

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

201849Orig1s000

PHARMACOLOGY REVIEW(S)

Signed off in DARRTS on 12/9/2014



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: 201-849
SERIAL NUMBER: 0018
DATE RECEIVED BY CENTER: 8/8/2014. Note that initially this original application was submitted on 10/30/2010, but it was not fileable; Sponsor resubmitted the application on 11/30/2011, the pharmacology /toxicology reviewed the submitted studies; found to be adequate and recommended approval. However, Clinical Pharmacology found deficiencies and this application has been resubmitted on 8/8/2014.

PRODUCT: Glucagon for injection
INTENDED CLINICAL POPULATION: Indicated for use as a diagnostic agent to temporarily inhibit movement of the gastrointestinal tract.

SPONSOR: Fresinus Kabi USA, LLC, Schaumburg, IL. Previously, it was APP Pharmaceuticals LLC, Schaumburg, IL.

DOCUMENTS REVIEWED: e-CTD submission (#0018).
REVIEW DIVISION: Division of Metabolism and Endocrinology Products
PHARM/TOX REVIEWER: Indra Antonipillai
PHARM/TOX SUPERVISOR: Karen Davis Bruno
DIVISION DIRECTOR: Jean Marc Guettier
PROJECT MANAGER: Elisabeth Hanan
Date of review submission to DARRTS: 12/9/14

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 201-849

Review number: 2

Sequence number/date/type of submission: 8/8/14 (sponsor's has provided response to the FDA's Complete Response letter dated September 27, 2012). This is an eCTD submission #0018. It is a 505(b)(2) application. Sponsor refers to the previous NDA of glucagon that has already been approved (NDA 20-918/ GlucaGen of Novo Nordisk).

Note that originally this application was submitted on 10/30/2010, we sent a refuse to file (RFT) letter to the Firm on 12/3/10 due to pharmacology/ toxicology deficiencies. Sponsor resubmitted the application on 11/30/2011. The pharmacology/ toxicology reviewed the submitted studies and found these to be adequate for the proposed indication and recommended this drug product for approval, see the review signed off in DARRTS on 7/26/12.

However, the Division sent a complete response to the company on 9/27/12 that it cannot be approved in the present form due to Clinical Pharmacology deficiencies; the Company has now resubmitted their application for the second time with the new repeated bioequivalence study (study # GLUC-002-CP1) and has removed a manufacturing facility that had deficiencies.

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Fresinus Kabi USA, LLC, Schaumurg. IL. Previous sponsor on this application was AAP Pharmaceuticals LLC, Schaumburg, IL.

Manufacturer for drug substance: The manufacturer of Glucagon drug substance is (b) (4)
The US office of API manufacturer is (b) (4)

Reviewer name: Indra Antonipillai

Division name: Division of Metabolism and Endocrinology Products (DMEP).

Review completion date: 12/1/2014

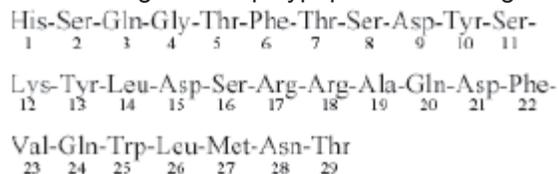
Drug:

Trade name: Glucagon for injection, 1 mg (1 IU)/ ml. Fresinus Kabi USA (previously APP Pharmaceuticals) is currently seeking intramuscular (IM) and intravenous (IV) routes of administration for their drug product to be used as a diagnostic aid during radiologic examinations.

Generic name: Glucagon

Code name: N/A

Chemical name: It is a single chain polypeptide containing 29 amino-acid residues



CAS registry number: 16941-32-5

Molecular formula/molecular weight: C₁₅₃H₂₂₅N₄₃O₄₉ / 3483

Relevant INDs/NDAs/DMFs: NDA 20-918 (GlucaGen, Novo Nordisk), NDA 20-928 (Glucagon, Eli Lilly). DMF # (b) (4) ((b) (4)) the manufacturer of the current drug substance).

Drug class: Peptide hormone. The current drug product is a synthetic glucagon.

Intended clinical population: The drug is intended to be used by intramuscular and intravenous route of administration as a diagnostic aid during radiologic exams to temporarily inhibit movement of the gastrointestinal tract. (b) (4)

(b) (4) they are asking for only one indication as a diagnostic aid during radiologic exam, as indicated above.

Clinical formulation: Glucagon for Injection, 1 mg / ml vial is supplied as a sterile lyophilized white powder in a 3 ml vial. (b) (4) Glucagon is soluble in water and reconstitutes in water to a pH range of 2.5-3.5. Glucagon as the hydrochloride salt, 1 mg dose corresponds to 1 IU.

Fresenius Kabi USA, LLC (FK USA) is providing this section to update information previously provided in this NDA as well as to remove reference to the Grand Island, NY manufacturing facility. The Grand Island, NY facility owned by FK USA will not be used for any release or stability testing of the drug substance or drug product. Additionally, the company name APP Pharmaceuticals, LLC has been revised to reflect the transfer of ownership to Fresenius Kabi USA, LLC.

The full address of the facility that manufactured the exhibit batches and will manufacture commercial Glucagon for Injection is shown below:

Production Site:	Fresenius Kabi USA, LLC
Address:	2020 Ruby Street Melrose Park, Illinois 60160
Name of Contact Person:	Cristina Fernandes, Director of QA
Telephone Number for Contact Person:	(708) 450-7527
Fax Number for Contact Person:	(708) 450-7525
Email Address:	cristina.fernandes@fresenius-kabi.com
Date of FDA's last inspection:	25 September 2013 to 11 December 2013

The testing sites for the glucagon for injection are described below:

Table 1. Fresenius Kabi USA, LLC Testing Sites for Glucagon for Injection

Tests	Exhibit Batches (Release and Stability)	Commercial Batches (Release and Stability)
<u>In-process Test</u> (b) (4)	Fresenius Kabi USA, LLC (FK USA) Quality Control Laboratories 2020 North Ruby Street Melrose Park, Illinois 60160 Establishment ID: 1450022 Contact: Cristina Fernandes, QA Director Phone: (708) 450-7527 Fax: (708) 450-7525 Email: cristina.fernandes@fresenius-kabi.com	FK USA Quality Control Laboratories 2020 North Ruby Street Melrose Park, Illinois 60160 Establishment ID: 1450022 Contact: Cristina Fernandes, QA Director Phone: (708) 450-7527 Fax: (708) 450-7525 Email: cristina.fernandes@fresenius-kabi.com
<u>Finished Product</u> Chemistry/Analytical	FK USA Product Development (PD) and Quality Control Laboratories, and Corporate Stability 2045 North Cornell Avenue Melrose Park, IL 60160 Contact: Sharon W. Ayd, Ph.D., MBA Global Vice President of I&D Phone: (847) 983-7023 Fax: (847) 983-7054 Email: sharon.ayd@fresenius-kabi.com	FK USA Quality Control Laboratories 2045 North Cornell Avenue Melrose Park, IL 60160 Contact: Robert Jacobus, Director Quality Control Phone: (708) 486-2924 Fax: (708) 486-2927 Email: Robert.jacobus@fresenius-kabi.com or (b) (4)

Sponsor states that the inactive ingredients of the proposed drug product are the same as that of the reference drug, GlucaGen®, held by Novo Nordisk (distributed by Bedford Laboratories).

The side by-side comparison of their drug vs the reference drug (RD) is provided below. Note that this is from the Pharmacology/toxicology review signed off in DARRTS on 7/26/2012:

1.12.12.5 Side-by-Side Comparison

(b) (4)

(b) (4)

Table provided lists information

(b) (4)

(b) (4)

Table 1. Side-by-Side Comparison of the Reference Listed and Proposed Drugs

	Reference Listed Drug	Proposed Drug Product
Name	GlucaGen®	Glucagon for Injection
Conditions of Use (Indications)	It is indicated for the treatment of severe hypoglycemic reactions which may occur in patients with diabetes treated with insulin, or for use as a diagnostic aid during radiologic examinations of the gastrointestinal system	It is indicated for use as a diagnostic aid during radiologic examinations of the gastrointestinal system
Dosage Form	Sterile lyophilized drug product	Sterile lyophilized drug product
Route of Administration	Subcutaneous (sc), intramuscular (im), or intravenous (iv) injection.	intramuscular (im) or intravenous (iv) injection.
Active Ingredient	Glucagon	Glucagon
Strength	1 mg	1 mg
Excipient (amount/1-mg vial) ¹		
Lactose Monohydrate	107 mg	107 mg
Hydrochloric Acid	As required for pH adjustment	As required for pH adjustment
Sodium Hydroxide	As required for pH adjustment	As required for pH adjustment
(b) (4)		
Bioequivalence	Refer to SECTION 5.3.1	Refer to SECTION 5.3.1
Labeling	Refer to SECTION 1.14	Refer to SECTION 1.14

¹ APP's excipients are all compendial grade materials.

(b) (4)

Route of administration: Intravenous and intramuscular.

Disclaimer: The tabular and graphical information is from sponsor's submission unless stated otherwise.

Studies reviewed within this submission: No new pharmacology /toxicology studies are submitted in the current submission dated 8/8/14, as these studies were submitted previously on 11/30/2011 and have been reviewed by us (see DARRTs 7/26/2012, recommending approval for Pharm/Tox). However in the current submission, sponsor has submitted the bioequivalent study of their glucagon to that of the reference drug GlucaGen (Novo Nordisk) following SC administration in healthy subjects under fasted conditions. In the last submission (dated 11/30/2011) the Firm had conducted the bioequivalent study by intra-muscular (IM) route of administration.

2.6.2 PHARMACOLOGY

Glucagon is a single chain polypeptide hormone containing 29 amino acids. Glucagon in a recombinant form has been approved before, as NDA 20-918 (Novo Nordisk) and NDA 20-928 (Eli Lilly) for both above indications, i.e. for the treatment of severe hypoglycemic reaction which may occur in patients with diabetes treated with insulin, as well as for a diagnostic aid during radiologic exam, as 1 mg/ml injection.

Fresinus Kabi Glucagon for injection 1 mg (1 IU) is a synthetic form of glucagon; it is recommended to be used for the intravenous and intra-muscular administration as a gastrointestinal motility inhibitor. This is a 505(b)(2) application, reference drug (RD) is NDA 20-918 (GlucaGen, Novo Nordisk).

Brief history: As indicated earlier, initially this application was submitted on 10/30/2010; it was not fileable (due to Pharmacology /toxicology deficiencies), then it was resubmitted on 11/30/2011, in which sponsor provided us the requested non-clinical toxicology studies. These studies included 28-day toxicity/TK study in rats and two geno-toxicity studies (Ames and chromosomal aberration assays); these were reviewed by us and were considered adequate. The pharmacology /toxicology recommended this application for approval, see the review signed off in DARRTS on 7/26/12.

However, the pivotal bioavailability study was not acceptable by the Clinical Pharmacology reviewer, as the glucose measurements in the study were not reliable. We issued a Complete Response letter to them on 9/27/2012. The current Class 2 Resubmission of the NDA is submitted to the Agency on 8/8/2014 with the repeated bioequivalence study and they have removed a manufacturing facility that had deficiencies.

The present sponsor of this drug product is Fresinus Kabi (FK) USA, LLC, Schaumburg, IL. Previously the holder of this NDA was APP Pharmaceuticals LLC, Schaumburg, IL. The current drug product is indicated only for use as a diagnostic aid (and not to treat severe hypo-glycemia, because it is not packaged with a syringe and diluent necessary for rapid preparation and administration during emergency outside of a health care facility).

Note that the Established Pharmacologic Class (EPC) "gastrointestinal motility inhibitor" is not currently included in the EPC e-list (it is a list of the related pharmacologic class MOA, PE, and CS indexing terms for use in SPL). This information was conveyed to Dr. Paul Brown, who communicated to us that the Labeling for GlucaGen currently is listed as a "anti-hypoglycemic agent and a gastrointestinal motility inhibitor". Both terms have been requested as separate EPCs, however these are not included in the current e-list, but these would be included in the next e-list.

Note that since the two terms will be separate EPCs, labeling of a glucagon product could contain one or the other term or both terms, as appropriate. Since in the current application, KB's glucagon is only indicated for inhibition of gastrointestinal motility, the "Indications and Usage section" of the label highlights it as "a gastrointestinal motility inhibitor".

Clinical pharmacology-bioequivalent studies in human subjects

In the current submission, the following bioequivalent study was conducted in healthy subjects by SC route of administration (study GLUC-002-CP1).

The primary objective of this study was to ascertain the pharmacokinetics (PK) and pharmacodynamics (PD) bioequivalence of an SC injection of 1 mg (1 IU) of Glucagon for Injection (Fresenius Kabi USA) in comparison to the reference product, GlucaGen® (Bedford Laboratories), 1 mg (1 IU), SC in healthy adult subjects.

Note that in a last submission (dated 11/30/11), they had conducted the above PK/PD study via IM route of administration.

Sponsor states that “Individual unadjusted and adjusted glucagon results are presented in Appendix 1.1. Tables 1 to 4 present the unadjusted plasma concentrations for each formulation and each administration (replicate), while Tables 5 to 8 present the unadjusted glucagon PK parameters. Results are also presented for the adjusted concentrations (Tables 9-12) and resulting PK parameters (Tables 13-16)”.

Results:

Note that the AUC exposures shown in the Table below are higher with the FK’s glucagon by about 24%, compared to the reference drug GlucaGen

In-Text Table 1 summarizes the least squares means, ratios of means and 90% confidence intervals (CIs) of ln-transformed baseline-adjusted glucagon data (Subject No. 7 excluded) for Test Product A versus Reference Product B.

In-Text Table 1: Fasted baseline adjusted glucagon PK parameter values (Subject No. 7 excluded)

Parameter	Least-Square Means		Ratio	90% Confidence Intervals	
	Test	Reference		Lower CI	Upper CI
Cmax	3477.69	3247.81	107.08	94.17	121.76
AUCt	2780.90	2189.55	127.01	114.19	141.27
AUCinf	2807.89	2263.60	124.05	112.15	137.20

In-Text Table 2 summarizes the potency corrected ratios of means and the 90% confidence intervals of ln-transformed baseline-adjusted glucagon data (Subject No.7 excluded) for Test Product A versus Reference Product B.

In-Text Table 2: Potency corrected ratio and 90% CIs for glucagon (Subject No. 7 excluded)

Parameter	Ratio	90% Confidence Intervals	
		Lower CI	Upper CI
Cmax	94.30	82.93	107.23
AUCt	111.85	100.56	124.41
AUCinf	109.24	98.76	120.82

In-Text Table 3 summarizes the least squares means, the ratios of means and the 90% confidence intervals of the ln-transformed baseline-adjusted glucose data (Subject No.7 excluded) for Test Product A versus Reference Product B. Results including Subject No. 7 were similar and the conclusions were the same (*i.e.*, also met BE criteria).

In-Text Table 3: Fasted baseline adjusted PD glucose parameter values (Subject No. 7 excluded)

Parameter	Least-Square Means		Ratio	90% Confidence Intervals	
	Test	Reference		Lower CI	Upper CI
Cmax	941.47	991.65	94.94	86.22	104.54
AUC0-2	921.54	927.54	99.35	85.64	115.26
AUC0-4	979.16	958.87	102.12	88.04	118.45

Sponsor's conclusions:

The PK results (glucagon) of this study indicate that BE criteria were met when 1 mg (1 IU/mL) synthetic glucagon for injection (Fresenius Kabi USA) and 1 mg (1 IU/mL) rDNA origin glucagon for injection (Bedford Laboratories) were administered by the SC route, and the potency of the two different formulations were taken into consideration and an outlying subject's data (Subject No. 7) was removed.

The PD results (glucose) of this study also indicate that BE criteria were met when 1 mg (1 IU/mL) synthetic glucagon for injection (Fresenius Kabi USA) and 1 mg (1 IU/mL) rDNA origin glucagon for injection (Bedford Laboratories) were administered by the SC route.

Therefore, these two products are considered to be bioequivalent *in vivo* when given through the SC route.

Reviewer's summary: Note that the above bioequivalent study will be reviewed by the clinical Pharmacology reviewer, but in the current submission using SC route of administration, the FK's glucagon appears to have 20-24% higher exposures vs the RD GlucaGen. It is not clear what factors contributed to the higher drug activity in the FK's Glucagon now (vs the previous bioequivalent study, where the IM route of administration showed that the two drugs were bioequivalent *i.e.* the current FK glucagon and the reference drug GlucaGen).

2.6.6 TOXICOLOGY

A 28-day rat toxicity study and gene-toxicity studies (Ames and Chromosome aberration assay in CHO cells) have been conducted under this NDA application, in each study comparing its glucagon product to the reference drug Glucagen

2.6.6.3 Repeat Dose Toxicity Studies

Following is a brief summary of the 28-day intra-muscular toxicity study in rats with the current drug product. For more details, please see the review signed off in DARRTS on 7/26/2012

Brief overview of nonclinical findings: The FK glucagon for injection drug product is manufactured (b) (4) vs the approved glucagon drug products (NDA 20-918, Novo Nordisk GlucaGen, and NDA 20-928, Eli Lilly) which (b) (4)

In a 28-day intramuscular (IM) toxicity study in rats, doses of 0, 1, and 5 mg/kg/day of the current drug product (Frezinus Kabi glucagon for injection) were administered to rats. The 4th group of rats were similarly administered the reference drug GlucaGen (5 mg/kg/day) for comparison. The exposures of the drug in general were similar with both drugs, on day-27 AUC exposures in males were 122, 502, 454 ng.hr/ml at 1, 5 mg/kg/day with the current glucagon, and 5 mg/kg/day of reference drug GlucaGen respectively; in females AUC values were 99, 557, 511 ng.hr/ml respectively. The subtle toxicity was noted with the current drug product, not noted with the reference drug (RD). These included lower body weight gains in males on days 22-29 at a high dose of 5 mg/kg/day (no effects on body weights/weight gains in females were noted). In male rats, absolute liver (10.9, 12.7, 13.6*, 11.9 g respectively), and in female rats, absolute kidney (1.5, 1.7*, 1.8*, 1.6 respectively) weights were increased by 25% and 20% respectively, but not with the RD. The target organs of toxicity may be heart in female rats (minimal to mild mineralization in 2/8 rats at a HD, not noted with the RD or controls), and kidney, in the male rats (bilateral chronic progressive nephropathy, minimal to mild in 3/8 males vs 0/8 with the RD and controls).

The total impurities that were tested in this 28-day toxicity study were up to (b) (4) % with the current drug product (FK glucagon for injection), and up to (b) (4) % with the reference drug (GlucaGen). The NOAEL or tolerated doses of the drug in this 28-day intramuscular toxicity study in rats could not be established, as histopathology findings in the heart (in females) and kidney (in males) were observed at a HD of 5 mg/kg/day. The lower dose of 1 mg/kg/day was not examined for histopathology findings. The sponsor does not consider any of these findings significant.

Thus the above 28-day toxicity study shows that the products are comparable. However differences are noted between the two glucagons (current glucagon and the RD GlucaGen) as noted above.

Note that these are daily IM injections, which represent significantly greater exposure in rats compared to the clinical use, as a diagnostic agent. The sponsor NOAEL of this drug was 5 mg/kg/day (or 30 mg/m²/day), note that this NOAEL (<5 mg/kg/day) provides the safety margin of <24 X in human subjects (the maximal recommended human dose is 2 mg/day or 1.23 mg/m²/day), based on body surface area.

Note that although no NOAEL could be established by us in the above toxicity study, histopathology findings are noted in one sex (e.g. heart findings in female rats: kidney findings in male rats), following daily repeated treatment in euglycemic healthy animals, the clinical indication is for single dose as a diagnostic agent, and therefore the heart or kidney findings are unlikely to have clinical significance, if the product is used as intended.

2.6.6.4 Genetic toxicology:

Following is a brief summary of gene-toxicity studies (Ames and chromosome aberration assay with KB glucagon), for more details, please see the review signed off in DARRTS on 7/26/2012

The **in vitro Ames assay** was negative for both the current glucagon (containing (b)(4) mcg of (b)(4)) and the reference drug GlucaGen; both compounds were tested at doses up to 2700 mcg/plate. The total combined impurities present in the current glucagon were (b)(4)% to (b)(4)% (lot # C108-002) used in the Ames assay, and in the reference drug GlucaGen were (b)(4)% (lot # AW60180).

The **'in vitro chromosome aberration assay'**, Fresnus Kabi's glucagon was positive in CHO cells at concentrations of 260 to 370 mcg/ml without S-9 activation (4 and 20-hour treatments); it was negative in the presence of S-9 metabolic activation. In contrast, the reference drug GlucaGen was negative in the above assay in CHO cells at concentrations of 370 mcg/ml with or without S-9 activation (at 4 and 20-hour treatments). In this assay in CHO cells, a lot number C109-002 of current glucagon was used, which had higher total impurities of (b)(4)% vs the reference drug GlucaGen (lot AW60180) which had total impurities of (b)(4)%.

Summary: The label for the FK glucagon will need to indicate the positive structural aberrations with this synthetic drug product as well as the clastogenicity labeling present in the approved recombinant-human glucagon.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

There are no outstanding pharmacology/toxicology issues and no new studies have been submitted in the present submission (dated 8/8/2014).

A human bioequivalent study provided in the current submission by SC route of administration results in higher drug exposures with FK glucagon (by about 24%) than the reference drug GlucaGen. The concern was whether the higher exposures in humans will pose increased toxicity.

In a preclinical 28-day toxicity study in rats with FK's glucagon, a NOAEL of <5 mg/kg/day (or 30 mg/m²/day), provides the safety margin of <24-fold in rats to humans, based on body surface area (the maximal recommended human dose is 2 mg/day or 1.23 mg/m²/day).

If there is 24% higher exposure with the FK glucagon in humans by SC administration, the safety margin at NOAEL of <5 mg/kg/day (or 30 mg/m²/day) in rats to humans will be 20-fold, based on body surface area (assuming the maximal recommended human dose will be 24% higher, i.e. 2.48 mg/day or 1.53 mg/m²/day). Thus there is sufficient safety margin in rats to humans, even with the higher exposures via SC administration of the current glucagon drug product.

In summary: From the pharmacology / toxicology point of view, this application is recommended for approval as stated in the previous review signed off in DARRTs on 7/26/2012, pending labeling changes, see below. Note that these labeling comments were not communicated to the sponsor in 2012, since the application was not approvable at that time.

Labeling Review: The pharmacology toxicology labeling in general is similar to the approved GlucaGen label (rDNA origin, Novo Nordisk label). In the current application, the submitted PLR label is reviewed and reviewer's recommended changes are stated below in bold letters:

Following is sponsor's proposed label (from 8/8/2014 submission):

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category B. Reproduction studies were performed in rats and rabbits at glucagon doses of 0.4, 2, and 10 mg/kg. These doses represent exposures of up to 100 and 200 times the human dose based on mg/m² for rats and rabbits, respectively, and revealed no evidence of harm to the fetus. There are, however, no adequate and well-controlled studies in pregnant women. Glucagon does not cross the human placenta barrier.

8.3 Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when glucagon is administered to a nursing woman. No clinical studies have been performed in nursing mothers, however, glucagon is a peptide and intact glucagon is not absorbed from the GI tract. Therefore, even if the infant ingested glucagon it would be unlikely to have any effect on the infant. Additionally, glucagon has a short plasma half-life thus limiting amounts available to the child.

Reviewer's recommended changes:

8.1 Pregnancy

Pregnancy Category B. Reproduction studies were performed in rats and rabbits at **GlucaGen (recombinant)** doses of 0.4, 2, and 10 mg/kg. These doses represent exposures of up to 100 and 200 times the human dose based on mg/m² for rats and rabbits, respectively, and revealed no evidence of harm to the fetus. There are, however, no adequate and well-controlled studies in pregnant women. Glucagon does not cross the human placenta barrier.

(b) (4)



(b) (4)

Reviewer's recommended changes:

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long term studies in animals to evaluate carcinogenic potential have not been performed.

(b) (4)

Synthetic glucagon was negative in the bacterial reverse mutation assay. The clastogenic potential of synthetic glucagon in the Chinese Hamster Ovary (CHO) assay was (b) (4) **positive in the absence of metabolic activation** (b) (4)

(b) (4) **Doses** of glucagon (100 and 200 mg/kg) of both pancreatic and recombinant origins gave (b) (4) slightly higher incidence of micronucleus formation in male mice but there was no effect in females. The weight of evidence indicates that synthetic and recombinant glucagon are not different (b) (4) and do not pose a genotoxic risk to humans.

Glucagon (rDNA or synthetic origin) was not tested in animal fertility studies. Studies in rats have shown that glucagon does not cause impaired fertility.

(b) (4)

Reviewer's recommended changes

(b) (4)

Justification for the changes: The animal pharmacology and/or toxicology do not have any added value in the label. Sponsor has already indicated some of the pharmacology information (in the first paragraph) in the clinical pharmacology section under 12.1 to 12.3 sections, therefore this section should be deleted from the label. In the second and third paragraph sponsor refers to the published literature, and again has no added value.

Recommendation: From the preclinical standpoint, approval of this application is recommended, pending labeling changes.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____

Concurrence Yes ___ No ___

cc: IND Arch
HFD-510
HFD-510/davisbruno/antonipillai/Hanan E/Yanoff.
Review code: AP
File name: nda 201849-2014 (glucagon, (b) (4))

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

/s/

INDRA ANTONIPILLAI

12/09/2014

From the Pharm/Tox point of view, approval of this application is recommended, pending labeling changes.

KAREN L DAVIS BRUNO

12/09/2014

Signed off in DARRTS on 7/26/12



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: 201-849
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 11/30/11 (initially this original application was submitted on 9/28/2007)
PRODUCT: Glucagon for injection
INTENDED CLINICAL POPULATION: Indicated for use as a diagnostic agent to temporarily inhibit movement of the gastrointestinal tract

SPONSOR: APP Pharmaceuticals LLC, Schaumburg, IL.

DOCUMENTS REVIEWED: e-CTD submission.
REVIEW DIVISION: Division of Metabolsim and Endocrinology Products

PHARM/TOX REVIEWER: Indra Antonipillai
PHARM/TOX SUPERVISOR: Karen Davis Bruno
DIVISION DIRECTOR: Mary Parks
PROJECT MANAGER: Meghna Jairath

Date of review submission to DARRTS: 7/26/12

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EXECUTIVE SUMMARY

I. Recommendations

- A. **Recommendation on approvability:** Pharmacology recommends approval of this application.
- B. **Recommendation for Nonclinical Studies:** No additional preclinical studies are required for this drug product. The sponsor in the current resubmission application has provided a 4-week toxicity study in rats and two in vitro geno-toxicity studies with their proposed drug product. The submitted studies are adequate for proposed use of a synthetic glucagon and provide a bridge to the Agency's prior approval decision of recombinant glucagon.
- C. **Recommendations on labeling:** See the draft label.

II. Summary of non-clinical findings

- A. **Brief overview of nonclinical findings:** The current glucagon for injection drug product is manufactured (b) (4) vs the approved glucagon drug products (NDA 20-918, Novo Nordisk GlucaGen, and NDA 20-928, Eli Lilly) which (b) (4). In a 28-day intramuscular (IM) toxicity study in rats, doses of 0, 1, and 5 mg/kg/day of the current drug product (APP glucagon for injection) were administered to rats. The 4th group of rats were similarly administered the reference drug GlucaGen (5 mg/kg/day) for comparison. The exposures of the drug in general were similar with both drugs, on day-27 AUC exposures in males were 122, 502, 454 ng.hr/ml at 1, 5 mg/kg/day of APP glucagon, and 5 mg/kg/day of reference drug GlucaGen respectively; in females AUC values were 99, 557, 511 ng.hr/ml respectively. The subtle toxicity was noted with the current drug product, not noted with the reference drug (RD). These included lower body weight gains in males on days 22-29 at a high dose of 5 mg/kg/day (no effects on body weights/weight gains in females were noted). In male rats, absolute liver (10.9, 12.7, 13.6*, 11.9 g respectively), and in female rats, absolute kidney (1.5, 1.7*, 1.8*, 1.6 respectively) weights were increased by 25% and 20% respectively, but not with the RD. The target organs of toxicity may be heart in female rats (minimal to mild mineralization in 2/8 rats at a HD, not noted with the RD or controls), and kidney, in the male rats (bilateral chronic progressive nephropathy, minimal to mild in 3/8 males vs 0/8 with the RD and controls). Note that total impurities that were tested in this 28-day toxicity study were up to (b) (4)% with the current drug product (glucagon for injection), and up to (b) (4)% with the reference drug (GlucaGen). The NOAEL or tolerated doses of the drug in this 28-day intramuscular toxicity study in rats could not be established, as histopathology findings in the heart (in females) and kidney (in males) were observed at a HD of 5 mg/kg/day. The lower dose of 1 mg/kg/day was not examined for histopathology findings. The sponsor does not consider any of these findings significant. This NOAEL of <5 mg/kg/day (or 30 mg/m²/day) provides the safety margin of <24 X in human subjects (the maximal recommended human dose is 2 mg/day or 1.23 mg/m²/day), based on body surface area. Although there is no NOAEL established, and histopathology findings are noted in one sex (e.g. heart findings in female rats: kidney findings in male rats), following daily repeated treatment in euglycemic healthy animals, the clinical indication is for single dose as a diagnostic agent, and therefore the heart or kidney findings are unlikely to have clinical significance, if the product is used as intended. Therefore, it is recommended for approval.

