CENTER FOR DRUG EVALUATION AND RESEARCH

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NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
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CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Applicant:	Serenity Pharmaceuticals, LLC
Review Division:	Division of Bone, Reproductive, and Urology Products
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List of Abbreviations

ADH	Anti-Diuretic Hormone
CPD	Cyclopentadecanolide
CPE-215	Cyclopentadecalactone
DDAVP	Desamino-8-D-Arginine Vasopressin
NE	Nocturnal Enuresis
RLD	Reference Listed Drug

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1 Executive Summary

1.1 Introduction

This 505(b)(2) application submitted by Serenity Pharmaceuticals, LLC., seeks to market their drug product SER120 under the trade name Noctiva™. Noctiva™ is a low dose intranasal reformulation of an FDA approved drug - DDAVP® Nasal Spray . The proposed new indication for Noctiva™ is the treatment of adult patients (age not specified) with nocturia. The active pharmaceutical ingredient in both Noctiva™ and the reference listed drug (RLD) - DDAVP® Nasal Spray is desmopressin. Desmopressin is the synthetic peptide analogue of the human anti-diuretic hormone (ADH) vasopressin. The mechanism of action is based on selective V2 agonist activity stimulating reabsorption of water from the lumen of renal collecting ducts.

The reference listed drug, DDAVP® Nasal Spray (desmopressin acetate), was approved in 1978 (NDA 17922) for the treatment of central cranial diabetes insipidus, and for the management of temporary polyuria and polydipsia following head trauma or surgery in the pituitary region. DDAVP® Nasal Spray was also approved for nocturnal enuresis in children aged 6-17 years old, but this indication was revoked by the Agency in December 2007 due an association with hyponatremia. The sponsor's drug, Noctiva™, is a lower dose reformulation of the approved desmopressin acetate (0.75 and 1.5 µg per dose spray of 100 µL, compared to 10 µg per dose spray of 100 µL of DDAVP® Nasal Spray). The proposed dosing regimen of Noctiva™ is an intranasal administration of a 100 µL dose via a metered spray once daily approximately 30 minutes prior to bedtime. There are no major concerns regarding the use of the active ingredient (desmopressin) in this reformulation based on long history of safe use in humans.

However, the sponsor's reformulation with Noctiva™ contains an excipient not previously used by the nasal route, cyclopentadecanolide (CPD), also known as CPE-215 or pentadecalactone. CPD has been included in the sponsor's formulation to facilitate absorption of desmopressin through the nasal mucosa to result in higher bioavailability. Although CPD is used in another FDA-approved drug, it is via different route of administration (transdermal product Testim®). Noctiva™ is the first proposed use of CPD in an *intranasal* formulation. Moreover, non-sponsor conducted chronic nonclinical toxicology studies by (b) (4) using intranasal CPD (under IND (b) (4)) revealed histopathology lesions (squamous metaplasia in the nasal cavity) of concern. Hence, the nonclinical evaluation includes a focus on the safety assessment of CPD via the intranasal route of administration, for which the sponsor has obtained a letter of authorization (IND (b) (4)).

1.2 Brief Discussion of Nonclinical Findings

Based on the long history of clinical use of intranasal desmopressin, and a proposed clinical dose for Noctiva™ that is 6 to 13 times lower than the RLD (DDAVP® Nasal Spray), there are no specific concerns regarding the use of the active pharmaceutical ingredient, desmopressin, in Noctiva™.

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However, Noctiva™ contains an excipient not previously used by the nasal route, cyclopentadecanolide (CPD). Noctiva™ is the first proposed use of CPD in an intranasal formulation. Nonclinical safety evaluation is primarily focused on the safety profile of this novel excipient, CPD.

CPD-related findings in a 39-week chronic nonclinical toxicology study in the dog were limited to histopathology lesions in the nose. These included minimal to slight hyperplasia of the nasal epithelium and mixed cell inflammation. Both lesions are consistent with an irritant response, but it was not considered to be dose-limiting. Based on nasal surface area, doses of (b) (4) mg/dog translate to dose multiples of 970, 2889, and 5789 times the proposed maximum clinical dose of 1.5 µg (see Section 11 for dose multiple calculations).

There were no CPD-related findings in the 26-week chronic nonclinical toxicology study in the rat. Based on nasal surface area, doses of (b) (4) mg/rat translate to dose multiples of 458, 1525, 4574, 9136 times the proposed maximum clinical dose of 1.5 µg (see Section 11 for the dose multiple calculation).

A 28-day rat bridging toxicology study comparing Noctiva™ with the marketed desmopressin product (DDAVP® Nasal Spray) did not reveal any remarkable findings. Based on nasal surface area, the dose of 150 ng/rat translates to a dose multiple that is approximately equivalent to the proposed maximum 1.5 µg clinical dose.

