and a third peak occurring after approximately 3 to 5 weeks. The initial peak is thought to occur through the release of exenatide loosely bound to the PLG microspheres. Both lots had measurable plasma concentrations for approximately 5 weeks. Exposure values (C_{max} and AUC_{0-t}) were slightly higher for Lot S426-3206AA compared with Lot 07-017-112. Overall, the plasma concentration versus time profiles for both exenatide LAR F17 lots had a similar pattern (Figure 1).

Summary of Antigenicity

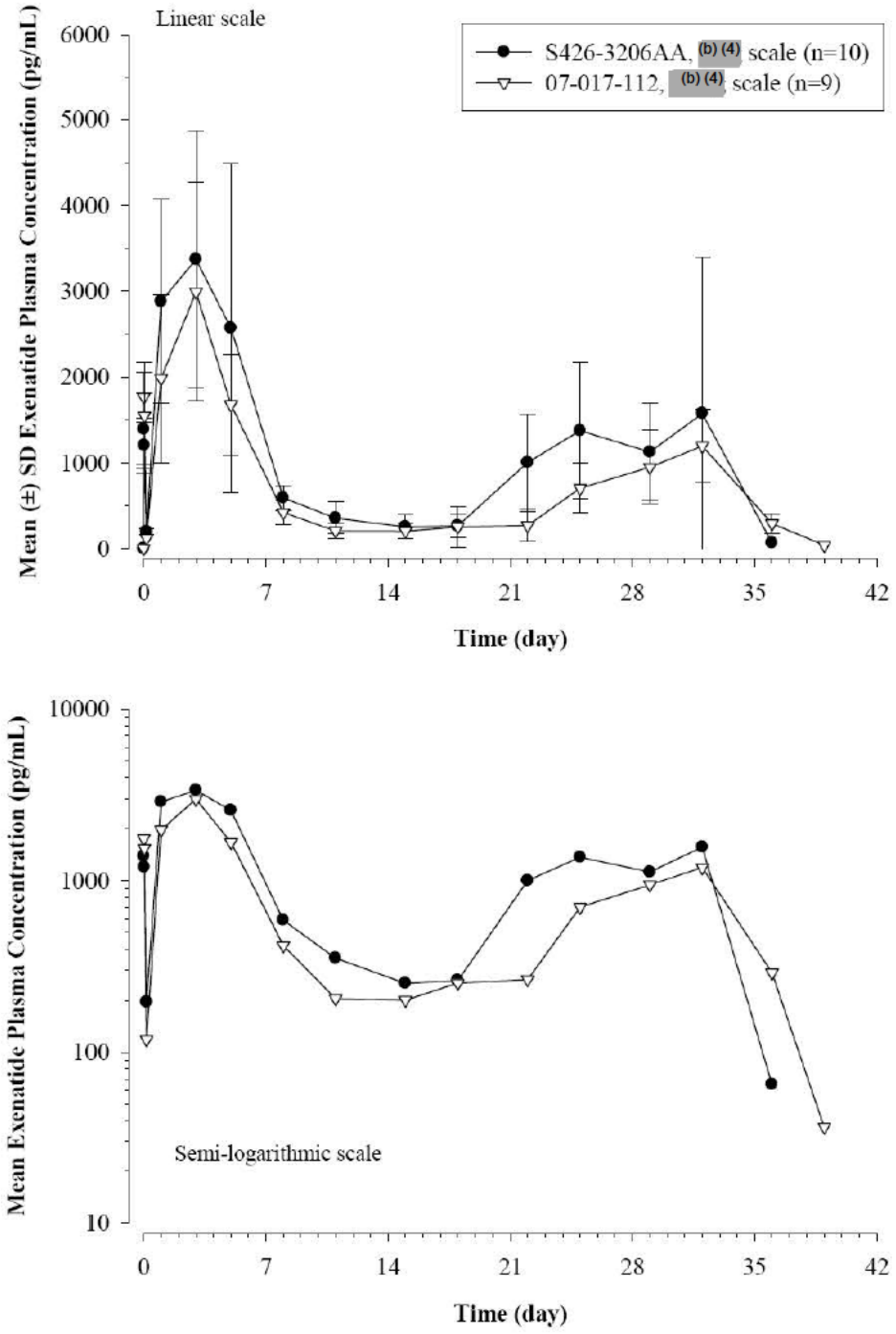
Group/ Test Article		Study Day		
		-7	24	53
1/ AC2993 LAR Lot S426-3206	Incidence of Positive Result (+/N)	0/15	3/15	5/15
	Range of Titers	NA	25-125	25-125
2/ AC2993 LAR Lot 07-017-112	Incidence of Positive Result (+/N)	0/15	1/14	6/14
	Range of Titers	NA	625	25-625

Mean Pharmacokinetic Parameters of Exenatide Following a Single Subcutaneous Administration of Exenatide LAR F17 Formulations Manufactured at (b) (4) or (b) (4) Scale (b) (4)) in Male Sprague-Dawley Rats Negative for Antibodies to Exenatide

Group	Formulation, Scale Lot No.		Initial $T_{max, 0-8h}$ (h)	Initial $C_{max, 0-8h}$ (pg/mL)	T_{max} (day)	C_{max} (pg/mL)	T_{last} (day)	AUC_{0-t} (pg·day/mL)	AUC_{0-t} (pg·h/mL)	% Bioavailability	
										Absolute	Relative
1	Alkermes F17 (b) (4) scale Lot S426-3206AA	N	10	10	10	10	10	10	10	10	10
		Mean	0.33	1,427	8	4,551	33	37,916	909,989	30	67
		SD	0.24	396	11	1,630	2	8,814	211,547	7	16
		CV%	73	28	131	36	5	23	23	23	23
2	Amylin F17 (b) (4) scale Lot 07-017-112	N	9	9	9	9	9	9	9	9	9
		Mean	0.50	1,900	6	3,306	34	27,447	658,726	22	48
		SD	0.38	405	10	900	6	5,790	138,971	5	10
		CV%	75	21	175	27	17	21	21	21	21
Combined		N	19	19	19	19	19	19	19	19	19
		Mean	0.41	1,651	7	3,961	33	32,957	790,970	26	58
		SD	0.31	459	10	1,448	4	9,088	218,114	7	16
		CV%	77	28	145	37	12	28	28	28	28

Abbreviations: AUC_{0-t} = Area under the plasma concentration-time curve from time zero through time of last quantifiable sample; C_{max} = Maximum observed plasma concentration over entire duration of study (up to 53 days); Initial C_{max} = Maximum observed plasma concentration in first 8 hours post dose; Initial T_{max} = Time of the Initial C_{max}; T_{max} = Time of C_{max}; T_{last} = Time of the last quantifiable sample.
Notes: Eleven animals had antibody titers ranging from 25 to 625 and were exeluded from pharmacokinetic analysis.

Figure 1: **Mean Exenatide Plasma Concentrations Following a Single Subcutaneous Administration of Exenatide LAR F17 Formulations Manufactured at (b) (4), or (b) (4), Scale (b) (4) mg/kg) in Male Sprague-Dawley Rats Negative for Antibodies to Exenatide (Study (b) (4) 843-053; REST070881)**



Exenatide LAR (AC2993-F17): Pharmacokinetics and Immunogenicity following Single-Dose Subcutaneous Administration in the Cynomolgus Monkey (Report REST04182R1)

The local tolerance, pharmacokinetics, and potential immunogenicity of exenatide LAR (AC2993-F17; Lot 278-0763) was investigated following a single subcutaneous dose. Three treatment groups of 3 (low and high doses) or 6 (mid-dose) male cynomolgus monkeys were administered 0.11, 0.44, or 1.10 mg/kg exenatide LAR via subcutaneous injection at dose volumes of 0.095, 0.109, and 0.136 mL/kg. Monkeys were observed for morbidity, mortality, and injury at least twice daily. Detailed clinical observations and injection site assessments were conducted twice on Day 1 and then weekly thereafter. Blood samples for plasma exenatide analysis were collected from all animals at various designated time points on Day 1 (predose and 0.5, 1, 2, 4, 8, 12, 16, and 24 hours postdose), and at a single time point on Days 3, 4, 8, 11, 15, 18, 22, 25, 29, 32, 36, 39, 43, 46, 50, 53, 57, 64, and 71 at approximately the same time each day. Blood samples for serum antibody analysis were collected on Day -5 and on Days 29, 43, 57, 64, and 71. After Day 71, no further assessments were conducted and the animals were returned to the laboratory's stock colony.

No treatment-related effects on survival, clinical signs, physical examinations, or body weights were noted during the study period. Occasional treatment-related injection site irritation was noted at a low incidence and findings of very slight erythema were noted at ≥0.44 mg/kg exenatide during Weeks 4, 5, and/or 6. Treatment-related local injection site enlargement was noted at 0.44 mg/kg (2/6 animals) and 1.10 mg/kg (3/3 animals), with incidence and severity diminishing in the latter part of the study. The size of enlargement ranged from small (1 to 5 mm) to medium (6 to 20 mm).

Anti-exenatide antibodies were noted as early as Day 29 at all doses and most animals had anti-exenatide antibodies by the end of the study, with titers ranging from 5 to 78,125 (sponsor-generated Table D below). Titers were not dose related, although the higher titers occurred at ≥0.44 mg/kg.

The PK data show that after a single subcutaneous injection of exenatide LAR, C_{max} and combined $AUC_{0\text{-}1704\ h}$ increased proportionally with dose (sponsor-generated Table A below). When comparing all animals versus the antibody negative subgroup for the mid-dose animals, mean AUC values were similar suggesting that the development of antibodies after a single administration did not have a significant impact on total exposure (sponsor-generated Table A and A1 below). However, TK data from repeat-dose toxicology studies indicate that the presence of antibodies during repeated dosing has more of an impact on exenatide exposure.

The results of this study show that a single subcutaneous (bolus) injection of exenatide LAR to male cynomolgus monkeys at doses up to 1.10 mg/kg were well tolerated, with minimal local irritation/enlargement at the injection site. Most animals developed anti-exenatide antibodies at all doses. Plasma exenatide concentrations increased with increasing doses of exenatide LAR.

Table D: Serum Anti-Exenatide Antibody in Cynomolgus Monkeys Following a Single Subcutaneous Injection of Exenatide LAR			
Dose Exenatide (mg/kg)	Number of Animals	Number Positive	Titer Range
0.11	3	2	25 to 125
0.44	6	5	5 to 78125
1.10	3	3	5 to >625

Table A: Pharmacokinetic Parameters in Cynomolgus Monkey Following a Single Subcutaneous Dose of Exenatide LAR				
Dose Exenatide (mg/kg)	$AUC_{0\text{-}1704h}$ (pg*h/mL)	SD	C_{max} (pg/mL)	SD
0.11	68588	22643	238	89
0.44	222778	93383	1342	965
1.10	707884	21482	2453	399
SD-Standard Deviation				

Table A1: Pharmacokinetics Parameters for Exenatide in Cynomolgus Monkeys That Remained Negative for Anti-exenatide Antibodies Following a Single Subcutaneous Dose of Exenatide LAR			
Dose (mg/kg)	Animal	AUC $_{0\text{-}1704\ h}$ (pg*h/mL)	Cmax (pg/mL)
0.11	101	42963	165
0.44	104[a]	176337	810
	107	263147	800
	108	140800	730
	Mean	**193428**	**780**
	SD	**62939**	**44**
1.10	111[b]	729877	2170

C_{max} - maximum concentration; AUC – area under the concentration curve.

[a]This animal was antibody positive at predose but antibody negative for all postdose time points (REST04211).

[b]This animal was antibody positive at Day 29, but antibody negative at predose and all other postdose time points [Days 43, 57, 64, and 71] (REST04211).

Effect of PLG MW on Persistence of AC2993LAR in Rats (Report 04104)
This study evaluated the subcutaneous persistence of exenatide-associated microspheres comprised of different molecular weight polylactide-co-glycolide (PLG) polymers in rats as well as the PK profile of exenatide when associated with each of the different molecular weight PLG polymers. The study evaluated four formulations composed of 2A (b) (4) kD), 2.5A (b) (4) kD), 3A ((b) (4) kD), and 4A (b) (4) kD) polymers. Each polymer contains a 50:50 lactide to glycolide ratio. The exenatide LAR formulation contains the (b) (4) polymer; the other three polymers were investigated as (b) (4) The diluent contained ingredients that are similar to the intended clinical formulation except that sodium phosphate was not included.

Male Sprague-Dawley rats (9-12/group) received a single subcutaneous 0.75 mL injection of resuspended exenatide/PLG, which contained approximately 1.0 mg exenatide. Subgroups (3/group) of animals were sacrificed at 14, 21, 28, and 49 days (4A polymer only) for macroscopic and microscopic examinations of the injection site to determine the prevalence of PLG used in each formulation. Blood samples were collected from each subgroup two days before, one day before, and on the day of necropsy to measure plasma exenatide concentrations for each of the PLG formulations.

The macroscopic and microscopic evaluations show that the lower the molecular weight of the polymer the shorter the duration of persistence in the subcutis. For example, by Day 28, injection sites containing the 2A polymer had very little residual polymer with some inflammatory cell infiltrate. In the 2.5A polymer group, the polymer bed was significantly reduced compared to previous days and inflammatory cell infiltration was still present. The microsphere bed for the 3A group was fully infiltrated by inflammatory cells but appeared smaller than at the 4A sites. For the 4A injections, large, prominent microspheres with moderate cellular infiltrate were observed. Therefore, polymer 2A appeared to have a markedly shorter duration compared with the 4A polymer and the 2.5A and 3A polymers had intermediate rates of degradation.

As expected, the PK profile of these formulations followed their degradation characteristics, with the lowest molecular weight polymer, 2A, releasing exenatide the fastest and the highest molecular weight polymer, 4A, having the longest duration of drug release (sponsor-generated Table 9). (b) (4)

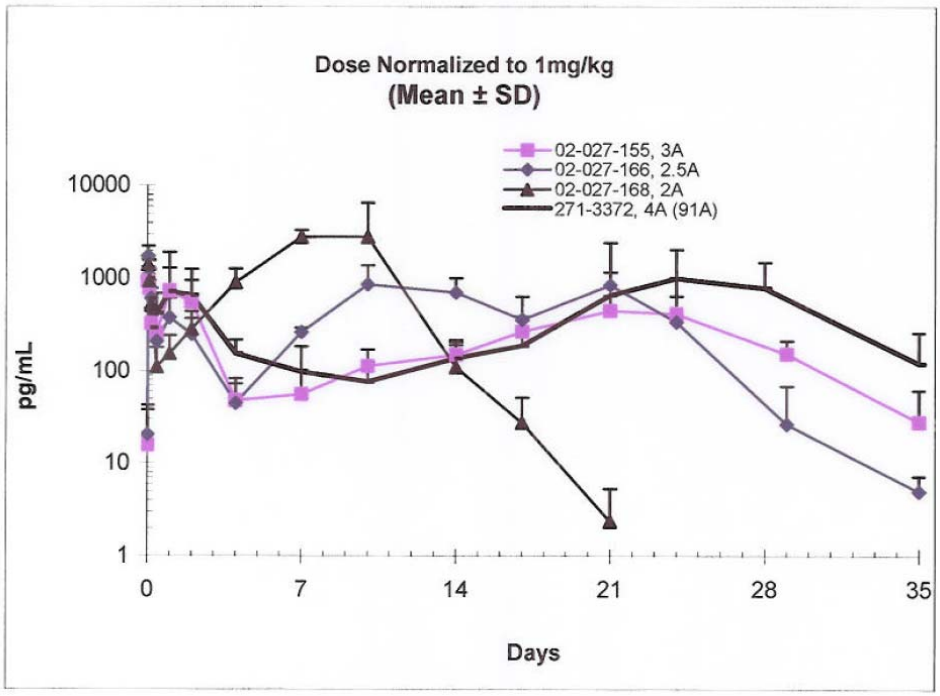
To further demonstrate how polymer size affects the PK profile of exenatide, the sponsor included Figure 8 (below) in the report (from Study AC2993-98) in which the same lower molecular weight polymers were compared with the 4A polymer (b) (4)

Table 9: Average IEMA Pharmacokinetic Data (pg/mL ± SD)

Group	Day 12	Day 13	Day14	Day 20	Day 21	Day 26	Day 27	Day 28	Day 47	Day 48	Day 49
2A	1977 ± 2130	849 ± 968	526 ± 477	343 ± 571	181 ± 313	7.5 ± 10.6	0	4.0 ± 6.8	-	-	-
2.5A	781 ± 369	877 ± 417	853 ± 366	521 ± 300	392 ± 270	107± 71.8	82 ± 56	64 ± 62	-	-	-
3A	565 ± 240	621 ± 184	662 ± 105	635 ± 677	755 ± 525	2023 ± 3106	1763 ± 2698	1373 ± 2115	-	-	-
4A	594 ± 438	766 ± 435	591 ± 228	1434 ± 1017	2381 ± 1334	1242 ± 536	1170 ± 356	1016 ± 337	1492 ± 1940	613 ± 467	476 ± 257

<10 = values treated as zero for purpose of averages
QNS = Quantity not sufficient were not included in the calculation of averages

Figure 8: Rat AC2993 Pharmacokinetic Profile



2.6.4.4 Distribution

Exenatide is a 39 amino acid peptide that, like other peptides, is believed to be metabolized throughout the body into peptide fragments or individual amino acids, thereby becoming available for incorporation into newly synthesized peptides and proteins. Because the incorporation of labeled amino acids into newly synthesized proteins would confound the interpretation of a tissue distribution study, the sponsor felt that whole body distribution studies were not technically feasible. Therefore whole body distribution studies were not conducted with immediate release exenatide or exenatide QW.

A human erythrocyte binding study conducted with immediate release exenatide demonstrated that approximately 82% of exenatide was associated with the plasma

fraction and 18% was associated with erythrocytes. Binding of exenatide to serum albumin and other plasma components was not assessed.

2.6.4.5 Metabolism

Studies investigating exenatide degradation were submitted and reviewed under NDA 21-773. When incubated with purified DPP-IV, exenatide was shown to be resistant to the proteolytic cleavage by DDP-IV, an enzyme involved in the degradation of several endogenous peptides including GLP-1. Studies using in vitro kidney membrane preparations from mouse, rat, rabbit, monkey, and human tissues showed the potential for metabolism of intact exenatide within renal tubules (Copley et al., 2006). In general, no significant fragments of exenatide were found in plasma following intravenous or subcutaneous injection of high doses (10 mg IV and 20 mg SC) of exenatide to rats. In renally ligated rats, three metabolites were identified in plasma at trace concentrations: exenatide 1-22, exenatide 23-39, and exenatide 1-20. Two of these metabolites, exenatide 1-22 and exenatide 23-39, were tested in an in vitro activity assay and found to have no biological activity. Characterization of exenatide metabolites from monkey and human plasma or urine was not conducted.

2.6.4.6 Excretion

Excretion studies were not conducted with exenatide QW but were conducted with exenatide in support of NDA 21-773. Exenatide clearance was studied in rat models of liver and kidney impairment to help determine the primary route of excretion. These studies showed that there was no significant difference in PK parameters in rat models of either acute or chronic liver injury versus controls indicating that hepatic excretion is not a major route of elimination. However, in rats with renal ligation, AUC, C_{max} and terminal t½ were significantly increased and clearance was decreased. These data suggest that exenatide is cleared predominantly by the kidneys. The relative absence of immunoreactive (full-length) exenatide in the urine of rats suggests that proteolytic degradation likely occurs in the renal tubule after filtration. As mentioned in Section 2.6.4.5, degradation studies performed using membrane preparations from rat, mouse, rabbit, monkey, and human kidneys support this hypothesis.

2.6.4.7 Pharmacokinetic drug interactions

Nonclinical PK interaction studies have not been conducted.

2.6.4.8 Other Pharmacokinetic Studies

No other pharmacokinetic studies were conducted with exenatide QW.

2.6.4.9 Discussion and Conclusions

Three single-dose PK studies were conducted in rats and monkeys to evaluate the delayed absorption properties due to the sustained release formulation of exenatide QW. The PK/TK characteristics of exenatide QW were also evaluated in repeat-dose toxicology studies (see individual study summaries in Section 2.6.6 for TK data). The bioavailability of exenatide QW was approximately 63% and 23% of immediate release exenatide in rats and monkeys, respectively. There were no consistent gender differences in exposure in rats or monkeys. On Day 1 of repeat-dose studies, rats demonstrated less

than dose proportional increases in exposure. Monkeys showed an increase in exposure that was proportional to dose level after a single subcutaneous dose. Based on TK data, repeated dosing resulted in accumulation relative to Day 1 in both rats (1.4 to 6.5 fold) and monkeys (8.8 to 28 fold) in animals that remained negative for anti-exenatide antibodies, with steady state concentrations achieved in approximately 1 to 2 months. In monkeys, plasma concentrations returned to undetectable concentrations within 2 months after the final dose administration. Once the exenatide peptide is absorbed from an injection of exenatide QW, the distribution, metabolism, and excretion of exenatide is not believed to be any different than it is for the immediate release formulation. Exenatide is resistant to the enzymatic cleavage by DPP-IV. No major metabolites have been observed in rat plasma. Renal filtration and subsequent proteolytic degradation within the renal tubules is believed to play a major role in the metabolism and excretion of exenatide.

2.6.4.10 Tables and Figures

(sponsor-generated tables)

Table 6: Comparison of Representative Derived Daily AUC in Repeated Dose Toxicology and Carcinogenicity Studies With BYETTA and Exenatide QW

					Derived Daily AUC (pg·h/mL) [1]	
Study Type	**Species**	**Duration**	**Dose [2]**	**Comment**	**BYETTA**	**Exenatide QW**
Repeated Dose	Rats	2 Months	9000 [3]	NOAEL	n/a	192,269 [4]
		3 Months	250	NOAEL	201,764	n/a
		4 Months	9000 [3]	NOAEL	n/a	146,914[5]
	Monkeys	3 Months	1100	NOAEL	n/a	104,777
		9 Months	150	NOAEL	1,000,708	n/a
		9 Months	1100	NOAEL	n/a	77,949
Carcinogenicity	Rats	2 Years	18 [6]	Low	20,188	n/a
			70 [6]	Mid	45,619	n/a
			250 [6]	High	201,764	n/a
			300 [3] [7]	Low	n/a	9483
			1000 [3] [7]	Mid	n/a	50,869
			3000 [3] [7]	High	n/a	140,546

AUC = area under the plasma concentration-time curve; n/a = 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 exenatide QW studies antibody-negative animals at end of study). Daily AUC estimates for BYETTA were determined by doubling the exposure following a single dose, and daily AUC estimates for exenatide QW were determined by dividing the exposure following a single dose by the dosing interval (days).

[2] Doses expressed in mcg/kg/day for BYETTA and mcg/kg/week for exenatide QW, unless otherwise specified.

[3] Dose administered once every other week.

[4] In this 2-month study (REST060307), the daily AUC value obtained at a dose of 3 mg/kg, the highest dose tested in the ongoing rat carcinogenicity with exenatide QW, was 74,676 pg·h/mL.

[5] In this 4-month study (REST050369), the daily AUC value obtained at a dose of 3 mg/kg, the highest dose tested in the ongoing rat carcinogenicity with exenatide QW, was 142,488 pg·h/mL.

[6] Data is from the 3 month rat study conducted at the same doses.

[7] Data is from TK animals within the study at the 6 month time point.

Summary of Derived Daily AUC Data from Key BYETTA Nonclinical Safety Studies

Study Type	Species	Duration	Route (Regimen)	Daily Dose (mcg/kg/day)	Derived Daily AUC (pg·h/mL) [1]	
					Day 1 [2]	Day last
Repeated Dose	Mice	6 Months	SC (BID)	18	15,490	21,124
				115	104,372	109,578
				760	859,596	1,077,340
	Rat	28 Days	SC (QD)	10	2558 ; 3721	3674 ; 2250
				100	94,293 ; 81,835	104,791 ; 119, 498
				1000	1,424,687 ; 1,211,247	1,653,257 ; 4,073,438
		3 Months	SC (QD)	18	20,188	10,178
				70	45,619	48,554
				250	201,764	268,094
	Monkey	9 Months	SC (BID)	2.2	10,243	16,634
				18	122,038	2,822,401
				150	1,000,708	2,062,782
DART	Mice		(BID)	6	6366 ; 6522	6852 ; 6500
				68	42,912 ; 46,812	113,398 ; 91,948
				760	928,814 ; 808,636	1,266,506 ; 953,152
	Rabbit		(BID)	0.2	456	NC
				2	24,328	NC
				22	429,766	NC
				156	2,973,334	NC
				260	7,221,500	NC
Carcinogenicity	Mice	2 Years [3]	SC (QD)	18	10,113	25,425
				70	32,508	58,403
				250	123,241	197,295
	Rat	2 Years [3]	SC (QD)	18	20,188	10,178
				70	45,619	48,554
				250	201,764	268,094

AUC = area under the plasma concentration-time curve; BID = twice daily; DART = developmental and reproductive toxicology; QD = once daily; SC = subcutaneous; NC = not calculated. QD = once daily; SC = subcutaneous

[1] Values presented are for male and female animals combined; when 2 values are presented, the first value refers to males and the second value refers to females. Daily AUCs are estimates for BID dosing determined by doubling the exposure following a single dose.

[2] For DART studies, value provided for mice is the Day 30 value and value provided for rabbit is the average value over gestational days 6, 9, 12 and 18.

[3] Exposure data from three month study at same doses, thus day last is approximately Day 90. C_{max} data was collected in carcinogenicity studies to confirm exposure.

Summary of Derived Daily AUC Data from Key Exenatide QW Nonclinical Safety Studies

					Derived Daily AUC (pg·h/mL) [1]		
Study Type	Species	Duration	Route (Regimen)	Dose (mcg /kg)	Day 1	Day last (All Animals)	Day last (Animals Negative for Antibodies)
Repeated Dose	Rat	2 Months [2]	SC (Bi-weekly)	300	3850	27,732	21,147
				3000	23,464	218,420	74,676
				9000	48,582	344,305	192,269
		4 Months	SC (Bi-weekly)	1000	9527	143,595	20,648
				3000	19,599	142,489	NC
				9000	29,513	176,761	146,914
	Monkey	3 Months	SC (QW)	110	1063	16,342	18,772
				440	4355	40,568	81,639
				1100	8512	139,109	104,777
		9 Months	SC (QW)	110	1122	32,525	15,063
				420	3982	159,378	50,173
				1100	4970	153,422	77,949
Carcinogenicity	Rat	6 Months	SC (Bi-weekly)	300	6730; 3653 (5191)	14,399; 10,022 (12,210)	11,300; 7666 (9483)
				1000	14,635; 8150 (11,392)	111,353; 120,417 (115,885)	55,948; 45,789 (50,869)
				3000	27,561; 34,678 (31,119)	175,407; 585,590 (380,498)	144,622; 136,470 (140,546)

AUC = area under the plasma concentration-time curve; QW = once weekly; NC = not calculated due to insufficient number of available pharmacokinetic samples; SC = subcutaneous.

[1] Daily AUCs are estimates for weekly dosing determined by dividing the exposure following a single dose by the number of days between doses. Thus Day 1 values represent average daily exposure following the first dose. Values presented are for male and female animals combined; when 2 values are presented, the first value refers to males, the second value refers to females, and the mean is presented in parenthesis.

[2] Day last is Week 6.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

(sponsor-generated table)

2.6.5.3 Pharmacokinetics: Absorption After a Single Dose – Antibody Negative Animals

Study Reference (Location)	Species, Strain (M/F)	Dose (mg/kg) Route	Mean Pharmacokinetic Parameters										Major Findings
			Initial C_{max} (pg/mL)		C_{max} (pg/mL)		T_{max} (h)		AUC_{0-t} (pg·h/mL)		BA (%)		
			mean	± SD	mean	± SD	mean	± SD	mean	± SD	Abs.	Rel.	
REST070794 [1] (Main study) REST070809 (PK analysis) (Section 2.6.4.3.2)	Rat, Sprague-Dawley (M)	2.4 [2] subcutaneous injection	600	246	6116	2782	124	195	1,003,422	185,374	33	74	2 lots of the F17 formulation mean exposure
REST070881 [1] (Main study) REST070887 (PK analysis) (Section 2.6.4.3.2)	Rat, Sprague-Dawley (M)	2.4 subcutaneous injection: (b) (4) scale [3]	1427	396	4551	1630	199	260	909,989	211,547	30	67	2 lots of the F17 formulation , different scales and locations
		2.4 subcutaneous injection: (b) (4) scale [3]	1900	405	3306	900	136	238	658,726	138,971	22	48	
REST04182R1 (Section 2.6.4.3.3)	Monkey, Cynomolgus (M)	0.11, 0.44, 1.10, subcutaneous injection	NC	NC	165 780 2170	NC 44 NC	NC	NC	42,963 193,428 729,877	NC 62,939 NC	13	23	C_{max} and $AUC_{(1-1704h)}$ increased proportionally with dose

Abs = absolute bioavailability; AUC_{0-t} = area under the serum concentration versus time curve from time zero to the time of the last measurable concentration; BA = Bioavailability; C_{max} = maximum serum concentration; Initial C_{max} = maximum serum concentration measured in first four hours; GLP = good laboratory practice; NC = not calculated; PK = pharmacokinetic; $T_{1/2}$ = elimination half-life; T_{max} = time at which maximum serum concentration is observed; Rel = Relative bioavailability; SD = standard deviation.

[1] Study conducted in compliance with GLP.

[2] 2 lots of F17 were examined; PK parameters for both lots were combined.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

A complete toxicology program was conducted to support the marketing approval of immediate release exenatide (Byetta). Summaries of these studies can be found in the pharmacology/toxicology review for NDA 21-773. The sponsor conducted additional toxicology studies with exenatide QW to supplement the Byetta toxicology program. These include repeat-dose toxicology studies up to 4 months in rats, a 3-month and 9-month toxicology study in monkeys, a carcinogenicity study in rats, and several in vitro genetic toxicology studies to qualify manufacturing changes for the drug substance. A list of toxicology studies conducted with immediate release exenatide and exenatide QW is shown in the sponsor-generated table below.

Table 1: Summary of Exenatide Once Weekly Nonclinical Program

Type of Study	Study	Species	Duration of Dosing	Dosing Regimen	Route	GLP Status
Exenatide (BYETTA)						
General Toxicology	Acute	Mouse	Single dose	QD	IV	Non-GLP
		Rat	Single dose	QD	SC	GLP
		Monkey	Single dose	QD	SC	GLP
	Repeated Dose	Mouse	3-Month	QD	SC	GLP
			3-Month	BID	SC	GLP
			6-Month	BID	SC	GLP
		Rat	14-Day	QD	IV	Non-GLP
			28-Day	QD	SC	GLP
			3-Month	QD	SC	GLP
		Monkey	5-Day	QD	SC	GLP
			28-Day	QD	SC	GLP
			3-Month	BID	SC	GLP
			9-Month	BID	SC	GLP
Genotoxicity	Ames (3 studies)	N/A	N/A	N/A	N/A	GLP
	Chromosomal Aberration (3 studies)	CHO	N/A	N/A	N/A	GLP
	Micronucleus	Mouse	Single Dose	QD	SC	GLP
Carcinogenicity		Mouse	2-year	QD	SC	GLP
		Rat	2-year	QD	SC	GLP
DART	Fertility	Mouse	As per ICH	BID	SC	GLP
	Embryofetal	Mouse (2 studies)	As per ICH	BID	SC	GLP
		Rabbit (2 studies)	As per ICH	BID	SC	GLP
	Peri/PostNatal	Mouse	As per ICH	BID	SC	GLP
Special Toxicology	Impurities & degradation products (3 studies)	Mouse	28-Day	BID	SC	GLP

Exenatide QW						
General Toxicology	Repeated Dose	Mouse [1]	28-Day	BID	SC	GLP
		Rat	8-Week	Every 2 weeks	SC	GLP
			8-Week	Every 2 weeks	SC	GLP
			4-Month	Every 2 weeks	SC	GLP
		Monkey	3-Month	QW	SC	GLP
			9-Month	QW	SC	GLP
Genotoxicity	Ames (3 studies) [1]	N/A	N/A	N/A	N/A	GLP
	Chromosomal Aberration (3 studies) [1]	CHO	N/A	N/A	N/A	GLP
Carcinogenicity[2]		Rat	2-year	Every 2 weeks	SC	GLP

BID = twice daily; CHO = Chinese Hamster Ovary Cells; DART = developmental and reproductive toxicology; GLP = Good Laboratory Practice; SC = subcutaneous; IV = intravenous; QD = once daily; QW = once weekly.

[1] A 28-day mouse study and in vitro genotoxicity studies (Ames and chromosomal aberration) were conducted with exenatide (drug substance qualification studies)

[2] Study ongoing; the audited draft report was previously submitted to the FDA (IND 67,092, Serial 0120). The final report will be submitted post-marketing as agreed with the Division at the pre-NDA meeting.

2.6.6.2 Single-dose toxicity

Single-dose toxicity studies were not conducted with exenatide QW. However, single-dose studies were conducted with exenatide in support of NDA 21-773. In mice, single intravenous doses up to 1,500 µg/kg did not cause mortality. Decreased grip strength was observed at ≥300 µg/kg and transient, dose-related reductions in spontaneous motor activity were observed at doses ≥30 µg/mL. In rats, a single subcutaneous injection at doses up to 30,000 µg/kg did not cause mortality or serious toxicity. Clinical signs included hunched posture and/or yellow staining of fur at doses ≥10,000 µg/kg and slightly lower body weights at 30,000 µg/kg. In monkeys a single subcutaneous dose did not induce mortality or serious toxicity at doses up to 5,000 µg/kg. Decreased food consumption was noted at doses ≥3,000 µg/kg.

2.6.6.3 Repeat-dose toxicity

Non-pivotal repeat-dose toxicology studies conducted with exenatide QW are summarized in the table below. One study was also conducted with immediate release exenatide to compare the toxicity profile of exenatide produced through two different synthetic methods. Summaries of repeat-dose toxicology studies conducted with immediate release exenatide can be found in the pharmacology/toxicology review for NDA 21-773.

Repeat-Dose Range-Finding Studies

Study Type Report No. GLP Status	Test Article	Species/strain Number/group	Dose Levels Regimen Route	Endpoints	Findings
28-day repeat-dose toxicity study REST04568 GLP	Exenatide-A: (b) (4) method Exenatide-B: (b) (4) method	Mice/CD-1 Main: 10M/10F TK: 10M/10F	0 (vehicle), 760 µg/kg/day exenatide-A, or 760 µg/kg/day exenatide-B Twice daily by subcutaneous injection	Mortality Clinical signs Body weight Food consumption Ophthalmoscopy Clinical pathology Toxicokinetics Ab assessment Organ weights Gross pathology Histopathology	• There were no treatment-related mortalities or effects on clinical signs, food consumption, ophthalmology, hematology, clinical chemistry, organ weighs, or gross pathology. • Main study animals treated with exenatide gained slightly more weight than vehicle control animals, although this effect was not observed for the TK groups. • Males and females receiving exenatide-B had increased occurrence of proteinuria. • The only noteworthy microscopic finding was an increased occurrence of focal basophilic hypertrophy of acinar cells in the parotid salivary glands. This finding was noted in 0/10, 3/10, and 7/10 males and 1/10, 9/10, 7/10 females receiving vehicle, exenatide-A, and exenatide-B, respectively. • All Day 30 titers for anti-exenatide Abs were BLQ except for one animal receiving exenatide-B, which had a titer of 5. • At 0.5 hours after the first dose on Day 28, respective mean exenatide concentrations were 317,200 and 331,800 pg/mL for females and males receiving exenatide-A and 327,600 and 249,400 for females and males receiving exenatide-B.

<table>
<tr><th>Study Type
Report No.
GLP Status</th><th>Test Article</th><th>Species/strain
Number/group</th><th>Dose Levels
Regimen
Route</th><th>Endpoints</th><th>Findings</th></tr>
<tr><td>8-week repeat-dose toxicity study with 12-week recovery

REST060307

GLP</td><td>Exenatide QW (AC2993-F17) ((b) (4) scale)</td><td>Rat/Sprague-Dawley

Main: 10M/10F
Recovery: 5M/5F
TK: 10M/10F or 3M/3F (controls)</td><td>0 (diluent), 0 (microspheres), 0.3, 3, and 9 mg/kg/dose

Once every 2 weeks by subcutaneous injection (4 doses)</td><td>Mortality
Clinical signs
Injection site rxn.
Body weight
Food consumption
Ophthalmoscopy
Clinical pathology
Toxicokinetics
Ab assessment
Organ weights
Gross pathology
Histopathology</td><td>

No toxicologically meaningful treatment-related effects were noted on survival, clinical observations, ophthalmoscopy, physical exams, hematology, serum chemistry, organ weights, or macroscopic or microscopic pathology.
Decreases in body weight gain (up to 37% and 43% less than diluent controls for males and females, respectively) and food consumption were observed. Compensatory increases in food consumption and body weight gain occurred during the recovery period.
Microsphere-related microscopic observations (including control group) included granulomatous inflammation characterized by focally dense aggregates of macrophages and/or multinucleated giant cells, typical of a foreign-body reaction.
19% to 36% of treated animals developed anti-exenatide abs with titers ranging from 25 to 15,625.
Based on the absence of systemic toxicity or severe local injection site injury, the NOAEL was considered to be the high dose of 9 mg/kg.
TK results at Week 6 are shown in the sponsor-generated tables below.

<table>
<tr><th>Sex</th><th>C_{max} (pg/mL)</th><th>AUC_{0-t} (pg·h/mL)</th><th>C_{avg} (pg/mL)</th></tr>
<tr><td colspan="4">0.3 mg/kg/dose exenatide</td></tr>
<tr><td>Male</td><td>8,960</td><td>503,096</td><td>1,497</td></tr>
<tr><td>Female</td><td>2,925</td><td>273,401</td><td>814</td></tr>
<tr><td colspan="4">3 mg/kg/dose exenatide</td></tr>
<tr><td>Male</td><td>29,334</td><td>4,829,039</td><td>14,372</td></tr>
<tr><td>Female</td><td>8,900</td><td>1,286,706</td><td>3,829</td></tr>
<tr><td colspan="4">9 mg/kg/dose exenatide</td></tr>
<tr><td>Male</td><td>25380</td><td>3,784,680</td><td>11,264</td></tr>
<tr><td>Female</td><td>65,480</td><td>5,855,867</td><td>17,428</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>Study Type
Report No.
GLP Status</th><th>Test Article</th><th>Species/strain
Number/group</th><th>Dose Levels
Regimen
Route</th><th>Endpoints</th><th>Findings</th></tr>
<tr><td>REST060307, continued</td><td></td><td></td><td></td><td></td><td>Antibody negative animals
<table>
<tr><th>Sex</th><th>C_{max} (pg/mL)</th><th>AUC_{0-t} (pg·h/mL)</th><th>C_{avg} (pg/mL)</th></tr>
<tr><td colspan="4">0.3 mg/kg/dose exenatide</td></tr>
<tr><td>Male</td><td>3,067</td><td>298,921</td><td>890</td></tr>
<tr><td>Female</td><td>3,333</td><td>293,196</td><td>873</td></tr>
<tr><td colspan="4">3 mg/kg/dose exenatide</td></tr>
<tr><td>Male</td><td>14,467</td><td>1,180,659</td><td>3,514</td></tr>
<tr><td>Female</td><td>9,575</td><td>1,189,931</td><td>3,541</td></tr>
<tr><td colspan="4">9 mg/kg/dose exenatide</td></tr>
<tr><td>Male</td><td>19,575</td><td>2,742,556</td><td>8,162</td></tr>
<tr><td>Female</td><td>15,850</td><td>2,640,973</td><td>7,860</td></tr>
</table></td></tr>
<tr><td>8-week repeat-dose toxicity study with 12-week recovery

REST080043

GLP</td><td>Exenatide QW (AC2993-F17) ((b) (4) scale)</td><td>Rat/Sprague-Dawley

Main: 10M/10F
Recovery: 5M/5F
TK: 10M/10F or 3M/3F (controls)</td><td>0 (diluent), 0 (microspheres), 0.3, 3, and 9 mg/kg/dose

Once every 2 weeks by subcutaneous injection (4 doses)</td><td>Mortality
Clinical signs
Injection site rxn.
Body weight
Food consumption
Ophthalmoscopy
Clinical pathology
Toxicokinetics
Ab assessment
Organ weights
Gross pathology
Histopathology</td><td>• No toxicologically meaningful treatment-related effects were noted on survival, clinical signs, ophthalmoscopy, clinical pathology, organ weights, or macroscopic pathology.
• Decreases in body weight gain (up to 20% for males and 17% for females) and food consumption were observed at all doses. Compensatory increases in food consumption and body weight gain occurred during the recovery period.
• Increased incidence and/or severity of foreign body granulomas and chronic inflammation was seen at 9 mg/kg with an increased incidence of fibroplasia and foamy pigmented macrophage accumulation at the end of recovery.
• 17% to 48% of treated animals developed anti-exenatide abs with titers ranging from 25 to 15,625.
• Based on the absence of systemic toxicity or severe local injection site injury, the NOAEL was considered to be the high dose of 9 mg/kg.
• TK results for Day 1 and Day 43 are shown in the sponsor-generated table below.</td></tr>
</table>

Study Type Report No. GLP Status	Test Article	Species/strain Number/group	Dose Levels Regimen Route	Endpoints	Findings					
REST080043, continued					Day 1 Exposure (all animals)		Day 43 Exposure (all animals)		Day 43 Exposure (animals negative for antibodies to exenatide)	
					C_{max} (pg/mL)	AUC_{0-t} (pg·h/mL)	C_{max} (pg/mL)	AUC_{0-t} (pg·h/mL)	C_{max} (pg/mL)	AUC_{0-t} (pg·h/mL)
					Group 8: 0.3 mg/kg/dose					
				Female	756	28,552	3,033	219,816	3,394	226,749
				Male	1,123	NC	4,051	237,771	3,414	190,181
				Mean	940	NC	3,542	228,794	3,404	208,465
					Group 9: 3 mg/kg/dose					
				Female	3,232	163,347	48,331	4,667,017	31,798	507,193
				Male	2,817	138,386	35,270	867,515	36,578	517,639
				Mean	3,024	150,866	41,800	2,767,266	34,188	512,416
					Group 10: 9 mg/kg/dose					
				Female	9,269	378,205	45,299	4,073,066	24,300	1,807,528
				Male	5,191	307,054	28,892	5,536,820	14,845	1,128,702
				Mean	7,230	342,630	37,096	4,804,943	19,572	1,468,115

Ab = antibody; AUC = area under the plasma concentration versus time curve; BLQ = below the limit of quantitation; C_{max} = maximum achieved plasma concentration; GLP = Good Laboratory Practices; F = female; M = male; NOAEL = no observed adverse effect level; QW = once weekly; rxn = reaction; TK = toxicokinetics.

Pivotal Studies

Study title: Exenatide LAR (F-17): 18-Week Biweekly Subcutaneous Injection [9 Doses] Toxicity and Toxicokinetic Study in Rats with a 3-Month Recovery

Note: An interim study report that did not include recovery animal data was previously reviewed by Dr. John Colerangle under IND 67,092, the review for which is presented below with some modification. This reviewer is in agreement with Dr. Colerangle's assessments and conclusions. Recovery animal data and final anti-exenatide antibody data were added to this final review.