- B. **Pharmacologic activity:** Glucagon for injection is a polypeptide hormone identical to human glucagon. It increases blood glucose and relaxes smooth muscle of gastrointestinal tract. It increases blood glucose through stimulation of glycogenolysis and gluconeogenesis. It is indicated as a diagnostic aid in the radiologic examination to temporarily inhibit the movement of gastrointestinal tract.
- C. Nonclinical safety issues relevant to clinical use: None

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW**2.6.1 INTRODUCTION AND DRUG HISTORY****NDA number:** 201-849**Review number:** 1

Sequence number/date/type of submission: 11/30/11. Originally this application was submitted on 9/30/2010. However due to the Pharmacology/toxicology deficiencies, this application was not fileable. We sent a refuse to file (RFT) letter to the Firm on 12/3/10. The sponsor has now provided complete response to RFT letter and have resubmitted the application on 11/30/2011.

This is an eCTD submission. It is a 505(b)(2) application. Sponsor refers to the previous NDA of glucagon that has already been approved (NDA 20-918/ GlucaGen of Novo Nordisk).

Information to sponsor: Yes () No (X)**Sponsor and/or agent:** AAP Pharmaceuticals LLC, Schaumburg, IL.

Manufacturer for drug substance: The manufacturer of Glucagon drug substance is (b) (4)
 The US office of API manufacturer is (b) (4)

Reviewer name: Indra Antonipillai**Division name:** Division of Metabolism and Endocrinology Products (DMEP).**Review completion date:** 7/23/2012**Drug:**

Trade name: Glucagon for injection, 1 mg (1 IU)/ ml. APP Pharmaceuticals is currently seeking intramuscular (IM) and intravenous (IV) routes of administration for the current drug product to be used as a diagnostic aid during radiologic examinations.

Generic name: Glucagon

Code name: N/A

Chemical name: It is a single chain polypeptide containing 29 amino-acid residues

His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-
 1 2 3 4 5 6 7 8 9 10 11

Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-
 12 13 14 15 16 17 18 19 20 21 22

Val-Gln-Trp-Leu-Met-Asn-Thr
 23 24 25 26 27 28 29

CAS registry number: 16941-32-5

Molecular formula/molecular weight: C₁₅₃H₂₂₅N₄₃O₄₉ / 3483

Relevant INDs/NDAs/DMFs: NDA 20-918 (GlucaGen, Novo Nordisk), NDA 20-928 (Glucagon, Eli Lilly). DMF # (b) (4) ((b) (4) the manufacturer of the current drug substance).

Drug class: Peptide hormone. The current drug product is a synthetic glucagon.

Intended clinical population: The drug is intended to be used as a diagnostic aid during radiologic exams to temporarily inhibit movement of the gastrointestinal tract. (b) (4)

(b) (4)
 they are asking for only one indication as a diagnostic aid during radiologic exam, as indicated above.

Clinical formulation: Glucagon for Injection, 1 mg / ml vial is supplied as a sterile lyophilized white powder in a 3 ml vial (b) (4) Glucagon is soluble in water and reconstitutes in water to a pH range of 2.5-3.5. Glucagon as the hydrochloride salt, 1 mg dose corresponds to 1 IU.

The current submission (NDA 201-849) is a synthetic form of the peptide (Glucagon for injection) as compared to the innovators in which recombinant glucagon was used. When sponsor submitted the initial application (9/30/2010), no pharmacology/ toxicology studies were conducted. We sent a refuse to file (RFT) letter to the Firm on 12/3/10. At that time in the application, two impurities were concerning, which were both identified by the sponsor as degradants, and these impurity limits were above the CDER's and ICH's identification and qualification thresholds of 0.50% and 1.00%. We recommended that sponsor conduct qualifying toxicity studies, i.e. in vitro genotoxicity (mutagenicity, clastogenicity) and a 2 to 12 week toxicity study in one species with the proposed drug product and reference drug (as per ICH Q3A and ICH Q3B). In the current resubmitted application (11/30/2011), sponsor has conducted 3 non-clinical toxicity studies. These include a 28-day toxicity/TK study in rats and two geno-toxicity studies (Ames and chromosomal aberration assay) with their own drug product and the reference drug GlucaGen.

Sponsor below states that the inactive ingredients of the proposed drug product are the same as that of the reference drug, GlucaGen®, held by Novo Nordisk (distributed by Bedford Laboratories).

Sponsor has provided the following side by-side comparison of their drug vs the reference drug (RD) below:

1.12.12.5 Side-by-Side Comparison (b) (4)
 (b) (4)

Table provided lists information (b) (4)
 (b) (4)

Table 1. Side-by-Side Comparison of the Reference Listed and Proposed Drugs

	Reference Listed Drug	Proposed Drug Product
Name	GlucaGen®	Glucagon for Injection
Conditions of Use (Indications)	It is indicated for the treatment of severe hypoglycemic reactions which may occur in patients with diabetes treated with insulin, or for use as a diagnostic aid during radiologic examinations of the gastrointestinal system	It is indicated for use as a diagnostic aid during radiologic examinations of the gastrointestinal system
Dosage Form	Sterile lyophilized drug product	Sterile lyophilized drug product
Route of Administration	Subcutaneous (sc), intramuscular (im), or intravenous (iv) injection.	intramuscular (im) or intravenous (iv) injection.
Active Ingredient	Glucagon	Glucagon
Strength	1 mg	1 mg
Excipient (amount/1-mg vial) ¹		
Lactose Monohydrate	107 mg	107 mg
Hydrochloric Acid	As required for pH adjustment	As required for pH adjustment
Sodium Hydroxide	As required for pH adjustment	As required for pH adjustment
Bioequivalence	Refer to SECTION 5.3.1	Refer to SECTION 5.3.1
Labeling	Refer to SECTION 1.14	Refer to SECTION 1.14

¹ APP's excipients are all compendial grade materials.
 (b) (4)

Route of administration: Intravenous and intramuscular.

Disclaimer: The tabular and graphical information is from sponsor's submission unless stated otherwise.

Studies reviewed within this submission: 4-week toxicity study and two in vitro gene-toxicity studies (Ames and chromosomal aberration assay) with the current APP drug product and the reference drug (RD) GlucaGen are reviewed here.

2.6.2 PHARMACOLOGY

Glucagon is a single chain polypeptide hormone containing 29 amino acids, it is a gastrointestinal motility inhibitor. It is indicated for use during radiologic exam to temporarily inhibit movement of the gastrointestinal tract. In the current application, it is recommended to be used for intramuscular and intravenous administration.

Glucagon and glucagon-like peptide-1 (GLP-1) are homologous peptide hormones with important functions in glucose metabolism. The receptors for glucagon and GLP-1 are homologous family B G-protein coupled receptors and they selectively recognize the homologous peptide hormones glucagon (29 amino acids) and GLP-1 (30-31 amino acids) respectively. The amino-terminal extracellular domain of the glucagon and GLP-1 receptors (140-150 amino acids) determines specificity for the carboxy terminus of glucagon and GLP-1 respectively.

Glucagon administered through a parenteral route relaxes smooth muscle of the stomach, (b) (4) small bowel, and colon. It increases blood glucose through stimulation of glycogenolysis and gluconeogenesis, and it is used to treat severe hypoglycemic reactions which occur in patients with diabetes treated with insulin. Glucagon (recombinant or rDNA origin) has been approved before as NDA 20-918 (Novo Nordisk, as GlucaGen 1 mg/1U) and NDA 20-928 (Eli Lilly) for both above indications as 1 mg/ml injection.

Note that the previous glucagon applications were either recombinant (a yeast derived human glucagon) or of natural origin. However, AAP Pharmaceuticals Glucagon for injection drug product was manufactured (b) (4) as compared to the innovators in which (b) (4)

Sponsor has submitted this application as a 505(b)(2), which relies on the previous approved glucagon NDA (NDA 20-918, Novo Nordisk). (b) (4) the current glucagon for injection drug product was manufactured (b) (4) vs the innovator's in which (b) (4)

Sponsor states the following in section 2.5.2

APP's proposed Glucagon for Injection drug product is a sterile lyophilized powder intended solely for administration by intramuscular (IM) or intravenous (IV) injection. (b) (4)
(b) (4)

(b) (4) Though, the following scientific information is known relative to the Glucagon peptide:

1. The active therapeutic moiety Glucagon, USP is composed of 29 amino acids.
2. Glucagon, USP is a linear peptide and therefore does not contain any quaternary or tertiary structures that could alter the pharmacological properties of the chemical moiety.
3. The active therapeutic moiety is fully characterized and has (b) (4) amino acid sequence in comparison to the RLD derived from recombinant means.
4. The current USP has a monograph for Glucagon as well as Glucagon for Injection. The current USP monograph contains an *in-vivo* potency determination test required for release of the Glucagon drug substance.
5. The chromatographic profile of the active therapeutic moiety (b) (4) to the RLD.
6. There are no known immunogenicity reactions reported in the scientific literature for Glucagon.

The amino acid sequences of the recombinant and synthetic (and human glucagon) products (b) (4)

APP has proposed the following dosing regimen for glucagon as a diagnostic aid: Gucagon for Injection should be reconstituted with 1 ml of Sterile Water for Injection. The usual diagnostic dose for relaxation of the stomach, (b) (4) small bowel is 0.2 mg to 0.5 mg given intravenously or 1 mg given intramuscularly; the usual dose to relax the colon is 0.5 mg to 0.75 mg intravenously and 1 mg to 2 mg intramuscularly.

See below the FDA concerns and sponsor's complete response to RFT letter (provided in the current resubmitted application):

In summary, and presented here-in, discussions, decisions and agreements made in the referenced RTF meeting between the Agency and APP included:

- 1) That a nonclinical bridge must be established between the APP Glucagon for Injection and the RLD, the Bedford GlucaGen[®]. APP has established this bridge through the definitive identification and the comparison of the impurity levels of the two products on stability.

In addition, three nonclinical studies have been conducted, comparing the APP product and the RLD (GlucaGen[®]). These three studies include an AMES test, a chromosomal aberration trial and a 28 day toxicology and toxicokinetics trial. These nonclinical trials and their results are included in their entirety within this response.

- 2) APP has made the decision (b) (4) (b) (4) only the gastrointestinal indication via the intravenous route of administration is now being pursued by APP.

APP believes that it has established the nonclinical bridge through the definitive identification of the impurities and the comparison of impurity levels, coupled with the *in-vitro* genotoxicity (AMES and chromosomal aberration studies) and the 4 week rat toxicology (with toxicokinetics) studies referenced above. The toxicology study included the drug product upon which we intend to rely (GlucaGen[®], Bedford). Given previous Agency comments, and subject to review of the CMC data comparing GlucaGen[®] to APP Glucagon for Injection, APP considers this nonclinical data set as a complete response to the NDA.

Note that the RD GlucaGen (from Novo Nordisk) is approved for not only IM and IV use (like the APP glucagon) but also for subcutaneous use, and it is approved for both hypoglycemia and diagnostic use (unlike present APP glucagon which is indicated for only diagnostic use).

The current drug substance (APP's glucagon for injection) is (b) (4)

The Glucagon API of this application is (b) (4) however, it meets the requirements of the current USP and API manufacturer/supplier specifications.

The compendial specifications for Glucagon are provided below:

Table 1. Compendial Specifications for Glucagon

Test	APP Specification	USP Limits
Identification by HPLC	The retention time of the major peak in the chromatogram of the Raw Material Sample Preparation corresponds to that in the chromatogram of the Standard Preparation, and the ratio (R) of the retention times is (b) (4)	The retention time of the major peak in the chromatogram of the Raw Material Sample Preparation corresponds to that in the chromatogram of the Standard Preparation
Water Content	NMT (b) (4)%	NMT 10.0%
Residue on Ignition	NMT (b) (4)	NMT (b) (4)%
Related Peptides (Chromatographic Purity)	<i>Any Other Individual Impurity</i>	NMT (b) (4)
	(b) (4)	NMT (b) (4)
	Total Impurities	NMT (b) (4)%
(b) (4)		
Assay	(b) (4) USP Glucagon Units/mg	0.8 – 1.25 USP Glucagon Units/mg
Residual Solvents	(b) (4)	Meets requirements per USP <467>

As far as the impurities are concerned, the drug substance has following impurity, which is summarized below:

The drug substance impurity is summarized in the table below:

Table 3. Impurities in Drug Substance

Impurity Name	Structure	Origin
(b) (4)		

The drug product

As for as the drug product formulation is concerned, sponsor states that the inactive ingredients of the proposed drug product are the same as that of the reference drug, GlucaGen®, held by Novo Nordisk (distributed by Bedford Laboratories). Components of the Product are shown below. All inactive ingredients used in the formulation of the proposed drug product comply with the current compendia. APP also tested the RLD drug product and found that APP’s stability test results, under the same storage conditions, are comparable. The route, and dosage strength are stated below:

The dosage form and strength of the proposed drug product are the same as those of the reference listed drug. The route of administration being sought by APP Pharmaceuticals under this original NDA filing does not, however, include subcutaneous administration (sc). The proposed drug product is a sterile, lyophilized drug product containing 1 mg/vial of Glucagon which is intended for intramuscular (im) or intravenous (iv) injection.

Sponsor states that the ICH impurities guidelines do not apply to peptides, so impurity limits are determined from the test data from the exhibit batch and reference listed drug (RLD). The proposed container/closure systems comply with the USP requirements, and all components used in this container/closure systems have been used in approved CDER products.

Complete details of the method of synthesis of Glucagon for Injection are provided in (b) (4) Type II DMF # (b) (4) submitted to the FDA. A copy of the letter from (b) (4) permitting FDA to cross-reference this DMF on behalf of APP Pharmaceuticals, LLC is provided in this NDA, refer to attached **LETTER OF AUTHORIZATION FROM** (b) (4) (b) (4) was last inspected on (b) (4) (b) (4)

Sponsor states that the excipients and their grades were selected based on the innovator’s drug product excipients. Compendial grades were chosen for quality. The proposed final formulation was optimized by meeting all acceptance criteria being proposed for the drug product. No changes will be made to the formulation of the drug product from the exhibit batch to commercial batches except for parameters due to scale-up. The manufacturing process was selected based on experience in manufacturing lyophilized drug products. (b) (4)

Sponsor provides the list of inactive ingredients in the current APP drug product and reference drug (GlucaGen), see below:

Table 1. Inactive Ingredient Comparison between RLD and APP Pharmaceuticals, LLC Formulation

APP’s Glucagon for Injection Inactive Ingredients	GlucaGen’s Inactive Ingredients	Function of Inactive Ingredients
Lactose Monohydrate, NF	Lactose Monohydrate	(b) (4)
Hydrochloric Acid, NF	Hydrochloric Acid	pH adjuster
Sodium Hydroxide, NF	Sodium Hydroxide	pH adjuster
(b) (4)		
(b) (4)		

Impurities in the drug product

As far the impurities in the drug product are concerned, sponsor (APP) states that they have revised the impurity limits in the finished drug product specifications to more adequately reflect the limits observed in the RLD GlucaGen® (Novo Nordisk) as well as those present in the Eli Lilly Glucagon finished drug product. The individual limits for glucagon related impurities and lactose related impurities were justified by data observed in GlucaGen® or Eli Lilly Glucagon. There are two impurities (b) (4) Glucagon and (b) (4) that are higher than the RLD for APP Lot # R107-002. The two APP lots C108- 002 and C109-002 have lower impurity profile than the RLD and marketed Eli Lilly product.

The R107-002 lot was manufactured at a lower pH (2.8) as compared to pH of 3.0 (in lot C108-002) and 2.9 (in lot C109-002). Subsequent to the original 505(b)(2) NDA submission, the pH target in (b) (4) has been tightened to (b) (4). This will provide a general impurity profile which is lower than that observed for the RLD and Eli Lilly glucagon. A toxicological assessment of the impurities was performed and further supportive genotoxicity studies (BACTERIAL REVERSE MUTATION ASSAY and IN VITRO MAMMALIAN CHROMOSOME ABERRATION study) and an IN VIVO 28 DAY REPEATED DOSE TOXICITY STUDY in rats were also performed to qualify the impurity limits of these two impurities. The limit for all Other Glucagon and Lactose Related Impurity were lowered to NMT (b) (4) %

The Chemist assigned to this NDA has provided the following Table, which shows the impurities in the current drug product vs the reference drug. Note that 3 drug lots numbers are provided below (R107-002, C108-002 and C109-002), sponsor has explained above as to why one drug lot (R107-002) has higher impurities. However, the two drug lots that have been used in the toxicity studies (C108-002 and C109-002) had lower impurities levels. As noted below, except for the (b) (4) impurity, most impurities are lower in the current drug product lots (C108-002 and C109-002) vs the RD. (b) (4) is present in APP glucagon at (b) (4)%, whereas levels of this impurity in GlucaGen are set at (b) (4)%. A 28-day rat toxicity study, as well as in vitro genotox studies were performed with APP glucagon containing (b) (4)% (b) (4). The outcome of these studies suggest similarity to Novo Nordisk’s GlucaGen and therefore the presence of higher (b) (4) (i.e. (b) (4)%) in the drug product does not appear to affect safety.

mpurity	R107-002 ¹	C108-002	C109-002	RD ²	Trending (b) (4)
[Redacted Table Content]					



(b) (4)

- 1 The applicant claims that this batch was adversely affected by (b) (4) at a lower pH relative to other batches, leading to larger impurity profile.
- 2 Four lots of glucagon for injection distributed by Bedford (Novo Nordisk) and two from Lilly were tested at receipt and near the expiry. Note that one batch, Eli Lilly Glucagon lot #A558900E was significantly more degraded than the other batches (~3-4X higher impurity levels). This represents the worst case estimate that the applicant bases their specifications.
- 3 Unlike the other glucagon related impurities, (b) (4) was uniformly higher for the for RD (see footnote #2 above).

2.6.6 TOXICOLOGY

2.6.6.3 Repeat Dose Toxicity Studies

In the current application, sponsor has submitted a 4-week toxicity study and two geno-toxicity studies to qualify the impurities/excipients in their drug product vs the innovators, these are reviewed below

Table below provides the toxicity studies conducted in the current application dated 11/30/11:

2.6.7.1: Toxicology		Overview			Test Article: Glucagon for Injection			
Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	GLP Compliance	Testing Facility	Study No.	Location in CTD
Repeat Dose Toxicity	SD Rat	Intramuscular	28-days	0, 1, 5	Yes	(b) (4)	AD29XE_AD30AZ.2M31.BTL	4.2.3.2
Genotoxicity <i>In Vitro</i>	S. typhimurium and E. coli	In Vitro	-	0.9 – 2700 µg/plate	Yes	(b) (4)	AD29XE.503.BTL and AD30AZ.503.BTL	4.2.3.3.1
Genotoxicity <i>In Vitro</i>	Chinese Hamster Ovary Cells	In Vitro	-	0.037 – 370 µg/mL	Yes	(b) (4)	AD29XE.331.BTL and AD30AZ.331.BTL	4.2.3.3.1

The sponsor states that residual substances in the test article, Glucagon for Injection drug product, were slightly higher than the reference listed drug product, GlucaGen®. The test article consisted of API lots/batches taken at the end of expiration (worst case) while the GlucaGen® lots/batches within expiration dates.

1) 28-Day Intra-muscular Toxicity Study of Glucagon in Fisher Rats (Study No. AD 29XE AD30AZ.2M31 (b) (4)).

Study no.: (b) (4) **Study Number:** AD29XE AD30AZ.2M31 (b) (4)
Volume #, and page #: eCTD submission on 11/30/11.

Conducting laboratory and location: (b) (4)

Date of study initiation: 5/17/11
GLP compliance: Yes
QA report: yes (X) no ().

Drug, lot #, and % purity:

Two lots of the current drug (APP glucagon for injection) were used in this 28-day study in rats. These lot numbers are described below:

- 1) C108-002 (which has (b) (4) # AD29XE 0001), and
 2) IC109-002 (which has (b) (4) # AD29XE 0002). The details are provided below:

(b) (4) Test Article Nos.:	AD29XE.0001 and .0002
Lot Number (sample 0001):	C108-002 (Test Dates 17 May and 05 August 11)
Lot Number (sample 0002):	C109-002 (Test Dates 14 June and 05 August 11)
Purity:	Average 90.4% per CofA for Lot No. C108-002 Average 90.6% per CofA for Lot No. C109-002

2. Similarly, two lots of the comparator RD (GlucaGen for injection) were used in this study, and details are provided below:

Comparator Drug Name:	GlucaGen® (rDNA Glucagon)
(b) (4) Comparator Drug No.:	AD30AZ.0001 and .0002
Lot Number (sample 0001):	AW60155
Lot Number (sample 0002):	AW60179
Purity:	94.6% per CofA for Lot No. AW60155 97.3% per CofA for Lot No. AW60179
Expiration Date:	30-Nov-2012

Formulation/vehicle: Sterile water for injection, USP

The formulation of Glucagon for Injection (APP Pharmaceuticals Test Article) and the comparator reference drug (RD), GlucaGen® (rDNA GlucaGen) were provided in a lyophilized vial, and were diluted with 0.3 ml of sterile water.

Methods (unique aspects):

Doses in administered units: 0, 1, and 5 mg/kg/day of the current drug (Glucagon for Injection) were administered to groups 1, 2, 3 respectively, and 5 mg/kg/day of RD (GlucaGen®, Novo Nordisk) to group 4. Doses were administered once daily by intramuscular (IM) injection.

The objective of this study was to investigate the potential toxicity of APP Glucagon for Injection (the current drug), and to compare it with the toxicity of the approved RD (GlucaGen) in the Fisher rats (n=8/sex/dose) for 28 consecutive days. The toxicokinetic (TK) group animals were similarly treated (n=3-5/sex/group) for 28 days.

Route, form, volume, and infusion rate: Intramuscular (IM), 2.5 ml/kg, given once daily for 28-days.

Species: Fisher rats. At initiation of dosing, the male rats were 8-9 weeks of age, and female rats of 9-10 weeks of age. The body weights of male rats were 240.9 to 290.4 grams, and the female rats were 187.8 to 238.5 grams.

Study design: This is shown below:

Group	Dose Levels (mg/kg/day)	Number of Animals		Number of Animals	
		Main Study		TK Study	
		Male	Female	Males	Female
Group 1	0	8	8	3	3
Group 2 Glucagon for Injection (Low Dose)	1	8	8	5	5
Group 3 Glucagon for Injection (High Dose)	5	8	8	5	5
Group 4 – GlucaGen [®] (rDNA Glucagon) (Comparator Drug)	5	8	8	5	5
Total		32	32	18	18

Groups 2 and 3 animals were administered the current drug (Glucagon for Injection, APP Pharmaceutical Test Article), and Group 4 animals received comparator drug GlucaGen[®] (rDNA Glucagon) as shown above.

Sponsor states the dose selection was based on the doses that were used in a 14-day subcutaneous toxicology study in rats, reviewed by FDA under NDA 20-928 (Eli Lilly), and a 4-week intravenous study in rats, reviewed by FDA under NDA 20-918 (Novo Nordisk).

The high dose selected for this study was 5 mg/kg, which is 150x the maximum dose indicated for GlucaGen[®] (2 mg), based on a body weight of 60 kg human (or 0.033 mg/kg) subject.

Note that the sponsor's safety margins stated above are based on the mg/kg weight in both rats and humans, and not on the body surface area.

Parameters evaluated for the toxicity study are shown below:

Toxicity assessment was based on viability, clinical signs, body weight, food consumption, ophthalmology, urinalysis, clinical pathology (hematology, clinical chemistry, and coagulation), selected organ weights, and complete macroscopic and microscopic evaluations. Microscopic evaluations of tissues were performed on Groups 1, 3 and 4 only (i.e. in controls and HD animals).

Gross pathology: At sacrifice on day 29.

Organs weighed: The adrenal glands, brain, heart, kidneys, liver, spleen, and testes/ovaries were weighed in the main study animals which were necropsied on Day-29.

Histopathology: This was performed at sacrifice in control and high dosed animals only (Glucagon for Injection, and the comparator RD GlucaGen[®]) in organs listed in the histopathology Table below.

Tissue	Tissue
Adrenal glands	Pancreas
Aorta	Parathyroid glands
Bone (femur and sternum)	Pituitary gland
Bone marrow (femur and sternum)	Prostate gland
Brain	Salivary gland
Epididymides	Sciatic nerve
Esophagus	Seminal vesicles
Eyes	Skeletal muscle (left and right thigh)
Gross lesions	Small intestine (duodenum, jejunum, and ileum)
Harderian gland	Spinal cord (cervical, thoracic, and lumbar)
Heart	Spleen
Kidneys	Stomach
Large intestine (cecum, colon, rectum)	Testes
Liver	Thymus
Lungs and bronchi	Thyroid glands
Lymph nodes (mesenteric and mandibular)	Trachea
Mammary gland (females)	Urinary bladder
Skin mammary area	Uterus
Nasal cavity	Vagina
Ovaries	Diaphragmatic muscle
Sternal muscle	

Toxicokinetic (TK) analysis: For toxico-kinetic evaluation, blood was collected from up to 2 TK animals/time-point/sex as indicated in the following Table.

Table 2. Blood collection for TK evaluation

Group	Number animals/time-point/sex	Time-points	Collection Day
1	First 2	15 min (\pm 2 min)	Day 1, Day 27
2 - 4	First 2 per group	Pre-dose, 30 min (\pm 2 min)	Day 1, Day 27
2 - 4	Second 2 per group	15 min (\pm 2 min), 2 h (\pm 5 min)	Day 1, Day 27

The Toxicokinetic and Bioanalytical Analysis Reports are included in [Appendix G](#) and [H](#), respectively. TK animals, including extra TK animals not bled, were sacrificed after completion of bleeding and discarded without necropsy.

Results:

Dose formulation analysis: Sponsor states that their product (glucagon for injection) had higher total combined impurities (i.e. (b)(4)%), than the RD GlucaGen (which had total combined impurities of (b)(4)%) as stated below:

All assay results met the acceptance criteria. The total combined impurity value for the APP product were about (b)(4)%, whereas the GlucaGen® had total combined impurity values up to (b)(4)%. The average assay concentration of dose formulation samples ranged from 2.3 to 2.5 mg/ml (94.2 to 99.7% of target concentrations) and %RSD (n=4) results ranged from 0 to 2%.

Following certificates of analysis (CAO) were provided in the appendix D for the two drug lots, used in the 28-day toxicity study in rats.

Table 1. Certificate of analysis for the current drug lot # C108-002 (APP's glucagon for injection, the (b)(4) # is AD29XE 0001). The drug was analyzed on 8/5/11. This drug lot # C108-002 had (b)(4)% of (b)(4) impurity. This is the same drug lot used for in vitro genotoxicity testing.

**CERTIFICATE OF ANALYSIS
INNOVATION AND DEVELOPMENT**

Glucagon for Injection (1 mg/vial)

Page 1 of 1
Version 2.0

NDC Code:	<u>NDC 63323-596-03</u>	Lot Number:	<u>C108-002</u>
Product			
Configuration:	<u>1 mg Glucagon / vial</u>	Expiry Date:	<u>N/A</u>
Manufactured By:	<u>APP Pharmaceuticals</u>	Distributed By:	<u>N/A</u>
	<u>Room Temperature,</u>		
Storage Condition:	<u>Upright</u>	Test Date:	<u>08/05/11</u>

TEST	RESULTS
Assay by HPLC	(b)(4)%
Residual Substances	(b)(4)

PREPARED BY: (b)(4) DATE: 09/06/11

Table 2. Certificate of analysis for the second drug lot # C109-002 (glucagon for injection, (b) (4) test article # is AD29XE 0002). The drug was analyzed on 8/5/11. This drug lot # had (b) (4) % of (b) (4) impurity

The above impurity is shown to be present at higher levels in the APP glucagon (i.e. (b) (4) %) than in the RD GlucaGen ((b) (4) %). Note that although sponsor has identified this impurity in the COA of GlucaGen; according to our chemist the sponsor may have identified (b) (4) because it has a similar retention time to a peak they see with their product and likely have not (b) (4) this impurity or compared it to a standard, it appears as a shoulder peak in HPLC. Therefore this impurity is not anticipated to be present in the recombinant glucagon (previously approved). However, this impurity has been qualified now, since sponsor has conducted a 28-day toxicity study and in vitro geno-toxicity with the current APP drug product.