All three toxicology studies included an emphasis on evaluation of nasal, oral, and pulmonary tissues based on concerns with non-sponsor conducted chronic nonclinical toxicology studies with CPD.

Although there has been concern for clinical hyponatremia with desmopressin products, potential imbalances in Na electrolyte impacting hyponatremia could not be evaluated since the 28-day bridging nonclinical toxicology study with Noctiva™ in the rat, conducted by the sponsor, did not include a measurement of electrolytes in the clinical chemistry panel.

Carcinogenicity studies with CPD were not conducted based on negative genetic toxicology data, limited systemic exposure, absence of accumulation based on nonclinical and clinical pharmacokinetic data, and negative histopathology data from the two chronic toxicology studies. A carcinogenicity waiver request was submitted by the sponsor. Following review of the toxicology studies, we concur that carcinogenicity studies are not required at this time.

There have been no long-term studies in animals to assess the impairment of fertility potential of Noctiva™ nasal spray.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical perspective, Noctiva™ appears to be reasonably safe for approval.

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1.3.2 Additional Non Clinical Recommendations

Based on lack of data regarding changes in Na electrolyte balances in the 28-day nonclinical bridging toxicology study in rats, close monitoring for hyponatremia is recommended.

1.3.3 Labeling

Sections 8 and 13 from the sponsor provided annotated label has been excerpted and tracked with the proposed changes as shown below.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk summary

(b) (4)

Data

(b) (4)

Animal Data

(b) (4)

8.2 Lactation

(b) (4)

(b) (4)

8.4 Pediatric Use

(b) (4)

8.5 Geriatric Use

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number
62357-86-2 (trihydrate)

Generic Name
Desmopressin acetate

Code Name
SER120

Chemical Name
1-(3-mercaptopropanoic acid)-8-d-arginine-vasopressin monoacetate
Trihydrate

Molecular Formula: (b) (4)
Molecular Mass: (b) (4)

Structure or Biochemical Description

Pharmacologic Class
Anti-diuretic Agent

2.2 Relevant INDs, NDAs, BLAs and DMFs

1) IND 76667
Product Name: Desmopressin (SER120)
Sponsor: Serenity Pharmaceuticals, LLC.
Indication: Adult Nocturia
Status: This is the corresponding IND for the sponsor's submitted NDA
Relevance: The three nonclinical toxicology studies were conducted under this IND.

2) NDA 17922
Product Name: DDAVP®
Sponsor: Ferring Pharmaceuticals, Inc.
Indication: Treatment of central cranial diabetes insipidus and for the management of temporary polyuria and polydipsia following head trauma or surgery in the pituitary region
Status: Active
Relevance: Reference Listed Drug (RLD)

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3) IND (b) (4)

(b) (4)

2.3 Drug Formulation

Table 1: Composition of Desmopressin Nasal Spray (SER120) – extracted from sponsor's report

Name of Ingredients/Quality	Composition (mg)		Function
	Strength: 7.5 µg/mL Per 100 µl Dose	Strength: 15 µg/mL Per 100 µl Dose	
Desmopressin acetate[a]/Manufacturer's specification	7.5 (b) (4)	15 (b) (4)	Active ingredient
Cyclopentadecanolide (CPD)/Manufacturer specification	(b) (4)		Permeation Enhancer
Cottonseed oil/NF			(b) (4)
Polysorbate 20/NF			
Sorbitan monolaurate/NF			
Citric acid, anhydrous/USP			
Sodium citrate Dihydrate/USP			
			(b) (4)
Water for Injection (WFI)/USP/EP			(b) (4)

[a] Composition refers to desmopressin free base; meets USP requirements

Reviewer Comment:

For nonclinical toxicology studies conducted with CPD (Section 6), concentrations of CPD included (b) (4) %. Based on the information from the sponsor, formulations with CPD concentrations above (b) (4) % do not share the same performance properties as described for lower concentrations. The sponsor states that "the formulation with (b) (4) % CPD appeared to be a suspension with particle sizes considerably larger than particle sizes in the (b) (4) % CPD formulations, and at refrigerated temperature formed precipitates that condensed into a solid". At room temperature, the (b) (4) % formulation appeared to remain a suspension and could be expressed through the metered dose nasal spray

pump. Hence, the storage conditions for CPD concentrations at (b) (4) % in the nonclinical toxicology studies were stored at room temperature (see Section 6).

2.4 Comments on Novel Excipients

All excipients in Noctiva™ formulation are listed in FDA's Inactive Ingredient Database and are present in other FDA-approved products with various routes of administration. However, three novel excipients (not present in formulations intended for intranasal administration) include CPD (b) (4), cottonseed oil (b) (4), and sorbitan monolaurate (b) (4).