Key study findings:

- A diluent control male rat was sacrificed in a moribund condition on Day 57 following blood collection. The cause of moribundity was not determined. A female rat given 3 mg/kg/dose was found dead on Day 55. Necropsy revealed a large, fluid-filled kidney, thickened urinary bladder, and large ureter. A toxicokinetic male given 1 mg/kg/dose and a female given 3 mg/kg/dose, died on Days 28 and 13, respectively, and were discarded without necropsy.
- Injection site swelling/palpable lumps (slight to moderate severity) were more noteworthy for animals given the control microspheres or the test article at 9 mg/kg and noted at a lesser severity (very slight to slight) for animals given 3 mg/kg. The swelling/palpable lumps were attributed to the physical presence of the microspheres. Very slight to slight redness was noted at a low incidence in the control microsphere and test article-treated groups and was attributed to the injection trauma. By the end of the recovery period, partial to complete reversibility was observed.
- A dose-related decrease in mean body weight was noted in males treated with the drug. While similar body weight decrements were noted in drug treated females, they were not dose related.
- Food consumption was minimally to slightly decreased in all drug-treated animals especially at Week 17 in males given 1 and 3 mg/kg.
- Decreased mean absolute organ weight was observed for liver and thymus from animals treated with ≥3 mg/kg, with no correlative histopathology; the effect on absolute organ weight was likely a secondary effect of the decreased mean body weights observed for exenatide-treated animals.
- Histopathology revealed foamy macrophages/fibroblasts, lymphocytic infiltrate and granuloma of minimal to slight severity at all injection sites. Lesions observed in the parotid salivary gland (atrophy), heart (hemorrhage), lung (mixed cell inflammation), mammary gland (corpora amylacea), stomach (erosion, lymphocytic infiltrate, hyperplasia) and uterus (dilatation) were of very low incidence (1/10) and minimal to slight in severity. These lesions were noted in the 9 mg/kg dose group.
- One HD female had renal tubular adenomas unilaterally with a focus of slight tubular hyperplasia in the contralateral kidney. One MD female had a renal tubular adenoma. One LD female and one female given the microsphere control had slight and minimal renal tubular hyperplasia, respectively. The sponsor stated that these tumors are incidental findings and not drug related because the study duration was short (18 weeks) and secondly, tumors of the renal tubules are typically slow growing with a

long latency for development. The sponsor also argued that even with some of the most potent renal carcinogens, a latency period of 6 to 12 months is required for tumors to develop (Hard, 1990; Hard et al., 1970).

- Anti-exenatide antibodies were observed at ≥1 mg/kg/dose with no apparent dose-response relationship for incidence. Higher titers occurred on Day 57 and generally decreased towards the end of treatment (Month 4). The highest titers were observed at the LD of 1 mg/kg.
- The MTD is 9 mg/kg (~27X MRHD, AUC for Ab negative rats and humans) based upon the absence of significant target organ toxicity at this dose.

Study no: REST050369
Volume # and page #: N/A, electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: October 31, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: AC2993-F17 Lot # 278-2565A; purity was not provided
Formulation/vehicle:
Control microsphere: 0 mg AC2993-F17 powder/kg, 0.39 ml Microsphere Diluent is injected into a vial containing no AC2993-F17 powder

LD: 1 mg AC2993-F17 powder/kg, 3.53 ml Microsphere Diluent (note: each vial of diluent contains 1.5 ml) is injected into a vial containing AC2993-F17 powder

MD: 3 mg AC2993-F17 powder/kg), 1.18 ml Microsphere Diluent is injected into a vial containing AC2993-F17 powder

HD: 9 mg AC2993-F17 powder/kg), 0.39 ml Microsphere Diluent is injected into a vial containing AC2993-F17 powder

Methods (unique aspects)**:**
Dosing:
Species/strain: Rat/Sprague-Dawley
Number/sex/group (main study): 10
Number/sex/group (recovery): 5
Number/sex/group (toxicokinetics): 9 or 3 (control groups)
Age: 46-52 days old at study initiation
Weight: 216-287 g (M); 140-207 g (F)
Doses in administered units: 1, 3 and 9 mg/kg once every other week
Route and volume: Subcutaneous injection; 0.75 ml/kg

Study Design

Group[a]	No. of Animals Male	No. of Animals Female	Dose Level[b] (mg/kg exenatide)	Dose Concentration[b] (mg/mL)
Toxicity Animals[c]				
1 (Vehicle Control)	15	15	0	0
2 (Control Microspheres)	15	15	0	0
3 (Low)	15	15	1	1.3
4 (Mid)	15	15	3	4.0
5 (High)	15	15	9	12
Toxicokinetic Animals				
6 (Vehicle Control)	3	3	0	0
7 (Control Microspheres)	3	3	0	0
8 (Low)	9	9	1	1.3
9 (Mid)	9	9	3	4.0
10 (High)	9	9	9	12

a Animals in Groups 1 and 6 received vehicle only, and animals in Groups 2 and 7 received control microspheres only (microsphere dose equivalent to high dose group); all animals were dosed on Days 1, 15, 29, 43, 57, 71, 85, 99, and 113 of the dosing phase.
b The dose volume was 0.75 mL/kg.
c Toxicity animals designated for recovery phase sacrifice (five animals/sex/group, dependent on survival) underwent 13 weeks of recovery following the final dose administration.

Injection sites were rotated among four distinct sites close to the middle of the back: left dorsal thorax (Site A; Days 1, 57, and 113), right dorsal thorax (Site B; Days 15 and 71), left dorsal lumbar (Site C; Days 29 and 85), and right dorsal lumbar (Site D; Days 43 and 99). Each site was marked to permit injection site observations and collection at termination.

Observations and times:

Clinical signs: Daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Conducted once predose and during the final week of dosing
EKG: Not conducted
Hematology: Blood samples for hematology evaluation were collected at terminal sacrifice
Clinical chemistry: Blood samples for clinical chemistry evaluation were collected at terminal sacrifice
Urinalysis: Not conducted
Gross pathology: Organs/tissues collected for gross examination are indicated in the histopathology table at the end of the repeat-dose toxicity section
Organs weighed: Organs weighed are indicated in the histopathology table
Histopathology: All tissues from toxicity animals in Groups 1 and 5 (diluent control and HD groups) and from the toxicity animals that died or were sacrificed at an unscheduled interval were processed for microscopic evaluation. In addition, the injection sites, pancreas, kidneys, adrenal gland, thyroid with parathyroid, thymus, parotid salivary gland, spleen, heart, liver, and macroscopic lesions from

each toxicity animal in Groups 2 through 4 were processed for microscopic evaluation.

Toxicokinetics: Blood samples for TK were collected on Days 1, 29, 57, 85, and 113 of the dosing phase.

On Days 1 and 113 (first and last dose-days) cohorts of 3 animals/sex/group from Groups 8 through 10 were bled predose and approximately 0.5, 2, 4, 10, 24, 48, 96, 168, 216, 288, and 336 hours postdose (336 hours postdose is predose for the following dose-day).

Three sets of animals were bled according to the following schedule:

Set 1: Predose and approximately 4, 48, and 216 hours postdose.
Set 2: Approximately 0.5, 10, 96, and 288 hours postdose.
Set 3: Approximately 2, 24, 168, and 336 hours postdose.

On Days 29, 57, and 85 cohorts of sets 1 and 2 (3 animals/sex/group) from Groups 8 through 10 were bled predose and 0.5 hours postdose.

On Days 1, 57, and 113 Group 6 and 7 animals (TK control groups) were bled predose and approximately 0.5 hours postdose.

Injection Site Observations: Injection sites were observed once daily for all toxicity animals (starting prior to dosing on Day 1 of the dosing phase) through Day 15 of the dosing phase for local tolerance including, but not necessarily limited to, swelling and redness. Beginning on Day 15 and for all remaining dosing days, the injection site to be dosed that day was scored predose and daily for 3 days following that dose. Scoring was discontinued if no indication of irritation was noted during those 3 days.
Antibody Determinations: Samples were taken predose on Days 1 and 57 of the dosing phase from all surviving animals, predose on Day 113 for all surviving toxicokinetic animals in Groups 6 through 10, and at the scheduled dosing phase sacrifice for all surviving animals in Groups 1 through 5 for anti-Exenatide antibody formation determinations.

Results:
Mortality: A diluent control male was sacrificed in a moribund condition on Day 57 following blood collection. The cause of moribundity was not determined. A female given 3 mg/kg/dose was found dead on Day 55. Necropsy findings included large, fluid-filled kidney, thickened urinary bladder, and large ureter. Two toxicokinetic animals, a male given 1 mg/kg/dose and a female given 3 mg/kg/dose, died on Days 28 and 13, respectively, and were discarded without necropsy. One microsphere control male was found dead on Day 87 of the recovery period; the cause of death was not determined. The deaths or moribund condition of these animals, although unclear, were not attributed to effects of the test article.

Clinical signs: There were no clinical signs of toxicity noted.

Injection site reactions: Injection site edema/swelling (slight to moderate severity) were most noteworthy for animals given the control microspheres or the test article at 9 mg/kg and noted at a lesser severity (very slight to slight) for animals given 3 mg/kg. The edema/swelling was attributed to the physical presence of the microspheres. Observations of very slight to slight redness/erythema were noted at a low incidence in the control microsphere and test article-treated groups and were attributed to the injection trauma. During the recovery phase, palpable lumps typically resolved within 4 weeks of the last dose in the control microsphere, 3 mg/kg, and 9 mg/kg groups.

Palpable lumps at injection sites A, B, C and D

Dose (mg/kg)	Diluent control		Control Microsphere		1		3		9	
N	15	15	15	15	15	15	15	15	15	15
Sex	M	F	M	F	M	F	M	F	M	F
Palpable lump - A	0	0	15	15	0	0	13	15	15	15
Palpable lump - B	0	0	15	15	1	0	14	12	15	15
Palpable lump - C	0	0	15	15	0	0	14	13	15	15
Palpable lump - D	0	0	15	15	0	0	14	13	15	15

Injection site observations:

Dose (mg/kg)	Diluent control		Control Microsphere		1		3		9	
N	15	15	15	15	15	15	15	15	15	15
Sex	M	F	M	F	M	F	M	F	M	F
Swelling (3)										
Site A	0	0	4	0	0	0	0	0	5	1
Site B	0	0	0	0	0	0	0	0	6	0
Site C	0	0	0	0	0	0	0	0	9	1
Site D	0	0	1	0	0	0	0	0	5	1
Swelling (2)										
Site A	0	0	15	6	0	0	3	1	15	14
Site B	0	0	9	4	0	0	11	1	13	13
Site C	0	0	8	5	3	0	5	0	12	10
Site D	0	0	13	3	0	0	4	2	12	5
Swelling (1)										
Site A	0	0	15	15	4	0	15	15	10	14
Site B	0	0	14	13	4	5	14	13	7	12
Site C	1	0	13	14	13	10	14	14	9	12
Site D	0	0	13	15	11	5	15	15	10	15
Redness (2)										
Site A		0		0		0		0		0
Site B		0		0		0		0		0
Site C		0		0		1		0		0
Site D		0		0		0		0		1
Redness (1)										
Site A	0	0	2	4	0	0	1	0	0	0
Site B	0	0	0	0	0	0	0	0	0	0
Site C	0	0	0	2	0	0	0	0	0	0
Site D	0	0	0	3	0	0	0	0	0	0

Redness: No redness – 0; Very slight redness (barely visible) – 1; Slight redness (edges well defined by distinct redness) – 2; Moderate redness (> slight, < severe) – 3; Severe redness (beet redness) – 4.
No swelling – 0; Very slight swelling (barely visible) – 1; Slight swelling (edges well defined by raising) – 2; Moderate swelling (raised up to approx 2 mm) – 3; Severe swelling (raise greater than approx 2 mm) – 4; Swelling with open drainage – 5.

Body Weights (g): Final body weights were lower for exenatide-treated animals compared with controls. The effects on body weight correlated with decreased food consumption and are attributed to the pharmacodynamic activity of the test article. At the end of recovery, body weights and food consumption were similar to control values.

Dose (mg/kg)	Diluent control		Control Microsphere		1		3		9	
N	15	15	15	15	15	15	15	15	15	15
Sex	M	F	M	F	M	F	M	F	M	F
Predose	247	176	247	176	247	176	248	176	247	176
Dosing Day 120	589	318	608	319	564	303	539*	296	523*	298
% ↓ in mean b. wt.	-	-	-	-	4	5	8	7	11	5

* $p<0.05$ (relative to diluent control)

Food consumption: (g/animal/day)

Dose (mg/kg)	Diluent control		Control Microsphere		1		3		9	
N	15	15	15	15	15	15	15	15	15	15
Sex	M	F	M	F	M	F	M	F	M	F
Predose Week	153	114	151	111	153	113	154	113	152	109
Dosing week 1	214	159	219	149*	193*	145*	169*	118*+	152*	112*+
Dosing week 9	248	166	245	166	223*	158	229*	162	233	164
Dosing week 17	253	174	256	173	231* 9%↓	167 4%↓	226* 11%↓	165 5%↓	239 6%↓	168 3%↓

* $p<0.05$ (relative to diluent control); + $p<0.05$ (relative to control microsphere)

Ophthalmoscopy: No treatment-related ophthalmoscopic findings were observed.

Electrocardiography: No data.

Hematology: There was no significant difference between hematology parameters of controls and drug treated animals.

Clinical chemistry: There were no treatment-related clinical chemistry changes.

Urinalysis: No data.

Organ weights: Terminal sacrifice. Some differences in organ weights were noted when compared with control values, however these effects were likely due to decreased body weights for exenatide-treated animals.

Gross pathology: Terminal sacrifice (also see injection site observations above)

Dose (mg/kg)	Diluent control		Control Microsphere		1		3		9	
N	10	10	10	10	10	10	10	10	10	10
Sex	M	F	M	F	M	F	M	F	M	F
Injection site A Thickened	0	0	8	10	2	0	7	8	1	9
Injection site C Thickened	0	0	2	8	2	0	7	4	8	8
Injection site D Thickened	0	0	9	10	2	0	9	8	10	10

Histopathology:
Tissues from terminal sacrifice toxicity animals from Groups 1 and 5 (diluent control and HD groups) and from the toxicity animals that died or were sacrificed at an unscheduled interval were processed for microscopic evaluation. In addition, the injection sites, pancreas, kidneys, adrenal gland, thyroid with parathyroid, thymus, parotid salivary gland, spleen, heart, liver, and macroscopic lesions from each toxicity animal in Groups 2 through 4 were processed for microscopic evaluation.

Histopathology revealed foamy macrophages/ fibroblasts and lymphocytic infiltrate at the injection sites of animals in all study groups; these effects are believed to have been due to injection trauma. Granulomas of minimal to slight severity were observed at injection sites for animals receiving microspheres, either with or without exenatide. This finding is thought to be the result of a foreign body response to the microspheres. After recovery, foamy macrophages and fibroblasts at injection sites were still observed, but were less prominent and a higher percentage of animals did not have this finding compared with the main group animals suggesting a trend toward recovery. A notable decrease in the number of granulomas, as well as the absence of microspheres, also indicated reversibility.

One HD female had renal tubular adenomas unilaterally with a focus of slight tubular hyperplasia in the contralateral kidney. One MD female had a renal tubular adenoma. One LD female and one microsphere control female had slight and minimal renal tubular hyperplasia, respectively. After recovery, one LD female had a renal tubular adenoma and one microsphere control female had atypical renal tubular hyperplasia. The sponsor contended that the tumors and hyperplasia were not drug-related because carcinogen-induced tumors of the renal tubules are typically slow growing with a long latency for development and the relatively short study duration (18-months) suggests that the adenomas were spontaneous occurrences. The sponsor added that even some of the most potent renal carcinogens have a latency period of 6 to 12 months before tumors develop (Hard, 1990; Hard et al., 1970). Additionally, there was no evidence of nephrotoxicity, which often precedes atypical tubular hyperplasia and renal tumorigenesis.

Lesions observed in the parotid salivary gland (atrophy), heart (hemorrhage), lung (mixed cell inflammation), mammary gland (corpora amylacea), stomach (erosion, lymphocytic infiltrate, hyperplasia) and uterus (dilatation) were of very low incidence (1/10) and minimal to slight in severity. A summary of histopathology findings is shown in the table below.

Dose (mg/kg)	Diluent control		Control Microsphere		1		3		9	
N	10	10	10	10	10	10	10	10	10	10
Sex	M	F	M	F	M	F	M	F	M	F
Parotid salivary gland Atrophy	0	0	0	0	0	0	1 1(2)	0	1 1(2)	0
Heart Hemorrhage	0	0							1 1(1)	0
Lung Mixed cell inflammation	0	0							1 1(1)	1 1(1)
Mammary gland Corpora amylacea		0								1 1(1)
Stomach, glandular Erosion	0	0							1 1(1)	1 1(1)
Epithelial hyperplasia	0	0							1 1(1)	0
Lymphocyte infiltrate	0	0							1 1(1)	0
Stomach, nonglandular Hyperplasia, squamous cell	0	0							0	1 1(1)
Kidney Atypical hyperplasia, tubule cell	0	0	0	1 1(1)	0	1 1(2)	0	0	0	1 1(1)
Adenoma (benign), tubule cell	0	0	0	0	0	0	0	1(2)	0	1(2)
Hyperplasia, transitional cell	0	0	0	0	0	0	0	1(3)	0	0
Injection site A Foamy macrophages/fibroblasts	10 9(1) 1(2)	8 7(1) 1(2)	10 9(1) 1(2)	10 10(1)	10 6(1) 4(2)	9 9(1)	10 7(1) 3(2)	10 6(1) 4(2)	5 5(1)	10 8(1) 2(2)
Muscle degeneration	1(1)	2(1)	0	0	0	0	0	0	0	1(1)
Granuloma	0	0	9 9(2)	9 9(2)	5 2(1) 3(2)	4 1(1) 3(2)	7 1(1) 6(2)	7 1(1) 6(2)	5 4(1) 1(2)	9 1(1) 8(2)
Lymphocyte infiltrate	0	0	0	0	4(1)	2(1)	0	0	1(1)	1(1)
Injection site B Foamy macrophages/fibroblasts	2 1(1) 1(2)	9 9(1)	8 7(1) 1(2)	9 9(1)	6 6(1)	10 10(1)	7 7(1)	10 10(1)	5 5(1)	8 8(1)
Granuloma	0	0	5 4(1) 1(2)	1 1(1)	1 1(1)	0	1 1(1)	3 2(1) 1(2)	5 1(1)	3 1(1) 2(2)
Lymphocyte infiltrate	0	2(1)	1(1)	0	3(1)	0	1(1)	2(1)	1(1)	0
Injection site C	9	8	8	9	8	7	10	10	7	10
Foamy macrophages/fibroblasts	6(1) 3(2)	7(1) 1(2)	8(1)	9(1)	7(1) 1(2)	7(1)	9(1) 1(2)	10(1)	6(1) 1(2)	10(1)
Lymphocyte infiltrate	2(1)	2(1)	0	0	1(1)	1(1)	0	0	1(1)	0
Granuloma	0	0	8 8(2)	7 7(2)	6 6(2)	3 2(1) 1(2)	9 9(2)	8 1(1) 7(2)	9 1(1) 8(2)	4 4(2)
Injection site D Foamy macrophages/fibroblasts	8 5(1) 3(2)	9 9(1)	10 8(1) 2(2)	10 10(1)	10 7(1) 3(2)	10 10(1)	10 8(1) 2(2)	10 10(1)	10 9(1) 1(2)	10 10(1)
Lymphocyte infiltrate	1(1)	2(1)	1(1)	0	0	1(2)	0	0	1(1)	0
Granuloma	0	0	10 10(2)	10 10(2)	3 1(1) 2(2)	5 4(1) 1(2)	10 1(1) 9(2)	9 1(1) 8(2)	10 10(2)	9 9(2)
Uterus Dilatation		2 1(1) 1(2)								4 1(1) 2(2) 1(3)

1 = minimal; 2 = slight; 3 = moderate

Toxicokinetics: (sponsor-generated table)
TK data indicate that steady-state concentrations are achieved by approximately 2 months of dosing. The presence of anti-exenatide antibodies correlated with higher plasma concentrations such that a dose-exposure relationship was not observed. However, when considering the data from antibody negative animals only, exposure did increase with increasing dose level. Because of the effects of antibodies on the exposure data, values from antibody-negative animals and humans were used to calculate exposure safety margins.

Mean Toxicokinetic Parameters of Exenatide following Single or Multiple Bi-weekly Subcutaneous Administration in Sprague-Dawley Rats (REST050369)

	Day 1 Exposure (all animals)		Day 113 Exposure (all animals)		Day 113 Exposure (antibody negative animals)	
	C_{max} (pg/mL)	AUC_{0-336h} (pg·h/mL)	C_{max} (pg/mL)	AUC_{0-336h} (pg·h/mL)	C_{max} (pg/mL)	AUC_{0-336h} (pg·h/mL)
1 mg/kg/dose						
Female	1823	112,684	12,823	1,048,608	2880	363,660
Male	2760	154,068	58,763	2,972,064	1730	214,490
Combined	2292	133,376	35,793	2,010,336	2305	289,075
3 mg/kg/dose						
Female	2243	233,005	33,067	2,903,585	NC	NC
Male	3460	315,772	7003	1,086,097	4455	787,858
Combined	2852	274,389	20,035	1,994,841	NC	NC
9 mg/kg/dose						
Female	7967	401,290	19,233	2,095,103	21,600	2,165,137
Male	5867	425,077	20,433	2,854,200	11,967	1,948,442
Combined	6917	413,183	19,833	2,474,652	16,783	2,056,789

Abbreviations:
AUC_{0-336h} = Area under the concentration-time curve for the dosing interval
C_{max} = maximum observed plasma concentration during the dosing interval
NC = Not calculated.

Antibody assessment: (sponsor-generated tables)
Anti-exenatide antibodies were observed at ≥1 mg/kg/dose with no apparent dose-response relationship for incidence. The higher titers occurred on Day 57 and generally decreased towards the end of treatment (Month 4, with positive titers remaining following the recovery period. On Day 57, titers ranged from 25 to 390,625 at 1 mg/kg, 25 to 15,625 at 3 mg/kg, and 25 to 625 at 9 mg/kg. The antibody titer for the combined main and toxicokinetic exenatide LAR-treated groups tended to show an inverse relationship to dose.

Summary of Anti-exenatide Reactive Antibody Results for Toxicity Animals

		Pretreatment		Treatment		End of Treatment				End of Recovery	
Study Day		Day 1		Day 57		Day 113		Week 18		Week 31	
Sex (M/F)		M	F	M	F	M	F	M	F	M	F
Dose Group 1 (0 mg/kg, Vehicle Control)	Incidence (+/N)	0/15	0/15	0/15	1/15	NA	NA	0/14	0/15	0/4	0/5
	Titer Range	NA	NA	NA	25	NA	NA	NA	NA	NA	NA
Dose Group 2 (0 mg/kg, Control Microspheres)	Incidence (+/N)	0/15	0/15	0/15	0/15	NA	NA	2/15	0/15	0/4	0/5
	Titer Range	NA	NA	NA	NA	NA	NA	25	NA	NA	NA
Dose Group 3 (1 mg/kg)	Incidence (+/N)	0/15	1/15	10/15	8/15	NA	NA	10/15	9/15	3/5	3/5
	Titer Range	NA	25	25-390,625	125-78,125	NA	NA	25-15,625	25-3,125	25-125	25-625
Dose Group 4 (3 mg/kg)	Incidence (+/N)	0/15	0/15	3/15	11/14[a]	NA	NA	2/15	7/14[a]	0/5	2/4
	Titer Range	NA	NA	25-3,125	25-3,125	NA	NA	25-3,125	125-3,125	NA	25-125
Dose Group 5 (9 mg/kg)	Incidence (+/N)	1/15	0/15	7/15	8/14[b]	NA	NA	5/15	6/15	1/5	5/5
	Titer Range	25	NA	25-625	25-625	NA	NA	25-625	125-625	125	25-125

[a] No specimens received for B52009, beyond Day 1.
[b] B52026 Day 57 specimen was reported as QNS (quantity was not sufficient to assay).
M = Male, F = Female, Incidence = incidence of positive results, "+" = Number of Positive Specimens, N = total number of specimens, NA = Not applicable.

Summary of Anti-exenatide Reactive Antibody Results for Toxicokinetic Animals

		Pretreatment		Treatment		End of Treatment				End of Recovery	
Study Day		Day 1		Day 57		Day 113		Week 18		Week 31	
Sex (M/F)		M	F	M	F	M	F	M	F	M	F
Dose Group 6 (0 mg/kg, Vehicle Control)	Incidence (+/N)	0/3	0/3	0/3	0/3	0/3	0/3	NA	NA	NA	NA
	Titer Range	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dose Group 7 (0 mg/kg, Control Microspheres)	Incidence (+/N)	0/3	0/3	0/3	0/3	0/3	0/3	NA	NA	NA	NA
	Titer Range	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dose Group 8 (1 mg/kg)	Incidence (+/N)	0/9	0/9	4/8[a]	3/9	4/8[a]	5/9	NA	NA	NA	NA
	Titer Range	NA	NA	625	25-3,125	25-3,125	25-625	NA	NA	NA	NA
Dose Group 9 (3 mg/kg)	Incidence (+/N)	0/9	0/9	3/9	6/8[b]	2/9	6/8[b]	NA	NA	NA	NA
	Titer Range	NA	NA	25-125	25-15,625	25-625	25-3,125	NA	NA	NA	NA
Dose Group 10 (9 mg/kg)	Incidence (+/N)	0/9	0/9	1/9	4/9	1/9	1/9	NA	NA	NA	NA
	Titer Range	NA	NA	125	25	25	25	NA	NA	NA	NA

[a] No specimens received for B51939 beyond Day 1.
[b] No specimens received for B52052 beyond Day 1.
M = Male, F = Female, Incidence = incidence of positive results, "+" = Number of Positive Specimens, N = total number of specimens, NA = Not applicable.

Toxicology conclusions:

Exenatide LAR was well tolerated following 18 weeks of bi-weekly (9 doses) subcutaneous administration. The primary findings were decreased body weights and food consumption, expected pharmacodynamic effects of exenatide, and microscopic findings at the injection sites, which were related to the physical trauma of the injections and foreign body reactions to the microspheres. There were no drug-related deaths or significant target organ toxicities. Hence, the MTD is 9 mg/kg (27X MRHD, AUC for Ab negative animals).

Study title: A 3-Month Toxicity Study of Exenatide LAR (AC2993-F17) Following Weekly Subcutaneous Administration in the Cynomolgus Monkey With a 13-Week Recovery Period

Note: this study was previously reviewed by Dr. John Colerangle under IND 67,092, the review for which is presented below with minor modifications. This reviewer is in agreement with Dr. Colerangle's assessments and conclusions. The study report was amended two times since the initial review; the TK report was amended to provide TK values for antibody negative animals. Overall, the changes did not affect the previous study conclusions.

Key study findings:

- Reversible decreases in body weight gain was observed in all treated animals relative to controls.
- Increases in AST (2-3X, not dose dependent) and ALT (1-4X, dose dependent in females) were observed in treated monkeys at the end of the recovery period. It is not clear if this is treatment-related or not. A slight but reversible increase in potassium was noted in females at 0.44 mg/kg.
- Mean absolute thymus weights were decreased by 45-50% in microsphere control males and males dosed at 0.44 and 1.10 mg/kg with no correlative histopathology. These decreases showed full to no recovery.
- Macroscopic lesions confined mainly to the injection sites were characterized by red or white discoloration, nodules, abscesses, and thickened tissue due to needle trauma. The nodules appear to increase in severity with increase in dose. Lobulation of the liver and red discoloration of the lung were noted with no dose-related pattern. At the end of the recovery period, the incidence of macroscopic lesions was drastically reduced suggestive of reversibility.
- Microscopic lesions confined mainly to the injection sites for all groups were characterized by chronic inflammation, abscesses, epidermal hyperplasia (correlates with the thickened sites), fibrosis, and hemorrhage. Granulomatous inflammation (minimal to severe) and foreign material were noted at the injection site of animals receiving microspheres with or without exenatide. At the end of the recovery period, the incidence of microscopic lesions was drastically reduced suggestive of reversibility. No microscopic lesions were observed in other tissues.
- Treatment-related injection site enlargement was noted during the treatment period for animals receiving microspheres. Most of the injection site enlargements were reversed early on in the recovery period while the few remaining were reversed towards the end of the recovery period. No evidence of sensitization was noted following a challenge dose of control microspheres (microspheres in diluent without exenatide) administered to a previously undosed site (Site 14) on all recovery animals.
- Anti-exenatide antibodies were evident as early as Day 29 at ≥0.44 mg/kg, and were evident in all exenatide LAR-treated groups by Day 92 with most animals positive at ≥0.44 mg/kg. On Day 29, positive results were observed in 3/8 animals with titers 5-625 and 1/8 animals with a titer of 3,125 at 0.44 and 1.10 mg/kg, respectively. On Day 92, positive results were observed in 2/8 animals with titers of 125-3,125; 7/8 animals with titers of 25-78,125; and 5/8 with titers of 25-3,125 at 0.11, 0.44, and

1.10 mg/kg, respectively. Following the recovery period, most exenatide LAR treated animals were still positive, though titers tended to reduce to a range of 25-625.

- The NOAEL was considered to be 1.10 mg/kg (19X the highest anticipated clinical dose, based on AUC values for Ab negative animals) due to lack of drug related microscopic lesions in any tissue.

Study no: REST04289R2
Volume # and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: July 8, 2004
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: AC2993-F17; Lot # 278-1414A; purity not provided
Formulation/vehicle: AC2993-F17 resuspended in microsphere diluent

Methods (unique aspects)**:**
Dosing:

Species/strain: Monkey/Cynomolgus
Number/sex/group (main study): 4/sex/group
Number/sex/group (recovery groups): 1/sex/group
Number/sex/group (TK): 4/sex/group (main animals used for TK)
Age: 1 year 11 months – 2 years 8 months
Weight: 1.8-2.0 kg (M); 1.7-2.8 kg (F)
Doses in administered units: 0.11, 0.44, 1.10 mg/kg
Route and volume: Subcutaneous injection; 0.088, 0.105 and 0.138 mL/kg for the LD, MD, and HD respectively

Study Design

Group Number	Number Males/Females	Dose (mg exenatide/kg)	Number of Males/Females per Termination Day	
			Day 92	Day 180[a]
1[b]	4/4	0 (vehicle; microsphere diluent)	3/3	1/1
2[b]	4/4	0 (control microspheres)	3/3	1/1
3	4/4	0.11	3/3	1/1
4	4/4	0.44	3/3	1/1
5	4/4	1.10	3/3	1/1

[a] One animal per sex per group in recovery phase.
[b] Two control groups. Plasma samples were analyzed for exenatide concentrations, but toxicokinetic analysis is limited to exenatide LAR-treated animals only (Groups 3 to 5).

Observations and times:

Clinical signs: Daily
Body weights: Measured pretest and weekly during the study
Food consumption: Quantitatively estimated daily during the study by offering 6 biscuits twice daily and recording the number of remaining biscuits at each feeding interval. Any remaining biscuits were removed prior to the next feeding interval

Ophthalmoscopy: Conducted pretest and prior to terminal and recovery necropsies
EKG: Not conducted
Hematology: Blood samples for hematology evaluation were collected from overnight fasted (had access to drinking water) animals pretest, Weeks 5, 9, 13, and 26 (recovery)
Clinical chemistry: Blood samples for clinical chemistry evaluation were collected from overnight fasted (had access to drinking water) animals pretest, Weeks 5, 9, 13, and 26 (recovery)
Urinalysis: Not conducted
Gross pathology: Organs/tissues collected for gross examination are indicated in the histopathology inventory table at the end of the toxicology section
Organs weighed: Organs weighed are indicated in the histopathology table
Histopathology: Standard tissues from all dose groups including injection sites were processed for microscopic examination - see histopathology table
Toxicokinetics: Blood samples for TK were collected predose, 0.5, 1, 2, 4, 8, 12, 24, 48, 96, 144, and 168 hours postdose beginning on Days 1 and 85. In addition, samples were collected predose on Days 15, 29, 43, 57, and 71 on all study animals, and in the AM on Days 120, 150, and 180 on all recovery animals (35, 65, and 95 days after the last dose)

Injection Site Irritation and Enlargement: The injection sites were graded for local irritation and each site was gently palpated to determine the presence and size of any local enlargement or swelling. These observations were conducted prior to injection, and then at approximately 1 and 24 hours postdose on dosing days (including the challenge dose), and weekly otherwise. The injection sites were scored using the Draize scale below:

Erythema and Eschar

0 - No erythema
1 - Very slight erythema (barely perceptible)
2 - Well-defined erythema
3 - Moderate to severe erythema
4 - Severe erythema (beet redness) to slight eschar formation (injuries in depth). Maximum possible = 4.

Serum Antibody Studies: Blood samples for serum antibody evaluation were collected at pretest, and on Days 29, 92, and 180 (recovery). Additional samples were collected from all animals at scheduled necropsy for possible future studies.

Dose Formulation Analysis: The exenatide concentration of dosing suspensions prepared for Weeks 1, 2, 3, 6, and 12 were found to be within the acceptance criteria, ranging from 98.8% to 110% of the nominal concentration.

Results:

Mortality: There were no mortalities.

Clinical signs: No treatment-related clinical findings occurred during either the dosing or recovery periods of the study. A single male (animal number 117) from the 1.10 mg/kg group exhibited a white discharge and/or ulceration at a few injection sites over the course of observations conducted on Days 34, 36, 43, 50, and 57. Since these findings were noted only in this particular animal, these findings were considered a likely artifact of a sensitivity of this particular animal to the injection procedures or to contamination of injection site, and not related to exenatide LAR (AC2993-F17).

Body weights (kg): There was no significant difference between controls and treated groups at the end of the treatment period.

Dose (mg/kg)	Vehicle Control		Control Microspheres		0.11		0.44		1.10	
Sex	M	F	M	F	M	F	M	F	M	F
Week -1	2.02	1.99	1.97	2.08	1.87	1.92	2.02	1.95	2.06	2.10
Week 13	2.27	2.10	2.06	2.18	2.03	1.99	2.03	1.92	2.05	2.10
Gain (kg)	0.22	0.11	0.09	0.10	0.16	0.07	0.01	-0.03	-0.01	0.00
Recovery Data (No Statistical analysis performed; n = 1)										
Week 13	2.27	2.10	2.06	2.18	2.03	1.99	2.03	1.92	2.05	2.10
Week 25	2.75	2.37	2.16	2.79	2.51	1.92	2.21	1.94	2.52	2.15

Food consumption: No treatment-related effects on food consumption were observed.

Ophthalmoscopy: No treatment-related ophthalmoscopic findings were noted.

Electrocardiography: Not conducted

Hematology: (Week 13 Data)

Dose (mg/kg)	Vehicle Control		Control Microspheres		0.11		0.44		1.10	
Sex	M	F	M	F	M	F	M	F	M	F
MCV (fl)	83.5		78.7* (6%↓)		80.9		81.3		78.2* (6%↓)	
Lymphocytes (10^3/ul)		5.4		7.6		9.2* (70%↑)		4.8		7.1
Recovery Data (No Statistical analysis performed; n = 1)										
MCV (fl)	79.7		75.2		80.1		79.1		75.1	
Lymphocytes (10^3/ul)		3.4		5.1		4.3		2.3		3.8

* $p<0.05$

Clinical chemistry: (Week 13 Data)

Dose (mg/kg)	Vehicle Control		Control Microspheres		0.11		0.44		1.10	
Sex	M	F	M	F	M	F	M	F	M	F
Potassium (mEq/l)		5.1		5.3		5.5		6.1* (20%↑)		6.0
GGT (U/l)	91	55	77	67	82	78	78	60	67	68
AST (U/l)	36	34	40	38	37	37	40	35	40	39
ALT (U/l)	38	34	36	40	36	44	38	33	37	39
Recovery Data (No Statistical analysis performed; n = 1)										
Potassium (mEq/l)		4.7		4.4		5.7		5.2		5.9
GGT (U/l)	105	58	98	74	112	80	40	80	93	89
AST (U/l)	44	33	75 (2X↑)	49	49	61 (2X↑)	129 (3X↑)	62 (2X↑)	88 (2X↑)	59 (2X↑)
ALT (U/l)	48	24	53 (1X↑)	54 (2X↑)	36	62 (3X↑)	63	71 (3X↑)	73 (2X↑)	99 (4X↑)

* $p<0.05$

Urinalysis: Not conducted.

Organ weights:

Dose (mg/kg)	Vehicle Control		Control Microspheres		0.11		0.44		1.10	
Sex	M	F	M	F	M	F	M	F	M	F
Thymus (g)	4.7	2.7	2.5* (47%↓)	2.5	2.9	3.2	2.4* (50%↓)	1.6 (41%↓)	2.6* (45%↓)	1.8 (33%↓)
Recovery Data (n = 1)										
Thymus (g)	8.5	4.3	3.8 (55%↓)	4.0	7.1	2.6 (40%↓)	2.0 (77%↓)	1.3 (70%↓)	6.3 26%↓)	2.8 (35%↓)

* $p<0.05$

Gross pathology

Dose (mg/kg)	Vehicle Control		Control Microspheres		0.11		0.44		1.10	
Sex	M	F	M	F	M	F	M	F	M	F
N	3	3	3	3	3	3	3	3	3	3
Injection site 1 Red discoloration	1 1(2)		0		0		0		0	
Injection site 2 Red discoloration	1 1(1)		0		0		0		0	
Injection site 3 White discoloration		0		0		0		1 1(1)		0
Injection site 4 Nodule	0		1 1(1)		0		0		0	
Injection site 5 Red discoloration		0		0		1 1(1)		0		0
Nodule	0		0		0		1(2)		1(2)	
Injection site 6 Red discoloration	1 1(1)		0		0		0		0	
White discoloration		0		0		1(2)		0		0
Nodule	0	0	0	1(2)	0	0	1(2)	0	0	1(1)
Injection site 7 Red discoloration	0		0		1 1(2)		0		0	
Nodule	0	0	2(2)	1(2)	0	0	1(2)	1(2)	3(2)	2(2)
Thickened	0		1(2)		0		0		0	
Injection site 8 Nodule	0	0	2 1(1) 1(2)	2 1(1) 1(3)	0	2 1(1) 1(2)	1 1(2)	3 3(2)	3 3(2)	3 2(2) 1(3)
Thickened	0		1(2)		0		0		0	
Injection site 9 Abscess	0		0		0		1		0	
Nodule	0	0	2 1(2) 1(3)	2 2(2)	1 1(1)	2 2(2)	1 1(2)	3 3(2)	3 3(2)	3 2(2) 1(3)
Injection site 10 Nodule	0	0	1 1(2)	3 3(2)	2 1(1) 1(2)	3 3(2)	2 2(2)	3 2(2) 1(3)	2 1(2) 1(3)	3 2(2) 1(3)
Thickened	0		1(1)		0		0		0	

Injection site 11 Nodule	0	0	2 1(2) 1(3)	3 2(2) 1(3)	2 1(1) 1(2)	2 2(2)	1 1(2)	3 2(2) 1(3)	3 3(2)	3 1(2) 2(3)
Thickened	0		1(2)		0		0		0	
White discoloration		1(2)		0		0		0		0
Injection site 12 Nodule	0	0	2 2(2)	3 2(2) 1(3)	2 1(1) 1(2)	2 1(1) 1(2)	1 1(2)	3 3(2)	3 3(2)	3 1(1) 1(2) 1(3)
Thickened	0		1(3)		0		1(2)		0	
Injection site 13 Abscess	0		0		0		1 1(2)		0	
Nodule	0	0	2 2(2)	2 2(2)	1 1(2)	1 1(1)	2 2(2)	2 1(2) 1(3)	3 1(1) 2(2)	3 2(2) 1(3)
White discoloration		1(2)		0		0		0		0
Thickened	0		1(2)		0		0		0	
Liver, Lobulation, accentuated	0	0	1 1(1)	0	0	0	1 1(2)	0	0	1 1(2)
Lung Red discoloration		0		0		1 1(2)		0		1 1(2)
Recovery Data (n = 1)										
Injection site 12 Nodule		0		0		1 1(1)		0		0
Injection site 14 Nodule		0		0		1 1(2)		0		0
Lung Adhesion		0		0		0		0		1 1(1)
Red discoloration		0		0		0		1(3)		0

1 = minimal; 2 = mild; 3 = moderate

Histopathology: Standard tissues from all dose groups including injection sites were processed for microscopic examination.

Dose (mg/kg)	**Vehicle Control**		**Control Microspheres**		**0.11**		**0.44**		**1.10**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Injection site 1 Exudate, epidermal surface	1 1(1)	1 1(2)	0	0	0	0	0	0	0	0
Erosion/ulcer		1(2)		0		0		0		0
Hyperplasia, epidermal	1(1)		0		0		0		0	
Chronic inflammation,	0	0	0	0	0	0	1(1)	1(1)	0	0
Necrosis		1(2)		0		0		0		0
Injection site 2 Hemorrhage	1 1(1)	0	0	0	0	0	0	0	0	0
Chronic inflammation	1(1)	0	0	0	0	0	1(1)	0	0	0
Granulomatous inflammation	0	0	0	0	0	0	0	0	1(1)	0
Injection site 3 Hyperplasia, epidermal	0	0	0	1 1(1)	0	0	0	0	0	0
Granulomatous inflammation		0		0		0		1(1)		2(1)
Injection site 4 Chronic inflammation	0	0	0	0	0	1 1(1)	0	0	1 1(1)	0
Granulomatous inflammation	0	0	2 1(1) 1(2)	0	0	0	1 1(1)	0	1 (1)	0
Injection site 5 Granulomatous inflammation	0	0	1	0	1 1(1)	0	1	1 1(1)	3 1(1)	1 1(1)
			1(2)				1(2)		1(2)	

Injection site 6 Granulomatous inflammation	0	0	1 1(2)	2 1(1) 1(2)	0	0	1 1(1)	3 3(1)	2 2(1)	2 1(1) 1(3)
Injection site 7 Hemorrhage	0	0	0	0	1 1(1)	0	0	0	0	0
Foreign material		0		1(1)		0		0		0
Granulomatous inflammation	0	0	3 1(2) 2(3)	3 1(1) 2(2)	1 1(1)	1 1(2)	1 1(2)	1 1(1)	3 2(2) 1(3)	2 1(1) 1(3)
Injection site 8 Granulomatous inflammation	0	0	2 1(2) 1(3)	3 1(1) 2(2)	0	2 1(1) 1(2)	1 1(2)	2 2(2)	3 2(2) 1(3)	3 2(2) 1(3)
Abscess	0	0	0	0	0	1(1)	0	0	0	0
Foreign material	0	0	0	1(1)	0	1(1)	0	0	0	0
Injection site 9 Foreign material	0	0	0	0	0	1 1(2)	1 1(2)	1 1(2)	0	0
Abscess		0		0		1(2)		1(2)		0
Granulomatous inflammation	0	0	2 1(2) 1(4)	3 1(2) 2(3)	0	3 2(2) 1(3)	2 1(1) 1(3)	3 3(2)	3 1(2) 2(3)	3 2(2) 1(3)
Injection site 10 Foreign material	0	0	0	0	0	0	1 1(2)	0	0	0
Granulomatous inflammation	0	0	2 1(2) 1(3)	3 3(2)	2 2(1)	3 3(1)	2 1(2) 1(3)	3 1(1) 1(2) 1(3)	2 2(3)	3 3(3)
Injection site 11 Foreign material	0	0	0	0	0	0	1 1(2)	1(1)	0	0
Abscess	0	0	0	0	0	1(1)	0	0	0	0
Hemorrhage	0	1(2)	0	0	0	0	0	0	0	0
Granulomatous inflammation	0	0	3 2(2) 1(3)	3 3(2)	2 2(1)	2 1(1) 1(2)	1 1(3)	3 3(2)	3 3(2)	3 1(2) 2(3)
Injection site 12 Foreign material	0	0	0	0	0	1 1(1)	1 1(3)	1 1(2)	0	0
Abscess	1 1(1)	0	0	0	0	1 1(2)	0	1 1(2)	0	0
Granulomatous inflammation	0	0	3 3(3)	3 1(1) 2(2)	2 2(1)	2 1(1) 1(2)	2 1(1) 1(4)	3 1(1) 1(2) 1(3)	3 3(3)	3 1(1) 2(2)
Fibrosis	0	1(2)	0	0	0	0		0	0	0
Injection site 13 Abscess	0	0	0	0	0	0	1 1(2)	1 1(2)	0	1 1(1)
Foreign material	0	0	0	0	0	0	2(1)	1(2)	0	0
Granulomatous inflammation	0	0	3 2(2) 1(3)	2 1(1) 1(2)	1 1(1)	1 1(1)	3 1(1) 1(2) 1(3)	2 1(2) 1(3)	3 1(1) 2(3)	3 1(1) 2(2)
Hemorrhage	0	1(2)	0	0	0	0	0	0	0	0

Recovery Data (n = 1)										
Injection site 11 Granulomatous inflammation	0	0	1 1(2)	0	0	0	0	0	0	0
Chronic inflammation	0	0	0	0	0	0	0	0	1(1)	0
Injection site 14 Granulomatous inflammation	1 1(3)	1 1(3)	0	0	1 1(3)	0	1 1(3)	0	1 1(2)	1 1(3)
Injection site 9 Granulomatous inflammation	0	0	1 1(1)	0	0	0	0	0	0	0
Injection site 10 Granulomatous inflammation	0	0	0	0	0	0	0	0	0	1 1(1)
Injection site 12 Granulomatous inflammation	0	0	0	0	0	0	0	0	0	1 1(1)
Injection site 13 Granulomatous inflammation	0	0	0	1 1(1)	0	0	0	0	0	0

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

Injection Site Irritation and Enlargement: With the exception of slight erythema at injection Site 3 on Day 29 and injection Site 5 on Day 43 in a single 0.44 mg/kg animal, all injection site irritation noted during the dosing period was limited to animal number 117 from the 1.10 mg/kg group. This animal, as discussed in the clinical findings section, exhibited the following incidences of injection site irritation during the dosing period (none of which persisted into recovery) as presented in the sponsor-generated table below.