APP is seeking a specification for (b) (4) of (b) (4) %, and as noted in the certificate of analysis below, only up to (b) (4) % of this impurity is tested in the 28-day toxicity study. All other impurity levels are comparable to those found in the reference drug.

**CERTIFICATE OF ANALYSIS
INNOVATION AND DEVELOPMENT**
Glucagon for Injection (1 mg/vial)

Page 1 of 1
Version 2.0

NDC Code:	<u>NDC 63323-596-03</u>	Lot Number:	<u>C109-002</u>
Product			
Configuration:	<u>1 mg Glucagon / vial</u>	Expiry Date:	<u>N/A</u>
Manufactured By:	<u>APP Pharmaceuticals</u>	Distributed By:	<u>N/A</u>
	<u>Room Temperature,</u>		
Storage Condition:	<u>Upright</u>	Test Date:	<u>08/05/11</u>

TEST	RESULTS
Assay by HPLC	(b) (4) %
Residual Substances	(b) (4)

PREPARED BY:	(b) (4)	DATE:	<u>09/06/11</u>
REVIEWED BY:		DATE:	<u>9/6/11</u>
APPROVED BY:		DATE:	<u>9/7/11</u>

Note that total impurities tested in the 28-day toxicity study were present up to (b) (4)% in the current APP drug product, and up to (b) (4)% in the RD GlucaGen. The specified impurities are shown below pre and post toxicity study in two lots of the APP glucagon (C108-002 and C109-002).

Table 3. Specified impurities in both lots used in the toxicity study (pre and post study) are provided below:

2.6.7.4: Toxicology		Test Article: Glucagon for Injection		
Batch No.	Purity (%)	Specified Impurities (%)		Type of Study
Lot #/ Storage		(b) (4)		
C108-002 (25C)	Pre-study (↑) (↓)	88.1 88.7	(b) (4)	
	Post-study (↑) (↓)	92.4 92.4		
C109-002 (25C)	Pre-study (↑)	88.9		
	Post-study (↑)	92.2		
				4-Week IM in Rats

Table 4. Certificate of analysis (CAO) for the reference drug or RD (GlucaGen). Note that two lot numbers were used here also. See the CAO for lot # AW 60155 below:

As indicated earlier in the COA below, the sponsor has identified (b) (4) in the reference drug GlucaGen, because it has a similar retention time peak they see with their (b) (4) in the APP glucagon, but likely have not (b) (4) this impurity, or compared it to a standard. The chemist states that it appears as a shoulder peak in the HPLC, and he doesn't anticipate this impurity to be present in the recombinant glucagon (previously approved).



**CERTIFICATE OF ANALYSIS
INNOVATION AND DEVELOPMENT
GlucaGen® (Glucagon for Injection, 1 mg/vial)**

Page 1 of 1
Version 1.0

NDC Code:	<u>NDC 55390-004-01</u>	Lot Number:	<u>AW60155</u>
Product Configuration:	<u>1 mg Glucagon / vial</u>	Expiry Date:	<u>11/2012</u>
Manufactured By:	<u>Novo Nordisk A/S</u>	Distributed By:	<u>Bedford Laboratories</u>
Storage Condition:	<u>Room Temperature</u>	Test Date:	<u>05/17/11</u>

TEST	RESULTS
Assay by HPLC	(b) (4)
Residual Substances	(b) (4)

PREPARED BY:	(b) (4)	DATE:	<u>06/14/11</u>
REVIEWED BY:	(b) (4)	DATE:	<u>06/15/11</u>
APPROVED BY:	(b) (4)	DATE:	<u>06/15/11</u>

Table 5. Certificate of analysis (CAO) for the reference drug (GlucaGen) continued, see the CAO for the second lot # AW 60179 below:

**CERTIFICATE OF ANALYSIS
INNOVATION AND DEVELOPMENT
GlucaGen® (Glucagon for Injection, 1 mg/vial)**

NDC Code: NDC 55390-004-01 Lot Number: AW60179
 Product
 Configuration: 1 mg Glucagon / vial Expiry Date: 11/2012
 Manufactured By: Novo Nordisk A/S Distributed By: Bedford Laboratories
 Storage
 Condition: Room Temperature Test Date: 06/14/11

TEST	RESULTS
Assay by HPLC	(b) (4) %
Residual Substances	(b) (4)

PREPARED BY:  DATE: 6/27/11
 REVIEWED BY:  DATE: 6/27/11
 APPROVED BY:  DATE: 6/28/11

Mortality: No treatment related mortality was observed.

Clinical signs: At a HD with the current drug (5 mg/kg/day of APP's Glucagon for Injection), one male rat had thin appearance (rat #1120). At a HD, with the RD (5 mg/kg/day of GlucaGen®), one male exhibited decreased motor activity on Study Day 6. No other drug related clinical signs were observed.

Body weights: The BWs in males were slightly reduced with both the current drug and the RD. On day 29 body weights were 317, 323, 312, 310 g at 0, 1, 5 mg of the current drug and 5 mg/kg/day of the RD GlucaGen respectively (reduced by approximately 2% at HD). The BWs in females were increased with both drugs, see Table 7 below.

Table 6. Group mean body weights in male rats in grams (g)

		Bodyweight (Grams)					

		Day numbers relative to Start Date					
Group	Sex		1	8	15	22	29
1	m	Mean	273.73	290.60	306.20	320.73	317.28
		S.D.	11.60	14.56	16.33	21.69	21.75
		N	9	8	8	8	8
		-----	-----	-----	-----	-----	-----
2	m	Mean	271.31	295.87	314.65	331.84	323.15
		S.D.	11.18	14.79	15.43	21.48	18.66
		N	9	8	8	8	8
		-----	-----	-----	-----	-----	-----
3	m	Mean	253.32	286.40	305.86	326.44	312.70
		S.D.	40.06	20.81	18.81	17.10	16.68
		N	9	8	8	8	8
		-----	-----	-----	-----	-----	-----
4	m	Mean	254.57	293.04	304.53	319.80	310.16
		S.D.	34.14	11.33	14.04	11.74	11.04
		N	10	8	8	8	8
		-----	-----	-----	-----	-----	-----

Statistical Analysis using Dunnett's T-Test did not reveal any significant differences when Groups 2-4 were compared to Group 1 or when Group 3 is compared to Group 4.

Group 1 - 0 mg/kg/day Group 2 - 1 mg/kg/day Group 3 - 5 mg/kg/day Group 4 - 5 mg/kg/day GlucaGen®

Table 7. Group mean body weights in female rats.

		Day numbers relative to Start Date					
Group	Sex		1	8	15	22	29
1	f	Mean	212.19	224.00	231.56	240.05	232.01
		S.D.	7.14	11.08	7.59	11.41	12.40
		N	8	8	8	8	8

2	f	Mean	211.66	236.85	248.93*	262.26*	249.76*
		S.D.	21.64	5.35	8.76	11.58	10.20
		N	10	8	8	8	8

3	f	Mean	212.14	230.75	243.71	248.95	243.26
		S.D.	12.92	9.04	10.63	12.52	14.61
		N	8	8	8	8	8

4	f	Mean	213.75	227.84	243.78	245.85	237.54
		S.D.	14.43	22.24	22.78	17.12	15.96
		N	8	8	8	8	8

Body weight gains

In males, the body weight gains were significantly lower on day 22-29 at a HD with current drug, but not with the RD (-3.5, -8.7, -13.8*, -9.5 g at 0, 1, 5 of the drug, and with 5 mg/kg/day of RD GlucaGen respectively. *p<0.05). However absolute weight gains (on day 1-29) were not significantly different at a HD with both drugs (43, 52, 47, 41 g respectively), see Table 8.

In females, the body weight gains at a HD with both drugs were not significantly different (see Table 9 below)

Sponsor states there were no statistically significant differences in body weights or bodyweight gains in both males and females in 5 mg/kg/day Glucagon for Injection when compared to GlucaGen (RD)

Table 8. Mean body weight gains in males (in grams)

Body Weight Gain (Grams)								
Day numbers relative to Start Date								
Group	Sex	Base Weight Day 1	From: To:	1	8	15	22	Abs Gain 1
				8	15	22	29	29
1	m	273.73	Mean	16.36	15.60	14.53	-3.45	43.04
		11.60	S.D.	5.14	2.75	7.47	6.41	11.90
		9	N	8	8	8	8	8
2	m	271.31	Mean	24.33	18.78	17.19	-8.69	51.60
		11.18	S.D.	4.59	4.59	6.88	8.56	8.95
		9	N	8	8	8	8	8
3	m	253.32	Mean	20.18	19.46	20.58	-13.74*	46.48
		40.06	S.D.	13.02	12.37	7.52	3.85	9.07
		9	N	8	8	8	8	8
4	m	254.57	Mean	23.31	11.49	15.28	-9.64	40.44
		34.14	S.D.	11.86	8.80	10.37	8.93	16.29
		10	N	8	8	8	8	8

* = Statistically significant using Dunnett's T-Test (p < 0.05) when compared to the vehicle control (Group 1).

Statistical Analysis using Dunnett's T-Test did not reveal any significant differences when Group 3 is compared to Group 4.

Abs Gain = absolute bodyweight gain between base period and end of the analysis period

Group 1 - 0 mg/kg/day Group 2 - 1 mg/kg/day Group 3 - 5 mg/kg/day Group 4 - 5 mg/kg/day GlucaGen®

Table 9. Mean body weight gains in females (in grams):

Body Weight Gain (Grams)								
Day numbers relative to Start Date								
Group	Sex	Base Weight Day 1	From: To:	1	8	15	22	Abs Gain 1
				8	15	22	29	29
1	f	212.19	Mean	11.81	7.56	8.49	-8.04	19.83
		7.14	S.D.	10.78	9.79	10.98	13.79	12.41
		8	N	8	8	8	8	8
2	f	211.66	Mean	20.18	12.08	13.34	-12.50	33.09
		21.64	S.D.	11.32	7.62	11.80	14.34	12.25
		10	N	8	8	8	8	8
3	f	212.14	Mean	18.61	12.96	5.24	-5.69	31.12
		12.92	S.D.	12.13	9.63	9.31	8.33	12.01
		8	N	8	8	8	8	8
4	f	213.75	Mean	14.09	15.94	2.08	-8.31	23.79
		14.43	S.D.	11.17	16.67	9.57	6.13	6.57
		8	N	8	8	8	8	8

Statistical Analysis using Dunnett's T-Test did not reveal any significant differences when Groups 2-4 were compared to Group 1 or Group 3 is compared to Group 4.

Abs Gain = absolute bodyweight gain between base period and end of the analysis period

Food consumption: In males, food consumption (FC) was significantly higher on days 15-28. with the current drug and RD, these values on day 28 in males was 548, 591, 651*, 621* g/animal/day at 0, 1, 5 mg/kg/day of the drug, and 5 mg/kg/day of RD respectively, *p<0.05 (increased by 19% with the current drug and 13% with RD at high doses). Food consumption was also increased in females with both drugs (435, 513*, 488*, 567* g/animal/day respectively).

Ophthalmoscopy: No drug related effects were observed.

Hematology: In males, both drug treated rats had increased platelet counts (468, 1080*, 1096*, 1106* 10^3 cells/ul respectively, *p<0.05), decreased reticulocyte counts (283, 214*, 202*, 199* 10^3 cells/ul respectively, *p<0.05), and decreased %- reticulocytes (3, 2*, 2*, 2* % respectively). These were not considered biologically relevant because 2/5 controls had platelet counts below reference range, and 2/5 blood samples in the control group were clotted, as seen in the Table 10 below:

Table 10. Hematological parameters in male rats

	WBC 10 ³ cells/ul	Neutrophil(#) 10 ³ cells/ul	Lymphocyte(#) 10 ³ cells/ul	Monocyte(#) 10 ³ cells/ul	Eosinophil(#) 10 ³ cells/ul	Basophil(#) 10 ³ cells/ul
Group 1						
1101	16.42	2.52	13.18	0.37	0.13	0.07
1102	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED
1103	16.65	2.16	13.26	0.47	0.3	0.15
1105	15.46	2.16	12.48	0.47	0.11	0.08
1106	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED
Mean	16.18	2.28	12.97	0.44	0.18	0.10
SD	0.63	0.21	0.43	0.06	0.10	0.04
N	3	3	3	3	3	3
Group 2						
1110	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED
1111	11.9	2.04	9.34	0.19	0.1	0.12
1112	14.63	1.14	12.72	0.29	0.14	0.19
1113	12.37	2.05	9.75	0.18	0.13	0.09
1114	11.97	1.8	9.68	0.24	0.08	0.05
Mean	12.72	1.76	10.37	0.23	0.11	0.11
SD	1.29	0.43	1.58	0.05	0.03	0.06
N	4	4	4	4	4	4
Group 3						
1117	12.87	1.6	10.54	0.4	0.08	0.15
1118	18.12	2.03	14.96	0.6	0.11	0.13
1120	11.33	1.24	9.6	0.21	0.09	0.07
1121	8.31	0.97	6.94	0.11	0.16	0.04
1122	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED
Mean	12.66	1.46	10.51	0.33	0.11	0.10
SD	4.10	0.46	3.34	0.22	0.04	0.05
N	4	4	4	4	4	4
Group 4						
1125	11.36	0.91	9.89	0.23	0.09	0.09
1126	8.28	1.15	6.75	0.21	0.05	0.05
1127	15.65	2.26	12.74	0.25	0.15	0.11
1129	10.49	1.48	8.48	0.23	0.14	0.06
1130	11.13	1.72	8.81	0.23	0.13	0.09
Mean	11.38	1.50	9.33	0.23	0.11	0.08
SD	2.68	0.52	2.21	0.01	0.04	0.02
N	5	5	5	5	5	5

* in the above table, if present, indicates statistically significant findings using Dunnett's T-test when test article treatment groups were compared to the vehicle control group (Group 1).

Hematological parameters in male rats.

Text Table 6– Male Hematology (Group 1 vs. Groups 2-4)

Parameter	Group No.	Statistically Significant Difference compared to Control (Group 1) ↑ or ↓
(Platelets) PLT	2-4	↑
Retic	2-4	↓
% Retic	3 & 4	↓

↑ = Statistically significantly (p < 0.05) increased when compared to Group 1.
↓ = Statistically significantly (p < 0.05) decreased when compared to Group 1.

Group 1 - 0 mg/kg/day
Group 3 - 5 mg/kg/day

Group 2 - 1 mg/kg/day
Group 4 - 5 mg/kg/day GlucaGen®

In the males, increased platelets (PLT) for Groups 2-4; decreased reticulocytes (Retic) for Groups 2-4; and decreased percentage of reticulocytes (%Retic) in Groups 3 and 4. For platelets, the increase in Groups 2-4 compared to Group 1 appears to be due to a relative decrease in Group 1, which is due to two of three animals having platelet counts well below reference ranges (195 and 178) and two of five having unread platelet values due to clotted samples. This produced a standard deviation up to four times as large as Groups 2-4. The artificially lowered mean for Group 1 produces a statistically significant increase for Groups 2-4 that is not toxicologically relevant. Reticulocytes are immature erythrocytes that typically increase in response to anemia and are an indication that the bone marrow is responding appropriately to a need for new erythrocytes. In the absence of other clinical signs of anemia, a decrease in reticulocytes and percent of reticulocytes for Groups 2-4 is not considered biologically relevant.

Similarly in female rats, the blood samples got clotted (in 1/5 controls and 3/5 of drug-treated rats at a HD, in groups 3 & 4), so some hematological parameters could not be measured as stated below:

In the females, blood samples were clotted and were not suitable for analysis from one animal in Group 1 and three in each of the Groups 3 and 4. Because of marked variations in sample sizes the statistical analysis of the hematology parameters was not performed.

Table 11. Hematological parameters in female rats

	WBC 10 ³ cells/ul	Neutrophil(#) 10 ³ cells/ul	Lymphocyte(#) 10 ³ cells/ul	Monocyte(#) 10 ³ cells/ul	Eosinophil(#) 10 ³ cells/ul	Basophil(#) 10 ³ cells/ul
Group 1						
1133	10.45	1.8	8.07	0.19	0.2	0.09
1134	10.11	1.14	8.46	0.22	0.05	0.06
1135	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED
1136	11.34	1.83	8.68	0.26	0.22	0.17
1137	9.95	0.94	8	0.25	0.1	0.15
Mean	10.46	1.43	8.30	0.23	0.14	0.12
SD	0.62	0.46	0.32	0.03	0.08	0.05
N	4	4	4	4	4	4
Group 2						
1141	10.63	1.01	8.7	0.2	0.13	0.18
1142	12.92	1.49	10.72	0.31	0.16	0.12
1144	12.6	1.22	10.65	0.3	0.13	0.1
1145	10.94	1.01	9.31	0.19	0.18	0.1
1147	12.39	1.61	9.91	0.44	0.13	0.13
Mean	11.90	1.27	9.86	0.29	0.15	0.13
SD	1.04	0.27	0.87	0.10	0.02	0.03
N	5	5	5	5	5	5
Group 3						
1149	13.35	2.23	10.48	0.29	0.14	0.1
1150	10.29	1.59	8.12	0.24	0.1	0.09
1151	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED
1152	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED
1153	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED
Group 4						
1157	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED
1158	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED
1159	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED	CLOTTED
1160	9.16	1.06	7.73	0.2	0.05	0.04
1161	9.6	1.22	8	0.16	0.07	0.04

Due to uneven sample size, statistical analysis was not performed.

Group 1 - 0 mg/kg/day
Group 3 - 5 mg/kg/day

Group 2 - 1 mg/kg/day
Group 4 - 5 mg/kg/day GlucaGen

Clinical chemistry:

In males, the current drug (Group 3) produced increased total protein (TP), values were 6.4, 6.6, 6.9* and 6.2 g/dl respectively, which was not noted with the RD. However, since the magnitude of this elevation is small (6.88 vs. 6.43 g/dl in controls), it is unlikely that this is toxicologically relevant.

Additionally in males, both the current and RD showed increased A/G ratio, albumin (ALB), and decreased cholesterol, globulin and blood urea nitrogen levels. For example, BUN levels were 19, 14* 13*, 15* mg/dl at 0, 1, 5 mg/kg/day of the drug, and 5 mg/kg/day of RD respectively, *p<0.05. Both drugs produced decreased phosphorus (Phos) levels. However, sponsor does not consider these changes toxicologically relevant, as the differences were small, and there were no histological correlates.

Table12. Clinical chemistry parameters in male rats

	CA mg/dL	Creat mg/dL	GLU mg/dL	TP g/dL	TRIGS mg/dL	BUN mg/dL
Group 1						
1101	11.6	0.6	92	6.5	35	20
1102	11.4	0.43	95	6.5	48	18
1103	11	0.4	96	6.4	41	18
1105	QNS	0.38	99	QNS	37	QNS
1106	11	0.38	88	6.3	49	19
Mean	11.25	0.44	94.00	6.43	42.00	18.75
SD	0.30	0.09	4.18	0.10	6.32	0.96
N	4	5	5	4	5	4
Group 2						
1110	QNS	0.41	100	6.9	41	13
1111	11	0.41	115	6.7	42	16
1112	QNS	0.43	110	6.5	31	15
1113	QNS	0.42	105	6.2	22	14
1114	11	0.45	116	6.6	27	12
Mean	11.00	0.42	109.20*	6.58	32.60	14.00*
SD	0.00	0.02	6.76	0.26	8.73	1.58
N	2	5	5	5	5	5
Group 3						
1117	11.5	0.42	112	7	50	15
1118	QNS	0.45	110	QNS	52	QNS
1120	11.3	0.44	103	6.9	48	11
1121	11	0.41	103	6.9	47	12
1122	10.9	0.43	113	6.7	51	13
Mean	11.18	0.43	108.20*	6.88*	49.60	12.75*
SD	0.28	0.02	4.87	0.13	2.07	1.71
N	4	5	5	4	5	4
Group 4						
1125	11.3	0.38	116	6.4	52	14
1126	10.3	0.42	119	5.9	35	16
1127	10.8	0.45	106	6.4	34	15
1129	10.7	0.43	120	6	46	16
1130	10.9	0.38	130	6.4	52	16
Mean	10.80	0.41	118.20*	6.22	43.80	15.40*
SD	0.36	0.03	8.61	0.25	8.84	0.89
N	5	5	5	5	5	5

* in the above table, if present, indicates statistically significant findings using Dunnett's T-test when test article treatment groups were compared to the vehicle control group (Group 1).

Group 1 - 0 mg/kg/day Group 2 - 1 mg/kg/day
 Group 3 - 5 mg/kg/day Group 4 - 5 mg/kg/day GlucaGen

Text Table 7 – Male Chemistry (Group 1 vs. Groups 2-4)

Parameter	Group No.	Statistically Significant Difference compared to Control (Group 1) ↑ or ↓
A/G Ratio	2-4	↑
Cholesterol	2-4	↓
Albumin (ALB)	2-4	↑
Alanine Aminotransferase (ALT)	4	↑
Aspartate Aminotransferase (AST)	4	↑
Glucose (GLU)	2-4	↑
Total Protein (TP)	3	↑
Urea Nitrogen (BUN)	2-4	↓
Potassium (K)	4	↓
Phosphorus (Phos)	3 & 4	↓
Globulin	2-4	↓

↑ = Statistically significantly ($p < 0.05$) increased when compared to Group 1.

↓ = Statistically significantly ($p < 0.05$) decreased when compared to Group 1.

Group 1 - 0 mg/kg/day

Group 2 - 1 mg/kg/day

Group 3 - 5 mg/kg/day

Group 4 - 5 mg/kg/day GlucaGen[®]

In females, the current drug at a HD (Group 3) showed increased triglycerides (TRIGS) levels, values were 36, 45, 52,*, 40 mg/dl respectively, * $p < 0.05$, which was not noted with the RD. However, the magnitude of this elevation is small (52 vs. 40 mg/dl with RD).

Also elevated calcium (11, 11.4*, 11.4*, 11.2 mg/dl respectively) and decreased BUN levels (20, 16*, 14*, 17 mg/dl respectively) were noted with the current drug in females as well (not noted with RD in females). All these differences were not considered significant, because there was no dose response, and no histological correlates. Other changes were similarly not considered significant.

Table 13. Clinical chemistry parameters in female rats

	Creat mg/dL	GLU mg/dL	TP g/dL	TRIGS mg/dL	BUN mg/dL	Na mmol/L
Group 1						
1133	0.56	114	6.5	34	19	142
1134	0.45	103	6.1	41	22	146
1135	0.46	112	6.1	35	18	145
1136	0.45	112	6.2	30	19	145
1137	0.49	117	6	39	21	145
Mean	0.48	111.60	6.18	35.80	19.80	144.60
SD	0.05	5.22	0.19	4.32	1.64	1.52
N	5	5	5	5	5	5
Group 2						
1141	0.56	100	6.8	42	20	147
1142	0.55	99	7.2	46	17	147
1144	0.52	102	6.8	42	15	143
1145	0.53	120	7.2	56	16	144
1147	0.58	119	7	38	14	147
Mean	0.55	108.00	7.00*	44.80	16.40*	145.60
SD	0.02	10.56	0.20	6.87	2.30	1.95
N	5	5	5	5	5	5
Group 3						
1149	0.55	96	7.4	56	16	148
1150	0.55	104	6.8	51	16	147
1151	0.49	107	6.4	56	14	145
1152	0.46	109	6.8	51	13	143
1153	0.55	93	7	48	12	149
Mean	0.52	101.80	6.88*	52.40*	14.20*	146.40
SD	0.04	6.98	0.36	3.51	1.79	2.41
N	5	5	5	5	5	5
Group 4						
1157	0.5	134	6.9	51	16	144
1158	0.53	106	6.8	34	17	144
1159	0.55	112	6.8	37	20	148
1160	0.53	119	6.8	41	15	147
1161	0.51	124	6.5	35	17	144
Mean	0.52	119.00	6.76*	39.60	17.00	145.40
SD	0.02	10.82	0.15	6.91	1.87	1.95
N	5	5	5	5	5	5

* in the above table, if present, indicates statistically significant findings using Dunnett's T-test when test article treatment groups were compared to the vehicle control group (Group 1).

Group 1 - 0 mg/kg/day
Group 3 - 5 mg/kg/day

Group 2 - 1 mg/kg/day
Group 4 - 5 mg/kg/day GlucaGen

Table 14. Clinical chemistry parameters in female rats (continued)

	Cholesterol mg/dL	ALT U/L	ALP U/L	AST U/L	TBili mg/dL	CA mg/dL
Group 1						
1133	107	52	69	123	0.1	11.2
1134	116	48	71	103	0.1	11
1135	109	43	63	107	0.1	11
1136	76	41	77	138	0.1	10.9
1137	105	39	63	125	0.1	10.8
Mean	102.60	44.60	68.60	119.20	0.10	10.98
SD	15.44	5.32	5.90	14.25	0.00	0.15
N	5	5	5	5	5	5
Group 2						
1141	47	28	75	96	0.1	11.3
1142	77	31	63	97	0.1	11.6
1144	81	38	47	95	0.1	11.3
1145	81	42	97	104	0.1	11.5
1147	97	38	51	87	0.1	11.4
Mean	76.60*	35.40	66.60	95.80	0.10	11.42*
SD	18.24	5.73	20.22	6.06	0.00	0.13
N	5	5	5	5	5	5
Group 3						
1149	77	47	77	131	0.1	11.6
1150	64	40	72	131	0.1	11.1
1151	73	51	65	114	0.1	11.2
1152	60	39	56	96	0.1	11.1
1153	68	40	62	116	0.1	11.9
Mean	68.40*	43.40	66.40	117.60	0.10	11.38*
SD	6.80	5.32	8.26	14.50	0.00	0.36
N	5	5	5	5	5	5
Group 4						
1157	66	58	95	113	0.1	11.5
1158	60	40	60	89	0.1	11.2
1159	42	54	50	266	0.1	11.3
1160	61	45	112	100	0.1	11.2
1161	54	47	59	131	0.1	10.8
Mean	56.60*	48.80	75.20	139.80	0.10	11.20
SD	9.21	7.19	26.81	72.26	0.00	0.25
N	5	5	5	5	5	5

* in the above table, if present, indicates statistically significant findings using Dunnett's T-test when test article treatment groups were compared to the vehicle control group (Group 1).

Group 1 - 0 mg/kg/day

Group 2 - 1 mg/kg/day

Group 3 - 5 mg/kg/day

Group 4 - 5 mg/kg/day GlucaGen

In the females, Groups 2-4 had decreased cholesterol and globulin and increased total protein, albumin, and A/G ratio; Groups 2 and 3 had elevated calcium and decreased BUN; and Group 3 had elevated triglycerides (TRIG). The elevated albumin, decreased globulin, and corresponding increased A/G ratio in females are not considered toxicologically relevant for the same reasons cited in males, which is mainly a lack of dose response and any histological or clinical correlation. In addition, as in the males the decreased cholesterol and BUN in

females (Groups 2 and 3) are unlikely toxicologically relevant due to a lack of supporting evidence of hepatic insufficiency. The total protein was elevated for Groups 2-4 females. There is no dose response, as Group 2 has a slightly higher mean than Group 3 (7.00 vs. 6.88) and the magnitude of the change is small. This finding is not considered toxicologically relevant. Finally, calcium (CA) was elevated for Groups 2 and 3 females. Here again, this is not considered toxicologically relevant because the magnitude of the change is small and there is no dose response (Group 2 mean is 11.42 vs. Group 3 mean 11.38).

Effects on clinical chemistry parameters in females:

Text Table 8 – Female Chemistry (Group 1 vs. Groups 2-4)

Parameter	Group No.	Statistically Significant Difference compared to Control (Group 1) ↑ or ↓
Cholesterol	2-4	↓
Calcium (CA)	2 & 3	↑
TP	2-4	↑
Triglycerides (TRIG)	3	↑
BUN	2 & 3	↓
ALB	2-4	↑
A/G Ratio	2-4	↑
Globulin	2-4	↓

↑ = Statistically significantly ($p < 0.05$) increased when compared to Group 1.