The sponsor has conducted chronic nonclinical toxicology studies (see Section 6.2) in two species to demonstrate the safety of the above three ingredients via intranasal administration. Specifically, CPD has been evaluated as the test article, and a separate control group includes an emulsion with cottonseed oil and sorbitan monolaurate. There were no adverse effects with any of the above excipients from the toxicology studies in either species.

2.5 Comments on Impurities/Degradants of Concern

Concentration of individual impurities in drug substance (DS) is well below the limit of detection using a HPLC method. No peaks (HPLC method) were observed during forced degradation (acidic conditions) of the drug product in stability samples stored under long term or accelerated conditions. Contribution from residual solvents from any of the process components is well below the ICH limits. Refer to Quality Assessment Review for complete details.

2.6 Proposed Clinical Population and Dosing Regimen

Adults (age not specified) with nocturia (awakening 2 or more times to void).

The proposed dosing regimen of Noctiva™ is an intranasal administration of a 100 µL dose via a metered spray once daily approximately 30 minutes prior to bedtime.

Reviewer Comment:
The age of the patient population is not clearly specified. Refer to Clinical Review for complete details.

2.7 Regulatory Background

At the meeting with the sponsor in 2010 (July 30, 2010, Type B Meeting), the FDA recommended that a 1 month bridging study be conducted to evaluate and compare the local and systemic effects using a standard toxicology tissue battery for histopathology evaluation between SER120 product with the RLD (DDAVP® Nasal Spray). Olfactory pathway related neuroanatomical areas were specifically requested based on the intranasal route of administration.

It was confirmed in a written response dated June 12, 2012, that if systemic exposure of CPD could not be demonstrated, then chronic nonclinical toxicology studies would be required to support the safety of CPD in SER120.

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In light of the evidence of histopathology lesions of concern documented in chronic nonclinical toxicology studies conducted by (b) (4) FDA reiterated the need for chronic nonclinical toxicology studies with CPD focused on local effects in the nasal and respiratory tracts in a Type C meeting in March 2015 (see Meeting Minutes dated April 9th, 2014).

3 Studies Submitted

3.1 Studies Reviewed

Toxicology:
Study 8297078: A 39-Week Intranasal Chronic Toxicity Study in Dogs Evaluating Cyclopentadecalactone in Bland Emulsions For Nasal, Oral, and Pulmonary Effects with an 8-Week Recovery Phase

Study 8297079: A 26-Week Intranasal Chronic Toxicity Study in Rats Evaluating Cyclopentadecalactone in Bland Emulsions For Nasal, Oral, and Pulmonary Effects with a 4-Week Recovery Phase

Study 8279849: A 28-Day Intranasal Toxicity and Toxicokinetic Study in Rats Evaluating SER120 Nasal Spray Compared to Commercial Desmopressin Nasal Spray Formulation with a 4-Week Recovery Phase

Pharmacokinetics:
Study vca11189: Validation of an LC-MS/MS Method for the Determination of Desmopressin in Rat Plasma (K_3EDTA)

Study 030517: Method Development for Low Level CPD in Plasma

Study 031109: Bioanalytical Method Feasibility of LC-MS/MS Assays for the Determination of CPD and 15-OH-Pentadecanoic Acid in Rat Plasma and Investigation of Stability of CPD and 15-OH-Pentadecanoic Acid in Plasma

Study 031331: In Vitro Stability of Cyclopentadecanolide (CPD) and 15-Hydroxyl-pentadecanoic Acid in Rat and Human Whole Blood

Study 031522: Bioanalytical Method Development and Qualification of LC-MS/MS Assays for the Determination of CPD and 15-OH-Pentadecanoic Acid in Rat Blood and in Human Blood

3.2 Studies Not Reviewed

Additional non-sponsor conducted supporting nonclinical toxicology studies on CPD were submitted with this NDA along with letters of authorization from respective sponsors (b) (4)

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(b) (4). These studies were not reviewed in detail, since the sponsor-conducted chronic nonclinical toxicology studies with CPD in two species with appropriate control groups and variables (pH, dose volume and dosing frequency) relevant to Noctiva™'s formulation were considered sufficient to evaluate nonclinical safety.

3.3 Previous Reviews Referenced

Dr.Herman Rhee's nonclinical review for IND (b) (4) dated October 17, 2006 (DARRTS) – for genotoxicity and male fertility studies with CPD. The sponsor has obtained a letter of authorization to reference IND (b) (4).

4 Pharmacology

4.1 Primary Pharmacology

No nonclinical studies were submitted for Noctiva™.
The pharmacological activity of desmopressin is well understood. Desmopressin selectively binds to the vasopressin V_2 receptor which is expressed at high concentrations in the cells of the renal collecting ducts. By interacting with the V_2 receptor, desmopressin, like ADH, increases water permeability of the kidney allowing for the reabsorption of water into the body.