Injection Site Erythema for Animal Number 117 During Dosing Period		
Day of Study	Site Affected	Severity
29	3	Slight erythema
36	3	Moderate erythema
43	3	Slight erythema
29	4	Slight erythema
36	4	Well-defined erythema
43	4	Slight erythema
36	5	Well-defined erythema
43	5	Slight erythema
35	6	Moderate erythema
36	6	Moderate erythema
43	6	Well-defined erythema
50	6	Slight erythema
42	7	Well-defined erythema
43	7	Well-defined erythema
44	7	Slight erythema
50	7	Well-defined erythema
85	12	Slight erythema

Anatomical description of injection sites was not provided

According to the sponsor, treatment-related injection site enlargement noted during the dosing period of the study, is an expected finding following subcutaneous injection of microspheres, and was also present in a fairly comparable manner in the group receiving control microspheres without exenatide. Of the groups receiving exenatide, the 0.11 mg/kg group was least affected in incidence, indicating a correlation to dose volume administered. Most of the enlargements noted were of the small variety (1-5 mm), with a

small number reaching medium size (6-20 mm). Animal number 117 exhibited a few enlargements of comparatively large size (>20 mm) in conjunction with the other findings noted above. The persistence of the enlargements generally varied during the dosing period for the earlier sites, but in some cases was as much as 4 to 6 weeks from onset in microsphere groups receiving the highest volumes (microsphere control and 1.10 mg/kg). Most of the injection-site enlargements were reversed early on in the recovery period (by Days 99, 106, 120, or 127), while the few remaining injection site enlargements were reversed towards the end of the recovery period (by Day 148).

Following a challenge dose of control microspheres (microspheres in vehicle without exenatide) administered to a previously undosed site (Site 14) on all recovery animals approximately 4 weeks into the recovery period (Day 120), no evidence of sensitization was noted. Injection-site irritation findings noted after this challenge dose were sporadic and limited to slight erythema (noted on Days 127 and 134 for the 1.10 mg/kg male and on Day 121 for the 0.44 mg/kg female). The sponsor stated that the course of injection-site enlargement following the Day 120 challenge was typical and expected, and exhibited a trend toward reversibility over the course of the remainder of the recovery period after the challenge dose.

Anti-exenatide antibodies: Anti-exenatide antibodies were evident as early as Day 29 at ≥0.44 mg/kg, and were evident in all exenatide LAR-treated groups by Day 92 with most animals positive at ≥0.44 mg/kg. On Day 29, positive results were observed in 3/8 animals with titers ranging from 5 to 625 and 1/8 animals with a titer of 3,125 at 0.44 and 1.10 mg/kg, respectively. On Day 92, positive results were observed in 2/8 animals with titers ranging from 125 to 3,125; 7/8 animals with titers ranging from 25 to 78,125; and 5/8 with titers ranging from 25 to 3,125 at 0.11, 0.44, and 1.10 mg/kg, respectively. Following the recovery period, most exenatide LAR treated animals were still positive, though titers tended to reduce to a range of 25 to 625. A summary of antibody results is shown the in sponsor-generated table below.

Summary of Anti-exenatide Antibody Results

Specimen Collection Time	Pretest		Day -7		Day 29		Day 92		Day 180	
	M	F	M	F	M	F	M	F	M	F
Dose Group 1 Positives	0	0	0	0	0	0	0	0	0	0
Dose Group 2 Positives	0	0	0	0	0	0	0	0	0	0
Dose Group 3 Positives	0	0	0	0	0	0	0	2	0	1
Dose Group 4 Positives	0	0	0	0	3	0	4	3	1	1
Dose Group 5 Positives	0	0	0	0	1	0	2	3	1	1
Total Positive Specimens (N)	0		0		4		14		5	
Total Specimens (N)	1		40		40		40		10	
Range of Titers	N/A		N/A		Neg-3125		Neg-78125		Neg-625	

M = Male, F = Female, N/A = Not Applicable (all specimens were negative) Neg = Negative.
Specimens were collected from 4-animals/sex/dose group for all time points except Pretest and Day 180.
For Day 180 specimens were collected from 1-animal/sex/dose group.

Toxicokinetics:
Exenatide concentrations characterized over the entire dosing period after dosing on Days 1 and 85 showed that the highest concentrations occurred within the first few hours after dosing. Predose plasma exenatide concentrations generally increased over the entire dosing period after each weekly administration, from less than the lower limit of quantitation (LLOQ = 10 pg/ml) on Day 1 to a plateau by approximately Day 56 in all treatment groups, indicating that steady-state concentrations were achieved in less than 2 months. Maximum plasma concentrations during the entire study were generally observed following the Day 85 dose.

Antibodies to exenatide, which occurred in approximately 58% of exenatide LAR-treated animals, altered the toxicokinetics. Low exenatide concentrations near or below the LLOQ were observed in two animals from the 0.44 mg/kg group and one from the 1.10 mg/kg group that had high antibody titers (3,125 to 78,125) following Day 85 treatment. The highest exenatide concentrations were observed in two animals from the 1.10 mg/kg group with intermediate titers (125 and 625). Regardless of antibody status, exenatide exposure increased with increasing dose and after repeated dosing in both males and females. There were no apparent gender effects on the TK profile. The ratio of maximal plasma concentrations to minimum plasma concentrations were markedly (~3 to 45 fold) greater on Day 1 than on Day 85, indicating that plasma levels become more consistent at steady state. Mean accumulation, as determined by comparing Day 85 exposures to Day 1, ranged from 12 to 16 fold in animals that remained negative for antibodies through the end of treatment. After discontinuation of dosing on Day 85, plasma concentrations in recovery animals remained elevated for at least 35 days and were near or below the LLOQ after 65 days, indicating that washout occurred within approximately 2 months. A summary of TK results is shown in the sponsor-generated table below.

Table 1: Mean Exposure Parameters in Cynomolgus Monkeys Treated by Weekly Subcutaneous Administration of Exenatide LAR (AC2993-F17) for 3 Months ((b) (4)843-032; REST04289)

Dose (mg/kg)	Day 1 (all TK animals, N=8)		Day 85 (all TK animals, N=8)		Day 85 (animals negative for antibodies to exenatide)		
	C_{max} (pg/mL)	$AUC_{0\text{-}168h}$ (pg·h/mL)	C_{max} (pg/mL)	$AUC_{0\text{-}168h}$ (pg·h/mL)	N	C_{max} (pg/mL)	$AUC_{0\text{-}168h}$ (pg·h/mL)
0.11	334	7,442	2,895	114,397	6	3,372	131,407
0.44	1,208	30,483	5,434	283,977	1	6,300	571,475
1.10	2,915	59,582	19,939	973,761	3	17,433	733,436

Abbreviations: $AUC_{0\text{-}168h}$ = Area under the concentration-time curve for the dosing interval; C_{max} = Maximum observed plasma concentration during the dosing interval; N = Number of animals.

($AUC_{0\text{-}168h}$ at the highest anticipated clinical dose for Exenatide QW (2 mg/week) is 38,230 pg·h/ml)

Summary of individual study findings: In a 13-week toxicity study, exenatide LAR (exenatide QW) was administered subcutaneously (at rotating injection sites) to cynomolgus monkeys as single weekly injections. Three groups of four monkeys/sex received exenatide LAR at dose levels of 0.11, 0.44, and 1.10 mg/kg/week. These doses represent 3X, 15X and 19X of the highest anticipated clinical dose (2 mg/week) based on AUC values of antibody negative animals and humans. Two control groups each

included four monkeys per sex receiving the microsphere diluent vehicle or the diluent plus microspheres (without exenatide). Following 13-weeks of administration, one animal/sex/group was maintained for a 13-week recovery period. During Week 18 of the recovery period a challenge dose of control microspheres was administered at a previously untreated site to all recovery animals.

A reversible decrease in body weight gain was observed in all treated animals relative to controls. Increases in AST (2-3X, not dose dependent in males) and ALT (1-4X, dose dependent in females) were observed in treated monkeys at the end of the recovery period without a microscopic correlate. It is not clear if this was treatment-related. Absolute thymus weights were decreased by 45-50% in microsphere control males and males dosed at 0.44 and 1.10 mg/kg with no correlative histopathology. These decreases showed full to no recovery. Macroscopic lesions, confined mainly to the injection sites, were characterized by red or white discoloration, nodules, abscesses, and thickened tissue due to needle trauma. The nodules appeared to increase in severity with increasing dose. Lobulation of the liver and red discoloration of the lung were noted at low incidence with no dose-related pattern. At the end of the recovery period, the incidence of macroscopic lesions was drastically reduced suggestive of reversibility.

Microscopic lesions confined mainly to the injection sites of animals from all groups were characterized by chronic inflammation, abscesses, epidermal hyperplasia (correlates with the thickened sites), fibrosis, and hemorrhage. Animals receiving microspheres, either with or without exenatide, also showed granulomatous inflammation (minimal to severe, not dose dependent) and foreign material at the injection site. At the end of the recovery period, the incidence of microscopic lesions was drastically reduced suggestive of reversibility. Microscopic lesions were not observed in other tissues. Treatment-related injection-site enlargement was noted during the treatment period. Most of the injection-site enlargements were reversed early on in the recovery period while the few remaining were reversed toward the end of the recovery period. No evidence of sensitization was noted following a challenge dose of control microspheres (microspheres in diluent without exenatide) to all recovery animals. Anti-exenatide antibodies were evident as early as Day 29 at ≥0.44 mg/kg and were evident in all exenatide LAR-treated groups by Day 92 with most animals positive at ≥0.44 mg/kg. On Day 92, positive results were observed in 2/8 animals with titers ranging from 125 to 3,125; 7/8 animals with titers ranging from 25 to 78,125; and 5/8 with titers ranging from 25 to 3,125 at 0.11, 0.44, and 1.10 mg/kg, respectively. Following the recovery period, most exenatide LAR-treated animals were still positive, although titers tended to have decreased to a range of 25 to 625.

Toxicology conclusions: Once weekly administration of exenatide LAR (AC2993-F17) to cynomolgus monkeys for 13 consecutive weeks at doses up to 1.10 mg/kg/week was well tolerated, with no systemic toxicity observed. According to the sponsor, the injection site findings (in-life and postmortem) observed was typical for administration of microspheres. The NOAEL was considered to be 1.10 mg/kg/week (19X the highest anticipated clinical dose, AUC of Ab negative animals) based on a lack of toxicologically meaningful drug-related microscopic lesions.

Study title: Exenatide LAR: 39-Week (One Injection/Week) Subcutaneous Injection Toxicity and Toxicokinetic Study in Cynomolgus Monkeys with a 3-Month Recovery

Key study findings:

- There were no mortalities or treatment-related effects on clinical observations, body weight, or clinical pathology parameters.
- Local reactions at the injection sites (erythema and edema) were noted in all groups and tended to increase in incidence and severity with increasing doses of microspheres. There appeared to be a slight increase in severity in the high-dose exenatide LAR group (very slight to severe). Some occurrences of open drainage were noted at all exenatide-treated groups.
- Macroscopic observations of subcutis thickening were observed at the injection site of animals receiving microspheres.
- Microscopically, minimal to moderate granulomas were noted in all groups receiving microspheres. Granulomas were well-circumscribed with minimal fibrosis and consisted of macrophages and multinucleated giant cells, often containing microspheres. Minimal to moderate foamy macrophages and fibroblasts in the subcutis were also observed. A few animals receiving microspheres also showed mononuclear cell inflammation, suppurative inflammation, fibrosis, hemorrhage, and/or draining tracts, which did not appear to be related to exenatide dose. Injection site effects had mostly resolved by the end of the 3-month recovery period.
- There were no microscopic findings considered to be related to the systemic exposure of exenatide LAR.
- Anti-exenatide Ab development was evident in most animals as early as Week 8, with a maximal response occurring between Weeks 20 and 24 in all exenatide treatment groups (~75% of all exenatide-treated animals). There were no apparent differences in antibody response between genders or between exenatide treatment groups. Ab titers tended to decrease throughout the recovery period, although approximately 66% of the exenatide treated animals still had titers ranging from 25 to 3,125 at the end of the 3-month recovery period.
- Steady-state plasma exenatide concentrations occurred after approximately four injections (i.e., 1 month). In a subset of antibody negative animals, plasma exposure generally increased with increasing dose. There were no apparent gender differences. Mean AUC accumulation for Ab negative animals ranged from approximately 9 to 28 fold. Once dosing ended, exenatide concentrations were below the LLOQ by Recovery Day 63, indicating that exenatide plasma exposure was cleared over the 2 month recovery period, and this was independent of antibody status. Plasma exenatide exposure was affected by the degree of anti-exenatide antibodies, resulting in either marked exposure increases (titer range of 125 to 625) or decreases (at titers ≥3,125).
- The NOAEL was considered to be 1.1 mg/kg/week (14X the highest anticipated clinical dose, AUC comparison for Ab negative animals and humans) based on a lack of target organ toxicity.

Study no.: (b) (4) Study #6870-113; Amylin Report # REST050370
Volume # and page #: NA, electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 14 November 2005
GLP compliance: Yes
QA statement: yes (X) no ( )
Drug, lot #, and % purity: (sponsor-generated table)

Test Article	Lot No.	Storage	Purity	Expiration Date	Reserve (Archive) Sample
AC2993-F17[a]	278-2565A	In a refrigerator set to maintain 2 to 8°C, protected from light	Pass	15 Sep 2006	Collected
	278-2785A		Pass	05 Oct 2006	Collected
	278-3465A		Pass	14 Dec 2006	Collected
	278-1076A		Pass	17 Apr 2007	Collected
	278-0396A		Pass	08 Feb 2007	Collected
	278-2695A		Pass	26 Sep 2006	Collected
	278-0586A		Pass	27 Feb 2007	Collected
	278-0516A		Pass	20 Feb 2007	Not Collected
	278-0326A		Pass	01 Feb 2007	Not Collected
	278-0166A		Pass	16 Jan 2007	Collected
	278-0096A		Pass	09 Jan 2007	Collected[b]
	278-1216A		Pass	02 May 2007	Collected

a AC2993-F17 is synonymous with exenatide LAR.
b See protocol deviations

Vehicle Control Article	Lot No.	Storage	Purity	Expiration Date	Reserve (Archive) Sample
Microsphere Diluent	804258A	At room temperature	Meets acceptance criteria	01 Jun 2007	Collected

Vehicle/formulation: AC2993-F17 resuspended in microsphere diluent.

Methods

Doses: (sponsor-generated table)

Group	No. of Animals[b] Male	Female	Dose Level[c] (mg/kg exenatide)	Dose Concentration[c] (mg/mL)
1 (Vehicle Control)[a]	6	6	0	0
2 (Control Microspheres)[a]	6	6	0	0
3 (Low)	6	6	0.11	1.1
4 (Mid)	6	6	0.42	4.2
5 (High)	6	6	1.1	11

a Group 1 animals were dosed with the vehicle control article; Group 2 animals were dosed with the control microspheres (microsphere dose equivalent to Group 5).
b Animals designated for recovery phase sacrifice (two animals/sex/group) underwent at least 3 months of recovery following the final dose administration.
c The dose volume was 0.1 mL/kg.

Species/strain: Monkey/cynomolgus
Route and regimen: Subcutaneous injection once per week for 39 weeks at 12 separate sites on the back
Age: Approximately 2 to 4 years
Weight: 2.2 to 3.3 kg (males) and 2.0 to 2.6 kg (females)
Analysis of dosing preparations: Content uniformity was verified by the manufacturer at the batch fill level for the vials filled for each lot of material used in this study and the results are included in the Certificate of Analysis for each lot. Homogeneity assessment of the reconstituted formulations could not be assessed as the final volume present in each vial was not sufficient for such analysis. Dosing formulations were collected immediately following preparations for Weeks 1, 17, 26, and 39 of the dosing phase to assess test article concentration.
Unique study design or methodology: None

Mortality:	Twice daily
Clinical signs:	One hour post-dose on dosing days and once daily on non-dosing days. Detailed observations were conducted at least once weekly
Detailed exams:	Conducted on anesthetized animals during the pre-dose phase, Weeks 13 and 26 of the dosing phase, and on the day of scheduled sacrifice and included measurements of heart rate, respiration rate, and body temperature.
Injection site:	Injection sites were observed for local tolerance pre-dose and approximately 4 and 48 hours following each dose administration and once weekly during the recovery phase. Observations included, but were not necessarily limited to, swelling and redness:

Redness:
0 No redness
1 Very slight redness (barely visible)
2 Slight redness (edges well defined by distinct redness)
3 Moderate redness (> slight, < severe)
4 Severe redness (beet redness)

Swelling:
0 No swelling
1 Very slight swelling (barely visible)
2 Slight swelling (edges well defined by raising)
3 Moderate swelling (raised up to approx 2 mm)
4 Severe swelling (raise greater than approx 2 mm)
5 Swelling with open drainage

Body weights:	Once weekly
Food consumption:	Once daily (qualitative)
Ophthalmoscopy:	Once during the predose phase, Week 39 of the dosing phase, and Week 13 of the recovery phase
EKG:	**Not conducted**

Hematology: Predose phase; Weeks 4, 13, 26, and 39 of the dosing phase; and Weeks 4 and 13 of the recovery phase (fasted). Standard hematology and coagulation parameters measured.

Clinical chemistry: Predose phase; Weeks 4, 13, 26, and 39 of the dosing phase; and Weeks 4 and 13 of the recovery phase (fasted). Standard clinical chemistry parameters measured.

Urinalysis: Predose phase; Weeks 4, 13, 26, and 39 of the dosing phase; and Weeks 4 and 13 of the recovery phase (fasted, overnight collection). Parameters included volume, pH, specific volume, appearance/color, protein, glucose, ketones, bilirubin, blood, urobilogen and microscopic examination of sediment.

Gross pathology: All animals

Organ weights: All animals; weighed organs are shown in the histopathology inventory table at end of section

Histopathology: All animals; examined organs/tissues are shown in the histopathology inventory table at end of section
Adequate Battery: yes (X), no ( )
Peer review: yes ( ), no (X)

TK sampling times: (all main group monkeys [non-fasted] were included for TK sampling at each time point)

Control - predose and 1 hour postdose on Days 1, 85, and 267 of the dosing phase and predose on Day 176 and 309.

Treated- predose and approximately 1, 3, 6, 12, 24, 48, 96, and 168 hours postdose on Dosing Days 1, 85, and 267 of the dosing phase and predose on Dosing Days 29, 120, 148, 176, 211, and 239 of the dosing phase and in the morning on Days 309, 337, and 365 (recovery phase).

Ab sampling times: Samples were taken 1 week prior to initiation of dosing; during Weeks 4, 8, 12, 16, 20, 24, 28, and 32 of the dosing phase; during Weeks 4 and 8 of the recovery phase; and at scheduled sacrifice. Animals were not fasted unless the blood collection was concurrent with clinical pathology or necropsy.

Results:

Mortality: No mortality or unscheduled sacrifice occurred during the study.

Clinical observations: No noteworthy clinical observations were observed during the treatment period.

Physical examination findings: Firm masses were noted in most animals receiving microspheres with or without the test article. These masses were not observed after the recovery period. There were not noteworthy differences between control and treated animals for heart rate, respiration rate, or body temperature.

Dose (mg/kg)	Diluent Control		Microsphere Control		0.11		0.42		1.1	
Sex	M	F	M	F	M	F	M	F	M	F
Skin, firm masses, dorsal	0	1	6	6	4	2	6	6	6	6

Injection sites: A summary of injection site findings is shown below in the sponsor-generated tables.

Text Table 1
Summary of Injection Site Erythema Scores in Males and Females, Incidence[a] (Occurrence/Observation)[b] - Treatment Phase

	Erythema Score							
	Very Slight		Slight		Moderate		Severe	
Dose Group	M	F	M	F	M	F	M	F
1	3/6 (4)	-	5/6 (8)	2/6 (2)	-	-	-	-
2	3/6 (9)	2/6 (2)	5/6 (9)	3/6 (5)	-	2/6 (2)	-	-
3	4/6 (6)	3/6 (35)	5/6 (11)	2/6 (25)	1/6 (1)	1/6 (6)	-	-
4	3/6 (23)	5/6 (66)	2/6 (16)	4/6 (42)	2/6 (6)	3/6 (9)	-	-
5	5/6 (141)	5/6 (44)	6/6 (106)	4/6 (48)	4/6 (51)	1/6 (20)	1/6 (1)	1/6 (4)

- = No occurrence.
a Incidence = Number of animals with at least one observation during the dosing phase.
b Occurrence/Observation = Total Number of observations from all animals/sex/group.

Text Table 2
Summary of Injection Site Erythema Scores in Males and Females, Incidence[a] (Occurrence/Observation)[b] - Recovery Phase

	Erythema Score							
	Very Slight		Slight		Moderate		Severe	
Dose Group	M	F	M	F	M	F	M	F
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	1/2 (1)	-	-	-	-
5	1/2 (1)	-	-	-	-	-	-	-

- = No occurrence.
a Incidence = Number of animals with at least one observation during the recovery phase.
b Occurrence/Observation = Total Number of observations from all animals/sex/group.

Text Table 3
Summary of Injection Site Edema Scores in Males and Females, Incidence[a] (Occurrence/Observation)[b] - Treatment Phase

	Swelling Score									
Dose	Very Slight		Slight		Moderate		Severe		Open Drainage	
Group	M	F	M	F	M	F	M	F	M	F
1	1/6 (3)	2/6 (4)	2/6 (2)	2/6 (3)	-	-	-	-	-	-
2	6/6 (1047)	6/6 (1094)	6/6 (1100)	6/6 (1078)	6/6 (856)	6/6 (405)	6/6 (179)	6/6 (52)	-	-
3	6/6 (442)	6/6 (263)	6/6 (283)	6/6 (115)	3/6 (138)	2/6 (95)	2/6 (31)	1/6 (46)	1/6 (1)	1/6 (5)
4	6/6 (1160)	6/6 (887)	6/6 (919)	6/6 (687)	6/6 (325)	6/6 (422)	6/6 (64)	3/6 (132)	-	2/6 (16)
5	6/6 (732)	6/6 (1087)	6/6 (794)	6/6 (1100)	6/6 (720)	6/6 (622)	6/6 (377)	6/6 (112)	5/6 (44)	1/6 (25)

- = No occurrence.
a Incidence = Number of animals with at least one observation during the dosing phase.
b Occurrence/Observation = Total Number of observations from all animals/sex/group.

Text Table 4
Summary of Injection Site Edema Scores in Males and Females, Incidence[a] (Occurrence/Observation)[b] - Recovery Phase

	Swelling Score									
Dose	Very Slight		Slight		Moderate		Severe		Open Drainage	
Group	M	F	M	F	M	F	M	F	M	F
1	-	-	-	-	-	-	-	-	-	-
2	2/2 (14)	2/2 (16)	2/2 (12)	2/2 (21)	2/2 (24)	2/2 (22)	2/2 (10)	-	-	-
3	2/2 (10)	-	2/2 (23)	-	2/2 (3)	-	1/2 (1)	-	-	-
4	2/2 (15	2/2 (23)	2/2 (9)	2/2 (13)	2/2 (7)	1/2 (8)	-	1/2 (1)	-	-
5	2/2 (10)	2/2 (20)	2/2 (19)	2/2 (15)	1/2 (1)	2/2 (11)	2/2 (3)	2/2 (8)	-	-

- = No occurrence.
a Incidence = Number of animals with at least one observation during the recovery phase.
b Occurrence/Observation = Total Number of observations from all animals/sex/group.

Body weights: There were no noteworthy differences in final weights or body weight gain between control and treated animals.

Dose (mg/kg)	Diluent Control		Microsphere Control		0.11		0.42		1.1	
Sex	M	F	M	F	M	F	M	F	M	F
Weight (kg) -Week 39	3.4	2.9	3.7	2.8	3.3	2.7	3.5	2.7	3.3	2.8
Diff. from control (kg)			0.3	-0.1	-0.1	-0.2	0.1	-0.2	-0.1	-0.1
% diff. from control			↑9%	↓3%	↓3%	↓7%	↑3%	↓7%	↓3%	↓3%

F = female; M = male.

Food consumption: Qualitative food consumption assessment data were comparable between test-article treated and diluent and microsphere control groups throughout the study.

Ophthalmoscopy: There were no treatment-related ophthalmic findings.

EKG: Not conducted

Clinical pathology: There were no treatment-related effects on hematology, clinical chemistry, or urinalysis parameters.

Gross pathology: Subcutaneous thickening at injection sites was noted in the microsphere control and exenatide QW groups.

Organ weights:

Dose (mg/kg)	Diluent Control		Microsphere Control		0.11		0.42		1.1	
Sex	M	F	M	F	M	F	M	F	M	F
Spleen (g)	2.823	-	3.122	-	2.441	-	3.000	-	3.464	-
Relative to BW	0.101	-	0.098	-	0.084	-	0.098	-	0.126	-
Adrenal (g)	0.431	-	0.492	-	0.522	-	0.542	-	0.622 ↑44%	-
Relative to BW	0.015	-	0.016	-	0.018	-	0.018	-	0.023 ↑53%	-
Thymus (g)	5.673	-	3.651	-	2.121*	-	2.745*	-	2.227* ↓61%	-
Relative to BW	0.202	-	0.114*	-	0.073*	-	0.096	-	0.080*† ↓60%	-
Thyroid (g)	-	0.340	-	0.359	-	0.318	-	0.371	-	0.441 ↑30%
Relative to BW	-	0.013	-	0.014	-	0.014	-	0.014	-	0.017 ↑31%

*p<0.05 compared with diluent control; †p<0.05 compared with microsphere control; "-" = no noteworthy difference from control; BW = body weightF = female; M = male.

Histopathology: (sponsor-generated tables)

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males					-- Females				
	Dosage group:	Ctls	2	3	4	5	Ctls	2	3	4	5
Tissues With Diagnoses	No. in group:	4	4	4	4	4	4	4	4	4	4
Thymus	Number examined:	4	4	4	4	4	4	4	4	4	4
	Unremarkable:	2	3	4	3	1	4	3	3	3	4
Depletion, Lymphocytes		0	1	0	1	3	0	1	1	1	0
Subcutan Site A	Number examined:	4	4	4	4	4	4	4	4	4	4
	Unremarkable:	4	2	0	1	1	3	0	1	1	0
Granuloma		0	1	2	3	3	0	4	3	3	4
Foamy Macrophages/fibroblasts		0	0	2	0	0	0	2	0	0	0
Inflammation, Mixed cell, Dermal adenxa		0	1	0	0	0	1	0	0	0	0
Hemorrhage, Subcutis		0	0	0	1	0	0	0	0	0	0
Fibrosis, Subcutis		0	0	0	1	1	0	0	1	2	1
Inflammation, Mononuclear cell, Subcutis		0	0	0	1	1	0	0	2	1	1
Inflammation, Suppurative, subcutis		0	0	0	0	0	0	0	1	2	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	0	0	0	0	0
Subcutan Site B	Number examined:	4	4	4	4	4	4	4	4	4	4
	Unremarkable:	4	0	0	2	1	4	0	1	0	2
Inflammation, Mixed cell, Dermal adnexa		0	1	0	0	0	0	0	0	0	0
Granuloma		0	1	3	2	3	0	4	3	4	2
Foamy Macrophages/fibroblasts		0	2	1	0	1	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	1	0	0	0	0	1	2	0
Myofiber degeneration, Panniculus carnosus		0	0	1	0	1	0	0	0	0	0
Fibrosis, Subcutis		0	0	0	1	1	0	0	1	1	0
Inflammation, Suppurative, Subcutis		0	0	0	1	1	0	0	1	1	0
Hemorrhage, Subcutis		0	0	0	0	1	0	0	0	0	0
Draining tract		0	0	0	0	1	0	0	0	1	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	0	0	0	0	0
Granuloma, Hair Shaft		0	0	0	0	0	0	0	0	0	0
Subcutan Site C	Number examined:	4	4	4	4	4	4	4	4	4	4
	Unremarkable:	3	0	1	1	0	4	0	2	1	0
Inflammation, Mixed cell, Dermal adnexa		1	0	0	0	0	0	0	0	0	0
Foamy macrophages/fibroblasts		0	4	0	1	1	0	0	0	0	0
Granuloma		0	3	3	2	4	0	4	1	3	4
Myofiber degeneration, Panniculus carnosus		0	0	1	0	2	0	0	0	0	0
Inflammation, Suppurative, Subcutis		0	0	0	1	2	0	0	0	1	1
Fibrosis, Subcutis		0	0	0	1	1	0	0	0	1	1
Hemorrhage, Subcutis		0	0	0	0	2	0	0	0	0	0
Granulation tissue, Subcutis		0	0	0	0	1	0	0	0	0	0
Epidermal hyperplasia		0	0	0	0	1	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	0	0	0	1	2	0

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --					-- Females --				
Tissues With Diagnoses	Dosage group:	Ctls	2	3	4	5	Ctls	2	3	4	5
	No. in group:	4	4	4	4	4	4	4	4	4	4
Subcutan Site D	Number examined:	4	4	4	4	4	4	4	4	4	4
	Unremarkable:	4	1	3	3	3	4	1	1	2	3
Granuloma		0	3	0	0	0	0	1	1	1	0
Foamy Macrophages/fibroblasts		0	2	1	1	1	0	2	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	0	0	0	3	1	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	0	1	0
Inflammation, Mixed cell, Dermal adnexa		0	0	0	0	0	0	0	0	0	1
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	0	0	0	0	0
Subcutan Site E	Number examined:	4	4	4	4	4	4	4	4	4	4
	Unremarkable:	4	1	2	2	0	3	2	2	1	1
Granuloma		0	2	0	1	2	0	0	0	1	1
Foamy Macrophages/fibroblasts		0	2	1	1	1	0	2	0	1	2
Inflammation, Mononuclear cell, Subcutis		0	0	1	0	0	0	0	2	2	1
Myofiber degeneration, Panniculus carnosus		0	0	0	0	1	0	0	0	0	0
Fibrosis, dermis		0	0	0	0	1	0	0	0	0	0
Inflammation, Mixed cell, Dermal adnexa		0	0	0	0	0	1	0	1	0	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	0	1	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	0	0	0	0	0
Subcutan Site F	Number examined:	4	4	4	4	4	4	4	4	4	4
	Unremarkable:	4	3	3	1	3	4	4	2	2	1
Inflammation, Mixed cell, Dermal adnexa		0	0	0	0	0	0	0	1	0	1
Myofiber degeneration, Panniculus carnosus		0	0	0	0	0	0	0	0	0	0
Granuloma		0	1	0	1	0	0	0	0	1	1
Foamy Macrophages/fibroblasts		0	0	1	1	1	0	0	1	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	0	1	0	0	0	1	1	2
Fibrosis, Subcutis		0	0	0	0	0	0	0	1	1	1
Hemorrhage, Subcutis		0	0	0	0	0	0	0	0	1	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	0	0	0	0	0
Subcutan Site G	Number examined:	4	4	4	4	4	4	4	4	4	4
	Unremarkable:	3	2	2	2	2	4	1	1	1	0
Inflammation, Mixed cell, Dermal Adnexa		1	0	0	1	0	0	0	0	1	1
Myofiber degeneration, panniculus carnosus		1	0	1	0	1	0	0	0	0	0
Granuloma		0	2	0	1	0	0	3	0	0	2
Foamy Macrophages/fibroblasts		0	0	1	0	1	0	0	2	2	0
Fibrosis, Subcutis		0	0	0	1	0	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	2	0	1	2	1	1
Fibrosis, Dermis		0	0	0	0	0	0	0	0	1	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males					-- Females				
Tissues With Diagnoses	Dosage group:	Ctls	2	3	4	5	Ctls	2	3	4	5
	No. in group:	4	4	4	4	4	4	4	4	4	4
Subcutan Site H	Number examined:	4	4	4	4	4	4	4	4	4	4
	Unremarkable:	3	2	0	0	0	4	2	2	1	0
Inflammation, Mixed cell, Dermal adnexa		1	1	0	0	1	0	0	0	0	0
Granuloma		0	1	2	3	0	0	2	1	2	2
Foamy Macrophages/fibroblasts		0	1	2	1	2	0	0	0	0	3
Fibrosis, Subcutis		0	0	0	1	0	0	0	1	1	1
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	1	0	0	2	2	1
Hemorrhage, Subcutis		0	0	0	0	0	0	0	0	1	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	0	0	0	0	0
Granuloma, Dermal		0	0	0	0	0	0	0	0	0	0
Subcutan Site I	Number examined:	4	4	4	4	4	4	4	4	4	4
	Unremarkable:	4	1	1	2	1	4	3	0	2	1
Granuloma		0	2	3	0	2	0	0	2	0	2
Foamy Macrophages/fibroblasts		0	0	0	1	0	0	0	2	1	1
Myofiber degeneration, panniculus carnosus		0	1	0	0	0	0	1	0	0	0
Inflammation, Mixed cell, Dermal Adnexa		0	0	0	1	1	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	1	0	0	2	2	1
Fibrosis, Dermis		0	0	0	0	0	0	1	1	0	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	0	0	0	0	0
Subcutan Site J	Number examined:	4	4	4	4	4	4	4	4	4	4
	Unremarkable:	3	1	0	0	1	3	1	2	1	0
Fibrosis, Dermis		1	0	0	0	0	0	0	0	1	0
Granuloma		0	3	4	3	1	0	3	2	3	4
Foamy Macrophages/fibroblasts		0	0	0	1	1	0	0	0	0	0
Inflammation, Mixed cell, Dermal adnexa		0	0	1	0	2	1	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	1	0	0	2	2	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	1	1	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	0	0	0	0	0
Subcutan Site K	Number examined:	4	4	4	4	4	4	4	4	4	4
	Unremarkable:	4	1	2	4	0	4	1	0	2	1
Granuloma		0	2	2	0	2	0	3	3	1	3
Foamy Macrophages/fibroblasts		0	0	0	0	1	0	0	0	0	0
Myofiber degeneration, Panniculus carnosus		0	1	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	1	0	0	2	1	0
Inflammation, Mixed cell, Dermal adnexa		0	0	0	0	1	0	0	0	0	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	1	1	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	0	0	0	0	0
Subcutan SIte L	Number examined:	4	4	4	4	4	4	4	4	4	4
	Unremarkable:	2	0	2	0	3	4	0	0	0	2
Inflammation, Mixed cell, Dermal adnexa		1	0	0	2	0	0	0	1	0	0
Myofiber degeneration, Panniculus carnosus		1	0	0	0	0	0	0	1	0	0
Granuloma		0	3	2	3	1	0	4	2	3	2
Foamy Macrophages/fibroblasts		0	2	0	0	0	0	0	0	1	0
Fibrosis, Dermis		0	0	0	1	0	0	0	0	0	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	1	2	0
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	0	0	0	2	1	0
Inflammation, Suppurative, Subcutis		0	0	0	0	0	0	0	0	1	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	0	0	0	0	0

RECOVERY (sponsor-generated tables)

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --					-- Females --				
	Dosage group:	Ctls	2	3	4	5	Ctls	2	3	4	5
Tissues With Diagnoses	No. in group:	2	2	2	2	2	2	2	2	2	2
Subcutan Site A	Number examined:	2	2	2	2	2	2	2	2	2	2
	Unremarkable:	2	0	1	1	0	1	0	2	1	1
Granuloma		0	0	0	0	0	0	0	0	0	0
Foamy Macrophages/fibroblasts		0	2	1	1	2	0	2	0	0	1
Inflammation, Mixed cell, Dermal adenxa		0	0	0	0	0	0	0	0	0	0
Hemorrhage, Subcutis		0	0	0	0	0	0	0	0	0	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	1	0	0	0	0	0	1	0
Inflammation, Suppurative, subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	1	0	0	1	0
Subcutan Site B	Number examined:	2	2	2	2	2	2	2	2	2	2
	Unremarkable:	2	1	0	0	1	0	0	2	0	1
Inflammation, Mixed cell, Dermal adnexa		0	0	0	0	0	0	0	0	0	0
Granuloma		0	0	0	0	0	0	0	0	0	0
Foamy Macrophages/fibroblasts		0	1	2	2	1	0	2	0	1	1
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	0	0	0	0	1	0
Myofiber degeneration, Panniculus carnosus		0	0	0	0	0	0	0	0	0	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Suppurative, Subcutis		0	0	0	0	0	0	0	0	0	0
Hemorrhage, Subcutis		0	0	0	0	0	0	0	0	0	0
Draining tract		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Dermis		0	0	1	0	0	1	0	0	1	0
Granuloma, Hair Shaft		0	0	0	0	0	1	0	0	0	0
Subcutan Site C	Number examined:	2	2	2	2	2	2	2	2	2	2
	Unremarkable:	2	0	1	0	0	2	0	2	0	0
Inflammation, Mixed cell, Dermal adnexa		0	0	0	0	0	0	0	0	0	0
Foamy macrophages/fibroblasts		0	2	1	2	2	0	2	0	2	2
Granuloma		0	0	0	0	0	0	0	0	0	0
Myofiber degeneration, Panniculus carnosus		0	0	0	0	0	0	0	0	0	0
Inflammation, Suppurative, Subcutis		0	0	0	0	0	0	0	0	1	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	0	0	0
Hemorrhage, Subcutis		0	0	0	0	0	0	0	0	0	0
Granulation tissue, Subcutis		0	0	0	0	0	0	0	0	0	0
Epidermal hyperplasia		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	1	0	0	0	0	0	1	0
Subcutan Site D	Number examined:	2	2	2	2	2	2	2	2	2	2
	Unremarkable:	2	0	0	2	1	2	0	2	1	1
Granuloma		0	0	0	0	0	0	0	0	0	0
Foamy Macrophages/fibroblasts		0	2	2	0	1	0	2	0	1	1

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --					-- Females --				
	Dosage group:	Ctls	2	3	4	5	Ctls	2	3	4	5
Tissues With Diagnoses	No. in group:	2	2	2	2	2	2	2	2	2	2
Subcutan Site D	Number examined:	2	2	2	2	2	2	2	2	2	2
	Unremarkable:	2	0	0	2	1	2	0	2	1	1
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	0	0	0	0	0	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mixed cell, Dermal adnexa		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	1	0	0	0	0	0	1	0
Inflammation, Mononuclear cell, Dermis		0	0	1	0	0	0	0	0	1	0
Subcutan Site E	Number examined:	2	2	2	2	2	2	2	2	2	2
	Unremarkable:	2	1	1	1	1	2	2	2	0	1
Granuloma		0	0	0	0	0	0	0	0	0	0
Foamy Macrophages/fibroblasts		0	1	1	1	1	0	0	0	0	1
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	0	0	0	0	2	0
Myofiber degeneration, Panniculus carnosus		0	0	0	0	0	0	0	0	0	0
Fibrosis, dermis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mixed cell, Dermal adnexa		0	0	0	0	0	0	0	0	0	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	1	0	0	0	1	0
Subcutan Site F	Number examined:	2	2	2	2	2	2	2	2	2	2
	Unremarkable:	1	0	0	2	1	1	0	2	0	1
Inflammation, Mixed cell, Dermal adnexa		0	0	0	0	0	0	0	0	0	0
Myofiber degeneration, Panniculus carnosus		0	0	0	0	0	0	0	0	0	0
Granuloma		0	0	0	0	0	0	0	0	0	0
Foamy Macrophages/fibroblasts		0	2	1	0	1	0	2	0	2	1
Inflammation, Mononuclear cell, Subcutis		0	0	1	0	0	0	0	0	2	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	0	0	0
Hemorrhage, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Dermis		1	0	1	0	0	1	0	0	1	0
Subcutan Site G	Number examined:	2	2	2	2	2	2	2	2	2	2
	Unremarkable:	2	0	1	1	2	2	1	2	1	0
Inflammation, Mixed cell, Dermal Adnexa		0	0	0	0	0	0	0	0	0	0
Myofiber degeneration, panniculus carnosus		0	0	0	0	0	0	0	0	0	0
Granuloma		0	0	0	0	0	0	0	0	0	0
Foamy Macrophages/fibroblasts		0	1	1	1	0	0	1	0	1	2
Fibrosis, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	0	0	0	0	1	0
Fibrosis, Dermis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Dermis		0	1	1	0	0	0	0	0	1	0

Controls from group(s): 1	Animal sex:	-- Animals -- Males --					-- Animals -- Affected -- Females --				
	Dosage group:	Ctls	2	3	4	5	Ctls	2	3	4	5
Tissues With Diagnoses	No. in group:	2	2	2	2	2	2	2	2	2	2
Subcutan Site H	Number examined:	2	2	2	2	2	2	2	2	2	2
	Unremarkable:	2	2	2	2	2	2	1	2	0	2
Inflammation, Mixed cell, Dermal adnexa		0	0	0	0	0	0	0	0	0	0
Granuloma		0	0	0	0	0	0	0	0	0	0
Foamy Macrophages/fibroblasts		0	0	0	0	0	0	1	0	0	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	0	0	0	0	1	0
Hemorrhage, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	0	0	0	1	0
Granuloma, Dermal		0	0	0	0	0	0	0	0	1	0
Subcutan Site I	Number examined:	2	2	2	2	2	2	2	2	2	2
	Unremarkable:	2	2	1	2	2	1	2	2	1	1
Granuloma		0	0	0	0	0	0	0	0	0	0
Foamy Macrophages/fibroblasts		0	0	0	0	0	0	0	0	0	1
Myofiber degeneration, panniculus carnosus		0	0	0	0	0	0	0	0	0	0
Inflammation, Mixed cell, Dermal Adnexa		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	1	0	0	0	0	0	0	0
Fibrosis, Dermis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	1	0	0	1	0
Subcutan Site J	Number examined:	2	2	2	2	2	2	2	2	2	2
	Unremarkable:	2	0	1	1	1	1	1	1	1	2
Fibrosis, Dermis		0	0	0	0	0	0	0	0	0	0
Granuloma		0	0	0	0	0	0	0	0	0	0
Foamy Macrophages/fibroblasts		0	2	1	1	1	0	1	0	1	0
Inflammation, Mixed cell, Dermal adnexa		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	1	0	0	0	0	0	0	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	1	0	1	1	0
Subcutan Site K	Number examined:	2	2	2	2	2	2	2	2	2	2
	Unremarkable:	2	0	2	2	2	2	1	2	1	1
Granuloma		0	0	0	0	0	0	0	0	0	0
Foamy Macrophages/fibroblasts		0	2	0	0	0	0	1	0	0	1
Myofiber degeneration, Panniculus carnosus		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mixed cell, Dermal adnexa		0	0	0	0	0	0	0	0	0	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	0	0	0	1	0
Subcutan SIte L	Number examined:	2	2	2	2	2	2	2	2	2	2
	Unremarkable:	2	0	1	2	1	1	1	2	2	0
Inflammation, Mixed cell, Dermal adnexa		0	0	0	0	0	0	0	0	0	0
Myofiber degeneration, Panniculus carnosus		0	0	0	0	0	0	0	0	0	0
Granuloma		0	0	0	0	0	0	0	0	0	0
Foamy Macrophages/fibroblasts		0	2	1	0	1	0	1	0	0	2
Fibrosis, Dermis		0	0	0	0	0	0	0	0	0	0
Fibrosis, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Suppurative, Subcutis		0	0	0	0	0	0	0	0	0	0
Inflammation, Mononuclear cell, Dermis		0	0	0	0	0	1	0	0	0	0

Toxicokinetics: TK results indicate that steady-state plasma concentrations occurred after approximately four injections (i.e., 1 month). In a subset of antibody (Ab) negative animals, plasma exposure generally increased with increasing dose. There were no apparent gender differences. Mean AUC accumulation for Ab negative animals ranged from approximately 9 to 28 fold on Day 267 compared with Day 1. After the final dose, plasma concentrations remained elevated in 1/3 of recovery animals (which included both Ab negative and positive animals) for at least 35 days. After 63 days of recovery, concentrations were below the LLOQ (10 pg/mL), indicating exenatide plasma exposure was cleared over the 2 month recovery period, independent of Ab status. Plasma exenatide exposure was affected by anti-exenatide antibodies, resulting in either marked increases (at titers between 125 and 625) or decreases (at titers ≥3,125) in exenatide exposure. The apparent decrease in exenatide exposure in animals with titers >3,125 suggests that the decrease may have been due to the presence of anti-exenatide antibodies.