↓ = Statistically significantly ($p < 0.05$) decreased when compared to Group 1.

Group 1 - 0 mg/kg/day Group 2 - 1 mg/kg/day
Group 3 - 5 mg/kg/day Group 4 - 5 mg/kg/day GlucaGen®

Coagulation Panel

Fibrinogen levels were lower at a HD with both drugs (males 271, 118, 143, 105 mg/dl respectively; females the values were 261, 146, 110, 128 mg/dl respectively). These were again not considered significant.

Text Table 9– Male Coagulation Panel (Group 1 vs. Groups 2-4)

Parameter	Group No.	Statistically Significant Difference compared to Control (Group 1) ↑ or ↓
Fibrinogen	2 & 4	↓

Text Table 10– Female Coagulation Panel (Group 1 vs. Groups 2-4)

Parameter	Group No.	Statistically Significant Difference compared to Control (Group 1) ↑ or ↓
Fibrinogen	2-4	↓

↑ = Statistically significantly ($p < 0.05$) increased when compared to Group 1.

↓ = Statistically significantly ($p < 0.05$) decreased when compared to Group 1.

Group 1 - 0 mg/kg/day Group 2 - 1 mg/kg/day
Group 3 - 5 mg/kg/day Group 4 - 5 mg/kg/day GlucaGen[®]

Fibrinogen was decreased in Groups 2-4 females and Groups 2 and 4 males. In males, there was no dose response, as Group 3 had a higher mean than Group 2 (142.6 vs. 118). In females, there is a dose response, but the reason is not clear. Hypofibrinogenemia is typically found during hypercoagulative states, when fibrinogen is used up to form clots. There are no other abnormal coagulation parameters and there was no histological correlation to support a clinically meaningful hypofibrinogenemia. Therefore, in both males and females, the decreased fibrinogen is not considered toxicologically relevant.

Urinalysis: In females, the urinary pH was lower in both drug treated groups compared to controls (7.35, 6.4, 6.4, 6.38 at 0, 1, 5, 5 mg/kg/day respectively). These values in males were similarly lower (7.0, 6.8, 6.8, 6.8 respectively).

In summary, the statistically significant clinical pathology findings in this study are not considered toxicologically relevant, because in general, they lack sufficient magnitude or fail to demonstrate an appropriate dose response and none have supporting histological data.

Gross pathology: At a HD with the current drug, one male (rat #1121) had bilateral dark discoloration of the adrenal glands which also histologically correlated to mild congestion. Additionally at the same HD with the current drug, one male (rat #1120) was observed to be thin, but sponsor states that there was no histological correlation to this finding.

Organ weights:

In male rats, the absolute liver (10.9, 12.7, 13.6*, 11.9 g respectively) and relative liver (3.5, 3.9, 4.4, 3.8 respectively) weights were increased with the current drug at a HD, but not with the RD.

Additionally in males, absolute heart weights (1.16, 1.34*, 1.40*, 1.39* g respectively, *p<0.05) and relative heart weights (0.37, 0.42*, 0.45*, 0.45* respectively) were increased at all doses with both drugs. The relative spleen weights were decreased with both drugs (0.24, 0.21*, 0.22, 0.21* respectively).

Table 15. Absolute organ weights in male rats

Day: 29 relative to Start Date

Group	Sex		Adrenal Glands W g	Ovaries Weight g	Spleen Weight g	Heart We g	Brain We g	Kidneys Weight g	Testes Weight g	Liver We g
1	m	Mean	0.07461	.	0.75206	1.16601	1.82778	2.24289	3.57133	10.98831
		S.D.	0.00950	.	0.06468	0.21351	0.07680	0.17626	0.23507	1.35955
		N	8	0	8	8	8	8	8	8
2	m	Mean	0.07079	.	0.66751	1.34305*	1.80784	2.39990	3.51870	12.72764
		S.D.	0.00985	.	0.06287	0.08653	0.06122	0.16973	0.22860	1.19815
		N	8	0	8	8	8	8	8	8
3	m	Mean	0.07450	.	0.68843	1.40005**	1.79834	2.34226	3.63778	13.59906**
		S.D.	0.00940	.	0.06727	0.12894	0.05772	0.16542	0.27503	1.47171
		N	8	0	8	8	8	8	8	8
4	m	Mean	0.07581	.	0.64540*	1.38765**	1.76163	2.21614	3.41709	11.90709
		S.D.	0.01644	.	0.08591	0.08583	0.05223	0.17608	0.21279	1.60517
		N	8	0	8	8	8	8	8	8

Statistics Test: Dunnett Test: * - 5% significance level;
 ** - 1% significance level;
 n - Data not appropriate for statistical analysis;
 n1 - This group has only one value;

Arithmetic Mean Values Presented

Group 1 - 0 mg/kg/ day Group 2 - 1 mg/kg/ day Group 3 - 5 mg/kg/ day Group 4 - 5 mg/kg/ day GlucoG en

Table 16. Relative Organ weights (normalized to body weights) in male rats

Day: 29 relative to Start Date

Group	Sex		Relative Adrenal	Relative Ovary We	Relative Spleen W	Relative Heart We	Relative Brain We	Relative Kidney W	Relative Testes W	Relative Liver We
1	m	Mean	0.02365	.	0.23704	0.36624	0.57819	0.70683	1.12650	3.45453
		S.D.	0.00388	.	0.01172	0.04878	0.04182	0.02539	0.04029	0.26377
		N	8	0	8	8	8	8	8	8
2	m	Mean	0.02198	.	0.20650*	0.41586*	0.56033	0.74295	1.08918	3.93809
		S.D.	0.00338	.	0.01322	0.01986	0.02120	0.03912	0.04394	0.26989
		N	8	0	8	8	8	8	8	8
3	m	Mean	0.02391	.	0.22061	0.44810**	0.57641	0.74914	1.16389	4.35989**
		S.D.	0.00352	.	0.02455	0.03817	0.03347	0.03894	0.07137	0.52803
		N	8	0	8	8	8	8	8	8
4	m	Mean	0.02433	.	0.20782*	0.44791**	0.56838	0.71416	1.10254	3.83421
		S.D.	0.00465	.	0.02487	0.03258	0.01940	0.04646	0.07289	0.46115
		N	8	0	8	8	8	8	8	8

In female rats, absolute (1.5, 1.7*, 1.8*, 1.6 respectively) and relative (at a HD 0.72* vs 0.66 in controls) kidney weights were increased with the current drug, but not with the RD (0.69 vs 0.66 in controls). Similarly relative spleen weights were decreased with the current drug (0.23* vs 0.26 in the controls), but not with the RD (0.24 vs 0.26 g in controls). However, none of these were considered significant by the sponsor.

Note that in female rats absolute heart weights (0.89, 1.1*, 1.0*, 1.1* g respectively, *p<0.05) and relative heart weights (0.38, 0.43*, 0.43*, 0.44* respectively) were increased at all doses with both drugs, as noted in male rats. Similarly absolute (7.9, 10.3*, 10.2*, 9.8* g respectively) and relative liver weights were generally increased with both drugs.

In summary, the statistically significant organ weight findings in this study are not considered toxicologically relevant, because in general, they lack sufficient magnitude or fail to demonstrate an appropriate dose response and none have supporting clinical pathology or histological data.

Table 17. Absolute organ weights in female rats

		Day: 29 relative to Start Date								
Group	Sex		Adrenal Glands W g	Ovaries Weight g	Spleen Weight g	Heart We g	Brain We g	Kidneys Weight g	Testes Weight g	Liver We g
1	f	Mean	0.08609	0.14363	0.59619	0.88824	1.73411	1.53648	.	7.98384
		S.D.	0.01078	0.01925	0.05663	0.04016	0.05207	0.08427	.	0.49758
		N	8	8	8	8	8	8	0	8
2	f	Mean	0.08938	0.15181	0.60770	1.07978**	1.71963	1.72246**	.	10.31420**
		S.D.	0.00843	0.02303	0.06094	0.09094	0.06937	0.07615	.	0.86404
		N	8	8	8	8	8	8	0	8
3	f	Mean	0.08279	0.14923	0.55408	1.03395**	1.72084	1.75391**	.	10.17365**
		S.D.	0.00597	0.02324	0.06085	0.09110	0.08010	0.12265	.	1.24357
		N	8	8	8	8	8	8	0	8
4	f	Mean	0.08734	0.15893	0.55639	1.05053**	1.66556	1.63804	.	9.77140**
		S.D.	0.00790	0.03287	0.04704	0.10080	0.04092	0.11979	.	1.03172
		N	8	8	8	8	8	8	0	8

Table 18. Relative organ weights (normalized to body weights) in female rats

Day: 29 relative to Start Date

Group	Sex		Relative Adrenal	Relative Ovary We	Relative Spleen W	Relative Heart We	Relative Brain We	Relative Kidney W	Relative Testes W	Relative Liver We
1	f	Mean	0.03718	0.06234	0.25705	0.38325	0.74934	0.66288	.	3.44261
		S.D.	0.00487	0.01111	0.02141	0.01570	0.04532	0.03267	.	0.15942
		N	8	8	8	8	8	8	0	8
2	f	Mean	0.03579	0.06109	0.24328	0.43200**	0.68984	0.69011	.	4.13480**
		S.D.	0.00329	0.01083	0.02085	0.02656	0.04521	0.02977	.	0.37672
		N	8	8	8	8	8	8	0	8
3	f	Mean	0.03414	0.06159	0.22744*	0.42490*	0.71011	0.72149**	.	4.17284**
		S.D.	0.00326	0.01035	0.01791	0.02481	0.06084	0.03806	.	0.35100
		N	8	8	8	8	8	8	0	8
4	f	Mean	0.03679	0.06700	0.23498	0.44291**	0.70334	0.69024	.	4.11148**
		S.D.	0.00278	0.01332	0.02390	0.03905	0.03966	0.03763	.	0.30232
		N	8	8	8	8	8	8	0	8

↑ = Statistically significantly (p < 0.05) increased when compared to Group 1.
 ↓ = Statistically significantly (p < 0.05) decreased when compared to Group 1.

Thus, in male rats, absolute liver (10.9, 12.7, 13.6*, 11.9 g respectively) and relative liver weights (3.5, 3.9, 4.4, 3.8 respectively) were increased with the current drug, but not with RD. In females absolute liver weights were increased with both drugs. In female rats, absolute kidney (1.5, 1.7*, 1.8*, 1.6 respectively) and relative kidney (at a HD 0.72* vs 0.66 in controls) weights were increased with the drug, but not with the RD (0.69 vs 0.66 in controls). Also in females, relative spleen weights were decreased with the current drug (0.23* vs 0.26 in the controls), but not with the RD (0.24 vs 0.26 g in controls). Other findings were noted with both drugs, such as increase in absolute heart weights (in males 1.16, 1.34*, 1.40*, 1.39* g respectively; in females 0.89, 1.1*, 1.0*, 1.1* g respectively, *p<0.05) and increase in relative heart weights in both sexes. However, none of these were considered significant by the sponsor

Histopathology: In female rats, minimal to mild mineralization in the heart was noted in 2/8 rats at a HD with the current drug (which was not noted with the RD or in controls), note that increased heart weights were noted with both drugs. In male rats, minimal to mild chronic progressive bilateral nephropathy in the kidney was noted in 3/8 rats at a HD with the current drug (which was not noted with RD or in the controls).

Other findings were noted with both drugs, including the findings in the kidney (proteinosis unilateral), and the mandibular lymph nodes (incidences of hyperplasia). Similarly with both drugs (the current drug, and in RD), minimal to mild chronic active inflammation was noted in sciatic nerve fascia, including in controls; sponsor explains that this is due to intramuscular injection.

Sponsor states that IM injection caused a similar (minimal to moderate) inflammatory reaction at the injection site in control and test article treated animals that spread along the fascial planes of the hind limb muscle groups, and was detected at the site of the sciatic nerve. There were no treatment-related changes in any of the other tissues examined

On the other hand, the RD alone produced findings in liver (mild necrosis and chronic active inflammation and hemorrhage in to 2/8 males + females vs 0/8 controls); these were not noted with the current drug.

Thus at a HD of 5 mg/kg/day, the target organ of toxicity with the current drug (glucagon for injection) was heart in the female rats (minimal to mild mineralization in 2/8 vs 0/8 in controls or the RD), and kidney in male rats (minimal to mild chronic progressive bilateral nephropathy in 3/8 rats (not noted with the RD or controls).

Table 19. Histopathology findings in rats with the current drug (glucagon for injection) and RD (glucaGen) in rats

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
AD29XE2M3 - AD29XE AD30AZ.2M31.BTL:28-Day Repeated Dose Intramuscular Toxicity Study of Glucagon for Injection and GlucaGen (rDNA Glucagon) in Sprague-Dawley Rat

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	5 mg/kg/day Glucog	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	5 mg/kg/day Glucog
Removal Reasons: All of those SELECTED								
Number of Animals on Study :	8	8	8	8	8	8	8	8
Number of Animals Completed:	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
esophagus;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(7)	(8)
Within Normal Limits.....	8	0	8	8	8	0	7	8
Not Examined: INSUFFICIENT TISSUE TO EVALUATE	0	0	0	0	0	0	1	0
eyes;								
Examined.....	(8)	(0)	(8)	(7)	(8)	(0)	(8)	(8)
Within Normal Limits.....	7	0	8	7	8	0	8	8
Not Examined: INSUFFICIENT TISSUE TO EVALUATE	0	0	0	1	0	0	0	0
infiltration; mesenchymal; cornea; unilateral	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0	0	0	0
harderian glands;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	7	0	8	8	8	0	8	8
mineralization; unilateral	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0	0	0	0
heart;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	6	0	7	6	7	0	5	8
inflammation; chronic	(1)	(0)	(1)	(1)	(1)	(0)	(1)	(0)
minimal	1	0	1	0	1	0	1	0
mild	0	0	0	1	0	0	0	0
inflammation; chronic-active	(1)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal	1	0	0	1	0	0	0	0
mineralization	(0)	(0)	(0)	(0)	(0)	(0)	(2)	(0)
minimal	0	0	0	0	0	0	1	0
mild	0	0	0	0	0	0	1	0
fibrosis	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0	0	0	0
intestine, cecum;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	8	0	8	8	8	0	8	8
kidneys;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	7	0	2	5	8	0	6	3
chronic progressive nephropathy; bilateral	(0)	(0)	(3)	(0)	(0)	(0)	(0)	(0)
minimal	0	0	2	0	0	0	0	0
mild	0	0	1	0	0	0	0	0
chronic progressive nephropathy; unilateral	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
minimal	0	0	0	0	0	0	0	1
congestion; bilateral	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
mild	1	0	0	0	0	0	0	0
proteinosis; bilateral	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(1)
minimal	0	0	1	0	0	0	0	1
proteinosis; unilateral	(0)	(0)	(2)	(3)	(0)	(0)	(2)	(3)
minimal	0	0	2	3	0	0	2	3
liver;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)

Table 19 continued. Histopathology findings in rats with the current drug (glucagon for injection) and RD (glucaGen) in rats.

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations								
AD29XE2M3 - AD29XE AD30AZ.2M31.BTL:28-Day Repeated Dose Intramuscular Toxicity Study of Glucagon for Injection and GlucaGen (rDNA Glucagon) in Sprague-Dawley Rat								
Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	5 mg/kg/day GlucoG	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	5 mg/kg/day GlucoG
Removal Reasons: All of those SELECTED	Number of Animals on Study : Number of Animals Completed:				Number of Animals on Study : Number of Animals Completed:			
Liver; (continued)								
Within Normal Limits.....	1	0	8	3	2	0	8	2
inflammation; chronic.....	(3)	(0)	(0)	(0)	(3)	(0)	(0)	(4)
minimal.....	3	0	0	0	3	0	0	4
inflammation; chronic-active.....	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
mild.....	0	0	0	1	0	0	0	0
necrosis.....	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
mild.....	0	0	0	1	0	0	0	0
vacuolation; lipid.....	(6)	(0)	(0)	(4)	(4)	(0)	(0)	(4)
minimal.....	3	0	0	2	3	0	0	2
mild.....	3	0	0	2	1	0	0	2
hemorrhage.....	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
mild.....	0	0	0	1	0	0	0	0
Lungs with bronchi;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	8	0	8	8	8	0	8	8
Lymph node, mesenteric;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	8	0	8	8	8	0	8	8
Lymph node, mandibular;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	7	0	5	7	4	0	5	5
hyperplasia.....	(1)	(0)	(3)	(1)	(4)	(0)	(3)	(3)
minimal.....	0	0	0	0	1	0	2	2
mild.....	1	0	2	1	3	0	1	1
moderate.....	0	0	1	0	0	0	0	0
mammary gland;								
Examined.....	(-)	(-)	(-)	(-)	(8)	(0)	(8)	(8)
Within Normal Limits.....	-	-	-	-	8	0	8	8
skeletal muscle, sternal;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	7	0	8	7	8	0	8	8
AD29XE2M3 - AD29XE AD30AZ.2M31.BTL:28-Day Repeated Dose Intramuscular Toxicity Study of Glucagon for Injection and GlucaGen (rDNA Glucagon) in Sprague-Dawley Rat								
Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	5 mg/kg/day GlucoG	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	5 mg/kg/day GlucoG
Removal Reasons: All of those SELECTED	Number of Animals on Study : Number of Animals Completed:				Number of Animals on Study : Number of Animals Completed:			
skeletal muscle, sternal; (continued)								
inflammation, granulomatous.....	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
minimal.....	1	0	0	0	0	0	0	0
inflammation, chronic.....	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal.....	0	0	0	1	0	0	0	0
skeletal muscle, diaphragm;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	8	0	7	7	7	0	8	8
hypertrophy; parietal.....	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal.....	0	0	1	1	0	0	0	0
inflammation; chronic-active; parietal cell.....	(0)	(0)	(1)	(0)	(1)	(0)	(0)	(0)
minimal.....	0	0	1	0	1	0	0	0
nerve, sciatic;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	3	0	2	3	0	0	1	2
inflammation; chronic-active.....	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
mild.....	0	0	0	0	0	0	0	1
inflammation; chronic-active; fascia.....	(5)	(0)	(6)	(5)	(8)	(0)	(7)	(5)
minimal.....	5	0	5	4	5	0	7	3
mild.....	0	0	1	1	3	0	0	2
fibroplasia; fascial.....	(4)	(0)	(3)	(3)	(8)	(0)	(6)	(4)
minimal.....	2	0	2	2	6	0	6	2
mild.....	2	0	1	1	2	0	0	2
hemorrhage.....	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal.....	0	0	0	1	0	0	0	0
hemorrhage; fascia.....	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
mild.....	0	0	0	1	0	0	0	0
ovaries;								
Examined.....	(-)	(-)	(-)	(-)	(8)	(0)	(8)	(8)
Within Normal Limits.....	-	-	-	-	8	0	8	8
pancreas;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	8	0	8	8	8	0	8	8

Table 19 continued. Histopathology findings in rats with the current drug (glucagon for injection) and RD (glucaGen) in rats continued

AD29XE2M3 - AD29XE AD30AZ.2M31.BTL:28-Day Repeated Dose Intramuscular Toxicity Study of Glucagon for Injection and GlucaGen (rDNA Glucagon) in Sprague-Dawley Rat

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	5 mg/kg/day GlucoG	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	5 mg/kg/day GlucoG
Removal Reasons: All of those SELECTED								
Number of Animals on Study :	8	8	8	8	8	8	8	8
Number of Animals Completed:	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
stomach;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	8	0	8	8	8	0	8	8
testes;								
Examined.....	(8)	(0)	(8)	(8)	(-)	(-)	(-)	(-)
Within Normal Limits.....	8	0	8	8	-	-	-	-
thymus;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	8	0	8	8	8	0	8	8
hemorrhage.....	(0)	(0)	(0)	(2)	(0)	(0)	(0)	(0)
minimal.....	0	0	0	1	0	0	0	0
mild.....	0	0	0	1	0	0	0	0
pigmentation, hemosiderin.....	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
mild.....	0	0	0	1	0	0	0	0
thyroid glands;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	8	0	7	7	7	0	7	7
cyst; keratinized; bilateral.....	0	0	0	0	0	0	1	0
cyst; keratinized; unilateral.....	0	0	1	1	1	0	0	1
trachea;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(8)	(8)
Within Normal Limits.....	8	0	8	8	8	0	8	8
urinary bladder;								
Examined.....	(8)	(0)	(8)	(8)	(8)	(0)	(7)	(8)
Within Normal Limits.....	7	0	7	7	8	0	7	8
Not Examined: NOT PRESENT.....	0	0	0	0	0	0	1	0
hyperplasia; papillary.....	(1)	(0)	(1)	(1)	(0)	(0)	(0)	(0)
mild.....	0	0	1	1	0	0	0	0
moderate.....	1	0	0	0	0	0	0	0
uterus;								
Examined.....	(-)	(-)	(-)	(-)	(8)	(0)	(8)	(8)

Sponsor's conclusions on histopathology are described below:

There were no treatment related microscopic findings in any dose groups in either sex.

When compared to control (Group 1) and to the comparator drug GlucaGen[®] (rDNA Glucagon, Group 4), intramuscular injection of the test article (Glucagon for Injection) at 5 mg/kg/day (Group 3) for twenty-eight consecutive days caused a similar (minimal to moderate) inflammatory reaction at the injection site in control and test article treated animals that spread along the fascial planes of the hind limb muscle groups and was detected at the site of the sciatic nerve. There were no treatment-related changes in any of the other tissues examined.

Toxicokinetics: Plasma concentration were increased in a dose proportional manner. Values on day 27 in males were 149, 582, 710 ng/ml at 1, 5 of the current APP drug, and 5 mg/kg/day of RD respectively. These values in females were 166, 630, 654 ng/ml respectively, thus plasma concentrations appear to be in general comparable with both drugs. The exposures of the drug in males and females were slightly higher on day 27 (males 122, 502, 454 ng.hr/ml at 1, 5, and 5 mg/kg/day respectively; in females were 99, 557, 511 ng.hr/ml respectively) than on day 1 (males 76, 355, 444 ng.hr/ml respectively, females 63, 306, 285 ng.hr/ml respectively).

Plasma concentrations for Glucagon for Injection and GlucaGen[®] were similar. T_{max} for Glucagon for Injection and GlucaGen[®] was estimated at 0.25 hr. Treatment with Glucagon for Injection as an intramuscular injection at 1 and 5 mg/kg/day for 27 days demonstrated slight but consistent increases in both C_{max} values and AUC values of Glucagon for Injection for both genders. Average C_{max} values were slightly less than dose proportional on Days 1 and 27. Comparing the 5 mg/kg dose to the 1 mg/kg dose the anticipated 5-fold increase was only 3.1 and 3.9 for male based values and 3.6 and 3.8 for female based values on Days 1 and 27, respectively.

Table 7. Toxicokinetic Data from Sprague Dawley Rats Dosed for 27 Days with Either Glucagon or GlucaGen by Intramuscular Injection

Group	Gender (M/F)	Day	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (hr)	$AUC_{(0-t)}$ (ng/mL [•] hr)	CL_{sys} (mL/kg/hr)
1	M	1	0	BLQ	NA	NC	NC
		27	0	BLQ	NA	NC	NC
	F	1	0	BLQ	NA	NC	NC
		27	0	BLQ	NA	NC	NC
2	M	1	1	140	0.25	75.9	549
		27	1	149	0.25	122	342
	F	1	1	115	0.25	63.3	659
		27	1	166	0.25	98.5	423
3	M	1	5	435	0.25	355	587
		27	5	582	0.25	502	415
	F	1	5	413	0.25	306	681
		27	5	630	0.25	557	374
4	M	1	5	462	0.25	444	470
		27	5	710	0.25	454	459
	F	1	5	398	0.25	285	730
		27	5	654	0.25	511	408

BLQ – below limit of quantification

NA – not applicable

NC – not calculable

2.6.7.3: Toxicokinetics		Overview of Toxicokinetics Data			
		Rats Day 27			
		Mean C _{max} (µg/mL)		Mean AUC _{0-2h} (µg·h/mL)	
Test Article	Daily Dose (mg/kg/day)	M	F	M	F
Glucagon for Injection	1	149	166	122	98.5
Glucagon for Injection	5	582	630	502	557
Reference Drug					
GlucaGen®	5	710	654	454	511

Sponsor states that the results of the toxicokinetic evaluations and a comparison of the plasma concentrations themselves demonstrate that the systemic exposures for animals treated with GlucaGen® and Glucagon for Injection are comparable.

The exposures of GlucaGen® compared to Glucagon for Injection, average AUC values for similar dose (5 mg/kg/day) were 125% and 90% for values calculated for Day 1 and Day 27, respectively for the male animals. The corresponding data for the female treated rats were 93% and 92% comparing the average AUC values for Days 1 and 27.

Following summary Table was provided on the current 28-day toxicity study by the sponsor:

Animal	Sprague-Dawley rats, 6-7 weeks and 7-8 weeks of age, males and females, respectively				
Group	1	2	3	4	
Test article	Sterile Water for Injection, USP	Glucagon for Injection (Low Dose)	Glucagon for Injection (High Dose)	GlucaGen [®] (rDNA Glucagon) (Comparator Drug)	
Dosage level (mg/kg/day)	0	1	5	5	
Number of Main Study animals (M:F)	8:8	8:8	8:8	8:8	
Mortality Main Study (M:F)	0:0	0:0	0:0	0:0	
Number of TK animals (M:F)	3:3	5:5	5:5	5:5	
Mortality TK Study (M:F)	0:0	0:0	0:0	0:0	
Clinical signs	-	-	-	-	
Body weights [Main Study] (M:F)	-	-	-	-	
Weekly Food consumption	-	F ↑Week 2 (21.1%)	M ↑Weeks 1, 3 & 4 (21.1, 31.5 & 22.3%) F ↑Week 2 (28.3%)	M ↑Weeks 3 & 4 (22.3 & 14.8%) F ↑Weeks 1 & 2 (25.6 & 60.4%)	
Ophthalmology	-	-	-	-	
Urinalysis	-	-	-	-	
Hematology	-	-	-	-	
Chemistry	-	-	-	-	
Coagulation	-	-	-	-	
Organ weights	-	-	-	-	
Gross Necropsy	-	-	-	-	
Histopathology	-	-	-	-	
Plasma concentration [M:F, Mean (n=2)]					
Average C _{max} (ng/mL) (M:F)	1 st	BLQ:BLQ	140:115	435:413	462:398
	27 th	BLQ:BLQ	149:166	582:630	710:654
Average Approx. T _{max} (hr) (M:F)	1 st	NA:NA	0.25:0.25	0.25:0.25	0.25:0.25
	27 th	NA:NA	0.25:0.25	0.25:0.25	0.25:0.25
Conclusion: Male and female Sprague-Dawley rats were administered intramuscularly with Glucagon for Injection (1 or 5 mg/kg/day) or GlucaGen [®] (5 mg/kg/day) for up to 28 days. There was no treatment related toxicity observed in any dose group. Treatment with Glucagon for Injection or GlucaGen [®] at 5 mg/kg/day showed similar safety profile.					

- No Effect

Toxicology summary: In a 28-day intra-muscular toxicity study in rats, doses of 0, 1, 5 mg/kg/day of glucagon for injection (the current drug) were administered to three groups of rats (n=8/sex/dose). The 4th group of rats were similarly administered the reference drug or RD, i.e. GlucaGen (5 mg/kg/day) for comparison. The TK in general were similar with both drugs. The exposures of the drug in males and females were slightly higher on day-27 (males 122, 502, 454 ng.hr/ml at 1, 5, and 5 mg/kg/day respectively; in females were 99, 557, 511 ng.hr/ml respectively) than on day-1 (males 76, 355, 444 ng.hr/ml respectively, females 63, 306, 285 ng.hr/ml respectively). No significant clinical signs were noted with both drugs (current or RD). In males at a HD, body weight gains were lower on days 22-29 with the current drug (but not with the RD (GlucaGen); no effects on body weights or weight gains in females were noted.

Food consumption was significantly increased with both (i.e. by 19% with the current drug and by 13% with RD). The current and RD glucagon had similar effects on hematological parameters (increased platelet counts, decreased reticulocyte counts & decreased %-reticulocytes), and both had no effects on ophthalmological parameters. The current drug produced some changes in clinical chemistry parameters, not noted with the RD, these in males included increases in total protein; in females increases in calcium & triglycerides, & decreases in BUN levels, but these were not toxicologically relevant as the differences were small, and no histological correlates were observed. Decreased fibrinogen levels and lower urinary pH were noted with both drugs (the current drug and RD).