4.2 Secondary Pharmacology

A well-known side-effect of desmopressin is hyponatremia.
Desmopressin has minimal to no binding affinity for the V_1 receptors.
In light of the concern for clinical hyponatremia with desmopressin products, the sponsor-conducted 28-day bridging nonclinical toxicology study with Noctiva™ did not include a measurement of electrolytes in the clinical chemistry panel. Hence, potential imbalances in Na electrolyte impacting hyponatremia could not be evaluated.

4.3 Safety Pharmacology

No safety pharmacology studies were submitted for Noctiva™.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Reviewer Comment:
No specific nonclinical studies were submitted to this NDA to demonstrate increased bioavailability of desmopressin with Noctiva™ compared to the RLD (DDAVP® Nasal Spray).

Sponsor-submitted pharmacokinetic nonclinical studies are summarized below:

Desmopressin:
Study vca11189: Validation of an LC-MS/MS Method for the Determination of Desmopressin in Rat Plasma (K_3EDTA)
Summary: This bioanalytical method showed an acceptable bioanalytical performance for the determination of desmopressin in rat plasma (K_3EDTA) over the

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calibration range of 4.00 to 250 pg/mL using an assay volume of 500 µL.

CPD:
Study 030517: Method Development for Low Level CPD in Plasma
Summary: In this study, an attempt was made to develop a gas chromatography-mass spectrometry (GC-MS) method for the analysis of the low level cyclopentadecanolide (CPD) in rat plasma with the ranges of (b) (4) µg/mL in plasma. Although GC-MS conditions to analyze CPD and internal standard at the limit of quantitation (LOQ) of (b) (4) ng/mL in a neat solvent was successful, extraction and analysis of the CPD from rat plasma was unsuccessful.

Study 031109: Bioanalytical Method Feasibility of LC-MS/MS Assays for the Determination of CPD and 15-OH-Pentadecanoic Acid in Rat Plasma and Investigation of Stability of CPD and 15-OH-Pentadecanoic Acid in Plasma
Summary: In this study, LC-MS/MS analysis conditions, as well as stability of cyclopentadecanolide (CPD) and its hydrolysis product, 15-OH-pentadecanoic acid were investigated in the development of bioanalytical assays for in rat plasma. Results indicated that:

1) The quantitation range for CPD with NaF treated plasma was linear only between (b) (4) ng/mL. CPD was unstable in untreated rat plasma since it was rapidly hydrolyzed by esterases. Recommendations for improving sensitivity and linearity included the use of NaF/ascorbic acid to better inhibit esterase hydrolysis of CPD and modification of chromatography conditions to reduce coelution of materials causing suppression.
2) The single product of esterase hydrolysis of CPD in rat plasma was 15-OH-Pentadecanoic acid (15-OH-PDA) that could serve as a potential surrogate for CPD absorption. The quantitation range for 15-OH-PDA was linear from (b) (4) ng/mL with no apparent issues that would prevent validation. 15-OH-PDA was stable in untreated rat plasma for up to 1 hour at 37 °C.

Study 031331: In Vitro Stability of Cyclopentadecanolide (CPD) and 15-Hydroxyl-pentadecanoic Acid in Rat and Human Whole Blood
Summary: In this study, the stability of cyclopentadecanolide (CPD) and 15-OH-pentadecanoic acid incubated in fresh rat and human whole blood was evaluated. whole blood at 37°C. 15-OH-pentadecanoic acid is stable in rat and human
Results showed that CPD is unstable in rat whole blood and marginally stable in human whole blood.

Study 031522: Bioanalytical Method Development and Qualification of LC-MS/MS Assays for the Determination of CPD and 15-OH-Pentadecanoic Acid in Rat Blood and in Human Blood
Summary: The objective of this study was to develop and qualify bioanalytical methods for the analysis of CPD and its hydrolysis product 15-OH-pentadecanoic acid in rat blood. The biological matrix for assay was changed from blood or plasma to acetonitrile precipitated blood (APB) to stop esterase hydrolysis of CPD present at blood sample collection, and to eliminate LC-MS/MS ionization suppression. The method for CPD in

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acetonitrile precipitated blood was linear from (b) (4) ng/mL (equivalent to (b) (4) ng/mL in blood).
The method for 15-OH-pentadecanoic acid was changed from that reported for Study 031109 to one based on (b) (4) in the negative ionization mode to improve sensitivity. Two quantitation ranges were evaluated to help compensate for the narrow linear dynamic range. The low quantitation range was linear from (b) (4) ng/mL (equivalent to (b) (4) ng/mL in blood), and the high quantitation range was linear from (b) (4) ng/mL (equivalent to (b) (4) ng/mL in blood). Stability in matrix was not of concern as 15-OH-PDA was the hydrolysis product of CPD.
During the course of this work, examination of control rat and control human plasma for the presence of 15-hydroxyl-pentadecanoic acid as an endogenous component was added to the objective. Analysis of control rat and human plasma for endogenous 15-OH-PDA in did not show any 15-OH-PDA greater than the response for (b) (4) ng/mL in plasma.