Summary of Exenatide Plasma Toxicokinetics

Dose Group	Study Day	Mean Cmax (pg/mL)	Mean AUC0-168h (pg·h/mL)
	Antibody Negative TK animals[a], M+F		
Group 3	Day 1 (N=5)	111	5929
0.11 mg/kg/dose	Day 85 (N=5)	1452	66,271
	Day 267 (N=5)	4418	105,443
Group 4	Day 1 (N=2)	785	40,474
0.42 mg/kg/dose	Day 85 (N=2)	5005	309,485
	Day 267 (N=2)	17,345	351,208
Group 5	Day 1(N=3)	1270	35,412
1.1 mg/kg/dose	Day 85 (N=3)	31,067	469,662
	Day 267 (N=3)	40,733	545,645
	All TK animals[b], M+F		
Group 3	Day 1 (N=12)	134	7856
0.11 mg/kg/dose	Day 85 (N=9)	1529	77,954
	Day 267 (N=9)	9099	227,674
Group 4	Day 1 (N=12)	683	27,875
0.42 mg/kg/dose	Day 85 (N=11)	8091	656,628
	Day 267 (N=9)	20,873	1,115,646
Group 5	Day 1 (N=12)	1276	34,788
1.1 mg/kg/dose	Day 85 (N=10)	18,100	537,064
	Day 267 (N=8)	36,538	1,073,952

Abbreviations: $AUC_{0\text{-}168h}$ = Area under the concentration-time curve for the dosing interval; C_{max} = maximum observed plasma concentration during the dosing interval; F= Female; M= Male; N = Number of animals included in calculation.

a Antibody negative animals were antibody negative on all TK collection days through end of treatment (I06877, I06878, I06879, I06906, and I06911 from Group 3; I06887 and I06915 from Group 4; I06891, I06918 and I06923 from Group 5);

b N < 12 on Day 85 and/or Day 267 due to some animals with high titers having exenatide concentrations near or below LLOQ following repeat dosing.

(sponsor-generated table)

Antigenicity:

During the study treatment phase, high titers (>3,125) of anti-exenatide Ab was evident in most animals as early as Week 8 and the maximal Ab response was observed between Weeks 20 and 24 in all exenatide treatment groups. Approximately 75% of exenatide-treated animals were Ab positive. There were no apparent differences in Ab response between genders or between exenatide treatment groups. Ab titers tended to decrease throughout recovery, although approximately 66% of the exenatide treated animals still had titers ranging from 25 to 3,125 at the end of the 3-month recovery period.

Table 1: Summary of Positive Specimens

Study Phase		Predose		Dosing											
Protocol Time point		Day 1		Week 4		Week 8		Week 12		Week 16		Week 20		Week 24	
Sex (M/F)		M	F	M	F	M	F	M	F	M	F	M	F	M	F
Group 1 (Vehicle Control)	Positives (+/N)	0/6	1/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
	Range of Titers	NA	25	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Group 2 (Control Microspheres)	Positives (+/N)	1/6	3/6	0/6	0/6	0/6	0/6	1/6	1/6	0/6	0/6	1/6	0/6	0/6	0/6
	Range of Titers	25	25	NA	NA	NA	NA	25	25	NA	NA	25	NA	NA	NA
Group 3 (0.11 mg/kg/week)	Positives (+/N)	0/6	1/6	0/6	1/6	2/6	2/6	3/6	2/6	3/6	3/6	3/6	4/6	3/6	4/6
	Range of Titers	NA	25	NA	25	3,125-15,625	3,125	125-78,125	3,125-78,125	125-78,125	25-78,125	125-78,125	125-78,125	125-15,625	125-78,125
Group 4 (0.42 mg/kg/week)	Positives (+/N)	0/6	1/6	1/6	0/6	3/6	3/6	4/6	4/6	3/6	4/6	5/6	5/6	5/6	5/6
	Range of Titers	NA	25	25	NA	25-3,125	25-15,625	25-625	125-78,125	125-15,625	25-78,125	25-78,125	125-390,625	25-78,125	125-78,125
Group 5 (1.1 mg/kg/week)	Positives (+/N)	2/6	0/6	2/6	1/6	4/6	3/6	4/6	3/6	4/6	3/6	4/6	4/6	5/6	4/6
	Range of Titers	25	NA	25-125	125	25-15,625	25-3,125	625-78,125	25-625	125-78,125	125-3,125	15,625-78,125	25-15,625	125-390,625	25-390,625

(sponsor-generated table)

Table 1: Summary of Postive Specimens (Continued)

Study Phase		Dosing				End of Dosing		Recovery					
Protocol Time point		Week 28		Week 32		Term		Week 4R		Week 8R		Week 13R	
Sex (M/F)		M	F	M	F	M	F	M	F	M	F	M	F
Group 1 (Vehicle Control)	Positives (+/N)	0/6	0/6	1/6	1/6	0/4	0/4	0/2	0/2	0/2	0/2	0/2	1/2
	Range of Titers	NA	NA	25	25	NA	NA	NA	NA	NA	NA	NA	25
Group 2 (Control Microspheres)	Positives (+/N)	0/6	1/6	1/6	2/6	1/4	0/4	0/2	0/2	0/2	0/2	0/2	1/1[a]
	Range of Titers	NA	25	25	25	25	NA	NA	NA	NA	NA	NA	25
Group 3 (0.11 mg/kg/dose)	Positives (+/N)	3/6	4/6	3/6	4/6	1/4	3/4	2/2	2/2	2/2	1/2	2/2	1/2
	Range of Titers	125-15,625	25-78,125	125-15,625	25-78,125	625	625-78,125	3,125-15,625	25-125	3,125	25	625-3,125	25
Group 4 (0.42 mg/kg/dose)	Positives (+/N)	5/6	5/6	5/6	5/6	3/4	3/4	1/2	2/2	1/2	2/2	1/2	2/2
	Range of Titers	25-15,625	25-78,125	25-15,625	125-78,125	625-15,625	625-78,125	125	3,125-15,625	125	625-15,625	125	625-3,125
Group 5 (1.1 mg/kg/dose)	Positives (+/N)	5/6	3/6	5/6	3/6	3/4	3/4	2/2	0/2	2/2	0/2	2/2	0/2
	Range of Titers	125-78,125	125-15,625	125-78,125	25-78,125	125-78,125	125-15,625	3,125-78,125	NA	625-3,125	NA	625	NA

[a] One specimen did not have sufficient quantity to obtain a reportable result.
Test article was administered once a week for 39 weeks.
Week 13R = dd 08Dec06 specimens.

(sponsor-generated table)

Histopathology Inventory for NDA #22-200

Study	**18 Week (#050369)**	**3 Month (#04289)**	**39 Week (#050370)**
Species	**RAT**	**MONKEY**	**MONKEY**
Adrenals	X*	X*	X*
Aorta	X	X	X
Brain	X*	X*	X*
Cecum	X	X	X
Colon	X	X	X
Duodenum	X	X	X
Epididymides	X	X*	X*
Esophagus	X	X	X
Eyes with optic nerves	X	X	X
Femur with bone marrow	X	X	X
Gall bladder		X	X
Gross lesions	X	X	X
Harderian gland			
Heart	X*	X*	X*
Ileum	X	X	X
Injection site	X	X	X
Jejunum	X	X	X
Kidneys	X*	X*	X*
Lachrymal gland			X
Larynx			
Liver	X*	X*	X*
Lungs	X*	X*	X*
Lymph node, mandibular	X	X	X
Lymph node, mesenteric	X	X	X
Lymph node, axillary			X
Lymph node, inguinal			X
Mammary gland (females only)	X	X	X
Ovaries	X*	X*	X*
Pancreas	X	X	X
Peyer's patch	X	X	
Pharynx			
Pituitary	X*	X*	X*
Prostate	X	X	X
Rectum	X	X	X
Salivary glands, mandibular and parotid	X	X	X
Sciatic nerve	X	X	X
Seminal vesicles	X	X	X
Skeletal muscle	X	X	X
Skin	X	X	X
Spinal cord (rat - thoracic and lumbar) (monkey - cervical, thoracic, and lumbar)	X	X	X
Spleen	X*	X*	X*
Sternum with bone marrow	X	X	X
Stomach	X	X	X
Testes	X*	X*	X*
Thymus	X*	X*	X*
Thyroid + parathyroid	X*	X*	X*
Tongue	X	X	X
Trachea	X	X	X
Urinary bladder	X	X	X
Uterus + cervix	X	X	X
Vagina	X	X	X
Zymbal gland			

X, histopathology performed; *, organ weight obtained

2.6.6.4 Genetic toxicology

Exenatide was previously found to be devoid of mutagenic or clastogenic activity in studies conducted to support NDA 21-773. The sponsor conducted additional in vitro genetic toxicology studies with both exenatide and exenatide QW to qualify the test article from various manufacturing sources. Under the conditions of these studies, exenatide and exenatide QW were devoid of mutagenic or clastogenic activity in vitro. These studies are briefly described below and sponsor-generated tabular summaries are presented in Section 2.6.7.

AC2993 ((b) (4) Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay Treat and Plate Method (Report REST04571)

Exenatide that was manufactured at a (b) (4) scale by Lonza by using a (b) (4) method was evaluated for mutagenicity in a bacterial reverse mutation assay. *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 plus the *Escherichia coli* strain WP2uvrA(pKM101) were exposed to exenatide (5000, 2000, 1000, 333, 100, and 33.3 µg/plate; 3 plates per dose) with and without metabolic activation with Aroclor-induced rat liver S9 mix. All assays used the pre-incubation method. Concurrent vehicle and positive controls were also tested. Initial results were confirmed in an independent experiment.

Under the conditions of this study, exenatide did not cause an increase in the mean number of revertants per plate with any of the tester strains in either the presence or absence of metabolic activation.

Bacterial Reverse Mutation Assay Using the Treat and Plate Method (Report REST060302)

Exenatide QW that was manufactured at a (b) (4) scale by Alkermes was evaluated for mutagenicity in a bacterial reverse mutation assay. *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 plus the *Escherichia coli* strain WP2uvrA(pKM101) were exposed to exenatide QW with and without metabolic activation with Aroclor-induced rat liver S9 mix. Two independent mutation tests were conducted by using the pre-incubation method, with the exception of the positive control with *E. coli* plus S9, which was tested by the plate incorporation method. In the initial assay exenatide QW concentrations ranged from 0.050 to 150 µg/plate. As no precipitate or appreciable toxicity was observed, concentrations of 5000, 1500, 500, 150, 50, and 15 µg/plate were used in the confirmatory assay. Vehicle and positive controls were also included.

Under the conditions of this study, exenatide QW manufactured at a (b) (4) scale by Alkermes did not cause an increase in the mean number of revertants per plate with any of the tester strains in either the presence or absence of metabolic activation.

Bacterial Reverse Mutation Assay (Report REST080228)

Exenatide QW that was manufactured at a (b) (4) scale by Amylin OH was evaluated for mutagenicity in a bacterial reverse mutation assay. *Salmonella typhimurium* strains

TA98, TA100, TA1535, and TA1537 plus the *Escherichia coli* strain WP2uvrA(pKM101) were exposed to exenatide QW with and without metabolic activation with Aroclor-induced rat liver S9 mix. Two independent mutation tests were conducted by using the pre-incubation method, with the exception of the positive control with *E. coli* plus S9, which was tested by the plate incorporation method. In the initial assay exenatide QW concentrations ranged from 0.75 to 2500 µg/plate. Concentrations of 2500, 750, 250, 75, and 25 µg/plate were used in the confirmatory assay. Concurrent vehicle and positive controls were also tested. No precipitate or appreciable toxicity was observed.

Under the conditions of this study, exenatide QW manufactured at a (b) (4) scale by Amylin OH did not cause an increase in the mean number of revertants per plate with any of the tester strains in either the presence or absence of metabolic activation.

AC2993 ((b) (4) Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells (Report REST04569)
Exenatide that was manufactured at a (b) (4) scale by Lonza by using a (b) (4) method was evaluated for its ability to induce chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells. The initial assay utilized a 3-hour treatment period with the cultures harvested 20 hours after initiation of treatment. Concentrations of 9.77, 19.5, 39.1, 78.1, 156, 313, 625, 1250, 2500, and 5000 µg/mL were tested with and without metabolic activation (rat liver S9) and the 625, 1250, 2500, and 5000 µg/mL concentrations were analyzed for chromosomal aberrations. In a confirmatory assay, the treatment period was 20 hours without metabolic activation and 3 hours with metabolic activation with culture harvest occurring at 20 hours after the initiation of treatment. Concentrations of 156, 313, 625, 938, 1250, 1880, 2500, 3750, and 5000 µg/mL were tested without metabolic activation and 625, 1250, 2500, 3750, and 5000 µg/mL were tested with metabolic activation. Cultures treated with concentrations of 1880, 2500, 3750, and 5000 µg/mL without metabolic activation and 1250, 2500, 3750, and 5000 µg/mL with metabolic activation were analyzed for chromosomal aberrations.

No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed. Under the conditions of this study, exenatide produced by Lonza using the (b) (4) method was considered negative for inducing structural chromosomal aberrations in CHO cells with and without metabolic activation.

In Vitro Mammalian Chromosome Aberration Test (Report REST060306)
Exenatide LAR (AC2993-F17) that was manufactured at a (b) (4) scale by Alkermes was evaluated for its ability to induce chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells. In a range-finding study, precipitate was noted at the highest concentration tested (66 µg/mL) initially after adding the test article to the culture media. At the end of the treatment period, all concentrations were found to be soluble. No substantial toxicity (i.e., at least 50% cell growth inhibition, relative to the solvent control) was observed at any dose level in all three exposure groups. Based on these findings, the doses chosen for the chromosome aberration assay ranged from 6.25 to

100 µg/mL. In the chromosome aberration assay, cells were treated for 4 and 20 hours without metabolic activation and for 4 hours in the S9-activated test system. Cells were harvested 20 hours after treatment initiation. Visible precipitate was observed in treatment medium at 100 µg/mL and dose levels ≤ 75 µg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. The results showed that the percentage of cells with structural or numerical aberrations in the exenatide LAR-treated groups was not significantly increased above that of the solvent control at any dose level. Treatment with the positive controls resulted in a statistically significant number of cells with chromosomal aberrations.

Under the conditions of this study, exenatide LAR was concluded to be negative for the induction of structural and numerical chromosome aberrations in CHO cells in both the absence and presence of metabolic activation.

In Vitro Mammalian Chromosome Aberration Test (Report REST080139)
Exenatide LAR (AC2993-F17) that was manufactured at a (b) (4) scale by Amylin OH was evaluated for its ability to induce chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells. In the preliminary toxicity assay, the maximum dose tested was 5000 µg/mL. Visible precipitate was observed in treatment medium at dose levels ≥500 µg/mL; dose levels ≤150 µg/mL were soluble in treatment medium at the beginning and end of the treatment period. Substantial toxicity (69% cell growth inhibition relative to the solvent control) was observed at 1500 µg/mL in 4-hour exposure group without metabolic activation but not in the 20-hour exposure group (23% inhibition). Growth inhibition at 5000 µg/mL for the 4-hour and 20-hour treatment groups was 48% and 3%, respectively. Substantial toxicity was not observed at any dose level in the S9-activated 4-hour groups. Based on these findings, the dose levels chosen for the chromosome aberration assay were 313, 625, 1250, 2500, and 5000 µg/mL for all three treatment groups.

The results of the dose formulation analysis from the initial chromosome aberration assay (B1) yielded very low recovery. Therefore, the chromosome aberration assay was repeated at dose levels of 25, 50, 100, 250, and 500 µg/mL. In the repeat chromosome aberration assay (B2), the cells were treated for 4 and 20 hours without metabolic activation and for 4 hours plus metabolic activation (rat liver S9). All cells were harvested 20 hours after treatment initiation. Visible precipitate was observed in treatment medium at dose levels ≥250 µg/mL. Dose levels ≤100 µg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. Based on the presence of precipitation, cultures treated with 50, 100, and 250 µg/mL were selected for microscopic analysis for each of the treatment conditions. Results showed that the percentage of cells with structural or numerical aberrations in the exenatide LAR-treated groups was not significantly increased relative to solvent control at any concentration. The positive and solvent control groups fulfilled the requirements for a valid test.

Under the conditions of this study, exenatide LAR was considered to be negative for the induction of structural and numerical chromosome aberrations in CHO cells in either the presence or absence of metabolic activation.

2.6.6.5 Carcinogenicity

Study title: Exenatide LAR: 104-Week Carcinogenicity Study Following Every Other Week Subcutaneous Administration in Rats

Key study findings:

- The overall mortality for exenatide QW-treated groups was similar to controls. There were no meaningful differences in the number of deaths due to tumors between treated and control groups.
- Treated groups weighed less than both diluent and microsphere control groups at the end of the treatment period, with the difference being greater for females. For the 0.3, 1, and 3 mg/kg dose groups, males weighed 8%, 13%, and 11% less and females weighed 17%, 21%, and 21% less than diluent controls, respectively. The effects on body weight correlated with decreased food consumption, a known pharmacological effect of GLP-1 receptor agonists.
- There were no hematology or gross pathology findings attributed to the test article.
- Non-neoplastic microscopic findings included an increase in **foreign body granulomas at the injection sites** in all male and female groups receiving PLG microsphere; a slight increase in **acinar cell atrophy of the pancreas** (minimal to moderate) for high-dose males and low-dose and high-dose females; a slight increase in **adipose tissue depletion** (mild to severe) in all exenatide-treated males, especially those that were found dead or sacrificed moribund; and an increase in **pelvic mineralization of the kidney** (minimal to moderate) for all exenatide-treated males.
- Neoplastic findings
  - The key neoplastic finding was **thyroid c-cell adenomas**, which was statistically significant for males for dose response and for pair-wise comparison vs. Control 1 and Control 2 at the MD and HD. For females the number c-cell adenomas were statistically significant for all dose groups for pair-wise comparison vs. Control 1 but not Control 2. Also, there was not a statistically significant increase when assessed for dose response vs. Control 1 or Control 2. The mean number of animals with c-cell adenomas for all exenatide-treated groups exceeded the high end of the historical control range (15.4% for males and 11.4% for females).
  - The number of **thyroid c-cell carcinomas** was only statistically significant for MD males when compared with Control 1, although the number of tumors was outside the background control range (0% to 1.7%) for that laboratory for the LD (2.9%), MD (7.1%), and HD (4.3%). For females, the number of c-cell carcinomas was statistically significant for dose response when compared with Control 1; only the HD group had a value (5.7%) that was outside the historical control range (0% to 4.0%), but was not statistically significantly different from either control group.
  - The number of **total thyroid c-cell tumors** (adenomas plus carcinomas) was statistically significantly increased for males for dose response and at MD and HD for pair-wise comparison versus Control 1 and Control 2. The p value for LD males was just slightly higher than the 0.01 threshold for significance. For females c-cell tumors were increased for dose response versus Control 1 only and

at all doses versus Control 1 and at HD versus Control 2 for pair-wise comparison.

- In early decedent animals, thyroid c-cell adenomas were first detected at Day 530, 484, 525, 441, and 306 in males and at Day 548, 553, 467, 440, and 499 in females receiving diluent control, microsphere control, 0.3 mg/kg, 1 mg/kg, and 3 mg/kg, respectively. Thyroid c-cell carcinomas were first detected at Day 691, 412, 574, and 630 in males and at Day 685, 729, 733, and 699 in females receiving microsphere control, 0.3 mg/kg, 1 mg/kg, and 3 mg/kg, respectively (c-cell carcinomas were not found in diluent control animals). None of the early deaths were attributed to the presence of thyroid c-cell tumors.
- **Fibromas of the skin**, subcutis, were found to be statistically significantly increased for males when assessed for dose response vs. Control 1 and increased in HD males when analyzed for pair-wise comparison vs. Control 1. The LD and HD groups (but not MD) had values (5.7% and 11%, respectively) greater than the historical control range (0% to 5%). Because the PLG microspheres probably contributed to the increase in fibromas due to foreign body response, a comparison to Control 2 does not seem as relevant as Control 1 for this particular tumor type.
- A potential increase in schwannomas (all tissues combined) was observed in males and an increase in kidney tubular cell carcinomas was observed in females. However, these tumors were likely not related to exenatide because of a lack of statistical significance at any dose level when compared to either control by pair-wise comparison.
- A potential increase in parathyroid benign adenomas and pancreatic islet cell adenomas were noted in males and females, respectively; however, these findings were not shown to be statistically significantly different from either control group when using trend analysis or pair-wise comparison.

- Approximately one third of exenatide-treated males had anti-exenatide antibodies at the 6-, 12-, and 18-month time points. The number of anti-exenatide antibody positive females ranged from 41% (low dose at 6 months) to 24% (high dose at 18 months). The number of females testing positive for antibodies tended to be inversely related to dose level. Generally, the percentage of animals with anti-exenatide antibodies was lower at 24 months, which could have been due to a smaller sample size because of decreased survival at two years.
- TK data show that exposure was slightly less than dose proportional between 0.3 and 1.0 mg/kg and 0.3 and 3.0 mg/kg; exposure was approximately dose proportional between 1.0 and 3.0 mg/kg. C_{max} increased in a less than dose proportional manner. Both C_{max} and AUC were increased by the presence of anti-exenatide antibodies.
- In conclusion, an increase in thyroid c-cell tumors (adenomas plus carcinomas) was observed at all doses for males and females, although the increase for low-dose males was not statistically significant. Based on TK data in antibody negative animals on Day 183, the mean exposure at the low dose is approximately 1.3 fold the maximum anticipated clinical exposure. An increase in skin fibromas was observed in males treated with 3 mg/kg, which is approximately 20-fold higher than the maximum clinical dose based on exposure.

Adequacy of the carcinogenicity study and appropriateness of the test model: The design and conduct of the carcinogenicity study was found to be adequate. (note: see ECAC meeting minutes for protocol dose selection and final data review in Appendices 1 and 2)

- Evaluation of tumor findings:
  - There was not a definitive tumorigenic effect elicited by the microspheres when comparing Control 1 vs. Control 2; however the Control 2 group males had slightly more fibromas of the skin than Control 1 males (3 vs. 0, respectively).

Males

- **Thyroid c-cell adenoma** (common tumor) - statistically significant ($p<0.005$) for dose response vs. Control 1 and Control 2; statistically significant at MD and HD for pair-wise comparison ($p<0.01$) vs. Control 1 and Control 2.
- **Thyroid c-cell carcinoma** (rare tumor) - statistically significant at only MD for pair-wise comparison vs. Control 1 only ($p<0.05$).
- **Thyroid c-cell adenoma plus carcinoma** - statistically significant ($p<0.005$) for dose response vs. Control 1 and Control 2; statistically significant at MD and HD for pair-wise comparison ($p<0.005$) vs. Control 1 and Control 2.
- **Fibroma of skin, subcutis, benign** (common tumor) - statistically significant ($p<0.005$) for dose response vs. Control 1 only; statistically significant at HD for pair-wise comparison ($p<0.01$) vs. Control 1 only.
- **Schwannoma**, malignant, all tissues combined (rare tumor) - statistically significant for dose response vs. Control 1 only ($p<0.025$).
- **Parathyroid, adenoma** (common tumor) - not statistically significant for dose response ($p>0.005$) or pair-wise comparison ($p>0.01$).

Females

- **Thyroid c-cell adenoma** (common tumor) - statistically significant at all doses for pair-wise comparison vs. Control 1 ($p<0.01$) but not vs. Control 2 ($p>0.01$). Also, not statistically significant for dose response ($p>0.005$) vs. Control 1 or Control 2.
- **Thyroid c-cell carcinoma** (rare tumor) - statistically significant for dose response vs. Control 1 only ($p<0.025$), but not statistically significant for pair-wise comparison vs. Control 1 or Control 2 ($p>0.05$).
- **Thyroid c-cell adenoma plus carcinoma** - statistically significant ($p<0.005$) for dose response vs. Control 1 only; statistically significant at all doses for pair-wise comparison ($p<0.01$) vs. Control 1 and at HD only vs. Control 2.
- **Kidney, tubular cell carcinoma** (rare tumor) - statistically significant for dose response vs. Control 1 ($p<0.025$) but not statistically significant for pair-wise comparison at any dose level ($p>0.05$).
- **Pancreas, islet cell adenoma** (common tumor) - not statistically significant for dose response ($p>0.005$) or pair-wise comparison ($p>0.01$).

Study no.: 843-040, amendment 1 (Sponsor Report #REST060229R1)
Volume # and page #: NA/electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 18 July 2006
GLP compliance: Yes
QA statement: yes (X) no ( )
Drug, lot #, and % purity:
(acceptance criteria range = (b) (4) mg AC2993 total peptide/100 mg microspheres)
AC2993-F17, Lot #05-016-051B, 4.9 mg/100 mg microspheres, impurities (b) (4)
AC2993-F17, Lot #05-016-051, 4.9 mg/100 mg microspheres, impurities =
AC2993-F17, Lot #05-016-052, 5.1 mg/100 mg microspheres, impurities =
AC2993-F17, Lot #05-016-053, 5.0 mg/100 mg microspheres, impurities =

CAC concurrence: Yes, sponsor used dose levels recommended by ECAC.

Methods

Study design:

Group	Treatment	Dose Level (mg/kg/dose exenatide)	Dose Volume (mL/kg)	Dose Conc. (mg/mL exenatide)	Dose Regimen	Number of Animals M	Number of Animals F
Main Study							
1	Diluent Control	0	0.5	0	104 wk, q 2 wk	70	70
2	Control Microspheres	0	0.5	0	104 wk, q 2 wk	70	70
3	Exenatide LAR	0.3	0.5	0.6	104 wk, q 2 wk	70	70
4	Exenatide LAR	1.0	0.5	2.0	104 wk, q 2 wk	70	70
5	Exenatide LAR	3.0	0.5	6.0	104 wk, q 2 wk	70	70
Toxicokinetic Groups							
6	Exenatide LAR	0.3	0.5	0.6	26 wk, q 2 wk	20	20
7	Exenatide LAR	1.0	0.5	2.0	26 wk, q 2 wk	20	20
8	Exenatide LAR	3.0	0.5	6.0	26 wk, q 2 wk	20	20

Basis of dose selection: Decreased body weight gain observed in 4-month study
Species/strain: Rat/Sprague-Dawley
Route: Subcutaneous - injection sites were rotated between six distinct sites on the dorsum; left craniothorax, right craniothorax, left caudal thorax, and right caudal thorax, left lumbar, and right lumbar regions, close to the midline.
Dosing regimen: Once every 2 weeks.
Formulation: Exenatide LAR powder was resuspended in diluent (purchased as premade solution) on each day of dosing.
Age: approximately 6 weeks at arrival

Weight: 226 to 303 g (males) and 176 to 216 g (females)
Animal housing: Animals were individually housed in suspended, stainless steel, wire-mesh type cages in an environmentally controlled room (12-hour light/dark cycle, 64 to79°F, and 30% to 70% humidity)
Restriction paradigm for dietary restriction studies: Dietary restriction was not implemented; animals were fed Meal Lab Diet (Certified Rodent Diet #5002) *ad libitum*.
Drug concentration analysis: Samples were collected for analysis during Weeks 1, 3, 5, 7, 13, 25, 37, 47, 49, 63, 75, 87, and 99. Samples were stored at -70°C until analyzed. The Week 1, 5, 7, 13, 75, 87, and 99 samples met the sample analysis criteria for average recovery (±15% of nominal) and precision (≤10% RSD) during the original sample analysis runs. No test article was detected in the diluent or microsphere control samples for these analyses. Samples from Week 3, 25, 37, 47, 49, 63 failed one or more runs and were reanalyzed. Upon reanalyzation, the 2.0 and 6.0 mg/mL concentrations passed acceptance criteria, whereas some of the 0.6 mg/mL samples did not pass. Samples from Week 37 were not reanalyzed so samples were collected on Week 47. Overall, samples for the 2.0 and 6.0 mg/mL concentrations were generally shown to be at the target nominal concentration. The 0.6 mg/mL samples had more variability, although over the course of the study, these samples were generally considered to be at the appropriate concentrations. A summary of results are shown in the sponsor generated table below.

Concentration			
Dose Level (mg/kg/dose)	Nominal Concentration (mg/mL)	Range of Mean Actual Concentrations (mg/mL)	% Recovery[a]
0.3	0.6	0.4027 to 0.7916	67.1 to 131.9
1.0	2.0	1.7412 to 2.2918	87.1 to 114.6
3.0	3.0	5.5418 to 7.9909	92.4 to 133.2
[a]Mean % Recovery was calculated from the nominal concentration.			

(note: error in sponsor table - the nominal concentration of 3.0 mg/mL should be 6.0 mg/mL)

Dual controls employed: Yes (diluent control and microsphere control)
Interim sacrifices: No
Deviations from original study protocol: Several minor deviations were noted. Examples are listed below. The fact that the microsphere control group received the high dose of exenatide is not ideal because exposure to the drug lasts for approximately 6 weeks; however this only occurred on a single occasion during the first half of the study and therefore, this error doubtfully had an impact on tumorigenicity for this group. Overall, the deviations noted are not expected to affect the quality or integrity of the study.

- On Week 37, the microsphere control animals mistakenly received the high-dose level of exenatide.
- There were several notations of protocol deviations regarding TK sample collection or processing being outside of the specified time range.
- There were some instances where some animals were found without food in their trays; food was immediately offered once discovered.
- There were some instances when hematology and Ab samples were not handled/ processed per the protocol's instructions.
- Occasionally, PK, hematology, or Ab samples were not collected/ processed for some animals.

Observation times

Mortality:	Twice daily
Cage-side observations:	Twice daily
Detailed observations:	Weekly
Body weights:	Weekly for first 16 weeks, then once every two weeks
Food consumption:	Weekly
Hematology:	At termination and before moribund sacrifice (when possible)
Gross Pathology:	All main study animals found dead, sacrificed moribund, and at scheduled sacrifice.
Histopathology:	All main study animals found dead, sacrificed moribund, and at scheduled sacrifice. Peer review: yes (X), no ( ); The peer review consisted of an examination of all tissues from 10% of the animals selected randomly from the control and high dose groups.

Organs and Tissues Collected for Microscopic Examination (sponsor table)

Adrenal gland	Peyer's patch
Aorta	Pituitary
Bone with bone marrow, femur, sternum	Prostate
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	Salivary gland, mandibular, parotid
Epididymis	Seminal vesicle
Esophagus	Skeletal muscle, quadriceps
Eye (with optic nerve)	Skin, ventral abdomen
Harderian gland	Small intestine, duodenum, ileum, jejunum
Head (nasal turbinates and nasopharynx)	Spinal cord, cervical, lumbar, thoracic
Heart	Spleen
Injection sites (N=6)	Stomach, glandular, nonglandular
Kidney	Testis
Lacrimal gland, exorbital	Thymus
Large intestine, cecum, colon, rectum	Thyroid gland (with parathyroid)
Larynx	Tongue
Liver	Trachea
Lung with bronchi	Urinary bladder
Lymph node, mandibular, mesenteric	Uterus with cervix
Mammary gland (females only processed)	Vagina
Nerve, sciatic	Zymbal's gland (auditory sebaceous gland)
Ovary	Gross lesions
Pancreas	Tissue masses with regional lymph node

Toxicokinetics:

Main study animals (all groups): Day 1 and 6, 12, and 18 months before dosing and 0.5 hours after dosing

TK animals (5/sex/group - exenatide treated only):

Day 1: 0, 0.5, 2, 4, 10, 24, 48, 96, 168, 216, 288, and 336 hours after dosing

Month 2 and 4: 0 (prior to dosing) and 0.5 hours after dosing

Month 6: 0, 0.5, 2, 4, 10, 24, 48, 96, 168, 216, 288, and 336 hours after dosing

Antibody Assessment: All surviving animals - once pretest and prior to dosing at 6, 12, 18, and 24 months and at termination of moribund animals (when possible). TK animals had samples taken pretest and prior to dosing at 2, 4, and 6 months.

Results

Mortality - Males

Dose (mg/kg) / Study Week	Number of Deaths				
	Diluent Control	Microsphere Control	0.3	1.0	3.0
1 - 13	0	0	1	2	0
14 - 26	1	0	2	0	0
27 - 39	0	2	1	0	1
40 - 52	3	1	0	1	4
53 - 65	3	5	2	4	2
66 - 78	10	7	8	6	5
79 - 91	15	14	16	13	14
92 - 104	17	13	12	14	22
105	0	0	0	0	1
Total Early Deaths	49	42	42	40	49
Number of surviving animals	21 (30%)	28 (40%)	28 (40%)	30 (43%)	21 (30%)
Summary of Animal Disposition					
Euthanized *in extremis*	24	12	20	18	20
Found dead	25	30	22	21	29
Died prior to euthanasia	0	0	0	1	0
Terminal necropsy	21	28	28	30	21
Deaths Suspected as Being Related to Tumors					
Adrenal gland tumor	0	0	0	1	0
Bone tumor	0	0	1	0	0
Brain tumor	1	1	1	0	0
Fibrosarcoma/fibroma	0	0	1	1	1
Fibrous histiocytoma	0	0	0	1	0
Gastrointestinal tumor	0	0	0	2	0
Hibernoma	2	1	1	2	1
Histiocytic sarcoma	1	1	0	2	2
Kidney tumor	0	0	0	0	1
Leukemia	0	1	0	0	0
Lung tumor	0	0	0	0	0
Lymphoid tumor	0	1	0	1	0
Mammary tumor	0	0	0	1	0
Mesothelioma	0	0	1	0	0
Neuroendocrine tumor	0	1	0	0	0
Nose/oral tumor	0	1	1	1	1
Pancreas tumor	1	1	0	0	0
Pituitary tumor	13	9	17	13	16
Schwannoma	0	1	0	0	2
Skin tumor	2	0	0	0	0
Thyroid tumor	0	0	0	0	0
Zymbals gland tumor	1	0	0	1	2
Undetermined	15	15	13	8	16

Mortality - Females

Dose (mg/kg) / Study Week	Number of Deaths				
	Diluent Control	**Microsphere Control**	**0.3**	**1.0**	**3.0**
1 - 13	0	0	1	1	1
14 - 26	0	0	0	0	0
27 - 39	1	3	0	1	0
40 - 52	1	4	1	3	4
53 - 65	7	9	4	4	4
66 - 78	11	6	9	9	12
79 - 91	24	13	14	18	14
92 - 104	11	16	18	15	20
105	0	0	0	0	0
Total Early Deaths	55	51	47	51	55
Number of surviving animals	15 (21%)	19 (27%)	23 (33%)	19 (27%)	15 (21%)
Summary of Animal Disposition					
Euthanized *in extremis*	43	34	41	39	40
Found dead	12	16	6	12	15
Died prior to euthanasia	0	1	0	0	0
Terminal necropsy	15	19	23	19	15
Deaths Suspected as Being Related to Tumors					
Adrenal gland tumor	1	1	0	0	0
Bone tumor	0	0	0	0	1
Brain tumor	0	1	1	0	0
Fibrosarcoma/fibroma	0	0	0	0	0
Fibrous histiocytoma	0	0	0	0	0
Gastrointestinal tumor	1	0	0	0	0
Hibernoma	1	1	1	0	1
Histiocytic sarcoma	2	0	0	1	0
Kidney tumor	0	1	0	1	0
Leukemia	0	0	0	0	0
Lung tumor	0	0	0	1	0
Lymphoid tumor	0	0	0	0	2
Mammary tumor	20	14	13	19	20
Mesothelioma	0	0	0	0	0
Neuroendocrine tumor	0	0	0	0	0
Nose/oral tumor	0	0	0	0	0
Pancreas tumor	0	0	0	0	0
Pituitary tumor	27	25	24	25	28
Schwannoma	0	0	0	1	0
Skin tumor	0	0	1	0	0
Thyroid tumor	0	0	0	0	0
Uterus tumor	0	0	3	0	0
Zymbals gland tumor	0	0	0	0	0
Undetermined	2	3	2	1	2

Mean survival over time is shown in the sponsor-generated figures below.

Figure 1 Mean Survival Estimate – MALE

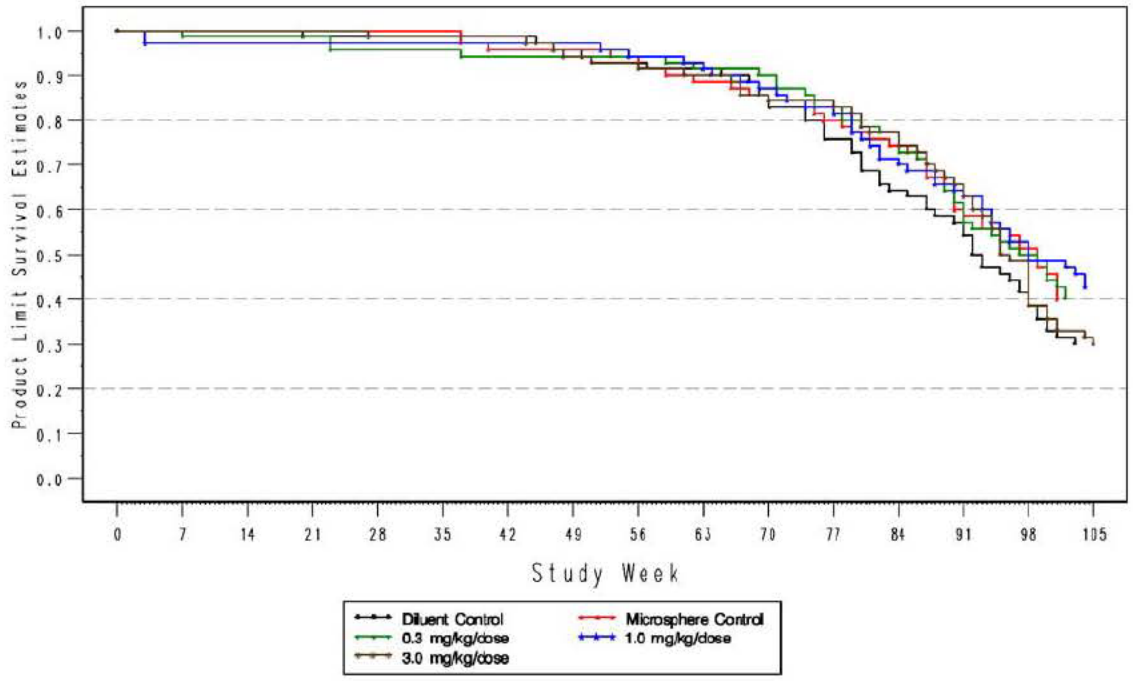
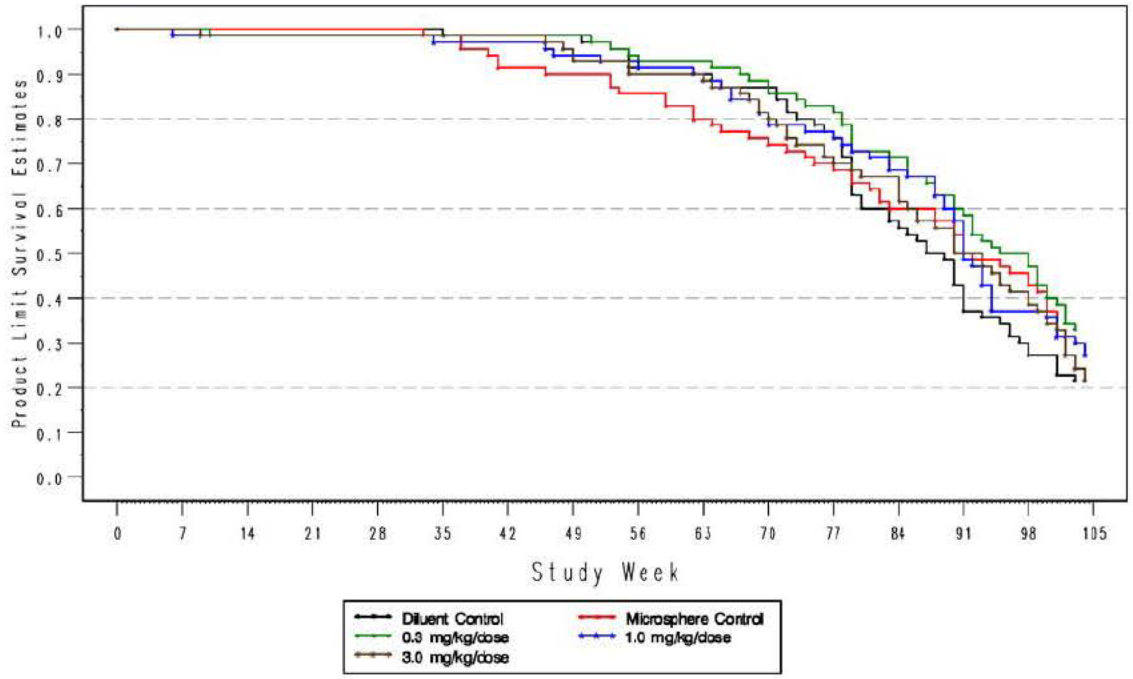


Figure 1A Mean Survival Estimate – FEMALE



Clinical signs: There were no clinical findings that were attributed to either the microspheres or exenatide QW. There were no definitive differences in palpable mass findings between control groups and treated groups.