In male rats, absolute liver (10.9, 12.7, 13.6*, 11.9 g respectively, by 25% at a HD), and in female rats, absolute kidney (1.5, 1.7*, 1.8*, 1.6 respectively, by 20% at a HD) weights were increased with the current drug by 25% and 20% respectively, but not with the RD. Similarly relative liver and kidney weights were increased in males and females respectively, but not with the RD. Other changes in organ weights noted were with both drugs, such as absolute heart weights which were similarly increased (males (1.2, 1.34*, 1.40*, 1.39* g respectively; in females 0.89, 1.1*, 1.0*, 1.1* g respectively, *p<0.05) and relative heart weights, these were increased with both drugs in both sexes. The target organs of toxicity may be heart in female rats, as minimal to mild mineralization in the heart was noted in 2/8 rats at a HD with the current drug (which was not noted in controls or the RD). In the kidney, chronic progressive nephropathy bilateral minimal to mild was noted in 3/8 males (vs 0/8 controls or RD). Note that in females, minimal chronic progressive nephropathy unilateral was noted in 1/8 female rats with the RD. Other findings were noted with both drugs, including in the kidney (unilateral proteinosis in males 0/8, 0/0, 2/8, 3/8; females 0/8, 0/0, 2/8, 3/8 respectively), mandibular lymph nodes (incidences of hyperplasia), and in sciatic nerve fascia (minimal to mild chronic active inflammation) which were noted with both drugs and also in controls; sponsor explains that this is due to intramuscular injection. They state that the "inflammatory reaction at the injection site in control and test article treated animals spreads along the fascial planes of the hind limb muscle groups, and was detected at the site of the sciatic nerve".

Note that total impurities that were tested in the 28-day toxicity study were up to (b) (4)% with the current drug product (glucagon for injection), while these were present up to (b) (4)% in the reference drug (GlucaGen). The recommended doses of the current drug product (glucagon for injection) are up to 2 mg/day. Therefore 2 mg (or 2000 mcg) will have up to (b) (4) mcg/day or (b) (4) mcg of (b) (4) (i.e. (b) (4)). In the 28-day toxicity, the doses of up to 5000 mcg/kg/day of the current drug product were used, which had up to (b) (4)% impurities, and of which (b) (4)% was (b) (4) (i.e. (b) (4)) was present in this 28-day toxicity study. According to our FDA chemist, the stability data (at 24 months) are similar to impurity levels seen in the repeat dose study here. Therefore levels of (b) (4) up to (b) (4)% have been tested and the toxicity profiles are similar between APP glucagon product and comparator.

The NOAEL or tolerated doses of the drug in a 4-week oral toxicity study in rats could not be established as histopathology findings were noted in the heart (in females), and in the kidney (in males) at a HD of 5 mg/kg/day with the current drug, not noted with the RD. The lower dose of 1 mg/kg/day was not examined for histopathology findings. The sponsor does not consider any of these findings significant. This NOAEL of <5 mg/kg/day (or 30 mg/m²/day) provides the safety margin of <24 X in human subjects (at the maximal recommended clinical dose of 2 mg/day or 1.23 mg/m²/day), based on body surface area.

Sponsor's conclusions are shown below:

CONCLUSION

Male and female Sprague-Dawley rats were administered intramuscularly with Glucagon for Injection (1 or 5 mg/kg/day) or GlucaGen[®] (5 mg/kg/day) for up to 28 days. There was no treatment related toxicity observed in any dose group. Treatment with Glucagon for Injection or GlucaGen[®] at 5 mg/kg/day showed similar safety profile.

2.6.6.4. Genetic toxicology: Following two gene-toxicity studies have been conducted with glucagon for injection.

- 1. Microbial mutagenesis assay** ((b) (4) study # AD29XE.503 (b) (4) and AD30AZ.503. (b) (4)).

This study was conducted in compliance with the testing guidelines of the ICH (1996 and 1997) and OECD (1998). The study was conducted by (b) (4)

The purpose of this study was to evaluate the mutagenic potential of the test article, APP Glucagon for Injection, in comparison to Reference Listed drug GlucaGen[®] by measuring their ability to induce reverse mutations at selected loci of several strains of Salmonella typhimurium and at the tryptophan locus of Escherichia coli strain WP2 uvrA in the presence and absence of Aroclor-induced rat liver S9.

Methods: The Ames assay (initial and confirmatory) was carried out using the plate incorporation method.

Test article lot number and description: APP Glucagon for Injection (batch C108-002) and the reference drug GlucaGen (batch number AW60180) were tested in the Ames assay. Same APP drug lot was used in a 28-day toxicity study in rats.

These test articles were received by (b) (4) on 17 June 2011 and were assigned the (b) (4) code numbers AD29XE (for APP's Glucagon / lot # C108-002) and AD30AZ (for RD GlucaGen/ lot # AW60180) respectively.

Sponsor states that the lots used in the toxicology testing program for Glucagon for Injection (the current APP drug) were at the end of expiration dating, to provide an exaggeration of the impurity testing. The post-study analysis of Glucagon for Injection when stored at 30°C, inverted, found the test article to be (b) (4) % pure, which was similar to the initial purity value reported on the pre-study Certificate of Analysis ((b) (4) %). The certificate of analysis (AOA) for these are provided below.

Table 1. Following is the certificate of analysis for the current drug lot # C108-002 (APP's glucagon for injection) used in the Ames assay. It had (b)(4) code number of AD29XE. The drug was analyzed on 8/5/11

**CERTIFICATE OF ANALYSIS
INNOVATION AND DEVELOPMENT
Glucagon for Injection (1 mg/vial)**

Page 1 of 1
Version 2.0

NDC Code:	<u>NDC 63323-596-03</u>	Lot Number:	<u>C108-002</u>
Product			
Configuration:	<u>1 mg Glucagon / vial</u>	Expiry Date:	<u>N/A</u>
Manufactured By:	<u>APP Pharmaceuticals</u>	Distributed By:	<u>N/A</u>
Storage Condition:	<u>30 °C, Inverted</u>	Test Date:	<u>08/05/11</u>

TEST		RESULTS
Assay by HPLC		(b)(4) %
Residual Substances		(b)(4)

PREPARED BY:	(b)(4)	DATE:	<u>9/6/11</u>
REVIEWED BY:	(b)(4)	DATE:	<u>9/6/11</u>
APPROVED BY:	(b)(4)	DATE:	<u>9/12/11</u>

Table 2. Certificate of analysis for the reference drug (GlucaGen), AW60180 Is provided below. It had (b) (4) code # AD30AZ,

**CERTIFICATE OF ANALYSIS
INNOVATION AND DEVELOPMENT
GlucaGen® (Glucagon for Injection, 1 mg/vial)**

NDC Code:	<u>NDC 55390-004-01</u>	Lot Number:	<u>AW60180</u>
Product		Expiry Date:	<u>11/2012</u>
Configuration:	<u>1 mg Glucagon / vial</u>	Distributed By:	<u>Bedford Laboratories</u>
Manufactured By:	<u>Novo Nordisk A/S</u>	Test Date:	<u>06/16/11</u>
Storage Condition:	<u>Room Temperature</u>		

TEST	RESULTS
Assay by HPLC	(b) (4) %
Residual Substances	(b) (4)

PREPARED BY:	(b) (4)	DATE:	<u>6/27/11</u>
REVIEWED BY:	(b) (4)	DATE:	<u>6/27/11</u>
APPROVED BY:	(b) (4)	DATE:	<u>6/28/11</u>

Dose formulation analysis: Sponsor states that their product (glucagon for injection) had higher total combined impurities of (b) (4) % vs the RD GlucaGen, which had total combined impurities of (b) (4) % as stated below.

The results are summarized in Tables I and II. Percent relative standard deviation (%RSD) was calculated on samples from APP product and GlucaGen. All assay results met the acceptance criteria. The total combined impurity values for the APP product were about (b) (4) %, whereas the GlucaGen had total combined impurity values of about (b) (4) %.

Table 3. Impurities were tested at the start of the study in APP glucagon (lot 108-002) and RD GlucaGen (lot AD30AZ) . All dose formulations were analyzed and injected in duplicates by HPLC.

TABLE I
Summary of Results - Concentration at the Start of the Study

Acceptance Criteria

Assay Limit: % Target Concentration 90 to 110%

%RSD (n=4): NMT (b) (4) %¹

Sample Name	Vial Number	Injection Number	Glucagon (mg/mL)	Glucagon Related Total Impurities (%)	Lactose Related Total Impurities (%)	Total Combined Impurities ² (%)
Vehicle Control	Vial 1	1	0	Not Applicable (NA)		
	Vial 2	1	0	NA		
	Mean		0	NA		
AD29XE Glucagon for Injection APP Lot # C108-002	Vial 1	1	2.363	(b) (4)		
		2	2.359			
	Vial 2	1	2.340			
		2	2.330			
	Mean		2.348			
	%RSD		1			
% Target Concentration³		101.5	NA			
AD30AZ GlucaGen RLD Lot # AW60180	Vial 1	1	2.492	(b) (4)		
		2	2.483			
	Vial 2	1	2.532			
		2	2.532			
	Mean		2.510			
	%RSD		1			
% Target Concentration⁴		98.0	NA			

¹ %RSD (n=4) was calculated from duplicate injections of two preparations.

² No criteria for impurities, but actual values are reported.

³ Target concentration is 2.314 mg/mL, which has been calculated using % Assay from C of A for Glucagon for Injection, Lot C108-002, 30 °C Inverted, and 300 µL reconstitution volume (Attachment I).

⁴ Target concentration is 2.562 mg/mL, which has been calculated using % Assay from C of A for GlucaGen, Lot AW60180, and 300 µL reconstitution volume (Attachment II).

Table 4. Impurities were tested at the end of the study

TABLE II
Summary of Results - Concentration at End of the Study

Acceptance Criteria

Assay Limit: % Target Concentration 90 to 110%
%RSD (n=4): NMT ^{(b) (4)}%¹

Sample Name	Vial Number	Injection Number	Glucagon (mg/mL)	Glucagon Related Total Impurities (%)	Lactose Related Total Impurities (%)	Total Combined Impurities ² (%)
Vehicle Control	Vial 1	1	0	Not Applicable (NA)		
	Vial 2	1	0	NA		
	Mean		0	NA		
AD29XE Glucagon for Injection APP Lot # C108-002	Vial 1	1	2.378	^{(b) (4)}		
		2	2.377			
	Vial 2	1	2.440			
		2	2.438			
	Mean		2.408			
	%RSD		1			
% Target Concentration ³		104.1	NA			
AD30AZ Glucagen RLD Lot # AW60180	Vial 1	1	2.698	^{(b) (4)}		
		2	2.700			
	Vial 2	1	2.720			
		2	2.770			
	Mean		2.722			
	%RSD		1			
% Target Concentration ⁴		106.2	NA			

¹ %RSD (n=4) was calculated from duplicate injections of two preparations.

² No criteria for impurities, but actual values are reported.

³ Target concentration is 2.314 mg/mL, which has been calculated using % Assay from C of A for Glucagon for Injection, lot C108-002, 30 °C Inverted and 300 µL reconstitution volume (Attachment I).

⁴ Target concentration is 2.562 mg/mL, which has been calculated using % Assay from C of A for Glucagen, lot AW60180 and 300 µL reconstitution volume (Attachment II).

Thus, impurities tested in the Ames assay were present up to ^{(b) (4)}% in the APP drug product (and up to ^{(b) (4)}% in the RD). For example the lot # C108-002 tested here was stored at 30 deg C, and according to our FDA chemist, these were beyond the 24 months of stability data, so impurity levels are higher than reported in the CoA for release.

The study details and results are provided below:

The test article, Glucagon for Injection (batch number C108-002), and the comparator/reference article, GlucaGen® (batch number AW60180), were tested in the Bacterial Reverse Mutation Assay using Salmonella typhimurium tester strains TA98, TA100, TA1535 and TA1537 and Escherichia coli tester strain WP2 uvrA in the presence and absence of Aroclor-induced rat liver S9. The assay was performed in two phases, using the plate incorporation method. The first phase, the initial toxicity-mutation assay, was used to establish the dose-range for the confirmatory mutagenicity assay. A copy of the Historical Negative and Positive Control Values is included in Appendix I. The post-study test article was (b)(4) % pure. Pre-study Certificate of Analysis shows similar purity ((b)(4) %)

A) Ames assay with APP's glucagon for injection with lot number C108-002 ((b)(4) code # AD29XE)

Doses tested of the current drug (glucagon for injection)

In the initial toxicity-mutation assay, the maximum dose tested was 2700 µg per plate; this dose was achieved using a concentration of 2.7 mg/mL and a 1000 µL plating aliquot. The nominal dose levels tested were 0.90, 2.7, 9.0, 27, 90, 270, 900 and 2700 µg per plate. The maximum nominal dose of 2700 µg per plate was the highest that could be achieved.

Criteria established for the positive Ames test are described below

For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article.

Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was greater than or equal to 3.0-times the mean vehicle control value. Data sets for tester strains TA98, TA100 and WP2 *uvrA* were judged positive if the increase in mean revertants at the peak of the dose response was greater than or equal to 2.0-times the mean vehicle control value.

An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response will be evaluated as negative, if it is neither positive nor equivocal.

Results

Initial Toxicity-Mutation Assay

The results of the initial toxicity-mutation assay are presented in Tables 5 through 7. These data were generated in Experiments B1 and B2.

In Experiment B1 (Initial Toxicity-Mutation Assay), the dose levels tested were 0.90, 2.7, 9.0, 27, 90, 270, 900 and 2700 µg per plate. The maximum nominal dose of 2700 µg per plate was the

highest that could be achieved. During protocol development, the Sponsor stated that reconstitution with 0.30 mL of SWI would yield a solution at 2.7 mg/mL, and this value was used throughout the raw data. Subsequently, the Sponsor indicated that the 2.7 mg/mL value was actually found to be 2.6 mg/mL. Nevertheless, formulation analysis confirmed that the actual glucagon content achieved was within the protocol-specified acceptance criterion of 85 to 115% of target, and the nominal dose levels are reported. The test article formed soluble and clear solutions in sterile water for injection from 0.00090 to 2.7 mg/mL. No positive mutagenic responses were observed with any of the tester strains in the absence of S9 activation and with tester strains TA98, TA100, TA1535 and WP2 *uvrA* in the presence of S9 activation. No precipitate was observed. Toxicity was observed beginning at 900 or at 2700 µg per plate with a few test conditions. Due to an unacceptable vehicle control value, tester strain TA1537 in the presence of S9 activation was not evaluated for mutagenicity but was retested in Experiment B2 based on the precipitate and toxicity profile observed. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the retest and confirmatory mutagenicity assays was 2700 µg per plate.

Note that in the initial Ames assay in the tester strain WP2 *uvrA* (in the presence of S9 activation), there was a contamination at 90 mcg/plate, and that plate was not counted as shown below.

In Experiment B2 (Retest of the Initial Toxicity-Mutation Assay), no positive mutagenic response was observed with tester strain TA1537 in the presence of S9 activation. The nominal dose levels tested were 9.0, 27, 90, 270, 900 and 2700 µg per plate. Neither precipitate, nor appreciable toxicity was observed.

- i) Table 5 below shows the results of initial Ames assay with APP glucagon without S-9 activation.

Table 5: Initial Toxicity-Mutation Assay with Glucagon for Injection without S9 activation

Study Number: AD29XE.503 (b) (4)
 Experiment: B1
 Exposure Method: Plate incorporation assay

Study Code: AD29XE
 Date Plated: 6/29/2011
 Evaluation Period: 7/3/2011 to 7/5/2011

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	Glucagon for Injection	2700 µg	9	1	0.6	10 ^A , 8 ^A
		900 µg	9	1	0.6	8 ^A , 9 ^A
		270 µg	19	11	1.3	11 ^A , 27 ^A
		90 µg	19	6	1.3	23 ^A , 14 ^A
		27 µg	16	3	1.1	14 ^A , 18 ^A
		9.0 µg	18	1	1.2	17 ^A , 19 ^A
		2.7 µg	12	4	0.8	9 ^A , 14 ^A
	0.90 µg	12	4	0.8	9 ^A , 14 ^A	
	Sterile Water for Injection	1000 µL	15	1		14 ^A , 15 ^A
TA100	Glucagon for Injection	2700 µg	87	13	1.1	96 ^M , 77 ^M
		900 µg	87	6	1.1	91 ^M , 83 ^M
		270 µg	63	5	0.8	66 ^M , 59 ^M
		90 µg	98	6	1.2	93 ^M , 102 ^M
		27 µg	96	4	1.2	98 ^M , 93 ^M
		9.0 µg	81	18	1.0	93 ^M , 68 ^M
		2.7 µg	99	1	1.2	98 ^M , 100 ^M
	0.90 µg	89	1	1.1	88 ^M , 90 ^M	
	Sterile Water for Injection	1000 µL	81	1		82 ^M , 80 ^M
TA1535	Glucagon for Injection	2700 µg	9	4	0.9	12 ^M 3, 6 ^M 3
		900 µg	4	2	0.4	2 ^M 3, 5 ^M 3
		270 µg	12	4	1.2	14 ^A , 9 ^A
		90 µg	14	4	1.4	17 ^A , 11 ^A
		27 µg	8	3	0.8	10 ^A , 6 ^A
		9.0 µg	12	4	1.2	14 ^A , 9 ^A
		2.7 µg	7	1	0.7	6 ^A , 8 ^A
	0.90 µg	11	3	1.1	9 ^A , 13 ^A	
	Sterile Water for Injection	1000 µL	10	2		11 ^A , 8 ^A

Key to Plate Postfix Codes

3 Moderately reduced background

Table 5 continues (APP glucagon without S-9 activation).

Table 5 cont.: Initial Toxicity-Mutation Assay with Glucagon for Injection without S9 activation

Study Number: AD29XE.503 ^{(b) (4)}
 Experiment: B1
 Exposure Method: Plate incorporation assay

Study Code: AD29XE
 Date Plated: 6/29/2011
 Evaluation Period: 7/3/2011 to 7/5/2011

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA1537	Glucagon for Injection	2700 µg	17	4	1.1	14 ^A , 19 ^A
		900 µg	17	4	1.1	20 ^A , 14 ^A
		270 µg	25	4	1.6	22 ^A , 27 ^A
		90 µg	25	2	1.6	26 ^A , 23 ^A
		27 µg	16	2	1.0	14 ^A , 17 ^A
		9.0 µg	22	4	1.4	19 ^A , 24 ^A
		2.7 µg	21	1	1.3	20 ^A , 22 ^A
	0.90 µg	16	2	1.0	14 ^A , 17 ^A	
	Sterile Water for Injection	1000 µL	16	2		17 ^A , 14 ^A
WP2 ^{InvrA}	Glucagon for Injection	2700 µg	24	5	1.0	20 ^A , 27 ^A
		900 µg	29	6	1.2	33 ^A , 24 ^A
		270 µg	29	4	1.2	32 ^A , 26 ^A
		90 µg	38	NA	1.5	CPN# ^a , 38 ^A
		27 µg	18	7	0.7	13 ^A , 23 ^A
		9.0 µg	17	4	0.7	19 ^A , 14 ^A
		2.7 µg	26	4	1.0	23 ^A , 28 ^A
	0.90 µg	28	2	1.1	26 ^A , 29 ^A	
	Sterile Water for Injection	1000 µL	25	4		22 ^A , 28 ^A
TA98	2NF	1.0 µg	161	52	10.7	124 ^A , 198 ^A
TA100	SA	1.0 µg	346	36	4.3	320 ^A , 371 ^A
TA1535	SA	1.0 µg	317	8	31.7	311 ^A , 323 ^A
TA1537	9AAD	75 µg	424	28	26.5	444 ^A , 404 ^A
WP2 ^{InvrA}	MMS	1000 µg	299	16	12.0	288 ^A , 310 ^A

Key to Positive Controls

2NF 2-nitrofluorene
 SA sodium azide
 9AAD 9-Aminoacridine
 MMS methyl methanesulfonate

Key to Plate Postfix Codes

CP Contaminated plate
 N# Not counted

Key to Automatic & Manual Count Flags

^M: Manual count ^A: Automatic count

^a The loss of this test article-treated plate does not invalidate the results since the dose levels above and below this one are not elevated compared with the negative control.

- ii) Table 6 below shows the results of initial Ames assay with APP glucagon with S-9 activation.

Table 6: Initial Toxicity-Mutation Assay with Glucagon for Injection with S9 activation

Study Number: AD29XE.503. (b) (4)
 Experiment: B1
 Exposure Method: Plate incorporation assay

Study Code: AD29XE
 Date Plated: 6/29/2011
 Evaluation Period: 7/3/2011 to 7/5/2011

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	Glucagon for Injection	2700 µg	3	1	0.1	3 ^M 3, 2 ^M 3
		900 µg	18	6	0.9	14 ^A 2, 22 ^A 2
		270 µg	16	2	0.8	17 ^A , 14 ^A
		90 µg	32	1	1.5	31 ^A , 32 ^A
		27 µg	22	0	1.0	22 ^A , 22 ^A
		9.0 µg	17	9	0.8	23 ^A , 10 ^A
		2.7 µg	21	1	1.0	22 ^A , 20 ^A
		0.90 µg	19	5	0.9	15 ^A , 22 ^A
	Sterile Water for Injection	1000 µL	21	3		23 ^A , 19 ^A
TA100	Glucagon for Injection	2700 µg	48	1	0.5	47 ^M 3, 49 ^M 3
		900 µg	138	27	1.4	157 ^A 3, 119 ^A 3
		270 µg	120	9	1.3	113 ^A , 126 ^A
		90 µg	101	17	1.1	89 ^A , 113 ^A
		27 µg	104	17	1.1	116 ^A , 92 ^A
		9.0 µg	111	1	1.2	112 ^A , 110 ^A
		2.7 µg	115	9	1.2	121 ^A , 108 ^A
		0.90 µg	106	1	1.1	106 ^A , 105 ^A
	Sterile Water for Injection	1000 µL	96	24		79 ^A , 113 ^A
TA1535	Glucagon for Injection	2700 µg	9	1	0.8	8 ^A , 9 ^A
		900 µg	11	1	0.9	11 ^A , 10 ^A
		270 µg	15	3	1.3	17 ^A , 13 ^A
		90 µg	8	4	0.7	11 ^A , 5 ^A
		27 µg	7	4	0.6	9 ^A , 4 ^A
		9.0 µg	10	5	0.8	6 ^A , 13 ^A
		2.7 µg	10	1	0.8	9 ^A , 11 ^A
		0.90 µg	15	7	1.3	20 ^A , 10 ^A
	Sterile Water for Injection	1000 µL	12	4		14 ^A , 9 ^A

Key to Plate Postfix Codes

3 Moderately reduced background
 2 Slightly reduced background

Key to Automatic & Manual Count Flags

^M: Manual count ^A: Automatic count

Table 6 continues (APP glucagon with S-9 activation).

Table 6 cont.: Initial Toxicity-Mutation Assay with Glucagon for Injection with S9 activation

Study Number: AD29XE.503 (b) (4)			Study Code: AD29XE			
Experiment: B1			Date Plated: 6/29/2011			
Exposure Method: Plate incorporation assay			Evaluation Period: 7/3/2011 to 7/5/2011			
Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
WP2uvrA	Glucagon for Injection	2700 µg	33	0	1.0	33 ^A , 2, 33 ^A , 2
		900 µg	47	5	1.5	43 ^A , 2, 50 ^A , 2
		270 µg	33	1	1.0	34 ^A , 32 ^A
		90 µg	33	10	1.0	40 ^A , 26 ^A
		27 µg	42	6	1.3	46 ^A , 38 ^A
		9.0 µg	23	0	0.7	23 ^A , 23 ^A
		2.7 µg	24	15	0.8	13 ^A , 34 ^A
		0.90 µg	24	0	0.8	24 ^A , 24 ^A
		Sterile Water for Injection	1000 µL	32	6	
	TA98	2AA	1.0 µg	260	10	12.4
TA100	2AA	2.0 µg	565	66	5.9	611 ^A , 518 ^A
TA1535	2AA	1.0 µg	65	8	5.4	59 ^A , 70 ^A
WP2uvrA	2AA	15 µg	144	18	4.5	131 ^A , 157 ^A
Key to Positive Controls			Key to Plate Postfix Codes			
2AA	2-aminoanthracene		2	Slightly reduced background		
Key to Automatic & Manual Count Flags						
M: Manual count			A: Automatic count			

Table 7 below shows the results of initial retest Ames assay with APP glucagon with S-9 activation in the tester strain TA1537, this is because due to an unacceptable vehicle control, this strain was not evaluated in the initial toxicity study (see page 51).

Table 7: Retest of the Initial Toxicity-Mutation Assay with Glucagon for Injection with S9 activation

Study Number: AD29XE.503 (b) (4)			Study Code: AD29XE			
Experiment: B2			Date Plated: 7/12/2011			
Exposure Method: Plate incorporation assay			Evaluation Period: 7/20/2011			
Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA1537	Glucagon for Injection	2700 µg	13	1	1.6	12 ^A , 13 ^A
		900 µg	8	0	1.0	8 ^A , 8 ^A
		270 µg	8	1	1.0	8 ^A , 7 ^A
		90 µg	3	2	0.4	1 ^A , 4 ^A
		27 µg	8	6	1.0	12 ^A , 4 ^A
		9.0 µg	10	4	1.3	7 ^A , 12 ^A
		Sterile Water for Injection	1000 µL	8	5	
	TA1537	2AA	1.0 µg	55	8	6.9
2AA 2-aminoanthracene			Key to Automatic & Manual Count Flags			
M: Manual count			A: Automatic count			

Table. This shows the summary of Ames assay with all the tester strains are also provided below with the current drug (APP's Glucagon for injection). The results from the initial assay in the absence of metabolic activation.

2.6.7.8A Genotoxicity: *In Vitro* Report Title: Bacterial Reverse Mutation Assay Test Article: Glucagon for Injection
 Comparator/Reference Article: GlucaGen®

Test for Induction of:	Reverse mutation in bacterial cells	No. of Independent Assays:	5	Study No.:	AD29XE.503 ^(b) ₍₄₎ and AD30AZ.503 ^(b) ₍₄₎
Species/Strain:	<i>S. typhimurium, E. coli</i>	No. of Replicate Cultures:	2 (#1 and 2)	Location in CTD:	
Metabolizing System:	Aroclor-induced rat liver S9			GLP Compliance:	Yes
Vehicles:	For Test Article: Sterile water for injection	For Positive Controls:	DMSO, except water for sodium azide		
Treatment:	Plate incorporation	Dates of Treatment:	29 June 2011 (#1); 12 July 2011 (#2)		
Cytotoxic Effects:	Toxicity was observed beginning at 900 or at 2700 µg per plate with a few test conditions in the initial toxicity-mutation assay with Glucagon for Injection (#1). No appreciable toxicity was observed in the retest of the initial assay with Glucagon for Injection (#2).				
Genotoxic Effects:	None				

Metabolic Activation	Test Article	Dose Level (µg/plate)	Initial Toxicity-Mutation Assay (#1) Revertant Colony Counts (Mean ±SD)					
			TA98	TA100	TA1535	TA1537	WP2uvrA	
Without Activation	Sterile Water for Injection	1000 µL	15 ± 1	81 ± 1	10 ± 2	16 ± 2	25 ± 4	
	Glucagon for Injection	0.90 µg	12 ± 4	89 ± 1	11 ± 3	16 ± 2	28 ± 2	
		2.7 µg	12 ± 4	99 ± 1	7 ± 1	21 ± 1	26 ± 4	
		9.0 µg	18 ± 1	81 ± 18	12 ± 4	22 ± 4	17 ± 4	
		27 µg	16 ± 3	96 ± 4	8 ± 3	16 ± 2	18 ± 7	
		90 µg	19 ± 6	98 ± 6	14 ± 4	25 ± 2	38 ± - ^a	
		270 µg	19 ± 11	63 ± 5	12 ± 4	25 ± 4	29 ± 4	
		900 µg	9 ± 1	87 ± 6	4 ± 2	17 ± 4	29 ± 6	
		2700 µg	9 ± 1	87 ± 13	9 ± 4	17 ± 4	24 ± 5	
	Positive Controls:							
		2-nitrofluorene	1.0 µg	161 ± 52				
		Sodium azide	1.0 µg		346 ± 36	317 ± 8		
	9-Aminoacridine	75 µg				424 ± 28		
	Methylmethanesulfonate	1000 µg					299 ± 16	

^a One replicate plate was not evaluated due to contamination. Therefore, standard deviation is not applicable when n=1.