6 General Toxicology

All toxicology studies reviewed in this section focus on the toxicity of the novel excipient (not previously used by the nasal route) in Noctiva™, namely, CPD.

6.1 Single-Dose Toxicity

None submitted

6.2 Repeat-Dose Toxicity

Study title: A 28-Day Intranasal Toxicity and Toxicokinetic Study in Rats Evaluating SER120 Nasal Spray Compared to Commercial Desmopressin Nasal Spray Formulation with a 4-Week Recovery Phase

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Study no.:	(b) (4) Study Number 8279849 Sponsor Reference Number SPC-SER120-Tox-201301
Study report location:	NDA 201656 SDN 1 Module 4.2.3.2 February 4, 2016
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 5, 2013
GLP compliance:	Yes FDA GLP Title 21 CFR Part 58 OECD GLP ENV/MC/CHEM(98)17
QA statement:	Yes
Drug, lot #, and % purity:	SER120 Lot No. 008007; Purity 105% Commercial desmopressin nasal spray (comparator arm) Batch No. JKL2390A Cyclopentadecalactone (placebo control) Lot No. 008005 (b) (4) %); Purity 98%

Reviewer Comment:
The Lot No. for SER120 in the (b) (4) Study Report is noted as 008007 whereas the Lot No. for the same in the Toxicology Tabulated Summary (Module 2.6.7) is noted as 00807. This was confirmed to be a typographical error based on communication with the sponsor dated August 12, 2016. The lot number used in Study number 8279849 was 008007.

Key Study Findings

Given the intranasal route of administration, the study design included special evaluation of nasal turbinates and neuroanatomical areas specific to olfactory pathways in the brain.

No remarkable findings were noted in this study based on a dose multiple (approximately 1.14 times) equivalent to the proposed clinical dose. Dose multiples are calculated based on interspecies dose comparisons of nasal cavity surface areas (see Section 11).

Although there has been concern for clinical hyponatremia with desmopressin products, the sponsor-conducted 28-day nonclinical toxicology study with Noctiva™ did not include a measurement of electrolytes in the clinical chemistry panel. Hence, potential changes in Na electrolyte balances impacting hyponatremia could not be evaluated.

Methods

Doses:	SER120: 150 ng/dose Desmopressin: 1000 ng/dose
Frequency of dosing:	Once daily
Route of administration:	Intranasal instillation
Dose volume:	10 µL
Formulation/Vehicle:	Placebo control (CPD (b) (4) % at pH 5.5±0.5) Saline control (0.9% sodium chloride)
Species/Strain:	Crl:CD(SD) Rats
Number/Sex/Group:	See experimental study design table below
Age:	7-8 weeks old
Weight:	195-297 g for males 173-243 g for females
Satellite groups:	Toxicokinetics
Unique study design:	No
Deviation from study protocol:	Cited deviations are included. None impacted overall study results or data interpretation

Table 2: Experimental Study Design (excerpted from the sponsor's submission)

Group	Subgroup[a]	No. of Animals[b] Male	No. of Animals[b] Female	Desmopressin Dose Level (ng/dose)	Dose Volume (µL/dose)
1 (Placebo Control)[c]	1 (Toxicity)	15	15	0	10
	2 (Toxicokinetic)	20	20	0	10
2 (Saline Control)[d]	1 (Toxicity)	15	15	0	10
	2 (Toxicokinetic)	3	3	0	10
3 (Untreated Control)[e]	1 (Toxicity)	15	15	NA	NA
4 (Desmopressin)	1 (Toxicity)	15	15	1000	10
	2 (Toxicokinetic)	20	20	1000	10
5 (SER120)	1 (Toxicity)	15	15	150	10
	2 (Toxicokinetic)	20	20	150	10

NA = Not applicable.

a For Groups 1, 4, and 5, toxicokinetic subgroups (Subgroup 2) included two extra animals/sex as possible replacement animals.

b Toxicity animals designated for recovery sacrifice (five animals/sex/group) underwent at least 4 weeks of recovery following dose administration. Group 3 animals followed the same schedule as the other groups.

c Group 1 received placebo control article (cyclopentadecalactone (b) (4) %) only.

d Group 2 received saline control article (sterile saline) only.

e Group 3 was not dosed.

Reviewer Comments:

Dose: The doses used in this nonclinical study was just equivalent to the proposed maximum clinical dose (1.5 mcg) where the dose multiple was calculated based on nasal surface area (see Section 11).

Dose Volume: The dose volume of 10 µL used in this nonclinical study was close to the volume of drug administered (13 µL) to obtain the same volume per surface area as in man (Gizurarson, 1990).