Body weights:

Dose (mg/kg)	Diluent Control		Microsphere Control		0.3		1.0		3.0	
Sex	M	F	M	F	M	F	M	F	M	F
Weight (g) -Day 721	686.7	507.3	670.6	483.4	631.7	423.4*	596.9**	403.1**	608.9**	399.2**
Diff from control (g)			-16.1	-23.9	-55.0	-83.9	-89.8	-104.2	-77.8	-108.1
% diff from control			↓2%	↓5%	↓8%	↓17%	↓13%	↓21%	↓11%	↓21%

*p<0.05; **p<0.01; F = female; M = male.

Mean body weights over time are shown in the sponsor-generated figures below.

Figure 2 Mean Body Weight Values – MALE

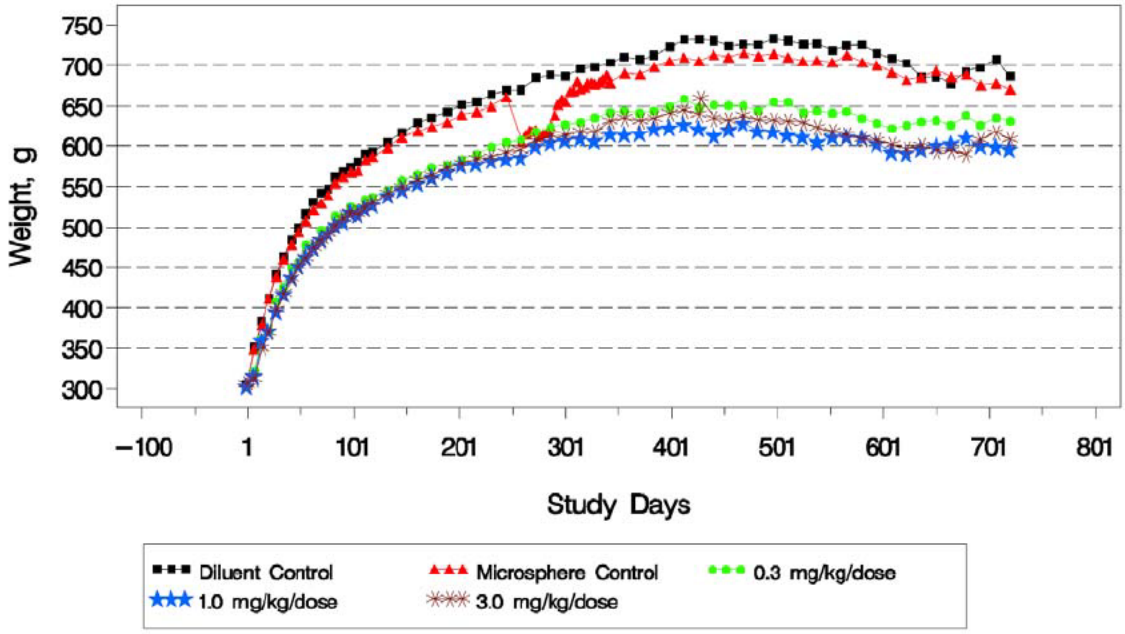


Figure 2A Mean Body Weight Values – FEMALE

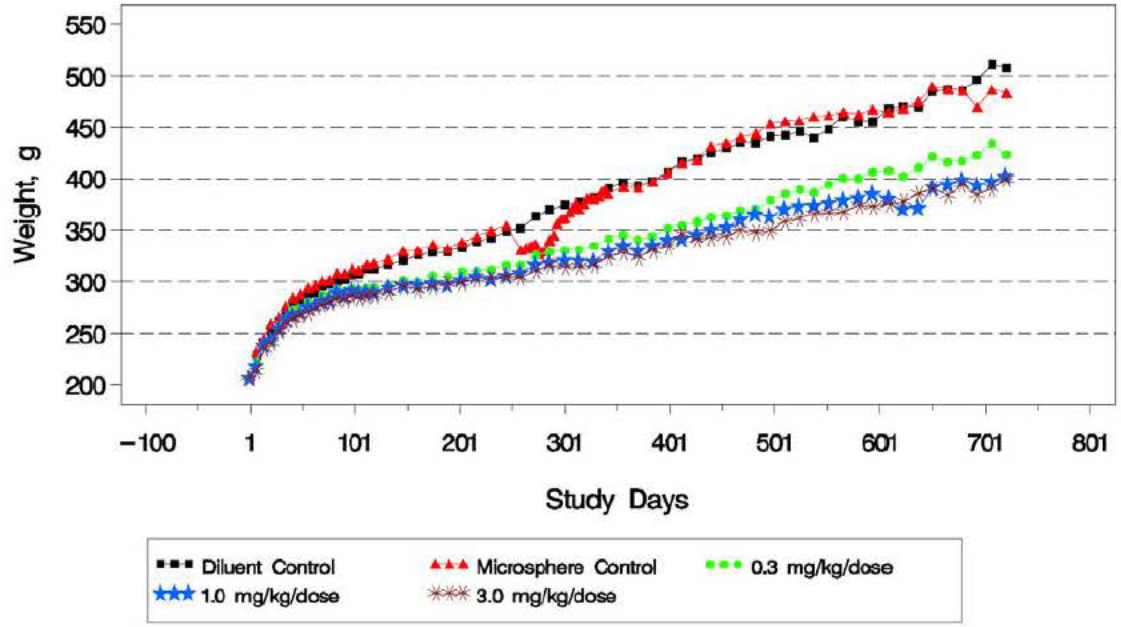


Figure 2B Mean Body Weight Values – MALE

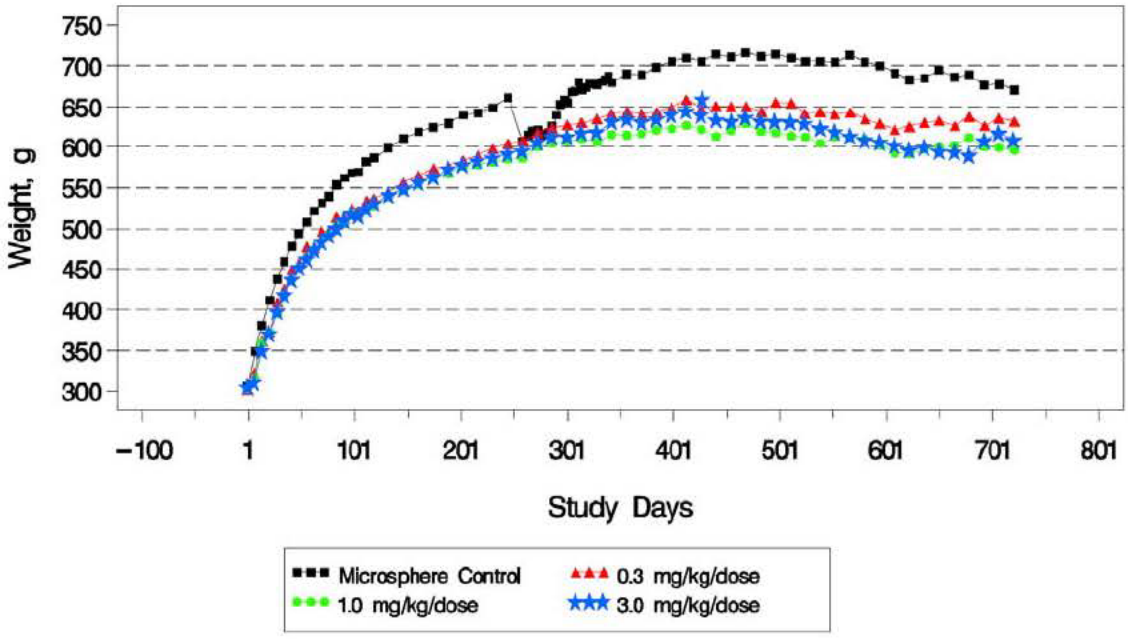
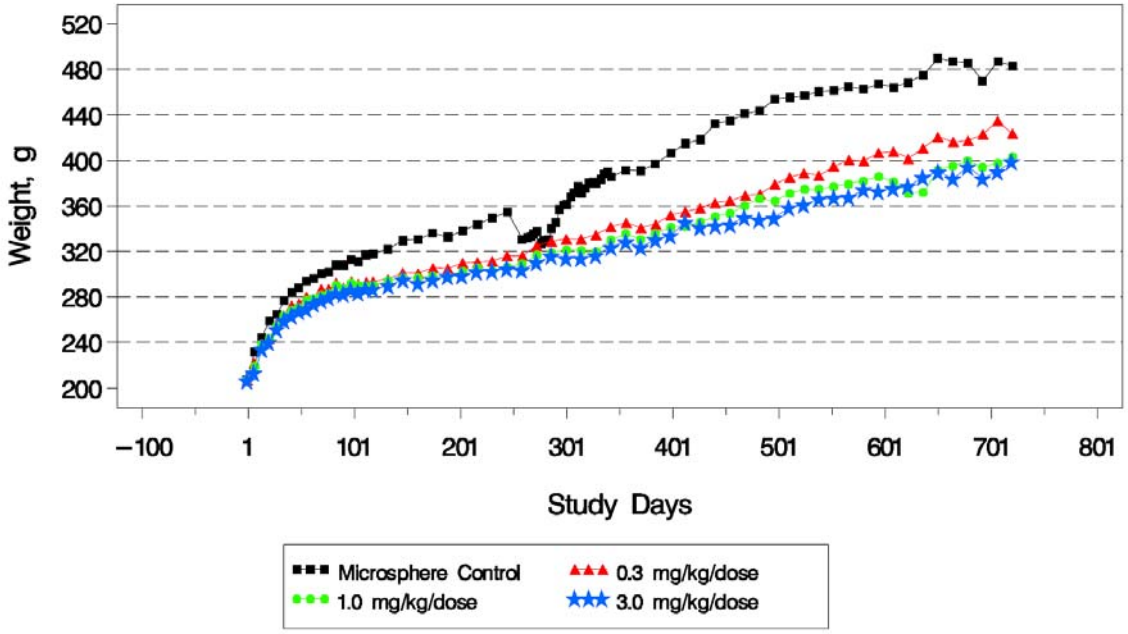


Figure 2C Mean Body Weight Values – FEMALE



Food consumption: Treatment-related and dose-dependent decreases in mean food consumption were noted in both sexes in all exenatide LAR groups when compared with diluent controls and microsphere controls (no microsphere-related effects), which correlated with the body weight effects.

Hematology: (erythrocyte count and leukocyte count [total and differential])
One high-dose male had elevated values for neutrophils, lymphocytes, eosinophils, and other cells that caused the high-dose male mean values for eosinophils and other cells to be elevated compared with controls.

Gross pathology: There were no macroscopic findings that were attributed to the microspheres or exenatide.

Histopathology:

Non-Neoplastic Findings - Males

Dose (mg/kg)	Diluent Control		Microsphere Control		0.3		1.0		3.0	
Survival status	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**
Number examined	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**
Adipose tissue, depletion	(5)	(0)	(3)	(1)	(8)	(1)	(7)	(1)	(11)	(0)
-mild	4	-	1	1	2	1	2	-	1	-
-moderate	-	-	-	-	5		3	1	5	-
-severe	1	-	2	-	1		2	-	5	-
Granuloma, foreign body										
Injection site 1	(0)	(0)	(6)	(0)	(2)	(0)	(5)	(0)	(3)	(1)
-minimal	-	-	4	-	2	-	5	-	-	1
-mild	-	-	2	-	-	-	-	-	3	-
Injection site 2	(0)	(0)	(9)	(2)	(0)	(1)	(6)	(4)	(7)	(1)
-minimal	-	-	4	2	-	1	5	4	3	1
-mild	-	-	5	-	-	-	1	-	4	-
Injection site 3	(0)	(0)	(7)	(8)	(3)	(1)	(5)	(5)	(6)	(4)
-minimal	-	-	7	8	3	1	5	5	5	4
-mild	-	-	-	-	-	-	-	-	1	-
Injection site 4	(0)	(0)	(7)	(11)	(2)	(0)	(4)	(1)	(9)	(3)
-minimal	-	-	7	9	2	-	4	1	5	2
-mild	-	-	-	2	-	-	-	-	4	1
Injection site 5	(0)	(0)	(7)	(0)	(3)	(0)	(8)	(0)	(7)	(0)
-minimal	-	-	3	-	3	-	8	-	7	-
-mild	-	-	4	-	-	-	-	-	-	-
Injection site 6	(0)	(0)	(10)	(0)	(3)	(0)	(3)	(0)	(7)	(0)
-minimal	-	-	9	-	3	-	2	-	7	-
-mild	-	-	1	-	-	-	1	-	-	-
Kidney,										
pelvic mineralization	(9)	(8)	(7)	(9)	(15)	(12)	(15)	(15)	(20)	(8)
-minimal	9	8	7	9	13	11	12	15	18	8
-mild	-	-	-	-	2	1	2	-	2	-
-moderate	-	-	-	-	-	-	1	-	-	-
Pancreas,										
Acinar atrophy	(6)	(3)	(3)	(5)	(2)	(10)	(3)	(9)	(8)	(9)
-minimal	4	3	3	5	2	9	2	9	7	6
-mild	2	-	-	-	-	1	1	-	-	2
-moderate	-	-	-	-	-	-	-	-	1	1

DOS = died or euthanized on study; SNC = scheduled necropsy.

Non-Neoplastic Findings - Females

Dose (mg/kg)	Diluent Control		Microsphere Control		0.3		1.0		3.0	
Survival status	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**
Number examined	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**
Granuloma, foreign body										
Injection site 1	(0)	(0)	(15)	(1)	(3)	(0)	(7)	(0)	(9)	(-)
-minimal	-	-	11	1	3	-	6	-	8	-
-mild	-	-	4	-	-	-	1	-	1	-
Injection site 2	(0)	(0)	(13)	(4)	(1)	(-)	(11)	(1)	(12)	(1)
-minimal	-	-	11	4	1	-	9	1	10	1
-mild	-	-	2	-	-	-	2	-	2	-
Injection site 3	(0)	(0)	(12)	(3)	(6)	(2)	(12)	(7)	(12)	(5)
-minimal	-	-	9	3	5	2	12	7	11	4
-mild	-	-	3	-	1	-	-	-	1	1
Injection site 4	(0)	(0)	(13)	(6)	(2)	(0)	(3)	(1)	(13)	(1)
-minimal	-	-	8	6	2	-	2	1	7	1
-mild	-	-	5	-	-	-	1	-	6	-
Injection site 5	(0)	(0)	(10)	(0)	(2)	(0)	(7)	(2)	(7)	(0)
-minimal	-	-	6	-	2	-	6	2	5	-
-mild	-	-	4	-	-	-	1	-	2	-
Injection site 6	(0)	(0)	(9)	(0)	(7)	(0)	(4)	(0)	(7)	(0)
-minimal	-	-	8	-	7	-	4	-	6	-
-mild	-	-	1	-	-	-	-	-	1	-
Pancreas,										
Acinar atrophy	(5)	(0)	(2)	(1)	(10)	(2)	(3)	(1)	(8)	(3)
-minimal	5	-	2	-	6	2	3	-	8	3
-mild	-	-	-	-	3	-	-	-	-	-
-moderate	-	-	-	1	1	-	-	-	-	-

DOS = died or euthanized on study; SNC = scheduled necropsy.

Neoplastic Findings - Males

Dose (mg/kg)	Diluent Control		Microsphere Control		0.3		1.0		3.0	
Survival status	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**
Number examined	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**
Thyroid, c-cell										
Hyperplasia, focal	(10)	(5)	(3)	(7)	(8)	(15)	(9)	(10)	(15)	(8)
-minimal	8	5	3	5	6	13	6	9	12	6
-mild	2	-	-	2	2	2	3	1	3	2
Adenoma, benign	5	4	7	2	13	7	12	20	19	14
Carcinoma, malignant	-	-	1	-	1	1	1	4	2	1
Adenoma + carcinoma†	(5)	(4)	(8)	(2)	(14)	(8)	(13)	(21)	(20)	(15)
Carcinoma, secondary										
-thoracic cavity	-	-	-	-	-	-	-	1	-	-
-parathyroid	-	-	-	-	-	-	-	-	-	-
Parathyroid, adenoma, benign	-	-	1	-	-	-	-	3	2	1
Schwannoma, malignant	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(1)	(3)	(0)
-eyes	-	-	-	-	-	-	-	-	1	-
-head	-	-	-	-	-	-	-	-	2	-
-skin	-	-	1	-	-	-	-	1	-	-
Skin, subcutis										
Fibroma, benign	-	-	1	2	2	2	1	1	5	3

DOS = died or euthanized on study; SNC = scheduled necropsy.
†animals having both an adenoma and carcinoma for the same tumor type (e.g., c-cell tumor) were only counted once for the combined (e.g., adenoma + carcinoma) tumor incidence.

Neoplastic Findings - Females

Dose (mg/kg)	Diluent Control		Microsphere Control		0.3		1.0		3.0	
Survival status	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**
Number examined	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**
Thyroid, c-cell										
Hyperplasia, focal	(8)	(5)	(7)	(5)	(21)	(10)	(19)	(10)	(29)	(11)
-minimal	8	3	6	3	19	9	9	7	17	6
-mild	-	2	1	2	2	1	10	3	11	5
-moderate	-	-	-	-	-	-	-	-	1	-
Adenoma, benign	4	1	6	3	11	11	13	6	16	5
Carcinoma, malignant	-	-	1	-	-	1	-	1	1	3
Adenoma + carcinoma†	(4)	(1)	(7)	(3)	(11)	(12)	(13)	(7)	(17)	(8)
Carcinoma, secondary										
-thoracic cavity	-	-	-	-	-	-	-	-	-	-
-parathyroid	-	-	-	-	-	1	-	1	-	-
Schwannoma,										
Benign	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
-abdominal cavity	-	1	-	-	-	-	-	-	-	-
Malignant	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(1)	(0)
-heart	-	-	-	-	-	-	-	-	1	-
-uterus	-	-	-	-	-	-	1	-	-	-
Kidneys, tubular cell	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(3)	(1)
-benign	-	-	-	-	-	-	-	-	-	1
-malignant	-	-	1	-	-	-	-	-	3	-
Pancreas, islet cell	(1)	(0)	(1)	(0)	(0)	(2)	(3)	(1)	(1)	(3)
-benign	1	-	-	-	-	1	2	1	1	3
-malignant	-	-	1	-	-	1	1	-	-	-

DOS = died or euthanized on study; SNC = scheduled necropsy.
†animals having both an adenoma and carcinoma for the same tumor type (e.g., c-cell tumor) were only counted once for the combined (e.g., adenoma + carcinoma) tumor incidence.

Summary of Tumor Incidence (Number of Affected Animals)

	Males					Females				
Dose (mg/kg)	**DC**	**MC**	**0.3**	**1.0**	**3.0**	**DC**	**MC**	**0.3**	**1.0**	**3.0**
Thyroid, c-cell										
Hyperplasia, focal	(15)	(10)	**(23)**	**(19)**	**(23)**	(13)	(12)	**(31)**	**(29)**	**(40)**
-minimal	13	8	19	15	18	11	9	28	16	23
-mild	2	2	4	4	5	2	3	3	13	16
-moderate	-	-	-	-	-	-	-	-	-	1
Adenoma	9	9	**20**	**32**	**33**	5	9	**22**	**19**	**21**
Carcinoma	-	1	2	**5**	**3**	-	1	1	1	**4**
Adenoma + carcinoma†	9	10	**22**	**34**	**35**	5	10	**23**	**20**	**25**
Carcinoma, secondary										
-thoracic cavity	-	-	-	1	-	-	-	-	-	-
-parathyroid	-	-	-	-	-	-	-	1	1	-
Skin, subcutis										
Fibroma, benign	-	3	4	2	**8**	-	-	-	-	-
Fibrosarcoma, malignant	-	-	-	-	-	-	1	-	-	-
Fibrous histiocytoma, malignant	-	1	-	1	-	-	-	-	-	1
Parathyroid, adenoma, benign	0	1	0	3	3	0	0	0	1	1
Schwannoma, Benign										
-abdominal cavity	-	-	-	-	-	1	-	-	-	-
Malignant										
-eyes	-	-	-	-	1	-	-	-	-	-
-head	-	-	-	-	2	-	-	-	-	-
-skin	-	1	-	1	-	-	-	-	-	-
-heart	-	-	-	-	-	-	-	-	-	1
-uterus	NA	NA	NA	NA	NA	-	-	-	1	-
Total (benign and malignant; all tissues)†	-	1	-	1	**3**	1	-	-	1	1
Kidneys, tubular cell										
-benign	-	-	-	-	-	-	-	-	-	1
-malignant	1	1	-	-	1	-	1	-	-	3
-benign + malignant†	1	1	-	-	1	-	1	-	-	**4**
Pancreas, islet cell										
-benign	8	12	12	3	3	1	-	1	3	4
-malignant	3	2	3	-	-	-	1	1	1	-
-benign + malignant†	11	14	15	3	3	1	1	2	**4**	**4**

DC = diluent control; MC = microsphere control.
†animals having both an adenoma and carcinoma for the same tumor type (e.g., c-cell tumor) were only counted once for the combined (e.g., adenoma + carcinoma) tumor incidence.

Summary of Tumor Incidence (Percentage of Affected Animals)

	Males						Females					
Dose (mg/kg)	**DC**	**MC**	**0.3**	**1.0**	**3.0**	**HIS**	**DC**	**MC**	**0.3**	**1.0**	**3.0**	**HIS**
Clinical Exposure Margin†			**2X**	**10X**	**26X**				**1X**	**8X**	**25X**	
Thyroid, c-cell Adenoma	14%	14%	29%	46%	47%	8.8% (1.9-15.4%)	7.1%	13%	31%	27%	30%	8.1% (2-11.4%)
Carcinoma	0%	1.4%	2.9%	7.1%	4.3%	0.6% (0-1.7%)	0%	1.4%	1.4%	1.4%	5.7%	0.6% (0-4.0%)
Skin, subcutis, Fibroma	0%	4.3%	5.7%	2.9%	11%	2.2% (0-5%)						
Parathyroid, adenoma	0%	1.4%	0%	4.3%	4.3%	NP						
Schwannoma, Total malignant (all tissues)	0%	1.4%	0%	1.4%	4.3%	NP						
Kidneys, tubular cell, Malignant							0%	1.4%	0%	0%	4.3%	0.6% (0-3.3%)
Pancreas, islet cell Benign + malignant							1.4%	1.4%	2.9%	5.7%	5.7%	NP

DC = diluent control; HIS = historical control data from 11 studies; MC = microsphere control; NP = not provided.
†Based on mean AUC values for antibody negative animals on Day 183 compared with mean AUC values for antibody negative humans.

The statistical analysis results for potentially drug-related tumors are shown in the tables below. For trend analysis, a significance level of $\alpha = 0.005$ was used for common tumors (≥1%) and $\alpha = 0.025$ for rare tumors (<1%), and for pair-wise comparison, a significance level of $\alpha = 0.01$ was used for common tumors and $\alpha = 0.05$ for rare tumors.

Statistical Analysis for Tumor Analysis - Males

Finding	Significant for dose response		Significant for pair-wise comparison	
	vs. Control 1	vs. Control 2	vs. Control 1	vs. Control 2
Thyroid c-cell adenoma	**p < 0.001***	**p < 0.001***	LD: p = 0.0380 **MD: p < 0.001*** **HD: p < 0.001***	LD: p = 0.0221 **MD: p < 0.001*** **HD: p < 0.001***
Thyroid c-cell carcinoma	No (p = 0.1636)	No (p = 0.2367)	**MD: p = 0.0363*** (HD: p = 0.1329)	No (p ≥ 0.1116)
Thyroid c-cell adenoma plus carcinoma	**p < 0.001***	**p < 0.001***	LD: p = 0.0185 **MD: p < 0.001*** **HD: p < 0.001***	LD: p = 0.0186 **MD: p < 0.001*** **HD: p < 0.001***
Skin, subcutis - fibroma, benign	**p = 0.0042***	No (p = 0.0343)	**HD: p = 0.0040***	No (p ≥ 0.1063)
Schwannoma, malignant - all tissues	**p = 0.0203***	No (p = 0.0637)	No (p ≥ 0.1369)	No (p ≥ 0.2525)
Parathyroid, benign adenoma	No (p = 0.0410)	No (p = 0.0837)	No (p ≥ 0.1329)	No (p ≥ 0.3087)

*Statistically significant; Statistical analysis was conducted by Dr. Atiar Rahman from the Division of Biometrics-6.

"No" indicates that p > 0.025 (rare tumors) or p >0.005 (common tumors) for dose-response and p > 0.05 (rare tumors) or p >0.01 (common tumors) for pair-wise comparisons.

Statistical Analysis for Tumor Analysis - Females

Finding	Significant for dose response		Significant for pair-wise comparison	
	vs. Control 1	vs. Control 2	vs. Control 1	vs. Control 2
Thyroid c-cell adenoma	No (p = 0.0237)	No (p = 0.0723)	**LD: p < 0.001*** **MD: p = 0.0028*** **HD: p < 0.001***	LD: p = 0.0158 MD: p = 0.0442 HD: p = 0.0152
Thyroid c-cell carcinoma	**p = 0.0139***	No (p = 0.0415)	No (HD: p = 0.0639)	No HD: p = 0.1874)
Thyroid c-cell adenoma plus carcinoma	**p = 0.0026***	No (p = 0.0163)	**LD: p < 0.001*** **MD: p = 0.0015*** **HD: p < 0.001***	LD: p = 0.0187 MD: p = 0.0499 **HD: p = 0.0036***
Kidneys, carcinoma, tubular	**p = 0.0156***	No (p = 0.0496)	No (p ≥ 0.1380)	No (p ≥ 0.3250)
Pancreas, adenoma, islet cell	No (p = 0.2021)	No (p = 0.0284)	No (p ≥ 0.5114)	No (p ≥ 0.5056)

*Statistically significant; Statistical analysis was conducted by Dr. Atiar Rahman from the Division of Biometrics-6.

"No" indicates that p > 0.025 (rare tumors) or p >0.005 (common tumors) for dose-response and p > 0.05 (rare tumors) or p >0.01 (common tumors) for pair-wise comparisons.

Toxicokinetics: (sponsor-generated table)

Mean Toxicokinetic Parameters of Exenatide Following Once Every Other Week Subcutaneous Administration of Exenatide LAR (F17) in Male and Female Sprague-Dawley Rats

Dose (mg/kg)	Sex	T_{max} (h)		C_{max} (pg/mL)		AUC_{0-t} (pg·h/mL)	
		Day 1	Day 183	Day 1	Day 183	Day 1	Day 183
		All Animals					
0.3	Male	24	24	1298	3020	94,215	201,583
	Female	24	24	1313	2775	51,137	140,308
	Mean	24	24	1306	2897	72,676	170,945
1.0	Male	48	24	2997	28,117	204,885	1,558,936
	Female	48	48	1678	18,607	114,093	1,685,831
	Mean	48	36	2338	23,362	159,489	1,622,383
3.0	Male	48	48	4392	10,662	385,851	2,455,698
	Female	48	24	4334	37,130	485,491	8,198,256
	Mean	48	36	4363	23,896	435,671	5,326,977
		Animals Negative for Antibodies to Exenatide					
0.3	Male	24	24	1298	2682	94,215	158,204
	Female	24	24	1313	2113	51,137	107,319
	Mean	24	24	1306	2398	72,676	132,762
1.0	Male	48	24	2997	4497	204,885	783,272
	Female	48	24	1678	4301	114,093	641,052
	Mean	48	24	2338	4399	159,489	712,162
3.0	Male	48	96	4392	10,586	385,851	2,024,703
	Female	48	96	4334	10,574	485,491	1,910,576
	Mean	48	96	4363	10,580	435,671	1,976,640

AUC_{0-t} – area under the concentration-time curve for the dosing interval (AUC_{0-336h}, or from time zero to the last quantifiable concentration).
C_{max} – maximum observed plasma concentration during the dosing interval.
T_{max} – time to the observed C_{max}.

Antibody Assessment: (sponsor-generated tables)

Males – Main Study

Group		Study Timepoint: Day -1 (pretest)	6 Month (Day 182)	12 Month (Day 364)	18 Month (Day 546)	24 Month (Day 729)
Diluent Control q 2 week	Incidence of Positive	0/70	1/67	4/66	3/53	2/21
	Result (+/N) and %	0%	1%	6%	6%	10%
	Range of Titers	NA	25	25-625	125	125
Microsphere Control q 2 week	Incidence of Positive	0/70	0/70	1/67	0/55	0/28
	Result (+/N) and %	0%	0%	1%	0%	0%
	Range of Titers	NA	NA	25	NA	NA
0.3 mg/kg/dose Exenatide LAR q 2 week	Incidence of Positive	0/70	24/67	23/66	18/56	3/28
	Result (+/N) and %	0%	36%	35%	32%	11%
	Range of Titers	NA	25-3125	25-3125	25-3125	25-625
1.0 mg/kg/dose Exenatide LAR q 2 week	Incidence of Positive	1/70	23/68	20/67	16/57	5/28
	Result (+/N) and %	1%	34%	30%	28%	18%
	Range of Titers	25	25-3125	25-15,625	25-15,625	25-3125
3.0 mg/kg/dose Exenatide LAR q 2 week	Incidence of Positive	0/70	25/69	20/65	17/58	4/21
	Result (+/N) and %	0%	36%	31%	29%	19%
	Range of Titers	NA	25-15,625	25-15,625	25-15,625	25-125

Females – Main Study

Group		Study Timepoint: Day -1 (pretest)	6 Month (Day 182)	12 Month (Day 364)	18 Month (Day 546)	24 Month (Day 729)
Diluent Control q 2 week	Incidence of Positive	0/70	0/67	1/68	1/50	0/15
	Result (+/N) and %	0%	0%	1%	2%	0%
	Range of Titers	NA	NA	25	25	NA
Microsphere Control q 2 week	Incidence of Positive	0/70	0/68	1/63	2/47	0/19
	Result (+/N) and %	0%	0%	2%	4%	0%
	Range of Titers	NA	NA	25	25	NA
0.3 mg/kg/dose Exenatide LAR q 2 week	Incidence of Positive	0/70	28/68	23/63	19/55	7/23
	Result (+/N) and %	0%	41%	37%	35%	30%
	Range of Titers	NA	25-15,625	25-15,625	25-15,625	25-3125
1.0 mg/kg/dose Exenatide LAR q 2 week	Incidence of Positive	1/70	19/67	22/65	17/52	5/18
	Result (+/N) and %	1%	28%	34%	33%	28%
	Range of Titers	125	25-15,625	25-15,625	25-3125	25-3125
3.0 mg/kg/dose Exenatide LAR q 2 week	Incidence of Positive	0/70	19/66	17/65	12/49	2/15
	Result (+/N) and %	0%	29%	26%	24%	13%
	Range of Titers	NA	25-15,625	25-15,625	25-3125	125-3125

Males – Toxicokinetic Animals					
		Study Timepoint			
Group		Day -1 (pretest)	2 Month (Day 56)	4 Month (Day 112)	6 Month (Day 182)
0.3 mg/kg/dose Exenatide LAR q 2 week	Incidence of Positive Result (+/N) and % Range of Titers	0/10 0% NA	3/10 30% 25-625	3/10 30% 125-3125	3/10 30% 625-3125
1.0 mg/kg/dose Exenatide LAR q 2 week	Incidence of Positive Result (+/N) and % Range of Titers	0/10 0% NA	6/10 60% 25-3125	4/10 40% 125-15,625	4/10 40% 625-78,125
3.0 mg/kg/dose Exenatide LAR q 2 week	Incidence of Positive Result (+/N) and % Range of Titers	0/10 0% NA	2/10 20% 25-125	3/10 30% 25-125	2/10 20% 125-625

Females – Toxicokinetic Animals					
		Study Timepoint			
Group		Day -1 (pretest)	2 Month (Day 56)	4 Month (Day 112)	6 Month (Day 182)
0.3 mg/kg/dose Exenatide LAR q 2 week	Incidence of Positive Result (+/N) and % Range of Titers	0/10 0% NA	3/10 30% 25-625	4/10 40% 125-625	4/10 40% 125-3125
1.0 mg/kg/dose Exenatide LAR q 2 week	Incidence of Positive Result (+/N) and % Range of Titers	0/10 0% NA	5/10 50% 125-3125	5/10 50% 25-3125	5/10 50% 125-3125
3.0 mg/kg/dose Exenatide LAR q 2 week	Incidence of Positive Result (+/N) and % Range of Titers	0/10 0% NA	5/10 50% 25-625	4/10 40% 25-3125	3/9 33% 125-3125

2.6.6.6 Reproductive and developmental toxicology

The effect of exenatide on reproduction and embryonic development was previously investigated by the sponsor in support of the marketing application for Byetta (NDA 21-773). Additional developmental and reproductive toxicology (DART) studies were not conducted with exenatide QW. A brief summary of DART study results is presented below, which is based on the review of NDA 21-773 by Dr. John Colerangle. Note that exposure margins between nonclinical NOAEL values and humans is based on exposures achieved with immediate release exenatide for all TK animals at Day 30 in a 3-month mouse study (REST99051) and during two teratology studies in rabbits (REST99061R2 and REST02022) compared with the mean exposure of antibody negative humans receiving the maximum recommended human dose (MRHD) of exenatide QW (2 mg) across three clinical studies (AUC_{0-24} = 5,461 pg·h/mL).

Fertility and Early Embryonic Development:
The effect of exenatide on fertility and early embryonic development was evaluated in mice. Mice received exenatide by subcutaneous injection at doses of at 3, 34 and 380 µg/kg twice daily (1X, 9X and 148X MRHD, AUC). Males were treated for 28 days before cohabitation through mating, and females were treated for 15 days prior to cohabitation through gestation day (GD) 7. Results showed that there were no exenatide-related adverse effects on estrus cycling, mating and fertility indices, numbers of corpora lutea, implantation, viable embryos, non-viable embryos, pre- or post-implantation viability, or cauda epididymal sperm motility, count, or density. Accordingly, the NOAEL for effects on male and female reproduction was the high dose of 380 µg/kd/dose (760 µg/kg/day; 148X MRHD).

Embryo-Fetal Toxicity
Mice
The potential of exenatide to cause reproductive or developmental toxicity was evaluated in mice and rabbits. In a mouse teratology study, exenatide doses of 3, 34, 230, and 380 µg/kg twice daily (1X, 9X, 92X, and 148X MRHD, AUC) were administered subcutaneously to pregnant mice (25/group) from GD 6 through 15. Consistent with the pharmacodynamic activity of exenatide, maternal body weight and food consumption were decreased compared with controls. One female in each of the 34 and 380 µg/kg/dose groups aborted on GD 15 and 16, respectively. One female in each of the 34, 230, and 380 µg/kg/dose groups delivered prematurely.

Developmental toxicity occurred in conjunction with maternal toxicity. The number of implantations, litter sizes, and live fetuses were significantly decreased for dams receiving 230 µg/kg/dose relative to control. Fetal body weights were decreased compared to controls at ≥230 µg/kg/dose for males and ≥68 µg/kg/dose for females. Skeletal variations associated with delayed fetal growth, including changes in the number of rib pairs or vertebral ossification sites, and wavy ribs were noted ≥230 µg/kg/dose. Five fetuses from the treated group and two from the control group had multiple findings. Cleft palate with or without hole was observed at 3 µg/kg/dose. Because the incidence of the multiple findings was greater in the treated group, a relationship to exenatide could not be ruled out. TK data showed that the potential of exenatide to cross the placental barrier is very low in mice. Because embryonic exposure is expected to be low, the observed fetal findings may have been a consequence of the dose-related reduced nutritional state of the dams during gestation or direct maternal toxicity. The maternal NOAEL was determined to be 3 µg/kg BID (1X MRHD) based on the observed abortions. The developmental NOAEL was also determined to be 3 µg/kg BID on the basis of decreased fetal body weights, cleft palate, and wavy ribs.

Rabbits
In a rabbit teratology study, timed pregnant female rabbits (20/group) were dosed subcutaneously at 0.1, 11, 78 and 130 µg/kg twice daily resulting in total daily doses of 0.2 (0.1X), 22 (79X), 156 (544X), or 260 µg/kg/day (1,322X MRHD, AUC). One female each from the 0.2- and 22-µg/kg/day dose groups was found dead on GD 10 and GD 19, respectively. The cause of deaths could not be determined at necropsy. One female

receiving 156 μg/kg/day aborted on GD 21 and one female receiving 22 μg/kg/day delivered prematurely on GD 29. These events were considered unrelated to the test article because they were not dose-dependent, the death of one doe appeared to be related to an injury, and the abortion and delivery for a single doe in a study was within the historical control incidence for the testing facility. Consistent with the pharmacological activity of exenatide, body weight gain that correlated to decreased food consumption was significantly decreased in all treated groups in a dose-dependent manner relative to control (GD 6 through 19). Morphological markers of fetal growth retardation were observed. These findings included umbilical hernias and skeletal variations of angulated hyoid, altered number of rib pair or vertebral bodies, and fused sternabrae at 22 μg/kg/day or greater. Fetal incidence of small gall bladder was significantly increased at 22, 156, and 260 μg/kg/day. The maternal NOAEL was determined to be 0.2 μg/kg/day (0.1X MRHD) based on dose-related decrease in weight gain during the treatment period. The developmental NOAEL is also 0.2 μg/kg/day (0.1X MRHD) based on the developmental toxicity (higher incidence of umbilical hernia, small gall bladder, angulated hyoid, delayed ossifications and fused sternal centra). TK data showed that the potential for exenatide to cross the placental barrier was also very low in rabbits. Therefore the fetal findings observed may be a consequence of the reduced nutritional state of the does during gestation or direct maternal toxicity.

Another rabbit teratology study was performed to better define the NOAEL with regard to fetal effects and to clarify the role of exenatide-related decreases in food consumption and body weight on developmental effects. In this study, pregnant rabbits were administered exenatide at twice daily doses of 1, 11, and 130 μg/kg, representing daily doses of 2 (4X MRHD) , 22 (79X MRHD), and 260 μg/kg/day (1,322X MRHD). Three additional groups were pair-fed (fed the same average daily amount of food) to match the three respective exenatide-dosed groups. Rabbits that were administered exenatide exhibited profound, dose-related decreases in food and water consumption and loss in body weight. Clinical indicators of starvation (β-hydroxybuterate and potassium) and body weight loss were more pronounced in the exenatide-treated groups than in the pair-fed groups. Based on the severity of the body weight loss and anorexia, the MTD in pregnant rabbits was exceeded at doses ≥22 μg/kg/day exenatide. As in the previous rabbit study, developmental toxicity occurred only at doses ≥22 μg/kg/day exenatide, doses that exceeded the MTD in pregnant rabbits. None of the fetuses from pair-fed dams and from the dams administered 2 μg/kg/day exenatide had umbilical hernias. Skeletal variations were observed at ≥2 μg/kg/d, but were also present in similar incidences in both exenatide and pair-fed groups, suggesting these effects were a consequence of compromised maternal condition. Thus, exenatide was not a developmental toxicant in rabbits; the NOEL for developmental toxicity was 2 μg/kg/day exenatide (4X MRHD).

Perinatal and Postnatal Developmental Toxicity

The effects of exenatide on gestation, parturition, lactation, and maternal behavior were evaluated in mice from implantation through lactation and weaning. The effects on development and fertility of the offspring were also evaluated. Pregnant mice (25/group) were administered exenatide at doses of 3, 34, and 380 μg/kg twice daily by

subcutaneous injection resulting in total daily doses of 6 (3X MRHD), 68 (50X MRHD), and 760 μg/kg/d (520X MRHD). One dam in each dose group died before or during delivery. The high-dose female died while delivering the pups and this death might have been drug-related because a delivery-related death only occurred in the high-dose group and the other high-dose mice had increased incidences of stillbirths and pup deaths on LD1 (Lactation Day 1). Although the cause of deaths could not be determined, the sponsor indicated that the deaths in the 6 and 68 μg/kg/day dose groups were not considered drug-related because they did not occur in a dose-dependent manner. The number of dams delivering stillborn pups was significantly increased in the 760 μg/kg/day group (24%) relative to control (0%). Dams (F_0) with all pups dying during days 1-4 postpartum was also significantly increased in the 760 μg/kg/day group (12%) relative to control (0%). The number of live births was significantly decreased in the 760 μg/kg/day group (92%) relative to control (100%). Still birth was significantly increased in the high-dose group (6%) relative to control (0%). The number of F_1 pups found dead/presumed cannibalized was significantly increased in the 6 μg/kg/day (3.2%) and 760 μg/kg/day groups (5.5%) relative to control during days 1-4 postpartum, and in the 68 μg/kg/day group (4.5%) during days 8-14 postpartum. All F_1 pup tissues appeared normal at necropsy. Viability index, surviving pups/litter, and pup weight/litter were significantly decreased in the 760 μg/kg/day group relative to control.

Post-weaning body weight was also slightly but significantly decreased in the 760 μg/kg/day F_1 females during precohabitation for mating of the F_1 generation on GD 0 and on GD 18 relative to control. There were no treatment-related effects on the number of corpora lutea, implantations, litter sizes and resorptions in cesarean-sectioned F_1 females. One of 297 low-dose F_2 fetuses had a cleft palate; one of 268 mid-dose F_2 fetuses had exencephaly, opened eyelids, and a cleft snout. Litter and fetal incidences of forked tail tip and flexed (downward) hindlimb were slightly increased (not SS) in F_2 litters/fetuses of high-dose F_1 parents. Maternal administration of exenatide at doses as high as 760 μg/kg/d did not affect the day of preputial separation or day of vaginal patency in the F_1 generation mice, learning or memory, mating or fertility, cesarean-sectioning parameters or the incidence of fetal alterations in F_2 generation mice.

The F_0 maternal NOAEL was less than 6 μg/kg/day (<1X MRHD) due to mortality at 6 μg/kg/day and greater. The NOAEL for fetal viability and growth was 6 μg/kg/d (1X MRHD) because of reduced preweaning pup body weights at 68 μg/kg/d (9X MRHD) and 760 μg/kg/d (148X MRHD) and increased perinatal mortality and reduced body weight gains postweaning at 760 μg/kg/day. There were no effects on the fertility of the F_1 dams or on the development of the F_2 fetuses.

2.6.6.7 Local tolerance

Separate local tolerance studies were not conducted with exenatide QW as assessments for reactions at the injection sites were integrated into the repeat-dose toxicology studies as summarized briefly below in the sponsor-generated table. These studies were conducted with the exenatide QW clinical formulation (F17).

Summary of Injection Site Changes in Exenatide QW Nonclinical Studies

Exenatide QW Study (Reference)	Injection Site Changes	Incidence	Severity	Recovery
8-Week Rat (REST060307)	Swelling	Microsphere dose-related; all animals in microsphere control and high dose groups	NA	Complete
	Histiocytic Infiltration	Similar across groups	Minimal to moderate, similar across groups	Partial
	Granulomatous Inflammation	Microsphere dose-related	Minimal to severe; microsphere dose-related	Partial to complete
8-Week Rat (REST080043)	Foreign body reaction	All microsphere-treated groups, with slight increase at high dose	Slight increase at high dose	Partial
18-Week Rat (REST050369)	Thickening	Microsphere dose-related	Microsphere dose-related	Complete
	Foreign body reaction	All groups	Mild	Partial to complete
3-Month monkey (REST04289R2)	Erythema/edema, inflammation with abscess and white discharge	One animal at high dose	Severe	Complete
	Foreign body reaction	Microsphere dose-related	Microsphere dose-related	Partial to complete
9-Month monkey (REST050370)	Erythema/edema	All microsphere-treated groups, with slight microsphere dose-related increase	Slight microsphere dose-related increase	Complete
	Swelling with open drainage	2/12 at low and mid dose; 6/12 at high dose	n/a	Complete
	Foreign body reaction	All microsphere-treated groups	Minimal to moderate	Partial

NA = not available

Additionally, two local tolerance studies were conducted in rabbits with sustained release formulations that differ from the clinical product. Study AT-23-01 was conducted with a formulation that contained a slightly smaller molecular weight range of PLG (b) (4) polymer; (b) (4) kD), 1% sucrose, and 0.7% exenatide. Study REST02027 was conducted with AC2993-F14, which contained the same molecular weight range of PLG (b) (4) polymer; (b) (4) kD) as the intended clinical formulation but contained (b) (4) % exenatide, and (b) (4) % sucrose. These reports were submitted to the IND and were previously reviewed by Dr. Colerangle but were not resubmitted in the NDA and are not being summarized here because the sponsor decided on a different formulation for the final product. For comparison purposes, the ingredients of the intended clinical formulation (F17) are shown in the table below.