Table. The results from the initial assay in the presence of metabolic activation.

Metabolic Activation	Test Article	Dose Level (µg/plate)	Initial Toxicity-Mutation Assay (#1) Revertant Colony Counts (Mean ±SD)					
			TA98	TA100	TA1535	TA1537 ^a	WP2uvrA	
With Activation	Sterile Water for Injection	1000 µL	21 ± 3	96 ± 24	12 ± 4		32 ± 6	
	Glucagon for Injection	0.90 µg	19 ± 5	106 ± 1	15 ± 7		24 ± 0	
		2.7 µg	21 ± 1	115 ± 9	10 ± 1		24 ± 15	
		9.0 µg	17 ± 9	111 ± 1	10 ± 5		23 ± 0	
		27 µg	22 ± 0	104 ± 17	7 ± 4		42 ± 6	
		90 µg	32 ± 1	101 ± 17	8 ± 4		33 ± 10	
		270 µg	16 ± 2	120 ± 9	15 ± 3		33 ± 1	
		900 µg	18 ± 6	138 ± 27	11 ± 1		47 ± 5	
		2700 µg	3 ± 1	48 ± 1	9 ± 1		33 ± 0	
	Positive Control:							
		2-aminoanthracene	1.0 µg	260 ± 10		65 ± 8		
		2-aminoanthracene	2.0 µg		565 ± 66			
	2-aminoanthracene	15 µg					144 ± 18	

^a Due to an unacceptable vehicle control value, tester strain TA1537 in the presence of S9 activation was not evaluated for mutagenicity but was retested in Experiment B2 (#2) based on the precipitate and toxicity profile observed in Experiment B1 (#1).

Toxicity: *In Vitro*

Test Article: Glucagon for Injection (continued)

Experiment B2 (#2)

<u>Metabolic Activation</u>	<u>Test Article</u>	<u>Dose Level (µg/plate)</u>	<u>Retest of the Initial Toxicity-Mutation Assay (#2)</u> <u>Revertant Colony Counts (Mean ±SD)</u>
			<u>TA1537</u>
With Activation	Sterile Water for Injection	1000 µL	8 ± 5
	Glucagon for Injection	9.0 µg	10 ± 4
		27 µg	8 ± 6
		90 µg	3 ± 2
		270 µg	8 ± 1
		900 µg	8 ± 0
		2700 µg	13 ± 1
	Positive Control		
2-aminoanthracene	1.0 µg	55 ± 8	

Confirmatory Mutagenicity Assay

The results of the confirmatory mutagenicity assay are presented in [Tables 8](#) and [9](#). These data were generated in Experiment B3.

In Experiment B3 (Confirmatory Mutagenicity Assay), no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. The nominal dose levels tested were 9.0, 27, 90, 270, 900 and 2700 µg per plate. Neither precipitate nor appreciable toxicity was observed.

iii) Table 8 below shows the results of the confirmatory Ames assay with APP glucagon without S-9 activation

Table 8: Confirmatory Mutagenicity Assay with Glucagon for Injection without S9 activation

Study Number: AD29XE.503 (b)(4)			Study Code: AD29XE			
Experiment: B3			Date Plated: 7/12/2011			
Exposure Method: Plate incorporation assay			Evaluation Period: 7/20/2011			
Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	Glucagon for Injection	2700 µg	8	1	0.7	8 ^A , 9 ^A , 7 ^A
		900 µg	17	6	1.4	15 ^A , 13 ^A , 24 ^A
		270 µg	16	4	1.3	20 ^A , 13 ^A , 16 ^A
		90 µg	15	4	1.3	20 ^A , 12 ^A , 13 ^A
		27 µg	15	3	1.3	19 ^A , 13 ^A , 13 ^A
	9.0 µg	13	5	1.1	8 ^A , 16 ^A , 16 ^A	
	Sterile Water for Injection	1000 µL	12	4		15 ^A , 8 ^A , 12 ^A
TA100	Glucagon for Injection	2700 µg	118	15	1.2	105 ^A , 114 ^A , 134 ^A
		900 µg	114	10	1.2	119 ^A , 120 ^A , 102 ^A
		270 µg	97	16	1.0	92 ^A , 115 ^A , 84 ^A
		90 µg	115	14	1.2	115 ^A , 129 ^A , 101 ^A
		27 µg	118	9	1.2	118 ^A , 110 ^A , 127 ^A
	9.0 µg	98	13	1.0	88 ^A , 107 ^A , WDN#	
	Sterile Water for Injection	1000 µL	95	8		103 ^A , 95 ^A , 88 ^A
TA1535	Glucagon for Injection	2700 µg	16	8	1.0	25 ^A , 9 ^A , 13 ^A
		900 µg	15	6	0.9	21 ^A , 9 ^A , 16 ^A
		270 µg	14	2	0.9	15 ^A , 12 ^A , 16 ^A
		90 µg	12	6	0.8	9 ^A , 19 ^A , 9 ^A
		27 µg	7	6	0.4	8 ^A , 1 ^A , 12 ^A
	9.0 µg	17	3	1.1	21 ^A , 16 ^A , 15 ^A	
	Sterile Water for Injection	1000 µL	16	4		12 ^A , 15 ^A , 20 ^A
Key to Plate Postfix Codes						
		WD	Water damaged plate			
		N#	Not counted			
Key to Automatic & Manual Count Flags						
		M:	Manual count		A: Automatic count	

iii) Table 8 below continues (confirmatory Ames with APP glucagon without S-9 activation)

Table 8 cont.: Confirmatory Mutagenicity Assay with Glucagon for Injection without S9 activation

Study Number: AD29XE.503 ^{(b) (4)}		Study Code: AD29XE				
Experiment: B3		Date Plated: 7/12/2011				
Exposure Method: Plate incorporation assay		Evaluation Period: 7/20/2011				
Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA1537	Glucagon for Injection	2700 µg	9	2	1.8	9 ^A , 7 ^A , 11 ^A
		900 µg	9	1	1.8	9 ^A , 8 ^A , 9 ^A
		270 µg	6	5	1.2	11 ^A , 5 ^A , 1 ^A
		90 µg	8	3	1.6	7 ^A , 5 ^A , 11 ^A
		27 µg	4	3	0.8	7 ^A , 1 ^A , 5 ^A
		9.0 µg	6	1	1.2	7 ^A , 5 ^A , 5 ^A
	Sterile Water for Injection	1000 µL	5	2		7 ^A , 4 ^A , 5 ^A
WP2 ^{uvrA}	Glucagon for Injection	2700 µg	43	8	1.3	45 ^A , 34 ^A , 50 ^A
		900 µg	43	10	1.3	48 ^A , 31 ^A , 49 ^A
		270 µg	44	14	1.3	53 ^A , 52 ^A , 28 ^A
		90 µg	39	4	1.1	42 ^A , 40 ^A , 34 ^A
		27 µg	30	1	0.9	29 ^A , 31 ^A , 29 ^A
		9.0 µg	34	6	1.0	29 ^A , 41 ^A , 31 ^A
	Sterile Water for Injection	1000 µL	34	5		36 ^A , 28 ^A , 38 ^A
TA98	2NF	1.0 µg	201	56	16.8	138 ^A , 243 ^A , 223 ^A
TA100	SA	1.0 µg	685	20	7.2	663 ^A , 699 ^A , 694 ^A
TA1535	SA	1.0 µg	688	86	43.0	740 ^A , 589 ^A , 735 ^A
TA1537	9AAD	75 µg	536	227	107.2	281 ^A , 613 ^A , 714 ^A
WP2 ^{uvrA}	MMS	1000 µg	460	9	13.5	450 ^A , 464 ^A , 467 ^A
Key to Positive Controls						
2NF	2-nitrofluorene					
SA	sodium azide					
9AAD	9-Aminoacridine					
MMS	methyl methanesulfonate					
Key to Automatic & Manual Count Flags						
^M : Manual count		^A : Automatic count				

iv) Table 9 below shows the results of the confirmatory Ames assay with APP glucagon with S-9 activation

Table 9: Confirmatory Mutagenicity Assay with Glucagon for Injection with S9 activation

Study Number: AD29XE.503 ^{(b)(4)}		Study Code: AD29XE				
Experiment: B3		Date Plated: 7/12/2011				
Exposure Method: Plate incorporation assay		Evaluation Period: 7/20/2011				
Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	Glucagon for Injection	2700 µg	16	6	0.7	17 ^A , 9 ^A , 21 ^A
		900 µg	14	3	0.6	12 ^A , 17 ^A , 13 ^A
		270 µg	13	5	0.5	11 ^A , 19 ^A , 9 ^A
		90 µg	12	1	0.5	13 ^A , 11 ^A , 13 ^A
		27 µg	15	3	0.6	11 ^A , 16 ^A , 17 ^A
	9.0 µg	13	2	0.5	16 ^A , 12 ^A , 12 ^A	
	Sterile Water for Injection	1000 µL	24	5		25 ^A , 28 ^A , 19 ^A
TA100	Glucagon for Injection	2700 µg	166	17	1.3	184 ^A , 150 ^A , 164 ^A
		900 µg	175	17	1.4	186 ^A , 184 ^A , 155 ^A
		270 µg	158	31	1.3	164 ^A , 125 ^A , 186 ^A
		90 µg	164	27	1.3	137 ^A , 191 ^A , 163 ^A
		27 µg	152	16	1.2	170 ^A , 141 ^A , 146 ^A
	9.0 µg	141	2	1.1	139 ^A , 141 ^A , 143 ^A	
	Sterile Water for Injection	1000 µL	125	9		118 ^A , 135 ^A , 122 ^A
TA1535	Glucagon for Injection	2700 µg	13	3	0.8	11 ^A , 11 ^A , 16 ^A
		900 µg	8	4	0.5	12 ^A , 8 ^A , 5 ^A
		270 µg	17	4	1.1	19 ^A , 19 ^A , 12 ^A
		90 µg	6	3	0.4	4 ^A , 9 ^A , 4 ^A
		27 µg	13	6	0.8	8 ^A , 19 ^A , 12 ^A
	9.0 µg	8	2	0.5	5 ^A , 9 ^A , 6 ^A	
	Sterile Water for Injection	1000 µL	16	5		11 ^A , 15 ^A , 21 ^A

Key to Automatic & Manual Count Flags

M: Manual count A: Automatic count

iv) Table 9 continues (confirmatory Ames with APP glucagon with S-9 activation)

Table 9 cont.: Confirmatory Mutagenicity Assay with Glucagon for Injection with S9 activation

Study Number: AD29XE.503 (b) (4)
 Experiment: B3
 Exposure Method: Plate incorporation assay

Study Code: AD29XE
 Date Plated: 7/12/2011
 Evaluation Period: 7/20/2011

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA1537	Glucagon for Injection	2700 µg	11	2	1.1	13 ^M , 12 ^M , 9 ^M
		900 µg	5	1	0.5	4 ^M , 6 ^M , 6 ^M
		270 µg	6	2	0.6	6 ^M , 5 ^M , 8 ^M
		90 µg	6	3	0.6	8 ^M , 3 ^M , 7 ^M
		27 µg	7	2	0.7	9 ^M , 5 ^M , 7 ^M
		9.0 µg	7	4	0.7	12 ^M , 5 ^M , 5 ^M
	Sterile Water for Injection	1000 µL	10	5		5 ^M , 13 ^M , 13 ^M
WP2uvrA	Glucagon for Injection	2700 µg	37	4	0.9	37 ^A , 33 ^A , 41 ^A
		900 µg	46	9	1.1	36 ^A , 53 ^A , 49 ^A
		270 µg	52	11	1.2	62 ^A , 41 ^A , 52 ^A
		90 µg	52	1	1.2	52 ^A , 52 ^A , 53 ^A
		27 µg	50	8	1.2	58 ^A , 42 ^A , 50 ^A
		9.0 µg	38	2	0.9	36 ^A , 40 ^A , 38 ^A
	Sterile Water for Injection	1000 µL	43	4		45 ^A , 46 ^A , 38 ^A
TA98	2AA	1.0 µg	335	60	14.0	267 ^A , 358 ^A , 379 ^A
TA100	2AA	2.0 µg	1259	353	10.1	851 ^A , 1456 ^A , 1469 ^A
TA1535	2AA	1.0 µg	328	122	20.5	435 ^A , 353 ^A , 195 ^A
TA1537	2AA	1.0 µg	90	45	9.0	49 ^A , 138 ^A , 82 ^A
WP2uvrA	2AA	15 µg	508	34	11.8	532 ^A , 524 ^A , 469 ^A

Key to Positive Controls

2AA 2-aminoanthracene

Key to Automatic & Manual Count Flags

^M: Manual count ^A: Automatic count

Table 10. The summary of confirmatory Ames assay in all the tester strains with the APP's Glucagon for injection is also provided below. The results are in the **absence** of metabolic activation.

2.6.7.8A Genotoxicity: *In Vitro* Test Article: Glucagon for Injection (continued)

Test for Induction of:	Reverse mutation in bacterial cells	No. of Independent Assays:	5	Study No.:	AD29XE.503 ^{(b) (4)} and AD30AZ.503 ^{(b) (4)}
Species/Strain:	<i>S. typhimurium, E. coli</i>	No. of Replicate Cultures:	3 (#3)		Location in CTD:
Metabolizing System:	Aroclor-induced rat liver S9			GLP Compliance:	Yes
Vehicles:	For Test Article:	Sterile water for injection		For Positive Controls:	DMSO, except water for sodium azide
Treatment:	Plate incorporation	Date of Treatment:	12 July 2011 (#3)		
Cytotoxic Effects:	No appreciable toxicity was observed in the confirmatory mutagenicity assay with Glucagon for Injection (#3).				
Genotoxic Effects:	None				

Metabolic Activation	Test Article	Dose Level (µg/plate)	Confirmatory Mutagenicity Assay (#3) Revertant Colony Counts (Mean ±SD)				
			TA98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
Without Activation	Sterile Water for Injection	1000 µL	12 ± 4	95 ± 8	16 ± 4	5 ± 2	34 ± 5
	Glucagon for Injection	9.0 µg	13 ± 5	98 ± 13	17 ± 3	6 ± 1	34 ± 6
		27 µg	15 ± 3	118 ± 9	7 ± 6	4 ± 3	30 ± 1
		90 µg	15 ± 4	115 ± 14	12 ± 6	8 ± 3	39 ± 4
		270 µg	16 ± 4	97 ± 16	14 ± 2	6 ± 5	44 ± 14
		900 µg	17 ± 6	114 ± 10	15 ± 6	9 ± 1	43 ± 10
		2700 µg	8 ± 1	118 ± 15	16 ± 8	9 ± 2	43 ± 8
	Positive Controls:						
	2-nitrofluorene	1.0 µg	201 ± 56				
	Sodium azide	1.0 µg		685 ± 20	688 ± 86		
9-Aminoacridine	75 µg				536 ± 227		
Methyl methanesulfonate	1000 µg					460 ± 9	

Table 10 continued. The confirmatory Ames assay with the APP's Glucagon for injection. The results are in the **presence** of metabolic activation.

2.6.7.8A Genotoxicity: *In Vitro* Test Article: Glucagon for Injection (continued)

Metabolic Activation	Test Article	Dose Level (µg/plate)	Confirmatory Mutagenicity Assay (#3) Revertant Colony Counts (Mean ±SD)				
			TA98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
With Activation	Sterile Water for Injection	1000 µL	24 ± 5	125 ± 9	16 ± 5	10 ± 5	43 ± 4
	Glucagon for Injection	9.0 µg	13 ± 2	141 ± 2	8 ± 2	7 ± 4	38 ± 2
		27 µg	15 ± 3	152 ± 16	13 ± 6	7 ± 2	50 ± 8
		90 µg	12 ± 1	164 ± 27	6 ± 3	6 ± 3	52 ± 1
		270 µg	13 ± 5	158 ± 31	17 ± 4	6 ± 2	52 ± 11
		900 µg	14 ± 3	175 ± 17	8 ± 4	5 ± 1	46 ± 9
		2700 µg	16 ± 6	166 ± 17	13 ± 3	11 ± 2	37 ± 4
	Positive Control:						
	2-aminoanthracene	1.0 µg	335 ± 60		328 ± 122	90 ± 45	
	2-aminoanthracene	2.0 µg		1259 ± 353			
2-aminoanthracene	15 µg					508 ± 34	

In conclusion the test was considered negative, i.e. the current drug product (APP glucagon for injection) was not mutagenic in the Ames assay.

B. The Ames assay was also conducted with the reference drug (RD) GlucaGen with lot number AW60180 ((b) (4) code # AD30AZ)

GlucaGen® (AD30AZ)

The results of the initial toxicity-mutation assay are presented in the summary tables below. These data were generated in Experiment B1.

In Experiment B1 (Initial Toxicity-Mutation Assay), the maximum dose tested was 2700 µg per plate; this dose was achieved using a concentration of 2.7 mg/mL and a 1000 µL plating aliquot. The nominal dose levels tested were 0.90, 2.7, 9.0, 27, 90, 270, 900 and 2700 µg per plate. The maximum nominal dose of 2700 µg per plate was the highest that could be achieved while still allowing the dosing to be completed within the established stability period for Glucagon for Injection. Furthermore, it was known prior to the start of the study that the nominal doses may not be achieved because of impurities in the reference article. During protocol development, the Sponsor stated that reconstitution with 0.30 mL of SWI would yield a solution at 2.7 mg/mL, and this value was used throughout the raw data. Subsequently, the Sponsor indicated that the 2.7 mg/mL value was actually found to be 2.6 mg/mL. Nevertheless, formulation analysis confirmed that the actual glucagon content achieved was within the protocol-specified acceptance criterion of 85 to 115% of target, and the nominal dose levels are reported. The test article formed soluble and clear solutions in sterile water for injection from 0.00090 to 2.7 mg/mL. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. No precipitate was observed. Toxicity was observed beginning at 900 or at 2700 µg per plate in the presence of S9 activation. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 2700 µg per plate.

The summary of Ames assay results are also provided with the RD, see Table below

i) Table 11 below shows the results of the initial Ames assay with the RD GlucaGen without S-9 activation

Table. The initial Ames assay with the reference drug (GlucaGen) are shown below using various tester strain. The results are in the **absence** of metabolic activation.

2.6.7.8A Genotoxicity: *In Vitro* Test Article: GlucaGen® (continued)

Test for Induction of:	Reverse mutation in bacterial cells	No. of Independent Assays:	5	Study No.:	AD29XE.503 (b) (4) and AD30AZ.503 (b) (4)
Species/Strain:	<i>S. typhimurium, E. coli</i>	No. of Replicate Cultures:	2 (#4)		Location in CTD:
Metabolizing System:	Aroclor-induced rat liver S9			GLP Compliance:	Yes
Vehicles:	For Test Article:	Sterile water for injection		For Positive Controls:	DMSO, except water for sodium azide
Treatment:	Plate incorporation			Date of Treatment:	29 June 2011 (#4)
Cytotoxic Effects:	Toxicity was observed beginning at 900 or at 2700 µg per plate in the presence of S9 activation in the initial toxicity-mutation assay with GlucaGen® (#4).				
Genotoxic Effects:	None				

Metabolic Activation	Test Article	Dose Level (µg/plate)	Initial Toxicity-Mutation Assay (#4) Revertant Colony Counts (Mean ±SD)				
			TA98	TA100	TA1535	TA1537	WP2uvrA
Without Activation	Sterile Water for Injection	1000 µL	10 ± 1	108 ± 4	10 ± 8	9 ± 1	31 ± 20
	GlucaGen®	0.90 µg	13 ± 7	96 ± 4	12 ± 4	19 ± 1	29 ± 4
		2.7 µg	10 ± 0	89 ± 6	10 ± 6	13 ± 9	20 ± 3
		9.0 µg	11 ± 6	89 ± 8	12 ± 1	17 ± 3	28 ± 1
		27 µg	13 ± 9	90 ± 3	11 ± 9	14 ± 5	29 ± 3
		90 µg	10 ± 1	98 ± 12	11 ± 3	11 ± 4	20 ± 1
		270 µg	13 ± 2	86 ± 18	11 ± 3	12 ± 1	25 ± 11
		900 µg	11 ± 3	85 ± 9	11 ± 4	13 ± 2	28 ± 1
		2700 µg	9 ± 1	89 ± 28	14 ± 5	8 ± 4	38 ± 1
	Positive Controls:						
	2-nitrofluorene	1.0 µg	185 ± 28				
Sodium azide	1.0 µg		359 ± 11	344 ± 40			
9-Aminoacridine	75 µg				271 ± 100		
Methyl methanesulfonate	1000 µg					227 ± 1	

Table below shows the results of the initial Ames assay with the RD GlucaGen with S-9 activation

Table 11. The initial Ames assay with the reference drug (GlucaGen). The results are in the **presence** of metabolic activation.

2.6.7.8A Genotoxicity: *In Vitro* Test Article: GlucaGen® (continued)

Metabolic Activation	Test Article	Dose Level (µg/plate)	Initial Toxicity-Mutation Assay (#4) Revertant Colony Counts (Mean ±SD)				
			TA98	TA100	TA1535	TA1537	WP2uvrA
With Activation	Sterile Water for Injection	1000 µL	11 ± 8	109 ± 1	9 ± 4	11 ± 1	28 ± 13
	GlucaGen®	0.90 µg	10 ± 0	109 ± 1	11 ± 6	12 ± 3	35 ± 2
		2.7 µg	7 ± 2	120 ± 14	8 ± 7	15 ± 6	26 ± 4
		9.0 µg	14 ± 5	125 ± 4	9 ± 1	19 ± 7	32 ± 1
		27 µg	5 ± 2	127 ± 2	10 ± 1	12 ± 4	31 ± 0
		90 µg	8 ± 2	121 ± 0	10 ± 1	8 ± 4	25 ± 4
		270 µg	10 ± 1	137 ± 8	23 ± 7	15 ± 5	35 ± 4
		900 µg	7 ± 4	73 ± 2	17 ± 4	10 ± 1	39 ± 3
		2700 µg	13 ± 4	52 ± 8	10 ± 4	6 ± 4	28 ± 8
	Positive Control:						
	2-aminoanthracene	1.0 µg	308 ± 35		87 ± 6	56 ± 7	
2-aminoanthracene	2.0 µg		820 ± 248				
2-aminoanthracene	15 µg					114 ± 28	

ii) Table below shows the results of the confirmatory Ames assay with the RD GlucaGen in various tester strains without S-9 activation

Confirmatory Mutagenicity Assay

The results of the confirmatory mutagenicity assay are presented in Tables 12 and 13. These data were generated in Experiment B2.

In Experiment B2 (Confirmatory Mutagenicity Assay), no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. The nominal dose levels tested were 9.0, 27, 90, 270, 900 and 2700 µg per plate. No precipitate was observed. Toxicity was observed beginning at 900 or at 2700 µg per plate with most tester strains in the presence of S9 activation.

Table 12. The confirmatory Ames assay with the reference drug (GlucaGen). The results are in the absence of metabolic activation.

2.6.7.8A Genotoxicity: *In Vitro* Test Article: GlucaGen® (continued)

Test for Induction of:	Reverse mutation in bacterial cells	No. of Independent Assays:	5	Study No.:	AD29XE.503 (b) (4) and AD30AZ.503 (b) (4)
Species/Strain:	<i>S. typhimurium, E. coli</i>	No. of Replicate Cultures:	3 (#5)	Location in CTD:	
Metabolizing System:	Aroclor-induced rat liver S9			GLP Compliance:	Yes
Vehicles:	For Test Article: Sterile water for injection	For Positive Controls:	DMSO, except water for sodium azide		
Treatment:	Plate incorporation	Date of Treatment:	12 July 2011 (#5)		
Cytotoxic Effects:	Toxicity was observed beginning at 900 or at 2700 µg per plate with most tester strains in the presence of S9 activation in the confirmatory mutagenicity assay with GlucaGen® (#5).				
Genotoxic Effects:	None				

Metabolic Activation	Test Article	Dose Level (µg/plate)	Confirmatory Mutagenicity Assay (#5) Revertant Colony Counts (Mean ±SD)				
			TA98	TA100	TA1535	TA1537	WP2uvrA
Without Activation	Sterile Water for Injection	1000 µL	13 ± 3	101 ± 8	9 ± 2	9 ± 2	40 ± 9
	GlucaGen®	9.0 µg	14 ± 3	90 ± 10	10 ± 2	5 ± 3	36 ± 2
		27 µg	17 ± 2	111 ± 1	10 ± 3	7 ± 3	46 ± 4
		90 µg	14 ± 2	82 ± 16	10 ± 4	7 ± 2	36 ± 8
		270 µg	14 ± 3	96 ± 10	11 ± 2	5 ± 3	43 ± 10
		900 µg	12 ± 5	85 ± 8	15 ± 2	6 ± 3	45 ± 4
		2700 µg	9 ± 3	107 ± 6	12 ± 3	8 ± 3	33 ± 4
	Positive Controls:						
	2-nitrofluorene	1.0 µg	162 ± 29				
	Sodium azide	1.0 µg		534 ± 46	436 ± 57		
9-Aminoacridine	75 µg				213 ± 65		
Methyl methane-sulfonate	1000 µg					412 ± 34	

Table below shows the results of the confirmatory Ames assay with the RD GlucaGen with S-9 activation

Table 13. The confirmatory Ames assay with the reference drug (GlucaGen). The results are in the presence of metabolic activation.

2.6.7.8A Genotoxicity: *In Vitro* Test Article: GlucaGen® (continued)

Metabolic Activation	Test Article	Dose Level (µg/plate)	Confirmatory Mutagenicity Assay (#5) Revertant Colony Counts (Mean ±SD)					
			TA98	TA100	TA1535	TA1537	WP2uvrA	
With Activation	Sterile Water for Injection	1000 µL	17 ± 5	142 ± 2	11 ± 3	8 ± 1	45 ± 4	
	GlucaGen®	9.0 µg	21 ± 1	143 ± 10	12 ± 3	6 ± 2	50 ± 6	
		27 µg	19 ± 2	146 ± 20	11 ± 4	7 ± 2	59 ± 9	
		90 µg	19 ± 5	133 ± 11	13 ± 3	9 ± 3	46 ± 11	
		270 µg	26 ± 5	160 ± 9	18 ± 1	10 ± 4	53 ± 8	
		900 µg	26 ± 7	186 ± 28	19 ± 7	6 ± 1	62 ± 3	
		2700 µg	29 ± 8	180 ± 9	18 ± 3	5 ± 1	45 ± 6	
	Positive Control:							
		2-aminoanthracene	1.0 µg	252 ± 5		190 ± 99	66 ± 9	
		2-aminoanthracene	2.0 µg		616 ± 41			
	2-aminoanthracene	15 µg					240 ± 6	

Sponsor’s conclusions are provided below:

All criteria for a valid study were met as described in the protocol. The results of the Bacterial Reverse Mutation Assay indicate that, under the conditions of this study, testing up to 2700 µg per plate, Glucagon for Injection and GlucaGen® did not cause positive mutagenic responses with any of the tester strains in either the presence or absence of Aroclor-induced rat liver S9.

Thus glucagon for injection was not mutagenic in the Ames assay. Sponsor could have used higher concentration in the Ames assay without metabolic activation, as no toxicity was noted at doses up to 2700 mcg/plate, but it is possible that they were limited by the solubility of the drug as they state that “The maximum nominal dose of 2700 µg per plate was the highest that could be achieved”. It is not clear if only certain limited amount of the drug was provided to the contract Laboratory (b)(4) or because the drug had limited solubility. Note that the reference drug GlucaGen (recombinant glucagon) was also not mutagenic in the Ames assay.

Note that (b)(4) is a novel or new impurity in the current drug product and is present at (b)(4)%. The maximal recommended doses of the current drug product (APP glucagon for injection) are up to 2 mg/day. Therefore 2 mg (or 2000 mcg) will have up to (b)(4) mcg/day of (b)(4)/day (i.e. (b)(4)). In the current Ames assay doses of up to 2700 mcg/plate were evaluated, which had up to (b)(4) mcg of (b)(4) in the assay (b)(4)

Conclusion: Both APP’s glucagon for injection (lot # C108-002/ (b)(4) code # AD29XE), and the reference drug GlucaGen (lot # AW60180 / (b)(4) code # AD30AZ) were not mutagenic in the Ames assay.