Drug Delivery Device: The dose was administered by using an adjustable micropipette, and a new pipette tip was used for each animal.

Dose Administration: Animals were held upright for a period of time (approximately 15 seconds (Day 1 of the dosing phase) or approximately 5 seconds (Days 2 through 15 of the dosing phase).

Note: *Protocol deviation was noted from Day 1-15 of the dosing phase where holding each animal upright following dose administration was not documented.*

Observations and Results

Mortality

All animals survived to the respectively scheduled terminal and recovery sacrifice.

Clinical Signs
Clinical signs noted were non-specific and/or sporadic changes not considered to be adverse or related to the administration of CPD.

Body Weights
No differences in body weight or body weight change were noted among the treatment groups during the dosing or recovery phase.

Feed Consumption
No differences in food consumption were noted among the treatment groups during the dosing or recovery phase.

Ophthalmoscopy

Vitreal hemorrhage in one male given the comparator drug, desmopressin (Group 4) was considered an unrelated

ECG

Not conducted.

Hematology

No SER120-related changes were present on Day 29 of the dosing phase or Day 29 of the recovery phase in hematology and coagulation test results of animals given 150 ng/dose compared with placebo, saline, or untreated controls and desmopressin-treated animals.

Clinical Chemistry

No SER120-related changes were present on Day 29 of the dosing phase or Day 29 of the recovery phase in clinical chemistry test results of animals given 150 ng/dose compared with placebo, saline, or untreated controls and desmopressin-treated animals.

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Urinalysis

No SER120-related changes were present on Day 29 of the dosing phase or Day 29 of the recovery phase in clinical chemistry test results of animals given 150 ng/dose compared with placebo, saline, or untreated controls and desmopressin-treated animals.

Gross Pathology

No macroscopic observations were attributed to administration of SER120 (150 ng desmopressin/dose) or desmopressin (1000 ng/dose).

Organ Weights

No organ weight differences were attributed to administration of SER120 (150 ng desmopressin/dose) or desmopressin (1000 ng/dose) at the terminal or recovery sacrifice.

Histopathology

Adequate Battery: Yes.
Protocol specified brain structures included olfactory bulbs, piriform cortex, amygdala, and entorhinal cortex.

Peer Review: No

Histological Findings
No microscopic observations were attributed to administration of SER120 (150 ng desmopressin/dose) or desmopressin (1000 ng/dose). In particular, no findings were noted in the nasal turbinates (site of administration) or protocol-specified brain structures involved in olfaction (olfactory bulbs, piriform cortex, amygdala, and entorhinal cortex).

Toxicokinetics

Samples from Group 1 (Placebo control containing (b) (4) % CPD at pH 5.5±0.5) were analyzed for CPD and its metabolite (15-hydroxyl-pentadecanoic acid - 15-OH-PDA) using liquid chromatography-tandem mass spectrometry (LC/MS-MS) assays. Neither CPD nor 15-OH- PDA was observed with a response sufficiently above the control plasma response from which to calculate a concentration or to make estimates of analyte concentrations. As a result, toxicokinetic values could not be calculated. Samples from Group 2, 4, and 5 were analyzed for systemic desmopressin content using a validated liquid chromatography/mass spectrometry assay. The analyses revealed very low concentrations of desmopressin in sporadic animals from Groups 4 and 5. In animals treated with commercial desmopressin (Group 4), a total of 9 of 36 samples had desmopressin ranging between 4.10 to13.1 pg/mL. In animals treated with SER120, a total of 3 of 36 samples had desmopressin ranging between 4.23 to 6.77 pg/mL. Desmopressin was not detected in Group 2 animals. Given the intermittent nature of the systemic levels, toxicokinetic values could not be calculated.
Group 3 was an untreated control group.

Reference ID: 3996414

Dosing Solution Analysis

All formulations were administered as provided by the sponsor or acquired commercially. Dose analysis was not conducted.

Study title: A 39-Week Intranasal Chronic Toxicity Study in Dogs Evaluating Cyclopentadecalactone in Bland Emulsions For Nasal, Oral, and Pulmonary Effects with an 8-Week Recovery Phase

Study no.:	(b) (4) Study Number 8297078 Sponsor Reference Number SPC-CPD-Tox-201401
Study report location:	NDA 201656 SDN 1 Module 4.2.3.2 February 4, 2016
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 12, 2014
GLP compliance:	Yes FDA GLP Title 21 CFR Part 58 OECD GLP ENV/MC/CHEM(98)17
QA statement:	Yes
Drug, lot #, and % purity:	Vehicle in the sponsor's formulation is the test article for this study. Cyclopentadecalactone (b) (4) % Lot # 400222; Purity 104% Cyclopentadecalactone (b) (4) % Lot # 400626; Purity 104%

Key Study Findings

The purpose of this study was to evaluate potential nasal, oral, and pulmonary effects of cyclopentadecalactone (CPD) in comparison with those of saline and a cottonseed oil-containing emulsion when administered daily via intranasal spray to dogs for at least 39 weeks, and to assess the reversibility of any CPD-induced effects after a recovery of at least 8 weeks.