Ratio of Ingredients for the Clinical Microsphere Formulation (AC2993-F17)

FORMULATION OF AC2993-F17	
Ingredient	**Composition (mg/100 mg microspheres)**
(b) (4) 50:50 Polylactide-co-glycolide (b) (4)	(b) (4)
Exenatide	
Sucrose	

2.6.6.8 Other toxicology studies

Safety of polylactide-co-glycolide (GLP) microspheres

The safe use of biodegradable polymers in medical products is well documented in the literature. Polyesters of the alpha-hydroxy carboxylic acids, including combinations of lactide (PLA) and glycolide (PGA) polymers as well as homo-polymers containing poly-L-lactide, poly-D-lactide, or polyglycolide have been used for medical devices such as sutures (Dexon® [100% PGA] and Vicryl®[90% PGA-10%PLA]) and orthopedic implants (e.g., screws, rods, and plates) for several decades. The pharmaceutical industry has utilized polymer microspheres for sustained release depot formulations for over 20 years. Examples of drugs containing polylactide-co-glycolide (PLG) microspheres at varying lactide to glycolide ratios that have received FDA marketing approval are presented in the table below (adapted from sponsor's submission). As shown in the table, the amount of PLG that will be injected at each dose administration of exenatide QW ranges from slightly greater than to (b) (4) times less than that of other products currently marketed.

List of Approved Products Containing Polylactide-co-Glycolide (PLG)

Products*	Year of Marketing Approval	Route of Administration	Microsphere Dose (mg/injection)	Fold Difference versus Exenatide QW
Exenatide QW	under review	SC injection	(b) (4)	
Nutropin Depot	1999**	SC injection		
Zoladex	1996	SC implant		
Risperdal Consta	2003	IM injection		
Lupron Depot	1989	IM injection		
Sandostatin LAR	1998	IM injection		
Vivitrol	2006	IM injection		

*Ratio of lactide to glycolide is 50:50 for exenatide QW and 75:25 for Risperdal Consta and Vivitrol. The ratio for the other products listed in the table is not described in their respective labels.

**Nutropin Depot was voluntarily discontinued by the sponsor in 2004. The sponsor stated that discontinuation was based on a corporate decision that was not related to the safety of the product.

(b) (4)

In vivo, PLG and the homopolymers degrade by hydrolysis of ester bonds into lactic acid and glycolic acid, which are then incorporated into metabolic pathways and excreted. The in vivo degradation rates of PLGs are inversely related to chain length. The safety and biocompatibility of PLA and PGA polymers has been well documented. A comprehensive review of literature reporting results from biocompatibility and toxicity testing is presented in Athanasiou et al. (1996). Based on the literature, there is no indication that PLG or its degradation products cause systemic toxicity, reproductive and developmental effects, genotoxicity, or carcinogenicity at clinically relevant doses. However, local inflammatory reactions can occur with minimal clinical significance.

In addition to information available in published literature, the sponsor has evaluated the systemic and local toxicity of PLG by conducting a 4-month toxicity study in rats, a

9-month toxicity study in monkeys, and a 2-year carcinogenicity study in rats with exenatide QW. The results of these studies did not indicate that PLG induces systemic toxicity or tumor formation. Local reactions were characterized by swelling, inflammation, and granulomas, which are consistent with reactions noted for other products containing PLG (Anthanasiou et al., 1996; Anderson and Shive, 1997). Injection site reactions observed in rats and monkeys due to the presence of PLG were shown to be reversible during a treatment-free period. Therefore, based on the available information in the literature and the results from studies conducted by the sponsor, there are no apparent safety concerns regarding the use of PLG as a sustained release drug delivery system for exenatide QW.

References

Anderson JM and Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. Advanced Drug Delivery Reviews. 1997;28:5-28.

Anthanasiou KA, Niederauer GG, and Agrawal CM. Sterilization, toxicity, bio-compatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. Biomaterials. 1996; 17:93-102.

2.6.6.9 Discussion and Conclusions

See Integrated Safety Summary and Conclusions

2.6.6.10 Tables and Figures

All relevant tables and figures have been presented in-text.

2.6.7 TOXICOLOGY TABULATED SUMMARY

2.6.7.1 Toxicology Overview

Test Article	Type of Study	Species/ Strain	Method of Administration	Duration of Dosing	Exenatide Doses (mg/kg/dose)	GLP	Testing Facility	Study Reference (Location)
Exenatide	Repeated-dose toxicity	Mouse / CD-1	SC Injection	28 days (twice daily)	0.76 mg/kg/day	Yes	(b) (4)	REST04568 (Section 2.6.6.3.1.1)
Exenatide Once Weekly	Repeated-dose toxicity	Rat / Sprague-Dawley	SC Injection	8 weeks (every other week) + 3 month recovery	0.3, 3, 9	Yes		REST060307 (Section 2.6.6.3.2.1)
	Repeated-dose toxicity	Rat / Sprague-Dawley	SC Injection	8 weeks (every other week) + 3 month recovery	0.3, 3, 9	Yes		REST080043 (Section 2.6.6.3.2.2)
	Repeated-dose toxicity	Rat / Sprague-Dawley	SC Injection	18 weeks (every other week) + 3 month recovery	1, 3, 9	Yes		REST050369 (Section 2.6.6.3.2.3)
	Repeated-dose toxicity	Monkey / Cynomolgus	SC Injection	13 weeks (QW) + 3 month recovery	0.11, 0.44, 1.1	Yes		REST04289R2 (Section 2.6.6.3.3.1)
	Repeated-dose toxicity	Monkey / Cynomolgus	SC Injection	39 weeks (QW) + 3 month recovery	0.11, 0.42, 1.1	Yes		REST050370 (Section 2.6.6.3.3.2)
	Genotoxicity (bacterial reverse mutation test)	S. typhimurium/ TA98, TA100, TA1535, TA1537 E. coli / WP2 uvrA	N/A (in vitro)	Plates were incubated for 52 hours	33.3, 100, 333, 1000, 2000, 5000 µg/plate (with and without S9 activation)	Yes		REST04571 (Section 2.6.6.4.1)

Test Article	Type of Study	Species/ Strain	Method of Administration	Duration of Dosing	Exenatide Doses (mg/kg/dose)	GLP	Testing Facility	Study Reference (Location)
Exenatide Once Weekly	Genotoxicity (bacterial reverse mutation test)	S. typhimurium/ TA98, TA100, TA1535, TA1537 E. coli / WP2 uvrA	N/A (in vitro)	Plates were incubated for 48-72 hours	15, 50, 150, 500, 1500, 5000 µg/plate (with and without S9 activation)	Yes	(b) (4)	REST060302 (Section 2.6.6.4.2)
	Genotoxicity (bacterial reverse mutation test)	S. typhimurium/ TA98, TA100, TA1535, TA1537 E. coli / WP2 uvrA	N/A (in vitro)	Plates were incubated for 48-72 hours	25, 75, 250, 750, 2500 µg/plate (with and without S9 activation)	Yes		REST080228 (Section 2.6.6.4.3)
	Genotoxicity (chromosome aberration)	Chinese Hamster Ovary Cells (CHO)	NA (in vitro)	3-hour treatment with 17-hour recovery (+ S9); 20-hour treatment (- S9)	3-hour treatment: 1250, 2500, 3750, 5000 µg/mL 20-hour treatment: 1880, 2500, 3750, 5000 µg/mL	Yes		REST04569 (Section 2.6.6.4.4)
	Genotoxicity (chromosome aberration)	Chinese Hamster Ovary Cells (CHO)	NA (in vitro)	4-hour treatment with 16-hour recovery (± S9); 20-hour treatment (- S9)	4-hour treatment: 12.5, 25, 75 µg/mL 20-hour treatment: 12.5, 25, 75, 100 µg/mL	Yes		REST060306 (Section 2.6.6.4.5)
	Genotoxicity (chromosome aberration)	Chinese Hamster Ovary Cells (CHO)	NA (in vitro)	4-hour treatment with 16-hour recovery (± S9); 20-hour treatment (- S9)	50, 100, 250 µg/mL	Yes		REST080139 (Section 2.6.6.4.6)
	Carcinogenicity	Rat / Sprague-Dawley	SC Injection	2 years (every other week)	0.3, 1, 3	Yes		REST060229 (Section 2.6.6.5.1)

GLP = Good Laboratory Practice; NA = not applicable; SC = subcutaneous.

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies

Test Article: Exenatide Once Weekly

Type of Study	Test System Species / Strain	Method of Administration	Exenatide Doses (mg/kg)	GLP Compliance	Study Reference (Location)
8-week toxicology	Rat / Sprague-Dawley	SC Injection	0.3, 3 ,9	Yes	REST060307 (Section 2.6.6.3.2.1)
8-week toxicology	Rat / Sprague-Dawley	SC Injection	0.3, 3 ,9	Yes	REST080043 (Section 2.6.6.3.2.2)
18-week toxicology	Rat / Sprague-Dawley	SC Injection	1, 3, 9	Yes	REST050369 (Section 2.6.6.3.2.3)
3-month toxicology	Monkey / Cynomolgus	SC Injection	0.11, 0.44, 1.1	Yes	REST04289R2 (Section 2.6.6.3.3.1)
9-month toxicology	Monkey / Cynomolgus	SC Injection	0.11, 0.42, 1.1	Yes	REST050370 (Section 2.6.6.3.3.2)
Carcinogenicity	Rat / Sprague-Dawley	SC Injection	0.3, 1, 3	Yes	REST060229 (Section 2.6.6.5.1)

GLP = Good Laboratory Practice; SC = subcutaneous.

2.6.7.3 Toxicokinetics: Overview of Toxicokinetics Data

Test Article: Exenatide Once Weekly

All Animals						
Exenatide Dose (mg/kg)	**AUC_{0-t} (pg·hr/mL)**			**C_{max} (pg/mL)**		
	Rats (M)	**Rats (F)**	**Monkeys (M+F)**	**Rats (M)**	**Rats (F)**	**Monkeys (M+F)**
0.11 [1]	NA	NA	7,442 114,397	NA	NA	334 2,895
0.11 [2]	NA	NA	5,929 66,271 105,443	NA	NA	111 1,452 4,418
0.3 [3]	46,253 503,204	61,537 273,401	NA	373 8,960	503 2,925	NA
0.3 [4]	NC 237,771	28,552 219,816	NA	1,123 4,051	756 3,033	NA
0.3 [5]	94,215 201,583	51,137 140,308	NA	1,298 3,020	1,313 2,775	NA
0.42 [2]	NA	NA	40,474 309,485 351,208	NA	NA	785 5,005 17,345
0.44 [1]	NA	NA	30,483 283,977	NA	NA	1,208 5,434
1.0 [6]	154,068 2,972,064	112,684 1,048,608	NA	2,760 58,763	1,823 12,823	NA
1.0 [5]	204,885 1,558,936	114,093 1,685,831	NA	2,997 28,117	1,678 18,607	NA
1.1 [1]	NA	NA	59,582 973,761	NA	NA	2,915 19,939
1.1 [2]	NA	NA	35,412 469,662 545,645	NA	NA	1270 31,067 40,733
3.0 [3]	332,122 4,829,039	324,866 1,286,706	NA	1,748 29,334	1,980 8,900	NA
3.0 [6]	315,772 1,086,097	233,005 2, 903,585	NA	3,460 7,003	2,243 33,067	NA
3.0 [4]	138,386 867,515	163,347 4,667,017	NA	2,817 35,270	3,232 48,331	NA
3.0 [5]	385,851 2,455,698	485,491 8,198,256	NA	4,392 10,662	4,334 37,130	NA

9.0 [3]	734,273 3,784,680	626,024 5,855,867	NA	4,925 25,380	3,470 65,480	NA
9.0 [6]	425,077 2,854,200	401,290 2,095,103	NA	5,867 20,433	7,967 19,233	NA
9.0 [4]	307,054 5,536,820	378,205 4,073,066	NA	5,191 28,892	9,269 45,299	NA
Antibody Negative Animals						
Exenatide Dose (mg/kg)	**AUC_{0-t} (pg·hr/mL)**			**C_{max} (pg/mL)**		
	Rats (M)	**Rats (F)**	**Monkeys (M+F)**	**Rats (M)**	**Rats (F)**	**Monkeys (M+F)**
0.11 [1]	NA	NA	7,442 131,407	NA	NA	334 3,372
0.11 [2]	NA	NA	7,856 77,954 227,674	NA	NA	134 1,529 9,099
0.3 [3]	46,253 300,372	61,537 293,196	NA	373 3,067	503 3,333	NA
0.3 [4]	NC 190,181	28,552 226,749	NA	1,123 3,414	756 3,394	NA
0.3 [5]	94,215 158,204	51,137 107,319	NA	1,298 2,682	1,313 2,113	NA
0.42 [2]	NA	NA	27,875 656,628 1,115,646	NA	NA	683 8,091 20,873
0.44 [1]	NA	NA	30,483 571,475	NA	NA	1,208 6,300
1.0 [6]	154,068 214,490	112,684 363,660	NA	2,760 1,730	1,823 2,880	NA
1.0 [5]	204,885 783,272	114,093 641,052	NA	2,997 4,497	1,678 4,301	NA
1.1 [1]	NA	NA	59,582 733,436	NA	NA	2,915 17,433
1.1 [2]	NA	NA	34,788 537,064 1,073,952	NA	NA	1,276 18,100 36,538
3.0 [3]	332,122 1,180,659	324,866 912,291	NA	1,748 14,467	1,980 8,433	NA
3.0 [6]	315,772 787,858	233,005 NC	NA	3,460 4,455	2,243 NC	NA

3.0 [4]	138,386 517,639	163,347 507,193	NA	2,817 36,578	3,232 31,798	NA
3.0 [5]	385,851 2,024,703	485,491 1,910,576	NA	4,392 10,586	4,334 10,574	NA
9.0 [3]	734,273 2,742,556	626,024 2,640,973	NA	4,925 19,575	3,470 15,850	NA
9.0 [6]	425,077 1,948,442	401,290 2,165,137	NA	5,867 11,967	7,967 21,600	NA
9.0 [4]	307,054 1,128,702	378,205 1,807,528	NA	5,191 14,845	9,269 24,300	NA

AUC_{0-t} = area under the plasma concentration versus time curve from time zero to the time of the last measurable concentration; C_{max} = maximum plasma concentration; NA = not available; NC = not calculable;

[1] The first value represents measurements taken on Week 1, and the second value represents measurements taken on Week 13; AUC values given represent AUC_{0-168h} (REST04289R2).

[2] The first value represents measurements taken on Week 1, the second value represents measurements taken on Week13, and the third value represents measurements taken on Week 39; AUC values given represent AUC_{0-168h} (REST050370).

[3] The first value represents measurements taken on Week 1, and the second value represents measurements taken on Week 7; AUC values given represent AUC_{0-336h} (REST060307).

[4] The first value represents measurements taken on Week 1, and the second value represents measurements taken on Week 7; AUC values given represent AUC_{0-336h} (REST080043).

[5] The first value represents measurements taken on Week 1, and the second value represents measurements taken on Week 27; AUC values given represent AUC_{0-336h} (REST060229).

[6] The first value represents measurements taken on Week 1, and the second value represents measurements taken on Week 17; AUC values given represent AUC_{0-336h} (REST050369).

2.6.7.4 Toxicology: Drug Substance

	Impurity Level (%)				
Manufacturer	Lonza	Lonza	Lonza	(b) (4)	Mallinckrodt
					(b) (4)

DS = drug substance; ID = identity; RRT = retention time of impurity/degradation product relative to exenatide.

[1] (b) (4) therefore appears twice in this table.

[2] 8-week rat toxicology study (REST060307)

[3] In vitro chromosomal aberration (REST060306) and Ames (REST060302)

[4] 2-year rat carcinogenicity study (REST060229)

[5] 8-week rat toxicology study (REST080043)

[6] In vitro chromosomal aberration (REST080139) and Ames (REST080228)

[7] 28-day mouse toxicology study (REST04568)

[8] In vitro chromosomal aberration (REST04569) and Ames (REST04571)

[9] 4-month rat toxicology study (REST050369)

[10] 9-month monkey toxicology study (REST050370)

[11] 3-month monkey toxicology study (REST04289R2)

[12] The drug substance lots listed were used to manufacture drug product lots used in nonclinical studies, with the exception of lot SF326-4AC1 which was tested in drug substance qualification studies.

2.6.7.4.1 Toxicology: Drug Product

Manufacturer	Scale	DP Lot #	Impurity Level (ID or RRT) (%) (b) (4)	Nonclinical Studies (b) (4)
Amylin OH				
Alkermes				
Alkermes				
Alkermes				
Alkermes				
Alkermes				
Alkermes				
Alkermes				
Alkermes				
Alkermes				
Alkermes				
Alkermes				
Alkermes				
Alkermes				
Alkermes				
Alkermes				
Alkermes				

DP = drug product; ID = identity; RRT = relative retention time; Multiple = peak consists of multiple components with the main components indicated.

[1] 8-week rat toxicology study (REST080043)

[2] In vitro chromosomal aberration (REST080139) and bacterial reverse mutation (REST080228) studies

[3] 8-week rat toxicology study (REST060307)

[4] In vitro chromosomal aberration (REST060306) and Ames (REST060302) studies

[5] 2-year rat carcinogenicity study (REST060229)

[6] 4-month rat toxicology study (REST050369)

[7] 3-month monkey toxicology study (REST04289R2)

[8] 9-month monkey toxicology study (REST050370)

2.6.7.6 Repeat Dose Toxicology: Nonpivotal Studies

Test Article: Exenatide Once Weekly

Species (Strain)	Method of Administration (Control)	Duration of Dosing	Animals/ Group	Exenatide Doses (mg/kg)	NOAEL (mg/kg/dose)	Noteworthy Findings	Study Reference (Location in CTD) (GLP Status)
Mouse (CD-1)	SC Injection (Placebo)	28 days (twice daily)	10M, 10F	0.76	0.76 mg/kg/day	• Increased incidence of basophilic foci in the parotid salivary glands of males and females rats; • All animals were negative for anti-exenatide antibodies, except one animal with a titer of 5.	REST04568 (Section 2.6.6.3.1.1) (GLP)
Rat (Sprague-Dawley)	SC Injection (Diluent and Microsphere controls)	8 weeks (every other week)	15M, 15F	0.3, 3, 9	9	• Microsphere-related swelling of injection site; • Microsphere-related mild inflammatory reaction typical of foreign-body reaction; • All changes showed partial or complete reversibility; • Anti-exenatide antibody formation was evident at ≥ 0.3 mg/kg as early as Week 6.	REST060307 (Section 2.6.6.3.2.1) (GLP)
Rat (Sprague-Dawley)	SC Injection (Diluent and Microsphere controls)	8 weeks (every other week)	15M, 15F	0.3, 3, 9	9	• Microsphere-related swelling of injection site; • Microsphere-related mild inflammatory reaction typical of foreign-body reaction; • All changes showed partial or complete reversibility; • Anti-exenatide antibody formation was evident at ≥ 0.3 mg/kg as early as Week 6.	REST080043 (Section 2.6.6.3.2.2) (GLP)
Rat (Sprague-Dawley)	SC Injection (Diluent and Microsphere controls)	18 weeks (every other week)	15M, 15F	1, 3, 9	9	• Microsphere-related thickening of injection site; • Microsphere-related mild inflammatory reaction typical of foreign-body reaction; • Decreased body weight gain and food consumption at ≥ 3 mg/kg (expected pharmacological effects); • All changes showed partial or complete reversibility; • Anti-exenatide antibody formation was evident at ≥ 1 mg/kg as early as Day 57.	REST050369 (Section 2.6.6.3.2.3) (GLP)

Species (Strain)	Method of Administration (Control)	Duration of Dosing	Animals/ Group	Exenatide Doses (mg/kg)	NOAEL (mg/kg/dose)	Noteworthy Findings	Study Reference (Location in CTD) (GLP Status)
Monkey (Cynomolgus)	SC Injection (Diluent and Microsphere controls)	13 Weeks (QW)	4M, 4F	0.11, 0.44, 1.1	1.1	• Severe injection site reaction (swelling, inflammation with abscess) in 1/8 animals at 1.1 mg/kg; • Microsphere-related enlargement of injection site; • Challenge dose of control microsphere 4 weeks into recovery did not elicit any local response; • All changes showed partial or complete reversibility; • Anti-exenatide antibody formation was evident as early as Day 29 at ≥ 0.44 mg/kg; most animals were still antibody positive following a 13 weeks recovery period.	REST04289R2 (Section 2.6.6.3.3.1) (GLP)

CTD = Common Technical Document; F = female; GLP = Good Laboratory Practice; M = male; NOAEL = no-observable-adverse-effect-level; QW = once weekly; SC = subcutaneous.

2.6.7.7 Repeat Dose Toxicology: Pivotal Studies

Test Article: Exenatide Once Weekly

Report Title: Exenatide LAR: 39-Week (One Injection/Week) Subcutaneous Injection Toxicity and Toxicokinetic Study in Cynomolgus Monkeys with a 3-Month Recovery		
Species/Strain: Monkey/Cynomolgus	**Duration of Dosing:** 39 weeks	**Study Reference:** REST050370
Initial Age: 2-4 years old	**Duration of Postdose:** 12 weeks recovery group	**Location in CTD:** Section 2.6.6.3.3.2
Date of First Dose: 13 December 2005	**Method of Administration:** SC injection (QW)	**GLP Compliance:** Yes
Special Features: None	**Vehicle/Formulation:** Microsphere Diluent/Control Microspheres	
No Observed Adverse Effect Level: 1.1 mg/kg		

Toxicokinetic Phase					
Weekly Exenatide Dose (mg/kg)	**Vehicle Control**	**Microsphere Control**	**0.11**	**0.42**	**1.1**
Sex	**Male + Female**	**Male + Female**	**Male + Female**	**Male + Female**	**Male + Female**
Toxicokinetics – Antibody Negative Animals					
Number of Animals			5	2	3
$AUC_{0\text{-}168}$ (pg-h/mL)					
Day 1	–	–	7856	27,875	34,788
Day 85	–	–	77,954	656,628	537,064
Day 267	–	–	227,674	1,115,646	1,073,952
C_{max} (pg/mL)					
Day 1	–	–	134	683	1276
Day 85	–	–	1529	8091	18,100
Day 267	–	–	9099	20,873	36,538
Toxicokinetics – All Animals					
Number of Animals			9-12	9-12	8-12
$AUC_{0\text{-}168}$ (pg-h/mL)					
Day 1	–	–	5929	40,474	35,412
Day 85	–	–	66,271	309,485	469,662
Day 267	–	–	105,443	351,208	545,645
C_{max} (pg/mL)					
Day 1	–	–	111	785	1270
Day 85	–	–	1452	5005	31,067
Day 267	–	–	4418	17,345	40,733

Weekly Dose (mg/kg)	Vehicle Control		Microsphere Control		0.11		0.42		1.1	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Toxicity Phase										
Number of Animals	6	6	6	6	6	6	6	6	6	6
Noteworthy Findings										
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0	0	0
Clinical Observations	–	–	–	–	–	–	–	–	–	–
Body Weight	–	–	–	–	–	–	–	–	–	–
Food Consumption	–	–	–	–	–	–	–	–	–	–
Ophthalmology	–	–	–	–	–	–	–	–	–	–
Hematology	–	–	–	–	–	–	–	–	–	–
Clinical Chemistry	–	–	–	–	–	–	–	–	–	–
Coagulation	–	–	–	–	–	–	–	–	–	–
Urinalysis	–	–	–	–	–	–	–	–	–	–
Number of Animals Assessed	6	6	6	6	6	6	6	6	6	6
Injection Sites Observations										
Erythema [incidence (occurrence)]										
Very Slight	3/6 (4)	-	3/6 (9)	2/6 (2)	4/6 (6)-	3/6 (35)	3/6 (23)	5/6 (66)	5/6 (141)	5/6 (44)
Slight	5/6 (8)	2/6 (2)	5/6 (9)	3/6 (5)	5/6 (11)	2/6 (25)	2/6 (16)	4/6 (42)	6/6 (106)	4/6 (48)
Moderate	-	-	-	2/6 (2)	1/6 (1)	1/6 (6)	2/6 (6)	3/6 (9)	4/6 (51)	1/6 (20)
Severe	-	-	-	-	-	-	-	-	1/6 (1)	1/6 (4)
Edema [incidence (occurrence)]										
Very Slight	1/6 (3)	2/6 (4)	6/6 (1047)	6/6 (1094)	6/6 (442)	6/6 (263)	6/6 (1160)	6/6 (887)	6/6 (732)	6/6 (1087)
Slight	2/6 (2)	2/6 (3)	6/6 (1100)	6/6 (1078)	6/6 (283)	6/6 (115)	6/6 (919)	6/6 (687)	6/6 (794)	6/6 (1100)
Moderate	-	-	6/6 (856)	6/6 (405)	3/6 (138)	2/6 (95)	6/6 (325)	6/6 (422)	6/6 (720)	6/6 (622)
Severe	-	-	6/6 (179)	6/6 (52)	2/6 (31)	1/6 (46)	6/6 (64)	3/6 (132)	6/6 (377)	6/6 (112)
Open Drainage	-	-	-	-	1/6 (1)	1/6 (5)	-	2/6 (16)	5/6 (44)	1/6 (25)
Number of Animals Assessed	4	4	4	4	4	4	4	4	4	4
Organ Weights	–	–	–	–	–	–	–	–	–	–
Gross Pathology										
Injection Sites (incidence)										
Thickening	–	–	2/4	2/4	1/4	–	1/4	2/4	2/4	4/4
Histopathology (incidence)										
Injection Sites										
Granulomas										
Minimal									1/4	1/4
Slight			2/4	1/4	4/4	4/4	2/4	3/4	1/4	1/4
Moderate			2/4	3/4			2/4	1/4	2/4	2/4
Fibrosis										

Weekly Dose (mg/kg)	Vehicle Control		Microsphere Control		0.11		0.42		1.1	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Minimal	1/4			1/4			1/4			
Slight						1/4	1/4	2/4	1/4	1/4
Moderate									1/4	
Foamy macrophages/fibroblasts										
Minimal			3/4	2/4	2/4	3/4		3/4	2/4	3/4
Slight			1/4	1/4	1/4		1/4		1/4	1/4
Moderate							1/4			
Antibody to Exenatide										
Incidence of Positive	0/4	0/4	1/4	0/4	1/4	3/4	3/4	3/4	3/4	3/4
Range of Titers	–	–	25	–	625	625 – 78,125	625 – 15,625	625 – 78,125	625 – 78,125	625 – 15,625
12-Week Recovery Results										
Number of Animals	2	2	2	2	2	2	2	2	2	2
Noteworthy Findings										
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0	0	0
Clinical Observations	–	–	–	–	–	–	–	–	–	–
Body Weight	–	–	–	–	–	–	–	–	–	–
Food Consumption	–	–	–	–	–	–	–	–	–	–
Ophthalmology	–	–	–	–	–	–	–	–	–	–
Hematology	–	–	–	–	–	–	–	–	–	–
Clinical Chemistry	–	–	–	–	–	–	–	–	–	–
Coagulation	–	–	–	–	–	–	–	–	–	–
Urinalysis	–	–	–	–	–	–	–	–	–	–
Number of Animals Assessed	2	2	2	2	2	2	2	2	2	2
Injection Sites Observations										
Erythema [incidence (occurrence)]										
Very Slight	-	-	-	-	-	-	-	-	1/2 (1)	-
Slight	-	-	-	-	-	-	-	1/2 (1)	-	-
Moderate	-	-	-	-	-	-	-	-	-	-
Severe	-	-	-	-	-	-	-	-	-	-
Edema [incidence (occurrence)]										
Very Slight	-	-	2/2 (14)	2/2 (16)	2/2 (10)	-	2/2 (15)	2/2 (23)	2/2 (10)	2/2 (20)
Slight	-	-	2/2 (12)	2/2 (21)	2/2 (23)	-	2/2 (9)	2/2 (13)	2/2 (19)	2/2 (15)
Moderate	-	-	2/2 (24)	2/2 (22)	2/2 (3)	-	2/2 (7)	1/2 (8)	1/2 (1)	2/2 (10)
Severe	-	-	2/2 (10)	-	1/2 (1)	-	-	1/2 (1)	2/2 (3)	2/2 (8)
Open Drainage	-	-	2/2 (14)	2/2 (16)	2/2 (10)	-	2/2 (15)	2/2 (23)	2/2 (10)	2/2 (20)

Weekly Dose (mg/kg)	Vehicle Control		Microsphere Control		0.11		0.42		1.1	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Number of Animals Assessed	2	2	2	2	2	2	2	2	2	2
Organ Weights	–	–	–	–	–	–	–	–	–	–
Gross Pathology	–	–	–	–	–	–	–	–	–	–
Histopathology (incidence)										
Injection Sites										
Granulomas										
Minimal	–	1/2	–	–	–	–	–	1/2	–	–
Slight	–	–	–	–	–	–	–	–	–	–
Moderate	–	–	–	–	–	–	–	–	–	–
Fibrosis										
Minimal	–	–	–	–	–	–	–	–	–	–
Slight	–	–	–	–	–	–	–	–	–	–
Moderate	–	–	–	–	–	–	–	–	–	–
Foamy macrophages/fibroblasts										
Minimal	–	–	2/2	2/2	2/2	–	2/2	2/2	2/2	2/2
Slight	–	–	–	–	–	–	–	–	–	–
Moderate	–	–	–	–	–	–	–	–	–	–
Antibody to Exenatide										
Incidence of Positive	0	1/2	0	1/1	2/2	1/2	1/2	2/2	2/2	0/2
Range of Titers	–	25	–	25	625 – 3,125	25	125	625 – 3,125	625	–

– = no noteworthy findings; $AUC_{0\text{-}168}$ = area under the serum concentration versus time curve from time zero to 168 hours post-dose; C_{max} = maximum serum concentration; CTD = common technical document; GLP = good laboratory practice; NA = not applicable/not available.

2.6.7.8 Genotoxicity: In Vitro

Test Article: Exenatide

Report Title: AC2993 (b) (4) Salmonella-Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay Treat and Plate Method		
Test for Induction of: Bacterial reverse mutation	**Number of Independent Assays:** 2	**Study Reference:** REST04571
Strains: *S. typhimurium* and *E. coli*	**Number of Replicate Cultures:** 3	**Location in CTD:** Section 2.6.6.4.1
Metabolizing System: Aroclor 1254-induced rat liver S9, 10% v/v	**Number of Cells Analyzed/Culture:** Total colonies/plate	**GLP Compliance:** Yes
Vehicle for Test Article: Deionized water	**Vehicle for Positive Controls:** Deionized water	**Date of Treatment:** 10 February 2005
Treatment: 60-minute pre-incubation + 52-hour treatment		**Additional Information:** None
Cytotoxic Effects: None		
Genotoxic Effects: None		

Assay #1

Metabolic Activation	Test Article	Dose Level (µg/plate)	Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2 *uvrA*
Without S9 Activation	Deionized water	0	9 ± 3	88 ± 12	9 ± 1	6 ± 4	109 ± 10
	Exenatide	33.3	9 ± 4	83 ± 15	12 ± 5	8 ± 1	105 ± 15
		100	10 ± 4	83 ± 8	11 ± 4	6 ± 1	129 ± 13
		333	8 ± 1	84 ± 3	8 ± 1	6 ± 2	70 ± 2
		1000	12 ± 3	80 ± 19	10 ± 4	7 ± 2	88 ± 7
		2000	12 ± 6	78 ± 4	10 ± 3	8 ± 2	83 ± 4
		5000	14 ± 4	80 ± 6	12 ± 3	9 ± 2	96 ± 16
	2-Nitrofluorene	15.4 µg/mL	623 ± 4	NA	NA	NA	NA
	N-methyl-N-nitro-N-nitrosoguanidine	1.54µg/mL	NA	827 ± 34	1034 ± 14	NA	NA
	ICR-191	3.08 µg/mL	NA	NA	NA	2832 ± 50	NA
	4-Nitroquinoline-N-oxide	3.08 µg/mL	NA	NA	NA	NA	1554 ± 79
With S9 Activation	Deionized water	0	11 ± 5	74 ± 7	9 ± 4	5 ± 1	122 ± 17
	Exenatide	33.3	13 ± 3	72 ± 5	8 ± 2	2 ± 1	123 ± 9
		100	13 ± 1	64 ± 4	9 ± 4	2 ± 2	121 ± 18
		333	12 ± 3	73 ± 11	12 ± 3	7 ± 1	113 ± 32
		1000	9 ± 2	91 ± 5	10 ± 1	5 ± 1	101 ± 11
		2000	12 ± 3	90 ± 6	14 ± 1	3 ± 1	98 ± 8
		5000	13 ± 3	87 ± 3	14 ± 6	6 ± 2	118 ± 9
	Benzo[a]pyrene	15.4 µg/mL	72 ± 14	NA	NA	NA	NA
	2-Aminoanthracene	15.4 µg/mL	NA	1421 ± 102	103 ± 16	117 ± 25	NA
		38.5 µg/mL	NA	NA	NA	NA	412 ± 24

Assay #2							
Metabolic Activation	**Test Article**	**Dose Level (µg/plate)**	**Revertant Colony Counts (Mean ± SD)**				
			TA98	**TA100**	**TA1535**	**TA1537**	**WP2 *uvr*A**
Without S9 Activation	Deionized water	0	14 ± 2	90 ± 14	13 ± 3	7 ± 5	98 ± 17
	Exenatide	33.3	9 ± 1	92 ± 8	15 ± 7	8 ± 2	117 ± 39
		100	13 ± 1	102 ± 22	8 ± 7	7 ± 1	87 ± 11
		333	10 ± 1	95 ± 4	11 ± 3	8 ± 5	106 ± 15
		1000	11 ± 2	97 ± 8	9 ± 3	5 ± 2	88 ± 11
		2000	11 ± 3	91 ± 18	9 ± 2	4 ± 2	74 ± 3
		5000	10 ± 3	94 ± 26	11 ± 5	6 ± 1	82 ± 12
	2-Nitrofluorene	15.4 µg/mL	954 ± 49	NA	NA	NA	NA
	N-methyl-N-nitro-N-nitrosoguanidine	1.54µg/mL	NA	354 ± 52	1124 ± 54	NA	NA
	ICR-191	3.08 µg/mL	NA	NA	NA	3272 ± 111	NA
	4-Nitroquinoline-N-oxide	3.08 µg/mL	NA	NA	NA	NA	1514 ± 261
With S9 Activation	Deionized water	0	12 ± 7	89 ± 14	9 ± 2	8 ± 2	143 ± 6
	Exenatide	33.3	17 ± 3	86 ± 8	8 ± 3	5 ± 2	144 ± 3
		100	17 ± 8	101 ± 5	7 ± 2	3 ± 2	174 ± 16
		333	11 ± 6	97 ± 14	10 ± 1	7 ± 3	175 ± 13
		1000	19 ± 6	100 ± 11	11 ± 7	5 ± 4	170 ± 34
		2000	19 ± 5	104 ± 6	8 ± 2	4 ± 3	140 ± 2
		5000	15 ± 3	95 ± 9	7 ± 5	5 ± 2	148 ± 28
	Benzo[a]pyrene	15.4 µg/mL	93 ± 20	NA	NA	NA	NA
	2-Aminoanthracene	15.4 µg/mL	NA	1103 ± 247	88 ± 4	120 ± 19	NA
		38.5 µg/mL	NA	NA	NA	NA	673 ± 60

CTD = common technical document; DMSO = dimethylsulfoxide; GLP = good laboratory practice; NA = not applicable.

2.6.7.8 Genotoxicity: In Vitro

Test Article: Exenatide Once Weekly

Report Title: Bacterial Reverse Mutation Assay Using the Treat and Plate Method		
Test for Induction of: Bacterial reverse mutation	**Number of Independent Assays:** 2	**Study Reference:** REST060302
Strains: *S. typhimurium* and *E. coli*	**Number of Replicate Cultures:** 2 (initial test), 3 (confirmatory test)	**Location in CTD:** Section 2.6.6.4.2
Metabolizing System: Aroclor 1254-induced rat liver S9, 10% v/v	**Number of Cells Analyzed/Culture:** Total colonies/plate	**GLP Compliance:** Yes
Vehicle for Test Article: DMSO	**Vehicle for Positive Controls:** water; DMSO	**Date of Treatment:** 10 October 2006
Treatment: 60-minute pre-incubation + 48-72-hour treatment (Assay #1); 48-72-hour treatment (Assay #2)		**Additional Information:** Treat and plate method was used, except for the positive control with E. coli in the presence of S9 activation (plate incorporation method)
Cytotoxic Effects: None		
Genotoxic Effects: None		

Metabolic Activation	Test Article	Dose Level (µg/plate)	Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2 *uvr*A
Assay #1 (Range-Finding)							
Without S9 Activation	DMSO	0	19 ± 3	92 ± 6	11 ± 1	7 ± 1	27 ± 9
	Exenatide Once Weekly	0.05	16 ± 6	102 ± 16	13 ± 2	5 ± 2	28 ± 0
		0.15	17 ± 1	89 ± 4	10 ± 1	7 ± 1	29 ± 4
		0.5	13 ± 6	91 ± 0	12 ± 4	8 ± 5	27 ± 4
		1.5	6 ± 3	84 ± 15	13 ± 3	8 ± 3	19 ± 5
		5	14 ± 2	86 ± 16	14 ± 1	5 ± 0	22 ± 1
		15	14 ±1	76 ± 11	12 ± 7	5 ± 1	26 ± 1
		50	12 ± 3	91 ± 8	15 ± 4	6 ± 1	22 ± 6
		150	16 ± 4	78 ± 2	14 ± --	8 ± 2	25 ± 4
	2-Nitrofluorene	15 µg/mL	238 ± 79	NA	NA	NA	NA
	N-methyl-N-nitro-N-nitrosoguanidine	2 µg/mL	NA	975 ± 35	925 ± 31	NA	NA
		5 µg/mL	NA	NA	NA	NA	199 ± 31
	ICR-191	3 µg/mL	NA	NA	NA	1191 ± 14	NA

Metabolic Activation	Test Article	Dose Level (µg/plate)	Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2 *uvrA*
With S9 Activation	DMSO	0	14 ± 6	85 ± 2	16 ± 3	8 ± 4	28 ± 4
	Exenatide Once Weekly	0.05	18 ± 1	86 ± 1	12 ± 2	8 ± 1	30 ± 1
		0.15	18 ± --	87 ± 9	16 ± 6	6 ± 3	25 ± 2
		0.5	16 ± 1	74 ± 13	17 ± 0	6 ± 3	18 ± 1
		1.5	14 ± 3	85 ± 2	11 ± 3	6 ± 3	29 ± 1
		5	13 ± 3	89 ± 2	11 ± 1	7 ± 0	25 ± 6
		15	15 ± 4	88 ± 5	16 ± 4	8 ± 5	27 ± 7
		50	12 ± --	94 ± 8	13 ± 5	12 ± 0	26 ± 1
		150	13 ± 5	89 ± 3	16 ± 2	8 ± 2	25 ± 8
	2-Aminoanthracene	15 µg/mL	650 ± 178	906 ± 6	NA	NA	NA
		50 µg/mL	NA	NA	79 ± 6	156 ± 2	NA
		10	NA	NA	NA	NA	89 ± 6
Assay #2							
Without S9 Activation	DMSO	0	11 ± 3	104 ± 16	8 ± 2	4 ± 2	19 ± 4
	Exenatide Once Weekly	15	14 ± 5	98 ± 13	12 ± 2	4 ± 2	15 ± 10
		50	16 ± 4	89 ± 2	13 ± 3	6 ± 1	25 ± 5
		150	10 ± 2	72 ± 5	10 ± 3	6 ± 3	19 ± 4
		500	10 ± 1	109 ± 6	11 ± 2	5 ± 2	16 ± 3
		1500	11 ± 4	88 ± --	8 ± 1	7 ± 1	-- ± --
		5000	8 ± 1	112 ± 21	5 ± 3	5 ± 2	17 ± 4
	2-Nitrofluorene	15 µg/mL	248 ± 23	NA	NA	NA	NA
	N-methyl-N-nitro-N-nitrosoguanidine	2 µg/mL	NA	594 ± 15	406 ± 74	NA	NA
		5 µg/mL	NA	NA	NA	NA	118 ± 12
	ICR-191	3 µg/mL	NA	NA	NA	1112 ± 124	NA
With S9 Activation	DMSO	0	12 ± 5	91 ± 9	11 ± 1	5 ± 1	17 ± 2
	Exenatide Once Weekly	15	15 ± 4	75 ± 3	10 ± 3	3 ± 2	18 ± 4
		50	13 ± 1	95 ± 17	10 ± 3	6 ± 2	20 ± 3
		150	12 ± 5	83 ± 3	9 ± 1	7 ± 3	22 ± 1
		500	14 ± 8	96 ± 12	12 ± 3	5 ± 1	14 ± 6
		1500	13 ± 3	91 ± 11	12 ± 5	4 ± 2	18 ± 4
		5000	10 ± 2	74 ± 5	11 ± 7	5 ± 2	22 ± 9
	2-Aminoanthracene	15 µg/mL	538 ± 21	623 ± 35	NA	NA	NA
		50 µg/mL	NA	NA	88 ± 11	87 ± 3	NA
		10	NA	NA	NA	NA	71 ± 35

CTD = common technical document; DMSO = dimethylsulfoxide; GLP = good laboratory practice; NA = not applicable.