2. Chomosomal aberrations in vitro in Chinese hamster Ovary (CHO) cells

The APP's glucagon (lot # C109-002 / (b) (4) code # AD29XE), and the reference drug GlucaGen (lot # AW60180 / (b) (4) code # AD30AZ) were tested in the chromosomal aberration assay. The above APP drug lot was also tested in a 28-day toxicity study in rats.

The purpose of this study was to evaluate the clastogenic potential of the test article, Glucagon for Injection (lot # C109-002) in comparison to the reference article GlucaGen® (lot # AW60180) by measuring their ability to induce chromosome aberrations in Chinese Hamster Ovary (CHO) cells. The study was conducted by (b) (4) in conformance with the testing guidelines of the ICH (1996 and 1997) and OECD (1998). The test article, Glucagon for Injection, and reference article, GlucaGen®, were received by (b) (4) on 17 June 2011 and were assigned the code numbers AD29XE (APP glucagon) and AD30AZ (RD GlucaGen), respectively.

Note that the lot number used for APP glucagon was C109-002 ((b) (4) # AD29XE) for the chromosomal aberration assay. The COA were provided for the current drug and RD, these are shown below:

Table. The COA for APP glucagon for injection was C109-002 (the (b) (4) code number was AD29XE)

CERTIFICATE OF ANALYSIS
INNOVATION AND DEVELOPMENT
Glucagon for Injection (1 mg/vial)

Page 1 of 1
Version 2.0

NDC Code: NDC 63323-596-03 Lot Number: C109-002
Product
Configuration: 1 mg Glucagon / vial Expiry Date: N/A
Manufactured By: APP Pharmaceuticals Distributed By: N/A
Storage Condition: 30 °C, Inverted Test Date: 08/05/11

TEST		RESULTS
Assay by HPLC		(b) (4) %
Residual Substances		(b) (4)

PREPARED BY: (b) (4) DATE: 9/6/11

Table. The COA of the reference drug GlucaGen was AW60180 (the (b) (4) code number was AD30AZ).

CERTIFICATE OF ANALYSIS
INNOVATION AND DEVELOPMENT
GlucaGen® (Glucagon for Injection, 1 mg/vial)

NDC Code:	<u>NDC 55390-004-01</u>	Lot Number:	<u>AW60180</u>
Product			
Configuration:	<u>1 mg Glucagon / vial</u>	Expiry Date:	<u>11/2012</u>
Manufactured By:	<u>Novo Nordisk A/S</u>	Distributed By:	<u>Bedford Laboratories</u>
Storage Condition:	<u>Room Temperature</u>	Test Date:	<u>06/16/11</u>

TEST	RESULTS
Assay by HPLC	(b) (4) %
Residual Substances	(b) (4)

Sponsor states that the impurities tested in the chromosomal aberration assay were present up to (b) (4) % in the current drug product, and up to (b) (4) % in the RD, see below.

The results are summarized in Table I. Percent relative standard deviation (%RSD) was calculated on samples from APP product and GlucaGen. All assay results met the acceptance criteria. The total combined impurity values for the APP product were about (b) (4) %, whereas the GlucGen had total combined impurity values of about (b) (4) %.

Table. This Table provides the specified impurities present in the lot numbers (C108-002 & C109-002) with the APP glucagon for injection. These lot numbers were used in two genotoxicity studies (Ames and chromosomal aberration assay).

2.6.7.4: Toxicology		Test Article: Glucagon for Injection		
Batch No.	Purity (%)	Specified Impurities (%)		Type of Study
Lot #/ Storage		(b) (4)		
C108-002 (30C)	Pre-study (↓)	85.5		Bacterial Reverse Mutation Assay
	Post-study (↓)	88.8		
C109-002 (30C)	Pre-study (↓)	84.3		In Vitro Mammalian Chromosome Aberration Assay
	Post-study (↓)	86.0		
Proposed Specs				
1 Process Impurity				

The post-study analysis of APP Glucagon for Injection found the test article to be (b) (4) % pure, which was similar to the initial purity value reported on the pre-study Certificate of Analysis (b) (4) %. Therefore, Glucagon for Injection was considered stable under the conditions of use in this study.

Table. The summary of total combined impurities present in the APP glucagon (lot # C109-002, (b) (4) # AD29XE) and reference drug GlucaGen (lot # AW60180 / (b) (4) # AD30AZ) used in chromosomal aberration assay are shown below in Table 1.

TABLE 1
Summary of Results - Concentration of Samples
Acceptance Criteria

Assay Limit: % Target Concentration 90 to 110%
%RSD (n=4): NMT (b) (4) %¹

Sample Name	Vial Number	Injection Number	Glucagon (mg/mL)	Glucagon Related Total Impurities (%)	Lactose Related Total Impurities (%)	Total Combined Impurities ² (%)
Vehicle Control	Vial 1	1	0	Not Applicable (NA)		
	Vial 2	1	0	NA		
	Mean		0	NA		
AD29XE Glucagon for Injection APP Lot # C109-002	Vial 1	1	3.166	(b) (4)		
		2	3.180			
	Vial 2	1	3.124			
		2	3.111			
	Mean		3.145			
	%RSD		1			
% Target Concentration ³		100.7	NA			
AD30AZ GlucaGen RLD Lot # AW60180	Vial 1	1	3.694	(b) (4)		
		2	3.704			
	Vial 2	1	3.668			
		2	3.664			
	Mean		3.683			
	%RSD		1			
% Target Concentration ⁴		104.9	NA			

¹ %RSD (n=4) was calculated from duplicate injections of two preparations.

² No criteria for impurities, but actual values are reported.

³ Target concentration is 3.122 mg/mL, which has been calculated using % Assay from C of A for Glucagon for Injection, Lot C109-002, 30 °C Inverted, and 200 µL reconstitution volume (Attachment I).

⁴ Target concentration is 3.511 mg/mL, which has been calculated using % Assay from C of A for GlucaGen, Lot AW60180, and 200 µL reconstitution volume (Attachment II).

Note that above lot (# C109-002) was stored under intermediate conditions (at 30 deg C), leading to elevated impurity levels, present up to (b) (4) % in the APP glucagon drug product, and up to (b) (4) % in the RD. The chemist on this NDA indicated that the COA in the CMC section for lot C109-002 was provided at the time of release (28-Jan-2009), explaining why there are differences in impurity levels with the same lot number, because these were analyzed at different times.

The chromosomal aberration assay with the current drug and RD

The test article, Glucagon for Injection, and the comparator/reference article, GlucaGen® were tested in the chromosome aberration assay using Chinese hamster ovary (CHO) cells in both the absence and presence of an Aroclor-induced rat liver S9 metabolic activation system.

Preliminary toxicity tests were performed using both test and reference articles to establish the dose range for the chromosome aberration assays.

Sponsor's Criteria for a Valid Test are described below

The frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical solvent control. The percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p < 0.05$, Fisher's Exact test) relative to the solvent control. The Historical Control Data are included in [Appendix IV](#).

A) Chromosomal aberration assay with the current drug, i.e. APP's Glucagon for Injection (lot # C109-992/ (b) (4) # AD29XE)

Methods

Initial assay: *In the preliminary toxicity assay using Glucagon for Injection, the maximum dose tested was 370 µg/mL. The test article was soluble in sterile water for injection and in the treatment medium at all dose levels tested at the beginning and conclusion of the treatment period.*

Substantial toxicity (i.e., at least 50% cell growth inhibition, relative to the solvent control) was not observed at any dose level in the non-activated and S9-activated 4-hour exposure groups. Substantial toxicity was observed at 370 µg/mL in the non-activated 20-hour continuous exposure group. Based on these findings, the doses chosen for the chromosome aberration assay ranged from 45 to 370 µg/mL for all three treatment groups.

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	45, 90, 180, 260, 370
	20 hr	0 hr	45, 90, 126, 180, 260, 295, 330, 370
S9-activated	4 hr	16 hr	45, 90, 180, 260, 370

Results: No results were provided on this initial assay, because there was an error in the dilution of the test article (APP glucagon), see below

Sponsor states that "in the chromosome aberration assay, due to a possible error in dilution of the test article, the dose formulations were out of specification. Therefore, the chromosome aberration assay was repeated at the same dose levels. Data collected from the initial assay will be maintained in the study file, but not reported".

Repeat assay with the current drug product:

Methods: In the repeat chromosome aberration assay using APP Glucagon for Injection, the cells were treated for 4 and 20 hours in the non-activated test system and for 4 hours in the S9-activated test system. All cells were harvested 20 hours after treatment initiation. The test article was soluble in sterile water for injection.

In the non-activated 20-hour exposure group, selection of doses for microscopic analysis was based on toxicity, with the highest dose selected producing at least 50% reduction in mitotic index plus two lower doses. This was due to excessive mitotic inhibition at dose levels with $\geq 50\%$ reduction in cell growth. In the non-activated and S9-activated 4-hour exposure groups, due to lack of both substantial toxicity and visible precipitate in the treatment medium, the highest dose evaluated was the highest dose tested in the chromosome aberration assay. Two lower doses were included in the evaluation. Toxicity of Glucagon for Injection (cell growth inhibition relative to the solvent control) in CHO cells when treated for 4 hours in the absence of S9 activation was 43% at 370 $\mu\text{g}/\text{mL}$, the highest test dose level evaluated for chromosome aberrations.

Table. The exposure times and toxicity noted at high doses is stated below with the APP Glucagon for injection.

Treatment Time	Recovery Time	Harvest Time	S9	Toxicity* at highest dose scored ($\mu\text{g}/\text{mL}$)	Mitotic Index Reduction **	LED ¹ for Structural Aberrations $\mu\text{g}/\text{mL}$	LED ¹ for Numerical Aberrations $\mu\text{g}/\text{mL}$
4 hr	16 hr	20 hr	-	43% at 370	16%§	370	None
20 hr	0 hr	20 hr	-	47% at 260	58%§	260	None
4 hr	16 hr	20 hr	+	32% at 370	20%§	None	None

*Cell growth inhibition

** Relative to solvent control at the highest dose evaluated for chromosome aberrations

§ Mitotic Index calculations are presented in Tables 4, 6 and 8.

¹ LED = lowest effective dose

Results: Data are provided for both the structural aberrations and for the numerical aberrations.

a) Aberrations in the non-activated 4-hour exposure: The %-of cells with structural aberration was higher at 370 mcg/ml compared to controls (0.5%, 0%, 3%, 8%** at 0, 180, 260, 370 mcg/ml respectively, ** $p < 0.01$). The numerical aberrations were not increased (1.5%, 0%, 1.5%, 2.5% respectively), as state below, and see Table 10.

The percentage of cells with structural aberrations in the non-activated 4-hour exposure group was statistically increased (8.0%) relative to solvent control at 370 $\mu\text{g}/\text{mL}$ ($p < 0.01$, Fisher's Exact test). The Cochran-Armitage test was also positive for a dose response ($p < 0.05$). The percentage of cells with numerical aberrations in the test article-treated group was not significantly increased relative to solvent control at any dose level ($p > 0.05$, Fisher's Exact test).

b) Aberrations in the activated 4-hour exposure: The structural aberrations were not increased at any dose (0%, 0.5%, 0%, 0.5% respectively). However, the %-of cells with **numerical aberrations** were higher at 370 mcg/ml compared to controls (3%, 5%, 5.5%, 7%* at 0, 180, 260, 370 mcg/ml respectively, ** $p < 0.05$), but these were not considered significant by the sponsor because they are within the historical solvent control range (as stated below). as stated below.

The percentage of cells with numerical aberrations in the test article-treated group was statistically increased relative to solvent control at 370 $\mu\text{g}/\text{mL}$ ($p < 0.05$, Fisher's Exact test). However, the Cochran-Armitage test was negative for a dose response ($p > 0.05$). In addition, the percentage of cells with numerical aberrations in the test article-treated group (7.0%) was within the historical solvent control range of 0.0% to 7.5%. Therefore, the increase in numerical aberrations was not considered to be biologically significant.

The percentage of cells with **structural aberrations** in the S9-activated 4-hour exposure group was not significantly increased relative to solvent control at any dose level ($p > 0.05$, Fisher's Exact test).

c) **Aberrations in the non-activated 20-hour exposure:** The %-of cells with structural aberrations were higher at 370 mcg/ml compared to controls (0.5%, 0%, 2.5%, 10.5%** at 0, 180, 260, 370 mcg/ml respectively, ** $p < 0.01$). However, the numerical aberrations were not increased (1%, 1.5%, 2.5%, 0.5% respectively), see Table 10 below.

The percentage of cells with structural aberrations in the non-activated 20-hour exposure group was statistically increased (10.5%) relative to solvent control at 260 $\mu\text{g}/\text{mL}$ ($p < 0.01$, Fisher's Exact test). The Cochran-Armitage test was also positive for a dose response ($p < 0.05$). The percentage of cells with numerical aberrations in the test article-treated group was not significantly increased relative to solvent control at any dose level ($p > 0.05$, Fisher's Exact test).

Table. chromosomal aberration assay with the current drug product (APP glucagon for injection)

TABLE 10
SUMMARY (Glucagon for Injection)

Treatment $\mu\text{g}/\text{mL}$	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
Sterile Water for injection	-S9	4	8.1	200	200	0.005	≈ 0.071	1.5	0.5
Glucagon for Injection									
180	-S9	4	6.6	200	200	0.000	≈ 0.000	0.0	0.0
260	-S9	4	6.5	200	200	0.030	≈ 0.171	1.5	3.0
370	-S9	4	6.8	200	200	0.130	≈ 0.759	2.5	8.0**
MMC 0.2	-S9	4	6.0	200	100	0.230	≈ 0.566	1.0	19.0
Sterile Water for injection	+S9	4	8.4	200	200	0.000	≈ 0.000	3.0	0.0
Glucagon for Injection									
180	+S9	4	9.0	200	200	0.005	≈ 0.071	5.0	0.5
260	+S9	4	8.7	200	200	0.000	≈ 0.000	5.5	0.0
370	+S9	4	6.7	200	200	0.005	≈ 0.071	7.0*	0.5
CP 10	+S9	4	2.4	200	100	0.280	≈ 0.753	2.5	17.0**
Sterile Water for injection	-S9	20	8.4	200	200	0.005	≈ 0.071	1.0	0.5
Glucagon for Injection									
90	-S9	20	6.5	200	200	0.000	≈ 0.000	1.5	0.0
180	-S9	20	5.7	200	200	0.070	≈ 0.720	2.5	2.5
260	-S9	20	3.5	200	200	0.140	≈ 0.512	0.5	10.5**
MMC 0.1	-S9	20	4.2	200	100	0.350	≈ 1.438	0.0	16.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using Fisher's Exact test.

Historical control data for the numerical aberrations are provided below

Following Table was provided for historical control values. Note that the mean values for the solvent control were 2.0 ±1.8% in the presence of S-9 activation (in the historical controls data). Additionally, sponsor does not provide the details of the assay, if these conditions were similar to the current assay, i.e. were the cells exposed to the 4-hr treatment time and 16 hours of recovery time; were they using same treatment medium, etc. In the current assay the mean value with the current drug product falls within these levels (the numerical aberration were increased to 7% with APP glucagon) and the historical control range was 0-7.5%.

IN VITRO MAMMALIAN CYTOGENETIC TEST USING
CHINESE HAMSTER OVARY (CHO) CELLS

HISTORICAL CONTROL VALUES
COMBINED NUMERICAL ABERRATIONS
(POLYPLOID AND ENDOREDUCATED CELLS)
2008-2010

NON-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control ² (%)
Mean	1.5	1.3
±SD ¹	1.2	1.3
Range	0.0-7.5	0.0-5.0

S9-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control ³ (%)
Mean	2.0	1.4
±SD ¹	1.8	1.5
Range	0.0-7.5	0.0-6.0

¹ SD = standard deviation.

² Positive control for non-activated studies, Mitomycin C (MMC, 0.05-0.3 µg/mL).

³ Positive control for S9-activated studies, cyclophosphamide (CP, 10-50 µg/mL).

Sponsor states that in vitro in this chromosomal aberration assay in the CHO cells, the test was considered positive for the structural aberrations (at 4 hour and 20 hour treatment time) in the absence of metabolic activation at 370 mcg/ml, but not for the numerical aberrations in the presence of metabolic activation (because it is in the range of historical control data). Note that at 370 mcg/ml of the APP's glucagon, the increase in the numerical aberrations was 2.5% (without S-9), and 7% (with S-9), which does fall in the historical background rates. The sponsor did not provide the historical control data (or background rates) for the structural aberrations.

B) Chromosomal aberration assay with the RD, i.e. GlucaGen® (AD30AZ)**Methods**

In the preliminary toxicity assay using GlucaGen®, the reference listed drug, the maximum dose tested was 370 µg/mL. The test article was soluble in sterile water for injection and in the treatment medium at all dose levels tested at the beginning and conclusion of the treatment period.

Substantial toxicity (i.e., at least 50% cell growth inhibition, relative to the solvent control) was not observed at any dose level in all three treatment groups. Based on these findings, the doses chosen for the chromosome aberration assay ranged from 45 to 370 µg/mL for all three treatment groups.

In the chromosome aberration assay, the cells were treated for 4 and 20 hours in the non-activated test system and for 4 hours in the S9-activated test system. All cells were harvested 20 hours after treatment initiation. The test article was soluble in sterile water for injection and in the treatment medium at all dose levels tested at the beginning and conclusion of the treatment period.

In the absence of either substantial toxicity or visible precipitate in the treatment medium, the highest dose evaluated was the highest dose tested in the chromosome aberration assay in all harvests. Two lower doses were included in the evaluation.

Based upon the results of the toxicity study, the dose levels selected for testing of RD in the chromosome aberration assay are shown below:

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	45, 90, 180, 260, 370
	20 hr	0 hr	45, 90, 180, 260, 370
S9-activated	4 hr	16 hr	45, 90, 180, 260, 370

Table. The dose selection and toxicity noted at high doses is stated below using GlucaGen®

Treatment Time	Recovery Time	Harvest Time	S9	Toxicity* at highest dose scored (370 µg/mL)	Mitotic Index Reduction **	LED ¹ for Structural Aberrations µg/mL	LED ¹ for Numerical Aberrations µg/mL
4 hr	16 hr	20 hr	-	16%	23% §	None	None
20 hr	0 hr	20 hr	-	33%	42% §	None	None
4 hr	16 hr	20 hr	+	6%	13% §	None	None

*Cell growth inhibition

** Relative to solvent control at the highest dose evaluated for chromosome aberrations.

§ Mitotic Index calculations are presented in Tables 14, 16 and 18.

¹LED = lowest effective dose

Results

The percentage of cells with structural or numerical aberrations in the GlucaGen®-treated groups was not significantly increased relative to solvent control at any dose level ($p > 0.05$, Fisher's Exact test) in the presence of absence of metabolic activation, see Table 20 below:.

TABLE 20
SUMMARY (GlucaGen®)

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
Sterile Water for Injection	-S9	4	11.4	200	200	0.000	≠0.000	0.5	0.0
GlucaGen®									
180	-S9	4	11.1	200	200	0.000	≠0.000	1.5	0.0
260	-S9	4	9.3	200	200	0.005	≠0.071	0.5	0.5
370	-S9	4	8.8	200	200	0.020	≠0.140	1.5	2.0
MMC 0.2	-S9	4	8.4	200	100	0.210	≠0.518	1.5	17.0**
Sterile Water for Injection	+S9	4	12.0	200	200	0.000	≠0.000	2.5	0.0
GlucaGen®									
180	+S9	4	12.4	200	200	0.000	≠0.000	1.5	0.0
260	+S9	4	12.7	200	200	0.000	≠0.000	1.5	0.0
370	+S9	4	10.5	200	200	0.005	≠0.071	5.0	0.5
CP 10	+S9	4	5.3	200	100	0.600	≠1.263	0.0	27.0**
Sterile Water for Injection	-S9	20	12.1	200	200	0.010	≠0.100	2.0	1.0
GlucaGen®									
180	-S9	20	8.8	200	200	0.000	≠0.000	1.0	0.0
260	-S9	20	8.1	200	200	0.015	≠0.122	0.5	1.5
370	-S9	20	7.0	200	200	0.010	≠0.100	1.5	1.0
MMC 0.1	-S9	20	6.1	200	100	0.250	≠1.048	1.5	16.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using Fisher's Exact test.

The chromosomal aberration assay is also summarized below:

Table. Summary of chromosomal aberration assay with the current APP drug product

2.6.7.8B Genotoxicity: *In Vitro* Report Title: *In Vitro* Mammalian Chromosome Aberration Test Test Article: Glucagon for Injection
 Comparator/Reference Article: GlucaGen®

Test for Induction of:	Chromosome aberrations	No. of Independent Assays:	2	(b) (4)	Study No.:	AD29XE.331 (b) (4) and AD30AZ.331
Strains:	Chinese Hamster Ovary Cells (CHO)	No. of Replicate Cultures:	2	Location in CTD:		
Metabolizing System:	Aroclor-induced rat liver S9	No. of Cells Analyzed/Culture:	Glucagon for Injection: 100 for structural, 100 for numerical Positive controls: 50 for structural, 100 for numerical			
Vehicles:	For Test Article: Sterile water for injection	For Positive Controls:	Water (MMC, CP)	GLP Compliance:	Yes	
Treatment:	20 hr without S9; 4 hr with 16 hr recovery period with and without S9		Date of Treatment:	26 July 2011 (Definitive Assay)		

Cytotoxic Effects:	In the chromosome aberration assay using Glucagon for Injection, substantial toxicity (i.e., at least 50% cell growth inhibition, relative to the solvent control) was not observed at any dose level in the non-activated and S9-activated 4-hour exposure groups. Substantial toxicity was observed at dose levels $\geq 295 \mu\text{g/mL}$ in the non-activated 20-hour continuous exposure group.
Genotoxic Effects:	Glucagon for Injection, was positive for the induction of structural chromosome aberrations and negative for the induction of numerical chromosome aberrations in CHO cells in the non-activated test system. Glucagon for Injection was negative for the induction of structural and numerical chromosome aberrations in CHO cells in the S9-activated test systems.

MMC: Mitomycin C

CP: Cyclophosphamide

2.6.7.8B Genotoxicity: *In Vitro*

Test Article: Glucagon for Injection (continued)

Metabolic Activation	Test Article #	Concentration $\mu\text{g/mL}$	Cytotoxicity ^a (% of Control)	Aberrant Cells		Aberrations Per Cell ^{b,d}	Total Polyploid Cells (Mean %) ^e
				Structural (Mean %) ^b	Numerical (Mean %) ^c		
20-hr Continuous Treatment Without Activation	Sterile water for injection	NA	NA	0.5	1.0	0.005 \pm 0.071	0.5
	Glucagon for Injection	90	7	0.0	1.5	0.000 \pm 0.000	1.5
	Glucagon for Injection	180	28	2.5	2.5	0.070 \pm 0.720	2.5
	Glucagon for Injection	260	47	10.5**	0.5	0.140 \pm 0.512	0.5
	MMC	0.1	17	16.0**	0.0	0.350 \pm 1.438	0.0
4-hr Treatment With 16 hr Recovery Without Activation	Sterile water for injection	NA	NA	0.5	1.5	0.005 \pm 0.071	1.5
	Glucagon for Injection	180	2	0.0	0.0	0.000 \pm 0.000	0.0
	Glucagon for Injection	260	22	3.0	1.5	0.030 \pm 0.171	1.0
	Glucagon for Injection	370	43	8.0**	2.5	0.130 \pm 0.759	2.5
	MMC	0.2	13	19.0**	1.0	0.230 \pm 0.566	1.0
4-hr Treatment With 16 hr Recovery With Activation	Sterile water for injection	NA	NA	0.0	3.0	0.000 \pm 0.000	2.0
	Glucagon for Injection	180	-3	0.5	5.0	0.005 \pm 0.071	1.5
	Glucagon for Injection	260	2	0.0	5.5	0.000 \pm 0.000	2.0
	Glucagon for Injection	370	32	0.5	7.0*	0.005 \pm 0.071	1.5
	CP	10	53	17.0**	2.5	0.280 \pm 0.753	2.5

MMC: Mitomycin C; CP: Cyclophosphamide; NA: Not Applicable; Fisher's Exact Test: * p \leq 0.5; ** p \leq 0.01

- a. Based on cell growth inhibition.
- b. Does not include cells with only gaps.
- c. Includes polyploid and endoreduplicated cells.
- d. Severely damaged cells counted as 10 aberrations.
- e. Does not include endoreduplicated cells.

Table. Summary of chromosomal aberration assay with the RD (Glucagen)

2.6.7.8B Genotoxicity: *In Vitro*

Report Title: *In Vitro* Mammalian Chromosome Aberration Test
 Comparator/Reference Article: GlucaGen®

Test Article: Glucagon for Injection

Test for Induction of:	Chromosome aberrations	No. of Independent Assays:	2	(b) (4)	Study No.:	AD29XE 331 and AD30AZ 331
Strains:	Chinese Hamster Ovary Cells (CHO)	No. of Replicate Cultures:	2	Location in CTD:		
Metabolizing System:	Aroclor-induced rat liver S9	No. of Cells Analyzed/Culture:	GlucaGen®: 100 for structural, 100 for numerical Positive controls: 50 for structural, 100 for numerical			
Vehicles:	For Test Article: Sterile water for injection	For Positive Controls:	Water (MMC, CP)	GLP Compliance:	Yes	
Treatment:	20 hr without S9; 4 hr with 16 hr recovery period with and without S9			Date of Treatment:	12 July 2011 (Definitive Assay)	

Cytotoxic Effects:	In the chromosome aberration assay using GlucaGen®, substantial toxicity (i.e., at least 50% cell growth inhibition, relative to the solvent control) was not observed at any dose level in all three treatment groups.
Genotoxic Effects:	GlucaGen® was negative for the induction of structural and numerical chromosome aberrations in CHO cells in both the non-activated and the S9-activated test systems.

MMC: Mitomycin C

CP: Cyclophosphamide

2.6.7.8B Genotoxicity: *In Vitro*

Test Article: GlucaGen® (continued)

Metabolic Activation	Test Article #	Concentration µg/mL	Cytotoxicity ^a (% of Control)	Aberrant Cells		Aberrations Per Cell ^{b, d}	Total Polyploid Cells (Mean %) ^c
				Structural (Mean %) ^b	Numerical (Mean %) ^c		
20-hr Continuous Treatment Without Activation	Sterile water for injection	NA	NA	1.0	2.0	0.010 ± 0.100	2.0
	GlucaGen®	180	11	0.0	1.0	0.000 ± 0.000	1.0
	GlucaGen®	260	27	1.5	0.5	0.015 ± 0.122	0.5
	GlucaGen®	370	33	1.0	1.5	0.010 ± 0.100	1.5
	MMC	0.1	5	16.0**	1.5	0.250 ± 1.048	1.5
4-hr Treatment With 16 hr Recovery Without Activation	Sterile water for injection	NA	NA	0.0	0.5	0.000 ± 0.000	0.5
	GlucaGen®	180	-9	0.0	1.5	0.000 ± 0.000	1.5
	GlucaGen®	260	-2	0.5	0.5	0.005 ± 0.071	0.5
	GlucaGen®	370	16	2.0	1.5	0.020 ± 0.140	1.5
	MMC	0.2	32	17.0**	1.5	0.210 ± 0.518	1.5
4-hr Treatment With 16 hr Recovery With Activation	Sterile water for injection	NA	NA	0.0	2.5	0.000 ± 0.000	1.5
	GlucaGen®	180	-20	0.0	1.5	0.000 ± 0.000	1.0
	GlucaGen®	260	-5	0.0	1.5	0.000 ± 0.000	0.5
	GlucaGen®	370	6	0.5	5.0	0.005 ± 0.071	1.5
	CP	10	56	27.0**	0.0	0.600 ± 1.263	0.0

MMC: Mitomycin C; CP: Cyclophosphamide; NA: Not Applicable; Fisher's Exact Test: * p<0.5; ** p<0.01

- a. Based on cell growth inhibition.
- b. Does not include cells with only gaps.
- c. Includes polyploid and endoreduplicated cells.
- d. Severely damaged cells counted as 10 aberrations.
- e. Does not include endoreduplicated cells.

In conclusion only one repeat chromosomal aberration was carried out, because in the initial assay there was error in the dilution of the APP glucagon, so no results were provided from the initial assay. In the repeat chromosomal aberration assay (in the absence of metabolic activation at 4 and 20 hours exposure time), the current APP glucagon drug product at 260 to 370 mcg/ml significantly increased the structural aberration, but it did not increase the numerical aberrations. The toxicity at this dose was 43% to 47%.