Based on nasal surface area, doses of (b) (4) mg/animal translate to dose multiples of 970, 2889, and 5789 times the proposed maximum clinical dose of 1.5 µg (see Section 11).

CPD-related findings were limited to histopathology in the nose. These included minimal to slight hyperplasia of the nasal epithelium and mixed cell inflammation consistent with an irritant response, and therefore not considered to be dose-limiting.
No CPD-related adverse effects were noted in animals treated with the emulsion control.

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Methods:

Doses:	(b) (4) mg/animal ((b) (4)
	See Reviewer Comments on Dose, Dose Volume, and Drug Delivery device below
Frequency of dosing:	Once daily
Route of administration:	Intranasal Spray
Dose volume:	100 µL in each nostril/day– Groups 1, 4, and 6 100 µL in a single nostril/day (alternate nostril per dose when possible) – Groups 2 and 3
Formulation/Vehicle:	Group 1 – Emulsion Control [cottonseed oil, polysorbate, and sorbitan in aqueous sodium citrate buffer at pH 5.6 (concentrations of each component equivalent to final concentrations in the CPD containing formulations)] Group 6 – Saline Control [0.9% Sodium Chloride for Injection, USP (sterile saline) without preservatives]
Species/Strain:	Purebred Beagle Dogs
Number/Sex/Group:	6/sex – Groups 1, 4, and 6 4/sex – Groups 2, 3, and 5
Age:	8-10 months old
Weight:	Males: 7.6-11 kg Females: 5.8-8.9 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Cited deviations are included. None impacted overall study results or data interpretation

Table 3: Experimental Study Design (excerpted from the sponsor's submission)

Group	No. of Animals[a]		CPD Dose Level (mg/animal)	CPD Concentration (mg/mL)
	Male	Female		
1 (Emulsion Control)[b]	6	6		(b) (4)
2 (Low CPD)[c]	4	4		
3 (Mid CPD)[c]	4	4		
4 (High CPD)[b]	6	6		
5 (Untreated Control)[d]	4	4		
6 (Saline Control)[b]	6	6		

CPD = Cyclopentadecalactone; NA = Not applicable.

a Animals designated for the recovery sacrifice (two animals/sex/group in Groups 1, 4, and 6) underwent 8 weeks of recovery following the dosing phase.

b Animals in Groups 1, 4, and 6 were dosed at a fixed dose volume of 100 µL/nostril in both nostrils once daily.

c Animals in Groups 2 and 3 were dosed at a fixed volume of 100 µL in one nostril once/day

d Group 5 was not dosed.

Reviewer Comments:
Dose: The (b) (4) % CPD formulations were stored at room temperature.
The (b) (4) % CPD formulations were stored in a refrigerator, set to maintain 2 to 8°C, until removed for dosing, and were used within 4 hours of removal from refrigerated storage. See Reviewer Comment under Section 2.3 for an explanation on differences in storage conditions for (b) (4) % CPD.

Dose Volume: The dose volume used in this nonclinical study reached the maximum dose volume for the dog of 100 µL per nostril in the dog (Gad et al, 2006). The dose volume in Groups 1, 4, and 6 is equivalent to the volume of drug (Group 4) administered to obtain the same volume per surface area as in man (Gizurarson, 1990).

Drug Delivery Device: As stated in the Nonclinical Overview (Module 2.6.1), the dose actuating device used in this nonclinical study for test article delivery via intranasal administration was confirmed to be the clinical delivery device, namely, the (b) (4) Nasal Spray Pump the clinical delivery device). Each device delivered 100 µL of spray/actuation cycle.

Dose Administration: For each nostril dosed, the head of the animal was held downward during dosing and for at least 30 seconds postdose (Day 1 through 3). For all other days of dosing, for each nostril dosed, the head of the animal was held downwards during dosing and was then released from restraint, and the animal was observed for at least 15 seconds postdose.

Results:

Mortality

All animals survived to the respective scheduled terminal and recovery sacrifice.

Clinical Signs

Clinical signs noted were non-specific and/or sporadic changes not considered to be adverse or related to the administration of CPD.

Body Weights

Statistical differences for treated groups was limited to changes in the high dose males (Group 4) treated with CPD ((b) (4) mg/animal) compared to the saline control group (Group 6).This is considered non-adverse based the marginally lower weights of the saline control groups relative to other groups, and on the unremarkable differences between the high dose males and the emulsion control group (Group 1). All other statistical differences were limited to changes between the control groups (Group 1, 5, and 6).