2.6.7.8 Genotoxicity: In Vitro

Test Article: Exenatide Once Weekly

Report Title: Bacterial Reverse Mutation Assay		
Test for Induction of: Bacterial reverse mutation	**Number of Independent Assays:** 2	**Study Reference:** REST080228
Strains: *S. typhimurium* and *E. coli*	**Number of Replicate Cultures:** 2 (initial test), 3 (confirmatory test)	**Location in CTD:** Section 2.6.6.4.3
Metabolizing System: Aroclor 1254-induced rat liver S9, 10% v/v	**Number of Cells Analyzed/Culture:** Total colonies/plate	**GLP Compliance:** Yes
Vehicle for Test Article: DMSO	**Vehicle for Positive Controls:** water; DMSO	**Date of Treatment:** 15 April 2008
Treatment: 60-minute pre-incubation + 48-72-hour treatment (Assay #1); 48-72-hour treatment (Assay #2)		**Additional Information:** Treat and plate method was used, except for the positive control with E. coli in the presence of S9 activation (plate incorporation method)
Cytotoxic Effects: None		
Genotoxic Effects: None		

Metabolic Activation	Test Article	Dose Level (µg/plate)	Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2 *uvr*A
Assay #1 (Range-Finding)							
Without S9 Activation	DMSO	0	12 ± 2	88 ± 11	6 ± 1	4 ± 1	12 ± 3
	Exenatide Once Weekly	0.75	11 ± 1	86 ± 9	4 ± 1	3 ± 1	14 ± 1
		2.5	9 ± 1	93 ± 12	6 ± 1	3 ± 1	11 ± 1
		7.5	8 ± 2	102 ± 7	8 ± 2	0 ± 0	11 ± 1
		25	9 ± 0	81 ± 8	8 ± 1	1 ± 0	13 ± 1
		75	12 ± 3	92 ± 6	6 ± 1	5 ± 1	11 ± 1
		250	12 ± 1	78 ± 11	7 ± 3	3 ± 1	12 ± 2
		750	8 ± 1	90 ± 11	7 ± 1	4 ± 2	12 ± 3
		2500	8 ± 2	84 ± 4	3 ± 1	5 ± 1	14 ± 1
	2-Nitrofluorene	15 µg/mL	222 ± 19	NA	NA	NA	NA
	N-methyl-N-nitro-N-nitrosoguanidine	2 µg/mL	NA	330 ± 159	1397 ± 64	NA	NA
		5 µg/mL	NA	NA	NA	NA	281 ± 6
	ICR-191	3 µg/mL	NA	NA	NA	2371 ± 107	NA

Metabolic Activation	Test Article	Dose Level (µg/plate)	Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2 *uvr*A
With S9 Activation	DMSO	0	12 ± 0	88 ± 11	9 ± 1	7 ± 1	16 ± 3
	Exenatide Once Weekly	0.75	11 ± 1	81 ± 6	8 ± 1	4 ± 1	12 ± 1
		2.5	11 ± 4	102 ±24	11 ±1	4 ± 1	16 ± 0
		7.5	9 ± 0	93 ± 13	10 ± 1	7 ± 1	14 ± 1
		25	10 ±0	93 ± 9	6 ± 2	4 ± 0	14 ± 4
		75	10 ± 1	83 ± 5	11 ±3	4 ± 2	13 ± 2
		250	11 ± 1	81 ± 13	11 ± 0	4 ± 0	18 ± 1
		750	9 ± 1	82 ± 8	8 ± 1	5 ± 1	14 ± 6
		2500	10 ± 1	79 ± 4	8 ± 0	5 ± 1	11 ± 3
	2-Aminoanthracene	15 µg/mL	463 ± ---	740 ± 50	NA	NA	NA
		50 µg/mL	NA	NA	162 ± 81	88 ± 21	NA
		10	NA	NA	NA	NA	263 ± 25
Assay #2							
Without S9 Activation	DMSO	0	10 ± 3	92 ± 4	8 ± 2	8 ± 2	13 ± 1
	Exenatide Once Weekly	25	11 ± 2	115 ±3	7 ± 2	4 ± 1	13 ± 1
		75	11 ± 2	108 ± 3	7 ± 2	6 ± 2	13 ± 2
		250	9 ± 1	100 ± 13	6 ± 1	5 ± 1	13 ± 3
		750	13 ±1	95 ± 11	7 ± 1	4 ± 3	13 ± 1
		2500	12 ± 2	97 ± 24	7 ± 3	7 ± 2	13 ± 4
	2-Nitrofluorene	15 µg/mL	1472 ± 1706	NA	NA	NA	NA
	N-methyl-N-nitro-N-nitrosoguanidine	2 µg/mL	NA	954 ± 60	1146 ± 759	NA	NA
		5 µg/mL	NA	NA	NA	NA	282 ± 67
	ICR-191	3 µg/mL	NA	NA	NA	1520 ± 78	NA
With S9 Activation	DMSO	0	13 ± 3	93 ± 4	7 ± 3	4 ± 1	12 ± 1
	Exenatide Once Weekly	25	10 ± 2	93 ± 10	6 ± 2	5 ± 1	12 ± 1
		75	13 ± 1	90 ± 8	8 ± 2	4 ± 2	12 ± 1
		250	11 ± 2	86 ± 8	8 ± 1	4 ± 2	15 ± 3
		750	10 ± 1	95 ± 22	8 ± 1	4 ± 1	14 ± 1
		2500	13 ± 1	85 ± 13	8 ± 2	6 ± 1	13 ± 1
	2-Aminoanthracene	15 µg/mL	586 ± 64	699 ± 29	NA	NA	NA
		50 µg/mL	NA	NA	202 ± 6	92 ± 20	NA
		10	NA	NA	NA	NA	490 ± 216

CTD = common technical document; DMSO = dimethylsulfoxide; GLP = good laboratory practice; NA = not applicable.

2.6.7.8 Genotoxicity: In Vitro

Test Article: Exenatide

Report Title: AC2993 ((b) (4) Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells		
Test for Induction of: Chromosome aberrations	**Number of Independent Assays:** 2	**Study Reference:** REST04569
Strains: Chinese hamster ovary cells (CHO-WBL)	**Number of Replicate Cultures:** 2	**Location in CTD:** Section 2.6.6.4.4
Metabolizing System: Aroclor 1254-induced rat liver S9, 1.5% v/v	**Number of Cells Analyzed/Culture:** 1000 cells for mitotic index, 100 metaphases for structural and numerical aberration analysis	**GLP Compliance:** Yes
Vehicle for Test Article: Water	**Vehicle for Positive Controls:** DMSO	**Date of Treatment:** 24 January 2005
Treatment: 3-hour treatment with 20-hour recovery in the presence and absence of S9; and 20-hour treatment in the absence of S9 (confirmatory assay only).	**Additional Information:** None	
Cytotoxic Effects: None		
Genotoxic Effects: None		

Initial Chromosome Aberration Assay

Metabolic Activation	Treatment Time	Test Article	Concentration (µg/mL)	Mitotic Index Reduction (%)	Average Number of Cells		# Cells With Structural Aberrations (%)	
					Polyploidy	Endoreplication	Without Gaps	With gaps
Without S9 Activation	3 hour	Culture medium	n/a	---	2.0	0.0	0 (0.0)	2 (1.0)
		Water	n/a	0	2.0	0.0	3 (1.5)	7 (3.5)
		Exenatide	625	ND	2.5	0.0	2 (1.0)	3 (1.5)
			1250	ND	2.5	0.5	2 (1.0)	5 (2.5)
			2500	ND	0.0	0.0	0 (0.0)	0 (0.0)
			5000	0	1.5	1.0	1 (0.5)	1 (0.5)
		Mitomycin C	0.75	ND	4.0	0.0	70 (70.0)**	72 (72.0)
With S9 Activation	3 hour	Culture medium	n/a	---	3.0	2.0	3 (1.5)	6 (3.0)
		Water	n/a	0	2.0	0.5	3 (1.5)	8 (4.0)
		Exenatide	625	ND	2.5	0.5	2 (1.0)	6 (3.0)
			1250	0	1.5	1.0	1 (0.5)	5 (2.5)
			2500	0	2.0	0.0	3 (1.5)	9 (4.5)
			5000	20	1.0	2.0	1 (0.5)	9 (4.5)
		Cyclophosphamide	7.5	ND	5.5	0.0	41 (41.0)**	45 (45.0)

Confirmatory Chromosome Aberration Assay								
Metabolic Activation	Treatment Time	Test Article	Concentration (µg/mL)	Mitotic Index Reduction (%)	Average Number of Cells		# Cells With Structural Aberrations (%)	
					Polyploidy	Endoreplication	Without Gaps	With gaps
Without S9 Activation	20 hour	Culture medium	n/a	---	2.0	0.5	3 (1.5)	4 (2.0)
		Water	n/a	0	2.5	0.0	1 (0.5)	5 (2.5)
		Exenatide	1880	0	2.5	0.0	4 (2.0)	5 (2.5)
			2500	16	5.0	0.0	3 (1.5)	8 (4.0)
			3750	15	3.0	0.5	2 (1.0)	4 (2.0)
			5000	17	2.0	0.0	2 (1.0)	6 (3.0)
		Mitomycin C	0.400	ND	2.5	0.5	60 (60.0)**	62 (62.0)
With S9 Activation	3 hour	Culture medium	n/a	---	1.5	0.0	3 (1.5)	8 (4.0)
		Water	n/a	0	2.5	0.5	1 (0.5)	9 (4.5)
		Exenatide	1250	ND	3.0	0.0	1 (0.5)	4 (2.0)
			2500	ND	1.0	1.0	3 (1.5)	10 (5.0)
			3750	ND	5.0	0.0	2 (1.0)	10 (5.0)
			5000	0	3.5	0.5	1 (0.5)	6 (3.0)
		Cyclophosphamide	12.5	ND	3.5	0.0	59 (59.0)**	61 (61.0)

CTD = common technical document; DMSO = dimethylsulfoxide; GLP = good laboratory practice; ND = not determined; v/v = volume per volume.
** Statistically significant when compared to the solvent control ($p \leq 0.01$; Fisher's Exact Test)

2.6.7.8 Genotoxicity: In Vitro

Test Article: Exenatide Once Weekly

Report Title: In vitro chromosomal aberration test		
Test for Induction of: Chromosome aberrations	**Number of Independent Assays:** 2	**Study Reference:** REST060306
Strains: Chinese hamster ovary cells (CHO-K_1)	**Number of Replicate Cultures:** 2	**Location in CTD:** Section 2.6.6.4.5
Metabolizing System: Aroclor 1254-induced rat liver S9, 2% v/v	**Number of Cells Analyzed/Culture:** 500 cells for mitotic index, 200 metaphases for structural and numerical aberration analysis	**GLP Compliance:** Yes
Vehicle for Test Article: DMSO	**Vehicle for Positive Controls:** DMSO	**Date of Treatment:** 19 September 2006
Treatment: 4-hour treatment with 16-hour recovery in the presence and absence of S9; and 20-hour treatment in the absence of S9.	**Additional Information:** Based on cell growth inhibition, 3 dose levels were selected for metaphase analysis except for the non-activated 20-hr exposure group for which 4 dose levels were selected. The lowest concentration causing cell growth inhibition of at least 50% was used as the highest dose level for the metaphase analysis.	
Cytotoxic Effects: None		
Genotoxic Effects: None		

Preliminary Toxicity Test (Range Finding)

Metabolic Activation	Treatment Time	Test Article	Concentration (µg/mL)	Cell Count (x10^6)	Cell Viability (%)	Viable Cells/Flask (x10^6)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Without S9 Activation	4 hour	DMSO	n/a	2.07	99	2.05	100	n/a
		Exenatide Once Weekly	0.0066	1.98	99	1.96	96	4
			0.0198	1.96	100	1.96	96	4
			0.066	1.96	98	1.92	94	6
			0.198	1.88	99	1.86	91	9
			0.66	1.83	98	1.80	88	12
			1.98	1.92	100	1.92	94	6
			6.6	1.83	99	1.81	88	12
			19.8	1.74	98	1.70	83	17
			66	1.80	97	1.74	85	15
	20 hour	DMSO	n/a	1.99	99	1.97	100	n/a
		Exenatide Once Weekly	0.0066	1.96	100	1.96	99	1
			0.0198	1.96	100	1.96	99	0
			0.066	1.95	98	1.92	97	3
			0.198	1.88	100	1.88	96	4
			0.66	1.93	98	1.89	96	4
			1.98	1.90	99	1.88	96	4
			6.6	1.86	98	1.83	93	7
			19.8	1.79	97	1.73	88	12
			66	1.79	96	1.72	87	13

With S9 Activation	4 hour	DMSO	n/a	1.99	100	1.99	100	n/a
		Exenatide Once Weekly	0.0066	1.96	99	1.94	98	2
			0.0198	1.90	100	1.90	96	4
			0.066	1.96	99	1.94	98	2
			0.198	1.89	100	1.89	95	5
			0.66	1.85	98	1.81	91	9
			1.98	1.87	98	1.83	92	8
			6.6	1.84	99	1.82	92	8
			19.8	1.81	97	1.76	88	12
			66	1.98	97	1.92	97	3

Concurrent Toxicity Test

Metabolic Activation	Treatment Time	Test Article	Concentration (µg/mL)	Flask	Cell Count Averages ($x10^6$)	Cell Viability (%)	Mean Viable Cells/Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Without S9 Activation	4 hour	DMSO	n/a	A	1.96	99	–	–	–
				B	1.97	100	1.95	100	n/a
		Exenatide Once Weekly	6.25	A	1.96	99	–	–	–
				B	1.97	100	1.95	100	0
			12.5	A	1.82	98	–	–	–
				B	1.83	99	1.80	92	8
			25	A	1.85	97	–	–	–
				B	1.83	98	1.79	92	8
			50	A	1.77	98	–	–	–
				B	1.76	96	1.71	88	12
			75	A	1.02	95	–	–	–
				B	1.05	94	0.97	50	50
			100	A	0.50	94	–	–	–
				B	0.46	93	0.45	23	77
		Mitomycin C	0.1	A	1.98	99	–	–	–
				B	1.98	100	1.97	101	-1
			0.2	A	1.94	98	–	–	–
				B	1.95	99	1.92	98	2
	20 hour	DMSO	n/a	A	1.95	99	–	–	–
				B	1.96	98	1.93	100	n/a
		Exenatide Once Weekly	6.25	A	1.88	99	–	–	–
				B	1.87	100	1.86	97	3
			12.5	A	1.81	98	–	–	–
				B	1.83	99	1.79	93	7

			25	A	1.75	99	–	–	–
				B	1.76	98	1.73	90	10
			50	A	1.46	98	–	–	–
				B	1.46	97	1.42	74	26
			75	A	1.03	96	–	–	–
				B	1.05	97	1.01	52	48
			100	A	0.42	93	–	–	–
				B	0.45	93	0.41	21	79
		Mitomycin C	0.1	A	1.59	98	–	–	–
				B	1.61	99	1.58	82	18
			0.2	A	1.69	100	–	–	–
				B	1.62	99	1.65	86	14
With S9 Activation	4 hour	DMSO	n/a	A	1.93	99	–	–	–
				B	1.96	100	1.93	100	n/a
		Exenatide Once Weekly	6.25	A	1.87	98	–	–	–
				B	1.89	99	1.85	96	4
			12.5	A	1.83	99	–	–	–
				B	1.83	98	1.80	93	7
			25	A	1.76	98	–	–	–
				B	1.77	100	1.74	90	10
			50	A	1.76	97	–	–	–
				B	1.77	99	1.73	90	10
			75	A	0.99	96	–	–	–
				B	0.96	97	0.94	49	51
			100	A	0.49	94	–	–	–
				B	0.55	93	0.49	25	75
		Cyclophosphamide	10	A	1.42	98	–	–	–
				B	1.42	99	1.40	72	28
			20	A	1.62	98	–	–	–
				B	1.65	99	1.61	83	17

Chromosome Aberration Test								
Metabolic Activation	**Treatment Time**	**Test Article**	**Concentration (µg/mL)**	**Mean Mitotic Index (%)**	**Aberrations Per Cell**		**Cells With Aberrations (%)**	
					Mean	**SD**	**Numerical**	**Structural**
Without S9 Activation	4 hour	DMSO	n/a	7.9	0.005	0.071	1.5	0.5
		Exenatide Once Weekly	12.5	7.7	0.010	0.100	2.0	1.0
			25	7.3	0.010	0.100	3.0	1.0
			75	6.8	0.020	0.140	1.5	2.0
		Mitomycin C	0.2	6.1	0.400	0.739	2.0	28.0**
	20 hour	DMSO	n/a	8.4	0.000	0.000	3.0	0.0
		Exenatide Once Weekly	12.5	7.7	0.000	0.000	3.0	0.0
			25	8.7	0.000	0.000	3.5	0.0
			75	7.5	0.005	0.071	3.5	0.5
			100	4.7	0.030	0.299	1.0	1.5
		Mitomycin C	0.1	6.8	0.240	0.431	2.5	24.0**
With S9 Activation	4 hour	DMSO	n/a	8.3	0.000	0.000	3.0	0.0
		Exenatide Once Weekly	12.5	7.8	0.000	0.000	3.5	0.0
			25	7.0	0.005	0.071	4.0	0.5
			75	7.0	0.005	0.071	3.5	0.5
		Cyclophosphamide	10	6.5	0.240	0.553	3.5	18.0**

CTD = common technical document; DMSO = dimethylsulfoxide; GLP = good laboratory practice; ND = not determined; v/v = volume per volume.
** Statistically significant when compared to the solvent control ($p \leq 0.01$; Fisher's Exact Test)

2.6.7.8 Genotoxicity: In Vitro

Test Article: Exenatide Once Weekly

Report Title: In vitro chromosomal aberration test		
Test for Induction of: Chromosome aberrations	**Number of Independent Assays:** 2	**Study Reference:** REST080139
Strains: Chinese hamster ovary cells (CHO-K_1)	**Number of Replicate Cultures:** 2	**Location in CTD:** Section 2.6.6.4.6
Metabolizing System: Aroclor 1254-induced rat liver S9, 2% v/v	**Number of Cells Analyzed/Culture:** 500 cells for mitotic index, 200 metaphases for structural and numerical aberration analysis	**GLP Compliance:** Yes
Vehicle for Test Article: DMSO	**Vehicle for Positive Controls:** DMSO	**Date of Treatment:** 25 March 2008
Treatment: 4-hour treatment with 16-hour recovery in the presence and absence of S9; and 20-hour treatment in the absence of S9.	**Additional Information:** Based on precipitation, 3 dose levels were selected for metaphase analysis (the dose with the least precipitation and two lower doses).	
Cytotoxic Effects: None		
Genotoxic Effects: None		

Preliminary Toxicity Test (Range Finding)

Metabolic Activation	Treatment Time	Test Article	Concentration (µg/mL)	Cell Count ($x10^6$)	Cell Viability (%)	Viable Cells/Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Without S9 Activation	4 hour	DMSO	n/a	1.50	99	1.48	100	n/a
		Exenatide Once Weekly	0.5	1.58	99	1.57	113	-13
			1.5	1.50	100	1.50	103	-3
			5	1.63	99	1.62	121	-21
			15	1.63	99	1.61	120	-20
			50	1.43	99	1.42	91	9
			150	1.42	99	1.40	88	12
			500	1.58	98	1.55	111	-11
			1500	1.14	90	1.03	31	69
			5000	1.16	100	1.16	52	48
	20 hour	DMSO	n/a	1.84	100	1.84	100	n/a
		Exenatide Once Weekly	0.5	1.94	100	1.94	110	-10
			1.5	1.96	99	1.94	109	-9
			5	2.11	98	2.07	122	-22
			15	1.92	99	1.90	106	-6
			50	1.86	99	1.84	100	0
			150	1.73	100	1.73	89	11
			500	1.93	98	1.89	105	-5
			1500	1.61	100	1.61	77	23
			5000	1.88	99	1.86	102	-2

With S9 Activation	4 hour	DMSO	n/a	1.37	100	1.37	100	n/a
		Exenatide Once Weekly	0.5	1.42	98	1.39	104	-4
			1.5	1.42	99	1.40	106	-6
			5	1.48	99	1.47	119	-19
			15	1.40	100	1.40	106	-6
			50	1.47	98	1.44	114	-14
			150	1.51	99	1.50	124	-24
			500	1.65	100	1.65	153	-53
			1500	1.38	98	1.35	97	3
			5000	1.53	99	1.52	128	-28

Concurrent Toxicity Test

Metabolic Activation	Treatment Time	Test Article	Concentration (µg/mL)	Flask	Cell Count Averages ($x10^6$)	Cell Viability (%)	Mean Viable Cells/Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Without S9 Activation	4 hour	DMSO	n/a	A	1.87	100	–	–	–
				B	1.80	99	1.83	100	n/a
		Exenatide Once Weekly	25	A	1.87	98	–	–	–
				B	1.79	99	1.80	98	2
			50	A	1.87	99	–	–	–
				B	1.72	98	1.77	96	4
			100	A	1.71	100	–	–	–
				B	1.70	99	1.70	90	10
			250	A	1.60	98	–	–	–
				B	1.65	98	1.60	83	17
			500	A	1.28	100	–	–	–
				B	1.49	100	1.39	67	33
		Mitomycin C	0.1	A	1.43	98	–	–	–
				B	1.38	99	1.38	67	33
			0.2	A	1.33	99	–	–	–
				B	1.45	99	1.37	66	34
	20 hour	DMSO	n/a	A	1.93	100	–	–	–
				B	1.89	99	1.90	100	n/a
		Exenatide Once Weekly	25	A	1.80	100	–	–	–
				B	1.83	98	1.79	92	8
			50	A	1.71	99	–	–	–
				B	1.73	98	1.69	85	15

			100	A	1.66	99	–	–	–
				B	1.71	100	1.67	84	16
			250	A	1.65	99	–	–	–
				B	1.60	98	1.60	78	22
			500	A	1.29	99	–	–	–
				B	1.56	100	1.42	66	34
		Mitomycin C	0.1	A	1.47	98	–	–	–
				B	1.45	98	1.43	67	33
			0.2	A	1.45	97	–	–	–
				B	1.47	100	1.44	67	33
With S9 Activation	4 hour	DMSO	n/a	A	1.80	99	–	–	–
				B	1.72	98	1.73	100	n/a
		Exenatide Once Weekly	25	A	1.70	97	–	–	–
				B	1.60	95	1.58	88	12
			50	A	1.57	97	–	–	–
				B	1.53	97	1.51	82	18
			100	A	1.42	97	–	–	–
				B	1.38	95	1.34	68	32
			250	A	1.43	96	–	–	–
				B	1.38	98	1.36	70	30
			500	A	1.36	95	–	–	–
				B	1.32	95	1.27	63	37
		Cyclophosphamide	10	A	1.18	97	–	–	–
				B	1.04	94	1.06	45	55
			20	A	0.93	95	–	–	–
				B	1.02	97	0.94	35	65

Chromosome Aberration Test								
Metabolic Activation	**Treatment Time**	**Test Article**	**Concentration (µg/mL)**	**Mean Mitotic Index (%)**	**Aberrations Per Cell**		**Cells With Aberrations (%)**	
					Mean	**SD**	**Numerical**	**Structural**
Without S9 Activation	4 hour	DMSO	n/a	6.9	0.005	0.071	0.0	0.5
		Exenatide Once Weekly	50	6.5	0.010	0.100	0.0	1.0
			100	7.2	0.005	0.071	0.0	0.5
			250	7.2	0.010	0.100	0.0	1.0
		Mitomycin C	0.2	6.3	0.350	0.809	0.0	20.0**
	20 hour	DMSO	n/a	5.9	0.010	0.100	0.0	1.0
		Exenatide Once Weekly	50	6.1	0.005	0.071	0.0	0.5
			100	4.4	0.015	0.122	0.0	1.5
			250	5.2	0.030	0.264	0.0	1.5
		Mitomycin C	0.1	4.1	0.280	0.570	0.5	22.0**
With S9 Activation	4 hour	DMSO	n/a	10.2	0.015	0.122	0.0	1.5
		Exenatide Once Weekly	50	10.0	0.010	0.100	0.5	1.0
			100	10.0	0.015	0.122	0.0	1.5
			250	8.5	0.020	0.140	0.0	2.0
		Cyclophosphamide	10	2.5	0.330	0.792	0.0	20.0**

CTD = common technical document; DMSO = dimethylsulfoxide; GLP = good laboratory practice; ND = not determined; v/v = volume per volume.
** Statistically significant when compared to the solvent control ($p \leq 0.01$; Fisher's Exact Test)

2.6.7.10 Carcinogenicity

Test Article: Exenatide Once Weekly

Report Title: Exenatide LAR: 104-week carcinogenicity study following every other week subcutaneous administration in rats		
Species/Strain: Rat/Sprague-Dawley	**Duration of Dosing:** 104 weeks	**Study Reference:** REST060229
Initial Age: 6-7 weeks old	**Method of Administration:** SC injection (every other week)	(IND 67,092, Serial 0120)
Date of First Dose: 03 August 2006	**Vehicle/Formulation:** Microsphere Diluent	**Location in CTD:** Section 2.6.6.5.1
	Treatment of Controls: Microsphere Diluent, Control Microspheres	**GLP Compliance:** Yes
Basis for High-Dose Selection: Toxicity-based endpoint		
Special Features: None		

Bi-Weekly Exenatide Dose (mg/kg/dose)	Vehicle Control		Microsphere Control		0.3		1.0		3.0	
Sex	M	F	M	F	M	F	M	F	M	F
Toxicokinetics – Antibody Negative Animals										
Number of Animals	–	–	–	–	20	20	20	20	20	20
$AUC_{0\text{-}336}$ (pg-h/mL)										
Week 1	–	–	–	–	94,215	51,137	204,885	114,093	385,851	485,491
Week 27	–	–	–	–	158,204	107,319	783,272	641,052	2,024,703	1,910,576
C_{max} (pg/mL)										
Week 1	–	–	–	–	1,298	1,313	2,997	1,678	4,392	4,334
Week 27	–	–	–	–	2,682	2,113	28,117	18,607	10,586	10,574
Toxicokinetics – All Animals										
Number of Animals	–	–	–	–	20	20	20	20	20	20
$AUC_{0\text{-}336}$ (pg-h/mL)										
Week 1	–	–	–	–	94,215	51,137	204,885	114,093	385,851	485,491
Week 27	–	–	–	–	201,583	140,308	1,558,936	1,685,831	2,455,698	8,198,256
C_{max} (pg/mL)										
Week 1	–	–	–	–	1,298	1,313	2,997	1,678	4,392	4,334
Week 27	–	–	–	–	3,020	2,775	28,117	18,607	10,662	37,130
Number of Animals										
Start of Treatment	70	70	70	70	70	70	70	70	70	70
Died/Sacrificed Moribund	49	55	42	51	42	47	40	51	49	55
Terminal Sacrifice	21	15	28	19	28	23	30	19	21	15
Cumulative Survival (%)	30	21	40	27	40	33	43	27	30	21
Mean Body Weight (g)										
Day -1	305	206	305	208	302	208	303	207	306	207
Week 53	707	393	689	391	641 [b]	341 [b]	616 [b]	331 [b]	632 [b]	324 [b]

Week 103	687	507	671	483	632	423 [a]	597 [b]	403 [b]	609 [b]	399 [b]
Mean Food Consumption (g/day)										
Week -1	26.6	19.8	26.2	19.4	21.0 [b]	16.4 [b]	17.6 [b]	14.9 [b]	18.1 [b]	13.5 [b]
Week 53	29.6	23.2	29.1	23.6	29.0	21.8 [b]	27.4 [b]	21.9 [a]	28.1 [a]	22.1 [a]
Week 103	29.6	26.1	26.7	26.4	29.5	24.0	27.5	25.0	27.0	25.3
Neoplastic lesions (Incidence)										
Thyroid Gland										
Adenoma, c-cell, ben, 1°	9	5	9	9	20	22 [d]	32 [e]	19 [d]	33 [e]	21 [d]
Adenoma, follicular cell, ben, 1°	1	1	5	0	1	1	1	0	1	2
Carcinoma, c-cell, mal, 1°	0	0	1	1	2	1	5	1	3	4 [f]
Carcinoma, follicular cell, mal, 1°	0	0	2	0	1	0	0	0	1	0
Adipose Tissue, Brown										
Hibernoma, ben, 1°	1	0	0	0	2	0	0	0	0	0
Hibernoma, mal, 1°	2	1	1	2	0	2	2	0	2	2
Adrenal Glands										
Adenoma, cortical, ben, 1°	0	2	1	1	2	0	2	2	3	1
Carcinoma, cortical, mal, 1°	0	0	0	1	0	0	0	0	0	1
Osteosarcoma, mal, 2°	0	0	0	0	1	0	0	0	0	0
Pheochromocytoma, ben, 1°	9	1	5	1	6	0	7	0	5	1
Pheochromocytoma, complex, ben, 1°	0	0	0	0	0	0	1	0	0	0
Pheochromocytoma, mal, 1°	2	1	3	3	1	0	1	1	0	0
Brain										
Astrocytoma, mal, 1°	1	1	0	0	2	1	0	0	0	0
Carcinoma, pars distalis, mal, 2°	0	7	1	7	0	9	1	6	2	5
Granular cell tumor, ben, 1°	0	0	1	1	0	0	1	1	0	0
Meningioma, ben, 1°	0	0	1	0	0	0	0	0	0	0
Cavity, Abdominal										
Adenocarcinoma, mal, 2°	0	0	0	0	0	0	1	0	0	0
Carcinoma, squamous cell, mal, 2°	0	0	0	1	0	0	0	0	0	0
Carcinoma, tubular cell, mal, 2°	0	0	0	0	0	0	0	0	1	0
Fibrosarcoma, mal, 1°	0	0	0	0	0	0	0	1	0	0
Hemangiosarcoma, mal, 1°	0	0	0	0	0	0	1	0	0	0
Lipoma, ben, 1°	0	0	0	0	0	0	1	0	0	0
Liposarcoma, mal, 2°	1	0	0	0	0	0	0	0	0	0
Mesothelioma, mal, 1°	0	0	0	0	1	0	0	0	0	0
Osteosarcoma, mal, 1°	0	0	0	0	1	0	0	0	0	0
Schwannoma, ben, 1°	0	1	0	0	0	0	0	0	0	0
Cavity, Oral										

Carcinoma, squamous cell, mal, 1°	0	0	1	0	1	0	0	0	0	0
Cavity, Thoracic										
Carcinoma, c-cell, mal, 2°	0	0	0	0	0	0	1	0	0	0
Carcinoma, tubular cell, mal, 2°	0	0	0	0	0	0	0	0	1	0
Hemangiosarcoma, mal, 1°	0	0	0	0	0	0	0	0	0	1
Mesothelioma, mal, 2°	0	0	0	0	1	0	0	0	0	0
Neuroendocrine tumor, mal, 1°	0	0	1	0	0	0	0	0	0	0
Epididymides										
Mesothelioma, mal, 1°	0	n/a	1	n/a	0	n/a	1	n/a	0	n/a
Eyes										
Schwannoma, mal, 1°	0	0	0	0	0	0	0	0	1	0
Harderian Glands										
Adenoma, ben, 1°	1	0	0	0	0	1	0	0	1	0
Head										
Schwannoma, mal, 1°	0	0	0	0	0	0	0	0	2	0
Heart										
Mesothelioma, mal, 1°	1	0	0	0	0	0	0	0	0	0
Schwannoma, mal, 1°	0	0	0	0	0	0	0	0	0	1
Injection Site #3										
Fibrous histiocytoma, mal, 1°	0	0	0	0	0	0	1	0	0	0
Kidneys										
Adenocarcinoma, mal, 2°	0	0	0	1	0	0	1	0	0	0
Adenoma, tubular cell, ben, 1°	0	0	0	0	0	0	0	0	0	1
Carcinoma, tubular cell, mal, 1°	1	0	1	1	0	0	0	0	1	3[c]
Hemangiosarcoma, mal, 1°	1	0	0	0	0	0	0	0	0	0
Lipoma, ben, 1°	0	1	0	0	0	1	0	0	0	1
Liposarcoma, mal, 1°	1	0	0	0	1	0	0	0	1	0
Nephroblastoma, mal, 1°	0	0	0	1	0	0	0	0	0	0
Osteosarcoma, mal, 1°	0	0	0	0	0	0	0	1	0	0
Osteosarcoma, mal, 2°	0	0	0	0	1	0	0	0	0	0
Papilloma, transitional cell, ben, 1°	1	0	0	0	0	0	0	0	0	0
Large Intestine, Cecum										
Fibroma, ben, 1°	0	0	0	0	0	0	0	1	0	0
Large Intestine, Rectum										
Leiomyosarcoma, mal, 1°	0	1	0	0	0	0	0	0	0	0
Liver										
Adenocarcinoma, mal, 2°	0	0	0	0	0	0	1	0	0	0
Adenoma, hepatocellular, ben, 1°	1	0	2	0	1	0	1	1	2	1

Carcinoma, hepatocellular, mal, 1°	0	0	1	0	0	0	1	0	1	0
Cholangiofibroma, ben, 1°	0	0	0	1	0	0	0	0	0	0
Cholangioma, ben, 1°	0	1	0	0	0	0	0	0	0	0
Hemangiosarcoma, mal, 1°	0	0	0	0	0	0	0	1	0	0
Pheochromocytoma, mal, 2°	0	1	0	0	0	0	1	0	0	0
Lung										
Adenocarcinoma, mal, 2°	0	0	0	2	0	2	2	1	0	1
Carcinoma, bronchiolar alveolar, mal, 1°	0	0	0	0	0	0	0	1	0	0
Carcinoma, cortical, mal, 2°	0	0	0	0	0	0	0	0	0	1
Carcinoma, squamous cell, mal, 2°	0	0	0	1	0	0	0	0	0	0
Carcinoma, tubular cell, mal, 2°	0	0	0	0	0	0	0	0	1	0
Hemangiosarcoma, mal, 2°	0	0	0	0	0	0	0	1	0	0
Hibernoma, mal, 2°	0	0	0	0	0	0	1	0	0	1
Osteosarcoma, mal, 2°	0	0	0	0	1	0	0	1	0	1
Pheochromocytoma, mal, 2°	0	1	0	2	0	0	1	1	0	0
Lymph Node, Hepatic										
Carcinoma, tubular cell, mal, 2°	0	0	0	0	0	0	0	0	1	0
Lymph Node, Iliac										
Hemangiosarcoma, mal, 2°	0	0	0	0	0	0	1	0	0	0
Lymph Node, Mandibular										
Carcinoma, squamous cell, mal, 2°	0	0	0	0	0	0	0	0	1	0
Osteosarcoma, mal, 2°	0	0	0	0	0	0	0	0	0	1
Lymph Node, Mediastinal										
Adenocarcinoma, mal, 2°	0	0	0	0	0	0	1	0	0	0
Carcinoma, bronchiolar alveolar, mal, 2°	0	0	0	0	0	0	0	1	0	0
Carcinoma, tubular cell, mal, 2°	0	0	0	0	0	0	0	0	1	0
Lymph Node, Mesenteric										
Adenocarcinoma, mal, 2°	0	0	0	0	0	0	2	0	0	0
Hemangioma, ben, 1°	0	0	0	0	0	0	0	0	1	0
Hemangiosarcoma, mal, 1°	0	0	0	0	0	0	1	0	0	0
Hemangiosarcoma, mal, 2°	1	0	0	0	0	0	0	0	0	0
Lymphangiosarcoma, mal, 1°	0	0	1	0	0	0	0	0	0	0
Lymph Node, Renal										
Carcinoma, squamous cell, mal, 2°	0	0	0	1	0	0	0	0	0	0
Mammary Gland										
Adenocarcinoma, mal, 1°	0	29	0	28	0	24	1	28	0	33
Adenoma, ben, 1°	1	3	0	1	0	1	0	2	0	0
Carcinoma, squamous cell, mal, 1°	0	0	0	1	0	0	0	0	0	0

Fibroadenoma, ben, 1°	2	21	2	29	1	24	0	20	1	25
Mesentery/Peritoneum										
Lipoma, ben, 1°	0	1	0	0	0	0	0	0	0	0
Multicentric Neoplasm										
Leukemia, granulocytic, mal	0	0	1	1	0	0	0	0	1	0
Leukemia, large granular lymphocyte, mal	1	0	0	0	0	0	0	0	1	1
Lymphoma, mal	0	0	1	0	0	0	1	1	0	1
Sarcoma, histiocytic, mal	2	2	2	0	0	0	2	1	2	0
Nose, Level A										
Carcinoma, squamous cell, mal, 2°	1	0	0	0	0	0	0	0	0	0
Odontoma, mal, 1°	0	0	0	0	0	0	0	0	1	0
Nose, Level B										
Adenoma, ben, 1	0	0	0	0	1	0	0	0	0	0
Carcinoma, squamous cell, mal, 1°	0	0	0	0	0	0	1	0	0	0
Carcinoma, squamous cell, mal, 2°	1	0	0	0	0	0	0	0	0	0
Osteosarcoma, mal, 1°	0	0	0	0	0	0	0	0	0	1
Ovaries										
Cystadenoma, ben, 1°	n/a	0	n/a	1	n/a	0	n/a	0	n/a	0
Sertoli cell tumor, ben, 1°	n/a	1	n/a	0	n/a	0	n/a	0	n/a	0
Pancreas										
Adenocarcinoma, mal, 2°	0	0	0	0	0	0	1	0	0	0
Adenoma, acinar cell, ben, 1°	1	0	0	0	0	0	0	0	0	0
Adenoma, islet cell, ben, 1°	8	1	12	0	12	1	3	3	3	4
Carcinoma, acinar cell, mal, 1°	1	0	0	0	0	0	0	0	0	0
Carcinoma, islet cell, mal, 1°	3	0	2	1	3	1	0	1	0	0
Parathyroid Glands										
Adenoma, ben, 1°	0	0	1	0	0	0	3	1	3	1
Carcinoma, c-cell, mal, 2°	0	0	0	0	0	1	0	1	0	0
Pituitary Gland										
Adenoma, pars distalis, ben, 1°	38	52	35	42	36	49	42	52	32	51
Adenoma, pars intermedia, ben, 1°	2	0	1	0	0	0	0	0	0	0
Carcinoma, pars distalis, mal, 1°	0	9	2	7	0	9	1	6	2	5
Pituicytoma, pars nervosa, ben, 1°	0	0	0	0	1	0	0	0	0	0
Schwannoma, mal, 2°	0	0	0	0	0	0	0	0	1	0
Prostate Gland										
Adenoma, ben, 1°	1	n/a	1	n/a	0	n/a	0	n/a	0	n/a
Seminal Vesicles										
Adenoma, ben, 1°	1	n/a	1	n/a	1	n/a	0	n/a	0	n/a

Skeletal Muscle										
Schwannoma, mal, 2°	0	0	0	0	0	0	0	0	1	0
Skeletal Muscle, Quadriceps										
Adenocarcinoma, mal, 2°	0	0	0	0	0	0	1	0	0	0
Carcinoma, squamous cell, mal, 2°	0	0	0	1	0	0	0	0	0	0
Skin										
Adenoma, basal cell, ben, 1°	1	0	0	0	0	0	0	0	0	0
Adenoma, sebaceous cell, ben, 1°	0	0	0	0	0	0	2	0	0	0
Carcinoma, sebaceous cell, mal, 1°	0	0	0	0	0	1	0	0	0	0
Carcinoma, squamous cell, mal, 1°	1	0	0	0	0	0	0	0	0	0
Hair follicle tumor, ben, 1°	0	0	0	0	1	0	1	0	0	0
Keratoacanthoma, ben, 1°	3	0	2	0	0	0	1	0	1	0
Papilloma, squamous cell, ben, 1°	0	0	1	0	0	0	0	0	1	0
Skin, Subcutis										
Fibroma, ben, 1°	0	0	3	0	4	0	2	0	8 [d]	0
Fibrosarcoma, mal, 1°	0	0	0	1	0	0	0	0	0	0
Fibrous histiocytoma, mal, 1°	0	0	1	0	0	0	1	0	0	1
Lipoma, ben, 1°	1	0	2	1	1	0	0	0	0	0
Schwannoma, mal, 1°	0	0	1	0	0	0	1	0	0	0
Small Intestine, Duodenum										
Adenocarcinoma, mal, 1°	0	0	1	0	0	0	1	0	0	0
Spleen										
Adenocarcinoma, mal, 2°	0	0	0	0	0	0	1	0	0	0
Stomach, Glandular										
Adenocarcinoma, mal, 1°	0	0	0	0	0	0	2	0	0	0
Adenocarcinoma, mal, 2°	0	0	0	0	0	0	1	0	0	0
Testes										
Adenoma, interstitial cell, ben, 1°	4	n/a	2	n/a	0	n/a	1	n/a	1	n/a
Mesothelioma, mal, 2°	0	n/a	1	n/a	0	n/a	0	n/a	0	n/a
Tongue										
Papilloma, squamous cell, ben, 1°	0	0	1	0	0	0	0	0	0	0
Urinary Bladder										
Carcinoma, transitional cell, mal, 1°	0	0	0	1	0	0	0	0	0	0
Leiomyoma, ben, 1°	0	0	0	0	0	0	0	0	0	1
Leiomyosarcoma, mal, 2°	0	1	0	0	0	0	0	0	0	0
Papilloma, transitional cell, ben, 1°	0	0	0	0	0	0	0	0	1	0
Uterus With cervix										
Fibroma, ben, 1°	n/a	1	n/a	0	n/a	0	n/a	0	n/a	0

Granular cell tumor, ben, 1°	n/a	3	n/a	2	n/a	4	n/a	2	n/a	3
Granular cell tumor, mal, 1°	n/a	0	n/a	0	n/a	1	n/a	0	n/a	0
Leiomyosarcoma, mal, 1°	n/a	0	n/a	0	n/a	2	n/a	0	n/a	0
Polyp, stromal, ben, 1°	n/a	3	n/a	6	n/a	4	n/a	1	n/a	4
Sarcoma, stromal, mal, 1°	n/a	0	n/a	0	n/a	1	n/a	1	n/a	0
Schwannoma, mal, 1°	n/a	0	n/a	0	n/a	0	n/a	1	n/a	0
Vagina										
Carcinoma, squamous cell, mal, 2°	n/a	0	n/a	1	n/a	0	n/a	0	n/a	0
Granular cell tumor, ben, 1°	n/a	2	n/a	1	n/a	2	n/a	4	n/a	3
Zymbal's Gland										
Carcinoma, squamous cell, mal, 1°	1	0	2	0	0	0	1	0	2	1

1° = primary; 2° = secondary; ben = benign; mal = malignant

[a] $p<0.05$ [b] $p<0.01$ vs. diluent control (group pair-wise comparisons, Levene's/ANOVA-Dunnet's/Welch's)

[c] $p<0.025$ [d] $p<0.0.005$ [e] $p<0.0001$ [f] $p=0.0248$ vs. diluent control (Peto's survival-adjusted trend test [Peto et al. 1980] and exact permutation trend test [Gart et al. 1986])

INTEGRATED SAFETY SUMMARY AND CONCLUSIONS

General Toxicology

The toxicity profile of exenatide was previously characterized as part of the nonclinical development program for immediate release exenatide (Byetta; NDA 21-733). The sponsor conducted additional repeat-dose studies with the sustained release formulation (exenatide QW) in rats (4 months) and monkeys (3 and 9 months), a rat carcinogenicity study, and several in vitro genetic toxicology studies to qualify manufacturing changes and to determine whether the toxicity profile of exenatide QW is similar to that of immediate release exenatide. A summary of key toxicology findings for both immediate release exenatide and exenatide QW is presented below.

Single doses of immediate release exenatide did not result in mortality or severe toxicity in mice at doses up to 1,500 μg/kg by intravenous injection, in rats at doses up to 30,000 μg/kg by subcutaneous injection, or in monkeys at doses up to 5,000 μg/kg by subcutaneous injection. Decreased grip strength and decreased motor activity were seen in mice at intravenous doses of ≥300 and ≥30 μg/kg, respectively. Hunched posture and fur staining was seen in rats at ≥10,000 μg/kg and decreased food consumption was observed in monkeys receiving ≥3,000 μg/kg.

Mouse was used as the rodent species for the Byetta toxicology program. Toxicology studies have not been conducted with exenatide QW in mice; however, the results from studies conducted with immediate release exenatide are considered to be relevant for exenatide QW. Based on the review of NDA 21-773 by Dr. Colerangle, mice administered exenatide twice daily by subcutaneous injection at doses up to 380 μg/kg/dose (760 μg/kg/day) for 6 months had microscopic findings in the eye (retinal atrophy, corneal mineralization, cataract), parotid salivary gland (basophilia), bone marrow hyperplasia, and injection site reactions (inflammation, hemorrhage, fibrosis, epithelial hyperplasia). The histopathology findings were primarily observed at the high dose, however parotid gland hyperplasia was observed in nearly all low-dose animals and pthisis bulbi (shrinkage and wasting of the eyeball) was observed in one low-dose male. Therefore, a NOAEL was not established for this study because of the findings at the low dose (~2X MHRD, AUC)

In an 18-week toxicity study, Sprague-Dawley rats received exenatide QW every other week by subcutaneous injection at doses up to 9 mg/kg. Slight decreases in body weight gain that generally correlated with decreased food consumption were observed at all doses, but particularly for males receiving 3 and 9 mg/kg. No systemic toxicity was observed microscopically. One HD female had renal tubular adenomas unilaterally with a focus of slight tubular hyperplasia in the contralateral kidney. One MD female had a renal tubular adenoma. One LD female and one female given the microsphere control had slight and minimal renal tubular hyperplasia, respectively. The sponsor stated that these tumors are incidental findings and not drug-related because the study duration is short (18 weeks) and secondly, tumors of the renal tubules are typically slow growing with a long latency for development. The sponsor also argued that even with some of the most potent renal carcinogens, a latency period of 6 to 12 months is required for tumors

to develop (Hard, 1990; Hard et al., 1970). Interestingly, a non-statistically significant increase (4/70 vs. 0/70 for diluent controls) in renal tubular cell tumors (adenomas and carcinomas) was noted for high-dose females (3 mg/kg biweekly) in the rat carcinogenicity study conducted with exenatide QW. Because the metabolism and excretion of exenatide is thought to primarily occur in the kidney, it is uncertain whether this rare occurrence of renal tubule tumors in female rats is related to exenatide treatment.