In the presence of metabolic activation, the current drug product (APP glucagon for injection) produced a significant increase in the numerical aberrations at a HD of 370 mcg/ml (7%* vs 3% in the controls, p<0.05), however these are within the historical control range (0-7.5%). Note that

the reference drug (GlucaGen) was negative in the chromosomal aberration assay, it did not increase structural or numerical aberrations at any dose in the presence or absence of metabolic activation.

Thus, the current drug was considered positive in the *in vitro* assay for chromosomal aberrations in CHO cells at concentrations up to 370 ug/ml without S-9 activation (4 and 20-hour treatments).

As stated before, the impurities tested in the current APP drug product were present up to (b) (4)%. The (b) (4) was present at (b) (4)%. The maximal recommended doses of the current drug product (APP glucagon for injection) are up to 2 mg/day. Therefore 2 mg dose (or 2000 mcg) will have up to (b) (4) mcg/day of (b) (4) (i.e. (b) (4)). In the current chromosomal aberration assay doses of up to 370 mcg/ml were evaluated, of which (b) (4) mcg was (b) (4) (b) (4). Note that the assay was positive at concentrations of 370 mcg/ml of the current APP drug product (containing the total drug impurities of up to (b) (4)%).

However, sponsor does not consider the present drug product to be positive in the chromosomal aberration assay significant, because they state that previously "in vitro glucagon assay" has shown positive mutagenic and clastogenicity responses under certain conditions as stated below, and this clastogenic effect is in the glucagon labels.

Sponsor's conclusions are stated below:

In the *in vitro* CHO chromosomal aberration assay, Glucagon for Injection was clastogenic the non-activated test system but was negative in the S9-activated test systems. The reference listed drug product, GlucaGen[®], was not clastogenic in the non-activated and the S9-activated test systems.

In vitro assays with GlucaGen[®] have reported positive mutagenic and clastogenic response under certain conditions. GlucaGen[®] was also reported to be clastogenic *in vivo* in a mouse micronucleus test at high doses in males but not females. The concluding remarks were that the weight of evidence indicates that GlucaGen[®] was not different from glucagon of pancreatic origin and did not pose a genotoxic risk to humans.

The impurity profile of Glucagon for Injection was similar to that GlucaGen[®] but the amounts present in the test article used in the toxicology program were slightly higher than that of GlucaGen[®]. The lots used in the toxicology testing program for Glucagon for Injection were at the end of expiration dating to provide an exaggeration of the impurity testing. There were no differences in the *in vivo* testing between Glucagon for Injection and GlucaGen[®]. There were no differences in the *in vitro* mutagenic response between Glucagon for Injection and GlucaGen[®]. The only difference that occurred was in the clastogenic response between Glucagon for Injection and GlucaGen[®]. The SBA and the package insert for GlucaGen[®] states that mutagenic potential tested in the Ames and human lymphocyte assays, was borderline positive under certain conditions for both glucagon (pancreatic) and glucagon (rDNA) origin.

Given the uncertain nature of testing biologic products in these assays, the *in vitro* clastogenic response noted with Glucagon for Injection does not pose a genotoxic risk to patients given the intended single dose clinical use.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The primary pharmacology of glucagon is well established. Glucagon stimulates glycogenolysis via the activation of adenylyl cyclase, the formation of cAMP and a cascade that results in active liver phosphorylase breaking down glycogen into usable glucose. A second effect of glucagon is the stimulation of gluconeogenesis from amino acids, an effect that takes longer to initiate but is of longer duration. The net result of both of these actions is a rise in serum glucose concentration. Glucagon (b) (4) when administered by the parenteral route is relaxation of the smooth muscles of the stomach, (b) (4) small bowel and colon. Therefore, glucagon is also indicated for use during radiologic examinations to temporarily inhibit movement of the gastrointestinal tract.

APP Pharmaceutical Glucagon for injection drug product was manufactured (b) (4) as compared to the innovators, in which (b) (4) (NDA 20-918/Novo Nordisk, and NDA 20-928/Eli Lilly).

Sponsor has submitted this application as a 505(b)(2), which relies on the previous approved glucagon NDAs (NDA 20-918 and NDA 20-982). The drug is intended be used as a diagnostic aid during radiologic exams to temporarily inhibit movement of the gastrointestinal tract. The usual dose to relax the colon is 0.5 mg to 0.75 mg intravenously and 1 mg to 2 mg intramuscularly. (b) (4)

Sponsor had initially submitted this as 505(b)(2) NDA application on 9/30/2010. However, pharmacology/toxicology refused to file the application because the impurity profile of the drug product was not identical to the marketed GlucaGen and there were no non-clinical studies submitted to address the differences in impurity profiles or allow scientific reliance on the listed drugs being referenced by APP. We sent the sponsor a refuse to file letter. The sponsor resubmitted this application on 11/30/2011 with complete response to RTF (see page 9). They have now provided additional studies including a 4-week toxicity study in rats and two in vitro geno-toxicity studies with the current APP drug product and the RD GlucaGen; these toxicity studies are reviewed here.

In a 28-day Intramuscular (IM) toxicity study of glucagon for injection in rats, doses of 0, 1, and 5 mg/kg/day of the current drug product (APP glucagon for injection) were administered to three groups of rats (n=8/sex/dose). The 4th group of rats was similarly administered the reference drug GlucaGen (5 mg/kg/day) for comparison. The TK in general were similar with both drugs. The exposures of the drug in males and females were slightly higher on day-27 (males 122, 502, 454 ng.hr/ml at 1, 5, mg/kg/day of APP glucagon and 5 mg/kg/day of RD GlucaGen respectively; these values in females were 99, 557, 511 ng.hr/ml respectively). AUC values on day 1 in males were 76, 355, 444 ng.hr/ml respectively, females were 63, 306, 285 ng.hr/ml respectively. No significant clinical signs were noted with both drugs (current or RD).

In males at 5 mg/kg/day, body weight gains were lower on days 22-29 with the current APP drug (but not with the RD GlucaGen); no effects on body weights/weight gains in females were noted. Food consumption was significantly increased with both (i.e. by 19% with the current drug and by 13% with the RD). The current and RD GlucaGen had similar effects on hematological parameters (increased platelet counts, decreased reticulocyte counts & decreased %-reticulocytes), and both had no effects on ophthalmological parameters. The current drug produced some changes in clinical chemistry parameters, not noted with the RD, these in males included increases in total protein; in females increases in calcium & triglycerides, & decreases in BUN levels, but these were not toxicologically relevant as the differences were small, and no

histological correlates were observed. Decreased fibrinogen levels and lower urinary pH were noted with both drugs (the current drug and RD).

In male rats, absolute liver (10.9, 12.7, 13.6*, 11.9 g respectively), and in female rats, absolute kidney (1.5, 1.7*, 1.8*, 1.6 respectively) weights were increased with the current drug by 25% and 20% respectively, but not with the RD. Similarly relative liver and kidney weights were also increased in males and females respectively, but not with the RD. Note that other changes such as increases in absolute heart weights, these were noted with both drugs (males (1.2, 1.34*, 1.40*, 1.39* g respectively; in females 0.89, 1.1*, 1.0*, 1.1* g respectively, *p<0.05) and similarly the relative heart weights were increased in both sexes with both drugs (APP glucagon and RD GlucaGen).

The target organs of toxicity besides liver, may be heart in female rats, as minimal to mild mineralization in the heart was noted in 2/8 rats at a HD with the current APP drug (which was not noted with the RD or in controls), and kidney in male rats, where bilateral chronic progressive nephropathy of minimal to mild degree was noted in 3/8 males (vs 0/8 with the RD or in controls). Note that in females, minimal chronic progressive nephropathy unilateral was noted in 1/8 female rats with the RD. Other histopathology findings were noted with both drugs, these included kidney (unilateral proteinosis in males 0/8, 0/0, 2/8, 3/8; females 0/8, 0/0, 2/8, 3/8 respectively), mandibular lymph nodes (increased incidences of hyperplasia), and sciatic nerve (fascia, minimal to mild chronic active inflammation noted with both drugs and also in controls); sponsor explains that this is due to intramuscular injection. They state that the *"inflammatory reaction at the injection site in control and test article treated animals spreads along the fascial planes of the hind limb muscle groups, and was detected at the site of the sciatic nerve"*.

Thus the subtle toxicity was noted with the current drug product, not noted with the RD. These include lower body weight gains in males on days 22-29 at a high dose of 5 mg/kg/day (no effects on body weights/weight gains in females were noted) and increases in total protein. In females, increases in calcium & triglycerides, and decreases in BUN levels were noted, but these were not toxicologically relevant as the differences were small, and no histological correlates were observed. In male rats, absolute liver (10.9, 12.7, 13.6*, 11.9 g respectively), and in female rats, absolute kidney (1.5, 1.7*, 1.8*, 1.6 respectively) weights were increased by 25% and 20% respectively, but not with the RD. The target organs of toxicity may be heart in female rats (minimal to mild mineralization in 2/8 rats at a HD, not noted with the RD or controls), and kidney, in the male rats (bilateral chronic progressive nephropathy, minimal to mild in 3/8 males vs 0/8 with the RD and controls). These subtle differences in toxicity profile may represent biological variability response to the API.

Note that total impurities that were tested in the 28-day toxicity study were up to (b) (4)% with the current drug product (APP glucagon for injection), and up to (b) (4)% with the reference drug (GlucaGen). This may account for the subtle toxicity differences.

In the 28-day toxicity, the doses of up to 5000 mcg/kg/day of the current drug product were used, which had up to (b) (4)% impurities, and of these (b) (4)% was (b) (4) (i.e. up to (b) (4) mcg/day of (b) (4) was present in this 28-day toxicity study).

The maximal recommended doses of the current drug product (APP glucagon for injection) are up to 2 mg/day. Therefore 2 mg (or 2000 mcg) will have up to (b) (4) mcg/day of (b) (4) (i.e. (b) (4)), which exceeds levels present in RD GlucaGen. Other impurities are comparable to those found in the RD GlucaGen.

The NOAEL or tolerated doses of the drug in a 4-week oral toxicity study in rats could not be established as histopathology findings were noted in the heart (in females), and in the kidney (in males) at a HD of 5 mg/kg/day with the current APP drug, not noted with the RD. The lower

dose of 1 mg/kg/day was not examined for histopathology findings. The sponsor does not consider any of these findings significant. This NOAEL of <5 mg/kg/day (or 30 mg/m²/day) provides the safety margin of <24 X in human subjects (2 mg/day or 1.23 mg/m²/day), based on body surface area.

In genotoxicity studies, The current drug (glucagon for injection) and reference drug (GlucaGen) were both tested in the *in vitro* bacterial reverse mutation assay and chromosomal aberration assay. Both the current APP drug and reference drug GlucaGen were negative in all tester strains of *Salmonella typhimurium* or *Escherichia coli* strains, at doses up to 2700 mcg/ml. However, the current drug (APP Glucagon for injection) was positive *in vitro* Chinese hamster ovary cell chromosomal aberration assay at doses of 260 to 370 mcg/ml (in the absence of metabolic activation at 4 and 24 hours treatment). *Note that* this positive chromosomal aberration assay is not considered to be significant by the sponsor because it has been shown before that glucagon due to either its particular property, or a methodological problem in conducting these studies at high doses can be positive in these assay, which was also noted with the previous recombinant and animal source glucagons and is described in the approved product labels.

Impurities in the APP's Glucagon: As far as the impurities in the drug product are concerned, the sponsor has conducted a comparative bridging toxicity study and *in vitro* gene-toxicity in support of this NDA.

The percentage of impurities qualified are provided below, and in the parenthesis the specification proposed by the sponsor are stated: 1) (b) (4) was qualified up to (b) (4) % (specification proposed by the sponsor is up to (b) (4) %); 2) total impurities qualified were (b) (4) % (specification proposed is (b) (4) % excluding lactose related impurities). Similarly proposed lactose related impurities specification by the sponsor was (b) (4) %, while lower percentages of these were qualified in the conducted toxicity studies. In the pharmacology /toxicology section, some of these impurities are listed only as HPLC retention times which means they have not even been structurally identified.

The lactose related impurities would not amount to any considerable toxicity, especially since we probably ingest these daily via diet and we could probably argue the same for the glucagon related impurities. The exception being that glucagon related peptides that are novel (not found in the listed drug) might elicit an immune response. Anything novel, not present in the listed drug or covered under the USP specification was considered for safety, i.e. generally anything exceeding ICHQ3 (or with a structural alert for genotoxicity that's above 1.5 mcg/day) would need qualification. Pharmacology /Toxicology can only comment on the levels that were tested and found safe in the toxicity studies.

Note that the FDA chemist had concerns only about one novel impurity, not structurally identified which appears as a shoulder at the same HPLC retention time (RT) as (b) (4) CMC has confirmed that all other impurities are present in Novo Nordisk's GlucaGen (the reference drug). (b) (4) and its shoulder peaks were tested in general toxicity and *in vitro* gene-toxicity tests, therefore qualifying combined levels up to (b) (4) %. The Firm (APP) has proposed a spec of (b) (4) % for (b) (4) which is unacceptable. On 6/27/12, the sponsors agreed to lower the specification of this impurity to (b) (4) %. All other impurity levels are comparable to those found in the reference drug.

Safety evaluation:

Thus APP Pharmaceuticals has performed a 28-day rat toxicity study, Ames assay and Chromosome aberration assay in CHO cells, in each study comparing its glucagon product to the reference drug GlucaGen.

The 28-day toxicity study shows that the products are comparable. However differences are noted between the two glucagons (APP glucagon and the the RD GlucaGen). For example with APP glucagon, heart mineralization in female rats (2/8) and nephropathy incidences in male rats (3/8) are noted, not seen with the GlucaGen comparator or controls. There are some sporadic findings in clinical chemistry which do not seem to correlate with the histopathology findings: males had increased plasma protein and females had increased plasma calcium and triglycerides and decreased BUN. Note that these are daily IM injections, which represent significantly greater exposure in rats compared to the clinical use as a diagnostic agent. It is anticipated that if this were a drug related effect, than both male and female would experience this toxicity.

The in vitro Ames assay was negative for both the APP's glucagon (containing (b) (4) mcg of (b) (4)) and the reference drug GlucaGen, both compounds were tested at doses up to 2700 mcg/plate. Note that the total combined impurities present in the APP glucagon were (b) (4) % (lot # C108-002) used in the Ames assay, and in the reference drug GlucaGen were (b) (4) % (lot # AW60180).

The in vitro chromosome aberration assay in CHO cells, a lot number C109-002 of APP glucagon was used, which had higher total impurities of (b) (4) % vs the reference drug GlucaGen (lot AW60180) which had total impurities of (b) (4) %, similar to the one used in the Ames assay. GlucaGen (the RD) with total impurities of (b) (4) % did not produce an increase in the numerical or structural aberrations when tested at dose (b) (4) up to 370 mcg/ml. APP glucagon did not significantly increase the numerical aberrations in the presence of metabolic activation (as the levels were within the historical control range), but it produced a statistically significant increases in structural aberrations, in the absence of metabolic activation at 370 mcg/ml (-S9 after 4h treatment) and at 260 mcg/ml (-S9 after 20 hr). Cytotoxicity was <50% at either of these concentrations. The sponsor acknowledges that this is a clastogenic effect but appears to dismiss this observation by noting that approved glucagon labeling indicates a positive mutagenic response in Ames and human lymphocytes (clastogenicity) for both pancreatic glucagon (endogenous) and recombinant glucagon. Note that in vivo micronucleus assays at 100-200 mg/kg glucagon gave a higher micronucleus formation in male but not female mice, indicating that glucaGen was not considered different from endogenous glucagon and doesn't pose a genotoxic risk to humans. Note that the diagnostic use for maximum recommended human dose (MRHD) is 2 mg=0.03 mg/kg.

In summary, APP glucagon was positive in the in vitro assay for chromosomal aberrations in CHO cells at concentrations of 260 to 370 mcg/ml without S-9 activation (4 and 20-hour treatments); it was negative in the presence of S-9 metabolic activation. In contrast, the reference drug GlucaGen was negative in the above assay in CHO cells at concentrations of 370 mcg/ml with or without S-9 activation (at 4 and 20-hour treatments). Thus, the label for the APP glucagon will need to indicate for the positive structural aberrations with this drug product as well as the clastogenicity labeling present in the approved recombinant-human glucagons.

In summary, from the preclinical standpoint, approval of this application is recommended, pending following labeling changes.

Labeling Review: The pharmacology toxicology labeling in general is similar to the approved GlucaGen label (rDNA origin, Novo Nordisk label). In the current application, the submitted PLR label is reviewed and reviewer's recommended changes are stated below:

A. Following is sponsor's proposed label (from 5/18/2012 submission):

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category B. Reproduction studies were performed in rats and rabbits at glucagon doses of 0.4, 2, and 10 mg/kg. These doses represent exposures of up to 100 and 200 times the human dose based on mg/m² for rats and rabbits, respectively, and revealed no evidence of harm to the fetus. There are, however, no adequate and well-controlled studies in pregnant women. Glucagon does not cross the human placenta barrier.

8.3 Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when glucagon is administered to a nursing woman. No clinical studies have been performed in nursing mothers, however, glucagon is a peptide and intact glucagon is not absorbed from the GI tract. Therefore, even if the infant ingested glucagon it would be unlikely to have any effect on the infant. Additionally, glucagon has a short plasma half-life thus limiting amounts available to the child. Glucagon does not cross the human placental barrier.

Reviewer's recommended changes:

8.1 Pregnancy

Pregnancy Category B. **There are no adequate and well controlled studies in pregnant women.** Reproduction studies were performed in rats and rabbits at **GlucaGen (recombinant)** doses of 0.4, 2, and 10 mg/kg. These doses represent exposures of up to 100 and 200 times the human dose based on mg/m² for rats and rabbits, respectively, and revealed no evidence of harm to the fetus. There are, however, no adequate and well-controlled studies in pregnant women. Glucagon does not cross the human placenta barrier.

B. Following is sponsor's proposed label:





(b) (4)

Reviewer's recommended changes:



(b) (4)

Justification for the changes:



(b) (4)

Recommendation: From the preclinical standpoint, approval of this application is recommended, pending labeling changes.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____

Concurrence Yes ___ No ___

cc: IND Arch
HFD-510
HFD-510/davisbruno/antonipillai/calish/stephens.O/Jairath.
Review code: AP
File name: nda 201849 (glucagon, (b) (4))

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/s/

INDRA ANTONIPILLAI

07/26/2012

From the pharmacology/toxicology point of view, approval of this application is recommended, pending labeling changes.

KAREN L DAVIS BRUNO

07/26/2012

Signed off in DARRTS on 2/1/2012

**45 Day Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY**

NDA 201849: This is a 505(b)(2) application, RLD is NDA 20-918 (GlucaGen, Novo Nordisk).

Submission date: 11/30/11. Initially this application was submitted 9/30/2010, however it was not fileable (see below).

Sponsor: APP Pharmaceuticals LLC, Schaumburg, IL.

Drug: Glucagon injection, synthetic 1 mg (1 IU). It is recommended to be used for the intravenous and intra-muscular administration for gastrointestinal indication. In the initial submission (9/30/10), the sponsor wanted to use this product for (b) (4) intramuscular and intravenous administration.

Indication: It is indicated to be used as a diagnostic aid during radiologic exam to temporarily inhibit movement of the gastrointestinal tract. Sponsor had initially submitted this application on 9/30/2010, and wanted it fo (b) (4) to use as it for the gastrointestinal indication.

Introduction: Glucagon is a single chain polypeptide hormone containing 29 amino acids. Glucagon in a recombinant form has been approved before, as NDA 20-918 (Novo Nordisk) and NDA 20-928 (Eli Lilly) (b) (4) (as 1 mg/ml injection).

The current submission (NDA 201-849) is a synthetic form of the peptide. (b) (4)
The current sponsor submits this application as 505(b) (2) based on previously approved reference listed drug NDA 20-918 (GlucaGen, Novo Nordisk).

Sponsor had initially submitted this application on 9/30/2010. However, pharmacology/toxicology refused to file the application because the impurity profile of the drug product was not identical to the marketed GlucaGen. We recommended that sponsor conduct qualifying toxicity studies, e.g. in vitro genotoxicity (mutagenecity, clastogenicity) and a 2 to 12 week toxicity study in one species with the proposed drug product and reference listed drug (as per ICH Q3A and ICH Q3B).

In the current application, sponsor has conducted 3 non-clinical toxicity studies. These studies include a 28-day toxicity/TK study in rats and two geno-toxicity studies (Ames and chromosomal aberration assay). As indicated earlier, no pharmacology/ toxicology studies were conducted in the initial application (9/30/2010), in which two impurities were concerning, which were both identified by the sponsor as degradants, and these impurity limits were above the CDER's and ICH's identification and qualification thresholds of 0.50% and 1.00%.

ITEM: NDA (b) (4)	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?	Yes		In the current application, appropriate pharmacology/ toxicology information is included. In the initial submission, minimal toxicology information was provided.
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	Yes		
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.)?	Yes		
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)	Yes		<p>For general toxicology studies, sponsor refers to the innovator's drugs (NDA 20-918, GlucaGen, Novo Nordisk). However, we refused to file the initial application (12/3/2010) due to the presence of new impurities, sponsor conducted additional toxicity studies i.e. in vitro geno-toxicity studies (Ames and chromosomal aberration assays), and a 28-day toxicity study in one species (i.e. rat) with the proposed drug product to qualify the impurities (as per ICH Q3A and Q3B). these studies have been provided in the current submission.</p> <p>Have electronic files of the carcinogenicity studies been submitted for statistical review?</p> <p>No carcinogenicity studies have been performed with the current drug or previously approved recombinant glucagon, based on the proposed acute intermittent use.</p>

ITEM	YES	NO	COMMENT
5) Were the studies adequately designed (i.e., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?	Yes		The 4-week study in rats and two in vitro geno-toxicity studies to qualify the impurities are adequately designed.
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 (i.e., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	Yes		The previously approved drug was manufactured using recombinant techniques. The present drug is a synthetic glucagon and contains impurities & degradants for which the qualifying studies have now been submitted. (b) (4) <div style="background-color: gray; width: 100%; height: 1em; margin-top: 5px;"></div>
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?	Yes		The route of administration in the previously approved NDAs (recombinant glucagon) was by injection. In the current application the drug is administered to animals by the intra-muscular (IM) route. The sponsor plans to use this drug product for intramuscular and intravenous administration.
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/m ² or comparative serum/plasma AUC levels?	Yes		The draft pharmacology /toxicology label with the current drug in general is similar to the approved RLD (NDA 20-918/S-012). The current label is in the SPL format. The draft labeling submitted in general is according to CFR, and data express human dose multiples in mg/kg/day and in mg/m ² .

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

Reviewing Pharmacologist: Indra Antonipillai

Supervisory Pharmacologist: Karen Davis-Bruno.

File Name: 201849 filing-11.

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/s/

INDRA ANTONIPILLAI

02/01/2012

From the pharmacology/toxicology point of view, this application is fileable.

KAREN L DAVIS BRUNO

02/01/2012

**45 Day Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY**

NDA 201849: This is a 505(b)(2) application, RLD is NDA 20-918 (GlucaGen, Novo Nordisk).

Submission date: 9/30/10, initial submission. 10/25/07, they provided the Structured product labeling.

Sponsor: APP Pharmaceuticals LLC, Schaumburg, IL.

Drug: Glucagon, synthetic 1 mg (1 IU). It is recommended to be used for (b) (4) intramuscular and intravenous administration.

Indication: It is indicated for (b) (4) to use as a diagnostic aid during radiologic exam to temporarily inhibit movement of the gastrointestinal tract.

Glucagon is a single chain polypeptide hormone containing 29 amino acids. Glucagon in a recombinant form has been approved before as NDA 20-918 (Novo Nordisk) and NDA 20-928 (Eli Lilly) (b) (4) (as 1 mg/ml injection).

The current submission (NDA 201-849) is a synthetic form of the peptide. (b) (4)

(b) (4) The current sponsor submits this application as 505(b) (2) based on previously approved reference listed drug NDA 20-918 (GlucaGen, Novo Nordisk).

No pharmacology/ toxicology studies have been conducted in this application, and no toxicology studies are mentioned in the designated section in the eCTD submission. However, in the drug product technical section of the NDA, there is a "Toxicity review and risk assessment" report for the glucagon-related degradants/impurities (section 3.2.P.5.6). This report is from (b) (4) (b) (4) This report is very poorly presented. Two impurities have been described in this report which are concerning; these are both identified by the sponsor as degradants; one is Lactose-Related Impurity (b) (4) they state that this one is also seen in the RLD (but details are not provided). The second is identified as lactose-related degradant, which is (b) (4) Both the peptide-related impurity limit and lactose-related impurity limit are above CDER's and ICH's identification and qualification thresholds of 0.50% and 1.00%.

The current sponsor (APP Pharmaceuticals LLC) needs to qualify the impurities in their drug product by the intended route of administration in humans. A 1-month quality assured/GLP toxicity study (by subcutaneous/ intravenous/ intramuscular route) in one species with the proposed drug product and the reference listed drug with toxico-kinetics will likely provide the necessary information as well as provide qualification of any differences in impurity/degradant profiles between the products. The toxicity studies should clearly identify target organs of toxicity and NOAELs.

Sponsor's DEREK analysis states that (b) (4) contains structural alerts that are potentially hepatotoxic and mutagenic, it is not clear what level of this impurity is present in the drug product. Sponsor needs to conduct geno-toxicity assays to characterize the genotoxic potential of the above impurities.

ITEM: NDA (b) (4)	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?		No	There is no pharmacology/toxicology section in the electronic submission under Common Technical Document. The minimal toxicology information is provided by the sponsor under 'Drug product technical section' (i.e. under justification of specifications {or section 3.2.P.5.6}).
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?		NO	No mention of pharmacology/toxicology information in the index section
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.)?		No	The toxicology data were not presented in an appropriate manner.
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)			<p>There were no previous pharmacology/toxicology discussions on this application. No toxicology studies have been conducted. Sponsor refers to the innovator's drug (NDA 20-918, Novo Nordisk).</p> <p>Pharmacology/tox is recommending that sponsor qualify the impurities in their drug product by the intended route of administration in humans.</p> <p>Have electronic files of the carcinogenicity studies been submitted for statistical review?</p> <p>No carcinogenicity studies have been performed with the current drug or previously approved recombinant glucagon, based on the proposed acute intermittent use</p> <p>.</p>

ITEM	YES	NO	COMMENT
<p>5) Were the studies adequately designed (i.e., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?</p>			<p>Not applicable, as no toxicity studies have been conducted.</p>
<p>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 (i.e., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?</p>	<p>Yes</p>		<p>The previously approved drug was manufactured using recombinant techniques. The present drug is a synthetic glucagon and contains impurities & degradants which need to be qualified. (b) (4)</p> <p>No information comparing the proposed drug to the RLD is provided.</p>
<p>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?</p>			<p>Not applicable, as no toxicity studies have been conducted in animals so far.</p>

<p>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?</p>	<p>Yes</p>	<p>The draft pharmacology /toxicology label with the current drug in general is similar to the approved RLD (NDA 20-918/S-012).</p> <p>The current label is in the SPL format vs the innovator's (Novo Nordisk), which is not in the SPL format.</p> <p>The draft labeling submitted in general is according to CFR. However data express human dose multiples in mg/kg/day (and not in mg/m² or AUC levels).</p>
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ITEM	YES	NO	COMMENT
<p>9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.</p>		<p>No</p>	
<p>10) Reasons for refusal to file:</p> <ol style="list-style-type: none"> 1. Sponsor needs to provide the pharmacology/toxicology information under the appropriate section(s). 2. The drug impurities/degradants need to be clearly identified and qualified 3. sponsor needs to provide the data (under section 3.2.P.5.5) that show the comparison of impurities/degradants in their product vs the innovator's (GlucaGen). 4. If the impurity profile with new manufacturing process is not identical to the marketed GlucaGen, sponsor will need to provide qualifying toxicity studies. These studies would include in vitro genotoxicity (mutagenicity, clastogenicity) and a 2-12 week toxicity study in one species with the proposed drug product and reference listed drug (as per ICH Q3A and ICH Q3B) with toxicokinetics. The toxicity studies should clearly identify target organs of toxicity and NOAELs, 			

Reviewing Pharmacologist: Indra Antonipillai

Supervisory Pharmacologist: Karen Davis-Bruno
 File Name: 201849 filing.

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/s/

INDRA ANTONIPILLAI

11/16/2010

From the Pharmacology/toxicology point of view, this application is not fileable. Please see the reasons for refusal to file in the filing review

KAREN L DAVIS BRUNO

11/16/2010

P/T RTF