Injection site findings were the primary treatment-related effect. Swelling/palpable lumps (slight to moderate severity) were relatively severe for animals given the control microspheres or the test article at 9 mg/kg and noted at a lesser severity (very slight to slight) for animals given 3 mg/kg. The swelling/palpable lumps were attributed to the physical presence of the microspheres. Very slight to slight redness was noted at a low incidence in the control microsphere and test article treated groups and was attributed to the injection trauma. Histopathology revealed foamy macrophages/ fibroblasts, lymphocytic infiltrate and granuloma of minimal to slight severity at all injection sites. Injection site findings reversed or showed a trend for recovery by the end of the 3-month recovery period. Anti-exenatide antibodies were observed at ≥1 mg/kg/dose with no apparent dose-response relationship for incidence. The presence of antibodies tended to increase exenatide exposure values and therefore, values from antibody negative animals were used for calculating clinical exposure margins. Other than more noteworthy findings at the injection sites due to the presence of the PLG microspheres, the results of this study are similar to those observed in rats receiving once daily injections of immediate release exenatide for 3 months. The NOAEL for this study was determined to be the high-dose of 9 mg/kg (~27X MRHD, Ab negative AUC) based on a lack of target organ toxicity.

The administration of exenatide QW to cynomolgus monkeys once weekly for 3 months by subcutaneous injection at doses up to 1.1 mg/kg injection primarily resulted in injection site reactions. Macroscopic lesions at the injection sites were characterized by red or white discoloration, nodules, abscesses, and thickened tissue due to needle trauma. The nodules appeared to increase in severity with increase in exenatide dose. Microscopically, the injection sites for all groups were characterized as having chronic inflammation, abscesses, epidermal hyperplasia, fibrosis, and/or hemorrhage, although these occurred at a lower incidence for diluent control monkeys. Granulomatous inflammation (minimal to severe) and foreign material were noted at the injection site of animals receiving microspheres with or without exenatide. Treatment-related injection site enlargement was also noted during the treatment period for animals receiving microspheres. At the end of the recovery period, the incidence of macroscopic and microscopic lesions was drastically reduced suggestive of reversibility. No microscopic lesions were observed in other tissues. No evidence of sensitization was noted following a challenge dose of control microspheres (microspheres in diluent without exenatide) administered to a previously undosed site on all recovery animals. Anti-exenatide antibodies were detected in all exenatide QW-treated groups by Day 92 with most animals positive at ≥0.44 mg/kg, with titers ranging from 25 to 78,125. The NOAEL for this study was considered to be 1.10 mg/kg (19X MRHD, AUC) on the basis of a lack of target organ toxicity.

Similar results were observed in a 6-month monkey study. Monkeys were administered exenatide QW once weekly by subcutaneous injection for 3 months at doses up to 1.1 mg/kg. Local reactions at the injection sites (erythema and edema) were noted in all groups and tended to increase in incidence and severity with increasing doses of microspheres. There appeared to be a slight increase in severity in the high-dose exenatide QW group (very slight to severe). Some occurrences of abscesses with drainage were noted for all exenatide-treated groups. Macroscopic observations of subcutis thickening were also observed at the injection site of animals receiving microspheres. Microscopically, minimal to moderate granulomas were noted in all groups receiving microspheres. Granulomas were well-circumscribed with minimal fibrosis and consisted of macrophages and multinucleated giant cells, often containing microspheres. Minimal to moderate foamy macrophages and fibroblasts were also observed in the subcutis. A few animals receiving microspheres also showed mononuclear cell inflammation, suppurative inflammation, fibrosis, hemorrhage, and/or draining tracts, which did not appear to be related to exenatide dose. Injection site effects had mostly resolved by the end of the 3-month recovery period. Treatment-related microscopic findings were not observed in other tissues.

Anti-exenatide antibodies were detected in approximately 75% of all treated animals, with the maximal response occurring between Weeks 20 and 24, with titers ranging from 25 to 78,125. Antibody titers tended to decrease during the recovery period, although approximately 66% of the exenatide treated animals still had titers ranging from 25 to 3,125 at the end of the 3-month recovery period. Steady-state plasma exenatide concentrations were achieved after approximately four injections (i.e., 1 month). Mean AUC accumulation for antibody negative animals ranged from approximately 9 to 28 fold. Exenatide exposures were generally below the limit of detection within 2 months after the final dose. Plasma exenatide exposure was affected by the degree of anti-exenatide antibodies, resulting in either marked exposure increases (titer range of 125 to 625) or decreases (at titers ≥3,125). The NOAEL was determined to be 1.1 mg/kg/week (14X MRHD, Ab negative AUC) based on a lack of target organ toxicity.

With the exception of injection site findings, the toxicity profile of exenatide QW in monkeys showed fewer potential target organs compared to monkeys treated with immediate release exenatide. As with rats, macroscopic and microscopic findings were more noteworthy in monkeys receiving microspheres, which resulted in findings that are generally consistent with foreign body reactions. In the 3-month monkey study conducted with immediate release exenatide, the NOAEL was determined to be the low dose (0.6 µg/kg BID; 2X MRHD, AUC) because of microscopic findings in the stomach (hemorrhage), lung (hemorrhage), and uterus (endometrial hemorrhage) at the higher dose levels. These specific findings were not observed in the 9-month monkey study conducted with immediate release exenatide. However, other microscopic findings were noted that were not observed in the 9-month monkey study conducted with exenatide QW that resulted in the NOAEL being placed at the low dose (1.1 µg/kg BID; 3X MRHD, Day 90 AUC). In that study, microscopic findings were noted in the brain (submeningeal hemorrhage, mononuclear cell infiltration of meninges and perivascular), thyroid (follicular distension, degeneration of follicular epithelium), adrenal gland

(mineralization, nodular hypertrophy in cortex), pancreas (cytoplasmic vacuolation, fibrosis, islet cell hypercellularity, mononuclear cell infiltrate), uterus (protein deposits), stomach (lymphoplasmacytic infiltrate, lymphoid hyperplasia) and injection sites (epidermal hyperplasia). All of these findings were considered minimal or mild. It is uncertain why there were more microscopic findings in animals treated with immediate release exenatide. It could be that many of the findings were incidental because many of the findings (with the exception of pancreas, stomach, and injection sites) occurred in only one or two animals out of six and occurred in only one gender. Alternatively, the difference in the PK characteristics between the two exenatide formulations (i.e., higher C_{max} and AUC values for immediate release) could have resulted in more target organ toxicity. A TK summary table is presented below that shows the difference in mean C_{max} and AUC values between the two 9-month monkey studies.

Comparative TK Data between 9-Month Monkey Studies Conducted with Exenatide and Exenatide QW

Exenatide			Exenatide QW		
Dose (µg/kg/day)	C_{max} (pg/mL)	AUC_{0-24} (pg·h/mL)*	Dose (mg/kg/week)	C_{max} (pg/mL)	AUC_{0-24} (pg·h/mL)†
2.2	3,858	16,858	0.11	1,529	11,136
18	49,941	580,822	0.42	8,091	93,804
150	221,080	1,472,576	1.10	18,100	76,723

*calculated by multiplying AUC_{0-12h} values by 2.
†calculated by dividing AUC_{0-168h} values by 7.
Note: because the TK data for the exenatide study did not distinguish between Ab negative and positive animals, data from all animals are being used but from an earlier time point, which is Day 90 for the exenatide study and Day 85 for the exenatide QW study. Values after these times appear to be significantly affected by the presence of antibodies.

Carcinogenicity and Genetic Toxicology

Results from a 2-year carcinogenicity study in which Sprague-Dawley rats received exenatide QW (0.3, 1, or 3 mg/kg) by subcutaneous injection once every other week showed an increase in thyroid c-cell tumors (adenomas plus carcinomas) at all doses (≥0.3 mg/kg; ≥1X MRHD, Ab negative AUC) for both males and females. The number of animals with thyroid c-cell hyperplasia was generally similar to controls, although there was a slight increase for high-dose males and low-dose females. A statistically significant increase in fibromas of the skin was observed in males treated with the high dose (3 mg/kg; 26X MRHD, Ab negative AUC). Fibromas were relatively acellular and were comprised primarily of bundles of collagen. The fibromas were not specifically noted as being at injection sites, but this was implied in the pathologists report. At the injection site for high-dose males, the amount of drug injected was approximately 10% less than the amount of exenatide administered clinically (~1.8 mg versus 2 mg), based on an average male rat weight of 0.6 kg. The concentration of the drug product once resuspended in diluent was approximately 2-fold higher at the high-dose compared with the concentration administered clinically. A non-statistically significant slight increase in renal tubular cell tumors (adenomas plus carcinomas) was observed in HD females (25X MRHD, Ab negative AUC). Two tubular cell adenomas were also observed in a 4-month rat study; a relationship to test article remains uncertain. Other findings typical of exenatide (reduced body weight) and PLG microspheres (foreign body granulomas at the

injection site) were also observed. A summary of tumor data with statistical values and historical control data is shown in the tables below.

Summary of Tumor Incidence in Males

Dose (mg/kg)	Diluent Control	Microsphere Control	0.3	1.0	3.0	Historical Control
Exposure Margin‡			**2X**	**10X**	**26X**	
Thyroid, c-cell hyperplasia	15/70 (21%)	10/70 (14%)	23/70 (33%)	19/70 (27%)	23/70 (33%)	NP
c-cell adenoma	9/70 (14%) **p<0.001†**	9/70 (14%) **p<0.001†**	**20/70** (29%) p=0.038	**32/70** (46%) **p<0.001***	**33/70** (47%) **p<0.001***	8.8% (1.9-15.4%)
c-cell carcinoma	0/70 (0%) p=0.164	1/70 (1.4%) p=0.237	2/70 (2.9%) p=0.268	**5/70** (7.1%) **p=0.036***	**3/70** (4.3%) p=0.133	0.6% (0-1.7%)
c-cell adenoma + carcinoma	9/70 (13%) **p<0.001†**	10/70 (14%) **p<0.001†**	**22/70** (31%) p=0.019	**34/70** (49%) **p<0.001***	**35/70** (50%) **p<0.001***	NP
Skin, subcutis, Fibroma	0/70 (0%) **p=0.004†**	3/70 (4.3%) p=0.034	4/70 (5.7%) p=0.069	2/70 (2.9%) p=0.273	**8/70** (11%) **p=0.004***	2.2% (0-5%)

Historical control data from 11 studies; NP = not provided.
*Statistically significant by pair-wise analysis compared with diluent control.
†Statistically significant for dose response.
‡Based on mean AUC values for antibody negative animals on Day 183 compared with mean AUC values for antibody negative humans.

Summary of Tumor Incidence in Females

Dose (mg/kg)	Diluent Control	Microsphere Control	0.3	1.0	3.0	Historical Control
Exposure Margin‡			**1X**	**8X**	**25X**	
Thyroid, c-cell hyperplasia	13/70 (19%)	12/70 (17%)	31/70 (44%)	29/70 (41%)	40/70 (57%)	NP
c-cell adenoma	5/70 (7.1%) p=0.024	9/70 (13%) p=0.072	**22/70** (31%) **p<0.001***	**19/70** (27%) **p=0.003***	**21/70** (30%) **p<0.001***	8.1% (2.0-11.4%)
c-cell carcinoma	0/70 (0%) **p=0.014†**	1/70 (1.4%) p=0.042	1/70 (1.4%) p=0.533	1/70 (1.4%) p=0.517	**4/70** (5.7%) p=0.064	0.6% (0-4.0%)
c-cell adenoma + carcinoma	5/70 (7%) **p=0.003†**	10/70 (14%) p=0.016	23/70 (33%) **p<0.001***	20/70 (29%) **p=0.002***	25/70 (36%) **p<0.001***	NP

Historical control data from 11 studies; NP = not provided.
*Statistically significant by pair-wise analysis compared with diluent control.
†Statistically significant for dose response.
‡Based on mean AUC values for antibody negative animals on Day 183 compared with mean AUC values for antibody negative humans.

In the rat carcinogenicity study conducted with immediate release exenatide (once daily subcutaneous injections), an increase in thyroid c-cell tumors was observed at all dose levels in females only, with an incidence of 8% (control 1), 5% (control 2), 14% (18 µg/kg/day), 11% (70 µg/kg/day), and 23% (250 µg/kg/day). Although these values were not statistically significantly significant from either control group based on pair-wise analysis, they were above of the historical control mean (5%) and upper range (0% to 10%) for the laboratory.

A comparison of rat the carcinogenicity results shows that exenatide QW was more potent at inducing thyroid c-cell tumors than immediate release exenatide even though the mean daily exenatide exposures were similar between the two studies. Additionally, some adenomas progressed to carcinomas in animals that received exenatide QW, whereas no carcinomas were observed in rats treated with immediate release exenatide. Through an assessment of available data for other GLP-1 receptor agonists, it is apparent that the induction of c-cell tumors is a common trait for this class of drugs. Additionally, agonists that have a long half life or that are formulated for sustained release, thereby allowing steady-state concentrations to be achieved, are more potent at inducing c-cell tumors than short-acting agonists such as immediate release exenatide. This difference is believed to be due, at least in part, to continuous GLP-1 receptor activation (presumably on thyroid c-cells) that occurs at steady-state compared with a more pulsatile receptor activation that occurs with shorter acting compounds. Therefore, the difference in c-cell tumor incidence in rats between immediate release exenatide and exenatide QW is likely due to the difference in PK profile between the two formulations and suggests that there is an apparent greater risk for the development of c-cell tumors in humans receiving Bydureon compared with Byetta.

Because the toxicology program for exenatide QW was meant to bridge to the immediate release exenatide program, which already included carcinogenicity studies in two rodent species, only a single carcinogenicity study was conducted. Therefore a mouse carcinogenicity study was not conducted with exenatide QW, which was supported by the Executive Carcinogenicity Assessment Committee (ECAC; See Appendices 1 and 2 for ECAC meeting minutes). In the mouse carcinogenicity study conducted with immediate release exenatide, no test-article related tumors were observed. The primary microscopic finding was an increase in basophilic hypertrophy of the parotid salivary gland at all dose levels. Based on mouse carcinogenicity results with other long-acting GLP-1 receptor agonists, this class of compounds also induces c-cell tumors in mice. However, the c-cell tumors generally occurred at higher clinical exposure margins and were mostly limited to adenomas. Based on these observations, if exenatide QW were to be tested in a mouse carcinogenicity study, it would be anticipated that thyroid c-cell adenomas would also be observed in mice.

There appear to be physiological differences between rats and mice with regard to c-cell effects, and therefore it is uncertain whether rats or mice, if either species, are more relevant for assessing human risk. C-cell hyperplasia and adenoma formation is a common, age-related effect in rats, whereas c-cell hyperplasia and adenomas are a rare background finding in mice. It is possible that GLP-1 agonists enhance or speed up the

process of c-cell hyperplasia that normally occurs in older rats. Understanding the difference in the mechanism of action for GLP-1 receptor agonist c-cell tumor induction between rats and mice could be an important step in determining the overall relevance of c-cell tumors to human risk.

Exenatide was found to be devoid of mutagenic or clastogenic activity in a standard battery of genetic toxicology studies (NDA 21-773). Additionally, data were submitted as part of this application demonstrating that exenatide QW is devoid of mutagenic activity in a reverse bacterial mutation assay and in an in vitro chromosomal aberration assay.

Developmental and Reproductive Toxicology (DART)

The effect of exenatide on reproduction and embryonic development was previously investigated by the sponsor in support of the marketing application for Byetta (NDA 21-773). Additional development and reproductive toxicology (DART) studies were not conducted with exenatide QW. Although it is uncertain whether the difference in PK profile between the two exenatide formulations could have an effect on DART results, it is felt that the results obtained with immediate release exenatide are relevant for exenatide QW.

The effect of exenatide on fertility and early embryonic development was evaluated in mice. Mice received exenatide by subcutaneous injection at doses of at 3, 34 and 380 µg/kg twice daily. Results showed that there were no exenatide-related adverse effects on estrus cycling, mating and fertility indices, numbers of corpora lutea, implantation, viable embryos, non-viable embryos, pre- or post-implantation viability, or cauda epididymal sperm motility, count, or density. Accordingly, the NOAEL for effects on male and female reproduction was the high dose of 380 µg/kd BID (760 µg/kg/day; 148X MRHD, AUC).

The effect on embryonic development was assessed in mice and rabbits. In a mouse teratology study, exenatide doses of 3, 34, 230, and 380 µg/kg twice daily (1X, 9X, 92X, and 148X MRHD, AUC) were administered subcutaneously to pregnant mice from GD 6 through 15. Consistent with the pharmacodynamic activity of exenatide, maternal body weight and food consumption were decreased compared with controls. One female in each of the 34 and 380 µg/kg/dose groups aborted on GD 15 and 16, respectively. One female in each of the 34, 230, and 380 µg/kg/dose groups delivered prematurely. Developmental toxicity occurred in conjunction with maternal toxicity. The number of implantations, litter sizes, and live fetuses were significantly decreased for dams receiving 230 µg/kg/dose relative to control. Fetal body weights were decreased at ≥230 µg/kg/dose for males and ≥68 µg/kg/dose for females. Skeletal variations associated with delayed fetal growth, including changes in the number of rib pairs or vertebral ossification sites, and wavy ribs were noted ≥230 µg/kg/dose. Cleft palate with or without hole was observed at ≥3 µg/kg/dose. TK data showed that the potential of exenatide to cross the placental barrier is very low in mice. Therefore, the observed fetal findings may have been a consequence of the dose-related reduced nutritional state of the dams during gestation or direct maternal toxicity. The maternal NOAEL was determined

to be 3 μg/kg BID (1X MRHD) based on the observed abortions. The developmental NOAEL was also determined to be 3 μg/kg BID on the basis of decreased fetal body weights, cleft palate, and wavy ribs.

A rabbit teratology study was conducted with twice daily exenatide doses ranging from 0.1 to 130 μg/kg/dose. Apparent maternal toxicity characterized by profound weight loss and reduced food consumption was observed at doses ≥11 μg/kg/dose. Morphological markers of fetal growth retardation were observed that included umbilical hernias and skeletal variations of angulated hyoid, altered number of rib pair or vertebral bodies, and fused sternabrae at ≥11 μg/kg/dose. Fetal incidence of small gall bladder was significantly increased at 11, 78 and 130 μg/kg/dose. The maternal NOAEL was determined to be 0.1 μg/kg/dose (0.1X MRHD) based on dose-related decrease in weight gain during the treatment period. The developmental NOAEL was also 0.1 μg/kg BID (0.1X MRHD) based on the developmental retardation. TK data showed that the potential for exenatide to cross the placental barrier was also very low in rabbits. Therefore the fetal findings observed may have been a consequence of the reduced nutritional state of the does during gestation or direct maternal toxicity.

A second rabbit teratology study was conducted in an effort to better define the fetal NOAEL and to investigate the effects of decreased maternal body weight on embryonic development by including pair-fed groups. In this study, pregnant rabbits were administered exenatide at twice daily doses of 1, 11, and 130 μg/kg/dose. Rabbits that were administered exenatide exhibited profound, dose-related decreases in food and water consumption and loss in body weight. Clinical indicators of starvation (β-hydroxybuterate and potassium) and body weight loss were more pronounced in the exenatide-treated groups than in the pair-fed groups. Based on the severity of the body weight loss and anorexia, the MTD in pregnant rabbits was exceeded at doses ≥11 μg/kg/dose exenatide. As in the previous rabbit study, developmental toxicity occurred only at doses ≥11 μg/kg/dose exenatide, doses that exceeded the MTD in pregnant rabbits. None of the fetuses from pair-fed dams and from the dams administered 1 μg/kg/dose exenatide had umbilical hernias. Skeletal variations were observed at ≥1 μg/kg/dose, but were also present in similar incidences in both exenatide and pair-fed groups, suggesting these effects were a consequence of compromised maternal condition. The NOEL for developmental toxicity was determined to be 1 μg/kg BID exenatide (4X MRHD).

The effects of exenatide on gestation, parturition, lactation, and maternal behavior were evaluated in mice from implantation through lactation and weaning. The effects on development and fertility of the offspring were also evaluated. Pregnant mice were administered exenatide at doses of 3, 34, and 380 μg/kg twice daily by subcutaneous injection. One high-dose female death that occurred during delivery may have been drug-related. The number of dams delivering stillborn pups was significantly increased in the 760 μg/kg/day group (24%) relative to control (0%). F_0 dams with all pups dying during days 1-4 postpartum was also significantly increased and the number of live births was significantly decreased in the 760 μg/kg/day group. Still birth was significantly increased in the high-dose group. F_1 pups found dead/presumed cannibalized was

significantly increased in the 6 and 760 µg/kg/day groups during days 1-4 postpartum and in the 68 µg/kg/day group during days 8-14 postpartum. All F_1 pup tissues appeared normal at necropsy. Viability index, surviving pups/litter, and pup weight/litter were significantly decreased in the 760 µg/kg/day group relative to control.

Post-weaning body weight was also slightly but significantly decreased in the 760 µg/kg/day F_1 females during precohabitation for mating of the F_1 generation on GD 0 and on GD 18 relative to control. There were no treatment-related effects on corpora lutea, implantations, litter sizes and resorptions in cesarean-sectioned F_1 females. Maternal administration of exenatide at doses as high as 760 µg/kg/day did not affect the day of preputial separation or day of vaginal patency in the F_1 generation mice, learning or memory, mating or fertility, cesarean-sectioning parameters or the incidence of fetal alterations in F_2 generation mice. The F_0 maternal NOAEL was less than 6 µg/kg/day (<1X MRHD) due to mortality at 6 µg/kg/day and greater. The NOAEL for fetal viability and growth was 6 µg/kg/d (1X MRHD) because of reduced preweaning pup body weights at 68 µg/kg/d (9X MRHD) and 760 µg/kg/d (148X MRHD) and increased perinatal mortality and reduced body weight gains postweaning at 760 µg/kg/day.

Local Tolerance

An assessment of local tolerance of exenatide QW at the injection sites was integrated into the repeat-dose toxicology studies, the results of which are summarized above. Briefly, injection sites were generally characterized by swelling, inflammation, and typical foreign body reactions in both rats and monkeys, with increased incidence and severity with increasing dose of microspheres. In monkeys administered exenatide QW for 9 months, swelling with open drainage and/or abscesses were observed in all groups receiving exenatide QW. Microscopically, granulomatous inflammation with foreign body giant cells and fibrosis were observed in rats and monkeys in animals receiving microspheres, with or without exenatide.

Overall, exenatide QW was well tolerated at the injection sites in both rats and monkeys. Injection site findings were mostly characterized as typical inflammation and foreign body reactions that are expected from the injection of PLG microspheres. Injection-site reactions showed partial to complete reversibility during recovery periods. In a 3-month monkey study, a challenge dose of microspheres administered during the recovery period demonstrated that the microspheres do not elicit an immune-mediated reaction.

Safety Margins

The sponsor-generated table below presents the key toxicology studies that have been conducted with exenatide and exenatide QW and corresponding human safety margins. Note, for the rat carcinogenicity study conducted with immediate release exenatide, the clinical safety margin based on AUC should be 9X rather than 37X because the c-cell adenomas observed in high-dose females are felt to be treatment related even though the incidence did not reach statistical significance. Additionally, it should be noted that the sponsor used a human body weight of 100 kg to calculate safety margins based on dose. Although many type 2 diabetic patients are overweight, the use of 100 kg potentially

overestimates the safety margin for patients in the lower end of the body weight range. Therefore, if 70 kg is used, safety margins are slightly lower than presented in the table.

Table 4: Summary of Human Safety Margins Achieved in the Nonclinical Safety Studies with Exenatide and Exenatide QW

Study Type	Species	Duration	Dose [1]	Human Safety Margins	
				Based on Dose [7]	Based on AUC [8]
Exenatide (BYETTA)					
Repeated Dose	Mice	6 Months	760 [2]	18	157
	Rat	28 Days	1000 [2]	48	222
		3 Months	250 [2]	12	37
	Monkey	9 Months	150 [2]	14	183
DART	Mice	As per ICH	6 [3]	0.1	1.2
			760 [4]	18	148
	Rabbit	As per ICH	2 [2]	0.2	4.5
Carcinogenicity	Mice	2 Years	250 [2]	6	23
	Rat	2 Years	250 [5]	12	37
Exenatide QW					
Repeated Dose	Rat	4 Months	9000 [2] [6]	31	27
	Monkey	9 Months	1100 [2]	15	14
Carcinogenicity	Rat	2 Years	300 [6]	10	1.4 – 2.1 [9]
			1000 [6]	33	8 – 10 [9]
			3000 [6]	100	25 – 26 [9]

Ab Neg = antibody negative; AUC = area under the plasma concentration-time curve; DART = developmental and reproductive toxicology.

[1] Dose expressed in mcg/kg/day for BYETTA and mcg/kg/week for exenatide QW, unless otherwise specified.

[2] No-observable-adverse-effect-level (NOAEL).

[3] No-observable-effect-level (NOEL) in the embryo-fetal and peri/postnatal development study.

[4] NOEL in the fertility study.

[5] Highest dose tested of 3 mg/kg/day (BYETTA label includes language about all doses tested).

[6] Dose administered once every other week.

[7] Based on allometric conversion between species, using a human body weight of 100 kg (approximate average body weight of subjects in clinical trials) and a human dose of 2 mg/week. Allometric conversion factor used for mice, rats, rabbit, monkeys and humans were 3, 6, 12, 12 and 44, respectively.

[8] Calculated by dividing the AUC obtained from all animals on Day 1 (BYETTA) or antibody-negative animals at end of study (exenatide QW), by the average AUC (38230 pg·h/mL divided by 7 days to give a daily derived AUC of 5461 pg·h/mL) obtained from antibody-negative patients at Week 30 in study 2993LAR-105 (Study 2993LAR-105, SDS 2.12.2.4) and patients receiving exenatide QW manufactured at the (b) (4) scale or the (b) (4) scale at Week 20 in study 2993LAR-105 Comparability (Study 2993LAR-105 Comparability, SDS 2.5.2.1). See Section 2.6.4 for comparative exposure between exenatide and exenatide QW nonclinical studies.

[9] Range of value represents value for female (first value) and male (second value) animals.

Conclusions: The administration of exenatide QW was generally well tolerated in rats and monkeys. Decreases in body weight gain and food consumption, expected pharmacodynamic effects of exenatide, were observed in rats. Macroscopic and microscopic findings at injection sites observed in rats and monkeys were primarily linked to the presence of PLG microspheres and have been documented for other marketed pharmaceutical products containing these polymers. In a rat carcinogenicity study conducted with exenatide QW, a statistically significant increase thyroid c-cell tumors (adenomas plus carcinomas) was observed at clinically relevant doses. This differs from the rat carcinogenicity findings for immediate release exenatide, in which non-statistically significant increases in c-cell adenomas were observed in females only, although the numbers were above the upper end of the historical background range. Males treated with the high-dose of exenatide QW also had a statistically significant increase in skin fibromas. With the exception of the rat tumorigenicity data, exenatide QW showed a similar or superior toxicity profile compared with immediate release exenatide.

Unresolved toxicology issues: Thyroid c-cell tumors were observed in rats at clinically relevant exposures. The sponsor has not provided data that demonstrate the thyroid c-cell tumors observed in rats are not relevant to human risk. Because the relevance to humans is currently not known, additional nonclinical data will be required to further evaluate these findings with regard to human risk. Please refer to the executive summary for suggested studies.

Recommendations: See the executive summary.

Suggested labeling: See the executive summary.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

Appendix 1

Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection Protocols

Application (IND/NDA) number: IND 67, 092.
Division: DMEP.
CAS#: N/A.
Drug name: Exenatide LAR.
Pharmacological Classification: Synthetic peptide, Antihypoglycemic.
Sponsor/Applicant: Amylin Pharmaceuticals, Inc.
Sponsor/Applicant contact name: Leslie Bennett
Sponsor/Applicant telephone and fax number: 858-642-7169 (T); 858-625-0737 (F).
Date submitted (stamp date): May 24, 2006.
45-day date (from submission stamp date): July 7, 2006.
P/T Reviewer(s): John Colerangle.
Date Review Completed: June 19, 2006.
Date of Exec CAC review: July 5, 2006.
CAC members: Abby Jacobs, John Leighton, Terry Peters, Karen Davis Bruno and Adele Siefried.

Background

The initial IND was submitted to the Division on March 13, 2003. On May 24, 2006, the sponsor submitted an 18-Week biweekly subcutaneous injection (9 Doses) toxicity and toxicokinetic study in rats with a 3-month recovery (Serial # 035 SX) to support the doses selected for the proposed 2-year carcinogenicity study.

A. Summary of Sponsor's Proposal for Review:

Species/strain: Rat/SD.
Number/sex/dose: 70.
Route: Subcutaneous injection.

	Male	**Female**
Doses proposed:	0, 0, 0.3, 0.8, 2.0 mg/kg bi-weekly	0, 0, 0.3, 0.8, 2.0 mg/kg bi-weekly
Basis of dose selection:		
MTD	_____	_____
AUC ratio	X	X
saturation	_____	_____
MFD	_____	_____
PD	_____	_____
other	_____	_____
Kinetics submitted:	**Rodent**	**Human**
pharmacokinetics	X	X
metabolism	X	X
protein binding	No data	No data

The sponsor proposed the use of MTD as a basis of dose selection. The selection of MTD (2 mg/kg bi-weekly) for exenatide LAR was based on toxicity endpoints determined from an 18-week bi-weekly subcutaneous injection toxicity study in rats with a 3-month recovery period. The sponsor had used body weight gain decrement ≥ 10% to establish the MTD. Based on the sponsor's calculations, body weight gain decrements of 7%, 15% and 19% (males) and 10%, 15% and 13% (females) were observed at 1, 3 and 9 mg/kg respectively relative to diluent control. Since the 3 mg/kg dose was associated with 15%

body weight gain decrement in both sexes, the sponsor selected 2 mg/kg as MTD and HD for the proposed carcinogenicity study.

Body Weights and Body Weight Gains After 4 Months Treatment in Sprague Dawley Rats Treated by Every Other Week Injections of Exenatide LAR

Dose Exenatide LAR (mg/kg/dose exenatide)	0 (Diluent)	0 (Control Microspheres)	1	3	9
	Males				
Male Mean Body Weight Day 1 (g)	249	248	248	251	248
Male Mean Body Weight Day 120 (g)	589	608	564	539	523
Male Body Weight Gain (g)	340	360	316	288	275
Male BW Gain % of Diluent Control	100	106	93	85	81
	Females				
Female Mean Body Weight Day 1 (g)	174	174	174	174	173
Female Mean Body Weight Day 120 (g)	318	319	303	296	298
Female Body Weight Gain (g)	144	145	129	122	125
Female BW Gain % of Diluent Control	100	101	90	85	87

BW = body weight

B. Summary of Reviewer's Recommendations to CAC:

	Male	Female
Doses recommended by reviewer:	0, 0, 1, 3, 9 mg/kg bi-weekly	0, 0, 1, 3, 9 mg/kg bi-weekly

C. Basis for Recommendation (experimental details from sponsor's submission):
The reviewer did not concur with the doses selected by the sponsor. The reviewer proposes the use of AUC ratio (≥ 25X MRHD) as the basis for dose selection. The battery of genetic toxicology tests performed were all negative and metabolism is similar in both human and rat.

Estimated Systemic Exposure ($AUC_{0\text{-}168h}$) in SD Rats and Exposure Multiples of MRHD - Males

Dose (mg/kg)	1	3	9
$AUC_{0\text{-}168h}$ (pg.h/ml)	1,486,032	543,049	1,427,100
Exposure Multiple	35X	13X	33X

SD or SR was not provided

Estimated Systemic Exposure ($AUC_{0\text{-}168h}$) in SD Rats and Exposure Multiples of MRHD - Females

Dose (mg/kg)	1	3	9
$AUC_{0\text{-}168h}$ (pg.h/ml)	524,304	1,290,773	1,047,552
Exposure Multiple	12X	30X	24X

SD or SR was not provided

The HD of 9 mg/kg recommended for the carcinogenicity study is 33X MRHD in males and 24X MRHD in females.

Toxicokinetics: Satellite animals will be used for collection of blood samples for TK on Day 1 and at 6 months (cohort 1) and at 2 and 4 months (cohort 2). Blood samples will be collected prior to dosing at pre-dose, 10, 48 and 216 hours post-dose (cohort 1) and at 0.5, 24, 96 and 336 hours post-dose (cohort 2).

Histopathology: Tissues from all animals will be processed for microscopic examination. A peer review of the pathology findings will be performed.

Questions for Executive CAC

1. Does ECAC concur that 9 mg/kg bi-weekly seems to be a reasonable HD for the proposed carcinogenicity study?

2. Since the proposed carcinogenicity study is a 104-Week biweekly subcutaneous injection [52 doses] study in rats and clinical dosing is weekly, does ECAC concur that the carcinogenic potential of exenatide LAR may not be adequately evaluated?

CAC Recommendations

- The committee did not concur with the doses proposed by the sponsor for the 2-year carcinogenicity study.
- The Committee recommends doses of 0 (diluent – 3% carboxymethyl cellulose sodium, 0.10% polysorbate 20, 0.90% NaCl and 96% H_20), 0 (microsphere control), 0.3, 1, and 3.0 mg/kg by bi-weekly subcutaneous injection, based on decreased body weight gain at 3 and 9 mg/kg and injection site effects at 9 mg/kg in the 18 week study.
- The Committee notes that in this case that a single study (in one specie) may be adequate based on other available data.

Appendix 2

Executive Carcinogenicity Assessment Committee Meeting Minutes
Rat Carcinogenicity Study Results (#REST060229R1)

Date of Meeting: January 19, 2010

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Lois Freed, Ph.D., DNP, Alternate Member
Karen Davis-Bruno, Ph.D., DMEP, Team Leader
Tim Hummer, Ph.D., DMEP, Presenting Reviewer

Author of Draft: Tim Hummer

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA #22-200
Drug Name: Bydureon (exenatide QW)
Sponsor: Amylin Pharmaceuticals

Background:
Exenatide is a glucagon-like peptide-1 (GLP-1) receptor agonist that is currently marketed as Byetta for the treatment of type 2 diabetes mellitus. Exenatide mimics several glucoregulatory actions of the endogenous incretin, GLP-1, including glucose-dependent enhancement of insulin synthesis and secretion, inhibition of glucagon secretion, and slowing of gastric emptying. Exenatide QW (Bydureon) is a sustained release formulation of exenatide that was developed by formulating exenatide with PLG microspheres (50:50 mix of poly(D,L-lactide-co-glycolide)), thereby allowing once weekly injections in the clinic rather than the twice daily injections required for Byetta. Amylin previously conducted a rat and mouse carcinogenicity study with immediate release exenatide to support the marketing approval of Byetta. The results of these studies indicated a slight increase in benign thyroid c-cell adenomas at 250 µg/kg/d in female rats only. The current study under review evaluated the carcinogenic potential of exenatide when formulated with PLG microspheres in rats with dosing every 2 weeks. The proposed clinical dosing is once weekly. Amylin has submitted the data from all three carcinogenicity studies to characterize the carcinogenic potential of exenatide QW and to support the marketing application for Bydureon.

GLP-1 receptor agonists as a class have shown a risk for the development of thyroid c-cell tumors in both rats and mice. Based on the available information regarding the carcinogenic potential of GLP-1 receptor agonists, the data indicate that long-acting GLP-1 agonists or formulations that allow a steady state exposure to be reached (in contrast to immediate release exenatide) have a higher risk for inducing thyroid c-cell tumors with a lower clinical exposure margin. This effect, at least in part, is thought to be due to the continuous exposure of c-cells to exenatide versus a pulsatile exposure observed with short-lived GLP-1 receptor agonists.

Rat Carcinogenicity Study:

The sponsor conducted a 2-year bioassay in Sprague-Dawley rats with the sustained release formulation of exenatide (exenatide QW). Rats (70/sex/group) were administered exenatide QW (0.3, 1, or 3 mg/kg), diluent vehicle, or diluent plus microspheres (without exenatide) once every 2 weeks by subcutaneous injection. The study was found to be adequately designed and conducted. Based on the review of the study report, neoplastic findings believed to be related to exenatide QW included thyroid c-cell tumors (adenomas plus carcinomas) at all doses in males and females (the value for low-dose males lacked statistical significance but was greater than the upper historical control range [the specific vehicle used for the historical control range was not provided]) and skin fibromas in high-dose males. Systemic exposures at the low-, mid-, and high-dose levels were approximately 2-, 9-, and 26-fold higher than the maximum anticipated clinical exposure, respectively. At the injection site for high-dose males, the amount of drug injected was approximately 10% less than the amount of exenatide administered clinically (~1.8 mg versus 2 mg), based on an average male rat weight of 0.6 kg. A summary of tumor incidence observed in males and females is shown in the tables below.

Summary of Tumor Incidence in Males

Dose (mg/kg)	Diluent Control	Microsphere Control	0.3	1.0	3.0	Historical Control
Thyroid, c-cell hyperplasia	15/70 (21%)	10/70 (14%)	23/70 (33%)	19/70 (27%)	23/70 (33%)	NP
c-cell adenoma	9/70 (14%) **p<0.001†**	9/70 (14%) **p<0.001†**	**20/70** (29%) p=0.038	**32/70** (46%) **p<0.001***	**33/70** (47%) **p<0.001***	8.8% (1.9-15.4%)
c-cell carcinoma	0/70 (0%) p=0.164	1/70 (1.4%) p=0.237	2/70 (2.9%) p=0.268	**5/70** (7.1%) **p=0.036***	**3/70** (4.3%) p=0.133	0.6% (0-1.7%)
c-cell adenoma + carcinoma	9/70 (13%) **p<0.001†**	10/70 (14%) **p<0.001†**	**22/70** (31%) p=0.019	**34/70** (49%) **p<0.001***	**35/70** (50%) **p<0.001***	NP
Skin, subcutis, Fibroma	0/70 (0%) **p=0.004†**	3/70 (4.3%) p=0.034	4/70 (5.7%) p=0.069	2/70 (2.9%) p=0.273	**8/70** (11%) **p=0.004***	2.2% (0-5%)

Historical control data from 11 studies; NP = not provided.
*Statistically significant by pair-wise analysis compared with diluent control.
†Statistically significant for dose response.

Summary of Tumor Incidence in Females

Dose (mg/kg)	Diluent Control	Microsphere Control	0.3	1.0	3.0	Historical Control
Thyroid, c-cell hyperplasia	13/70 (19%)	12/70 (17%)	31/70 (44%)	29/70 (41%)	40/70 (57%)	NP
c-cell adenoma	5/70 (7.1%) p=0.024	9/70 (13%) p=0.072	**22/70** (31%) **p<0.001***	**19/70** (27%) **p=0.003***	**21/70** (30%) **p<0.001***	8.1% (2.0-11.4%)
c-cell carcinoma	0/70 (0%) p=0.014	1/70 (1.4%) p=0.042	1/70 (1.4%) p=0.533	1/70 (1.4%) p=0.517	**4/70** (5.7%) p=0.064	0.6% (0-4.0%)
c-cell adenoma + carcinoma	5/70 (7%) **p=0.003†**	10/70 (14%) p=0.016	23/70 (33%) **p<0.001***	20/70 (29%) **p=0.002***	25/70 (36%) **p<0.001***	NP

Historical control data from 11 studies; NP = not provided.
*Statistically significant by pair-wise analysis compared with diluent control.
†Statistically significant for dose response.

Executive CAC Conclusions:

- The Committee agreed that the study was valid.
- The Committee found that the study was positive for drug-related thyroid c-cell tumors (adenomas plus carcinomas) in males and females at all doses tested and for fibromas of the skin in high dose males.
- The Committee noted that a mouse carcinogenicity study with exenatide QW was not warranted.

David Jacobson Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DMEP
KDavisBruno, DMEP
THummer, DMEP
JBishai, DMEP
/ASeifried, OND IO

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22200	ORIG-1	AMYLIN PHARMACEUTICALS INC	EXENATIDE LAR

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/s/

ADELE S SEIFRIED
02/01/2010

DAVID JACOBSON KRAM
02/01/2010

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22200	ORIG-1	AMYLIN PHARMACEUTICALS INC	EXENATIDE LAR

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/s/

BRIAN T HUMMER
02/22/2010

KAREN L DAVIS BRUNO
02/22/2010
see pharm/tox supervisor memo

NDA Number: 22-200
Drug Name: BYDUREON (exenatide once weekly)
Stamp Date: 05 May 2009
45-Day Meeting Date: 15 June 2009
Reviewing Toxicologist: B. Timothy Hummer, PhD, DABT

45 Day Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

ITEM	YES	NO	COMMENT
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	X		
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	X		
3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	X		
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA (genotox, reprotox, adequate duration of chronic tox, carcinogenicity).	X		To bridge to the BYETTA nonclinical program, 2- and 4- month studies in rats, and 3- and 9-month studies in monkeys were conducted. Additionally, in vitro genotoxicity studies (Ames and chrom ab) and a carcinogenicity study in rats were conducted. An audited draft report has been submitted. With regard to the draft report, all information appears to be present with the exception of TK and Ab data and the SAS data sets. Per a pre-NDA meeting agreement, the sponsor stated that they will be submitting the final report for the carcinogenicity study post marketing. However, because the incidence of C-cell adenomas increased and progressed to C-cell carcinomas in the rat carc study with BYDUREON compared with the rat study conducted with BYETTA, the sponsor will be asked in the 74-day letter to submit the final report within a specified amount of time from the filing date rather than wait until after marketing. Two local irritation studies in rabbits were submitted under IND 67,092 and previously reviewed by Dr. John Colerangle. These reports were not submitted as part of the NDA submission.

ITEM	YES	NO	COMMENT
5) Were the studies adequately designed (ie., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?	X		
6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (ie., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	X		Drug substance for exenatide QW may be produced by (b) (4) . The substance made by Bachem and Mallinckrodt are already approved suppliers for BYETTA. The material made by (b) (4) Lonza, was tested in in vitro genotox studies, an 8-week rat toxicity study, and a carcinogenicity study in rats. The test article tested in the toxicology studies was formulated in the same manner (e.g., same ingredients and same manufacturer) as the clinical material. Sponsor plans to switch manufacturing sites, but this will not affect the ingredients to be used for the clinical formulation.
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	X		
8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m2 or comparative serum/plasma AUC levels?	X		Draft labeling was submitted. No information was added regarding increased C-cell tumors, including carcinomas, observed in the rat carc study with BYDUREON. This information will need to be added. For the carcinogenicity study, AUC data are not yet available, but the Division will request that the final study report be submitted before a marketing decision is made. Additionally, exposure should be similar to that achieved in the 4-month rat study, and dose extrapolation based on body surface area can also be calculated.
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	X		
10) Reasons for refusal to file:			

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/s/

Brian T Hummer
6/18/2009 02:17:00 PM
PHARMACOLOGIST

Karen Davis-Bruno
6/19/2009 10:10:46 AM
PHARMACOLOGIST
P/T filing memo