Feed Consumption

Mean food consumption of animals given ≤(b) (4) mg/animal CPD were similar to those of emulsion control during the dosing and recovery phases.

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Ophthalmoscopy

Abnormalities were limited to a single incidence of epiphora (watery eye) in one saline control female and one high dose female. These findings were considered incidental and not related to the administration of CPD.

Hematology and Coagulation

No CPD-related hematology and coagulation effects were present during the dosing or recovery phase in animals given CPD compared with those given emulsion or saline control.

Clinical Chemistry

No CPD-related clinical chemistry effects were present during the dosing or recovery phase in animals given CPD compared with those given emulsion or saline control.

Urinalysis

No CPD-related urinalysis effects were present during the dosing or recovery phase in animals given CPD compared with those given emulsion or saline control.

Toxicokinetics

Plasma analyses revealed sporadic, low levels of desmopressin in the animals treated with desmopressin or SER120. Neither CPD nor 15-OH- PDA (metabolite of CPD) was observed with a response sufficiently above the control plasma response from which to calculate a concentration or to make estimates of analyte concentrations. As a result, no toxicokinetic parameters could be calculated from these data for any analyte.

Organ Weights

No CPD-related organ weight effects were noted at the terminal or recovery sacrifice.

Gross Pathology

No CPD-related macroscopic findings were noted at the terminal or recovery sacrifice.

Histopathology

Adequate Battery

Histopathology was limited to nasal, oral, and pulmonary tissues. The focus on select tissues was based on concurrence with FDA (Type C Meeting Minutes dated March 9, 2014 under IND 76667).

At the terminal sacrifice, the following tissues were processed to histologic slides and examined: the larynx (three sections), lung with large bronchi (one cross-section from each of the four major lobes), nares, nasal turbinates (including nasal sinuses), nasopharynx, tongue, trachea, and macroscopic lesions.

At the recovery sacrifice, only the nasal turbinates of females were examined microscopically (since CPD-related microscopic findings were limited to the nasal turbinates of terminal sacrifice females).

For chronic systemic toxicity evaluation, the full battery of routine tissues for

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histopathology was agreed to be unnecessary based on the sponsor's right of reference for IND (b) (4) (which includes chronic rat and dog studies with complete histopathology).

Peer Review
Yes

Histological Findings
CPD related findings were limited to two rostral nasal sections. Histopathological lesions included minimal to slight hyperplasia of the transitional epithelium (three high dose females; Animal Nos. H05917, H05919, and H05921), and minimal hyperplasia of the squamous epithelium (one high dose female; Animal No. H05921) in the most rostral (first) section. These were characterized by increased epithelial thickness and number of cellular layers. Inflammatory infiltrates were often collocated with areas of hyperplastic epithelium. Although foci of minimal mixed cell or neutrophilic inflammation were present in the rostral nasal turbinates of many animals, including one or more of the control groups, as a background finding, slight nasal turbinate mixed cell inflammation was noted in one high dose female (Animal No. H05919) given 12 mg/animal and was collocated with hyperplastic transitional epithelium; therefore, the increased severity was considered CPD-related. Slight mucus cell hyperplasia (two high dose females; Animal Nos. H05919 and H05921) was characterized by a more diffuse increase in the apparent number of mucus (goblet) cells in the mucociliary respiratory epithelium, within the most rostral (first) section and particularly in the ventral aspect of the second section, and was not restricted to foci of inflammation.

Table 4: Incidence and Severity of Test Article-Related Microscopic Findings in Nasal Turbinate – Terminal Sacrifice (excerpted from the sponsor's report)

	CPD											
Sex	Males						Females					
Dose Level (mg/animal)	(b) (4)											
Number Examined	4	4	4	4	4	4	4	4	4	4	4	4
Nasal Turbinates												
Hyperplasia, transitional epithelium												
Minimal	0	0	0	0	0	0	0	0	0	1	0	0
Slight	0	0	0	0	0	0	0	0	0	2	0	0
Hyperplasia, squamous epithelium												
Minimal	0	0	0	0	0	0	0	0	0	1	0	0
Hyperplasia, mucus cell												
Slight	0	0	0	0	0	0	0	0	0	2	0	0
Inflammation, mixed cell												
Minimal	0	1	1	1	1	1	0	2	1	1	0	1
Slight	0	0	0	0	0	0	0	0	0	1	0	0

CPD – Cyclopentadecalactone; NA – Not applicable.
a Emulsion control.
b Undosed control.
c Saline control.

At the recovery sacrifice, in females given (b) (4) mg/animal complete resolution was exhibited from CPD-related nasal turbinate microscopic findings present in the terminal sacrifice females at this dose.

Reviewer Concurrence with sponsor's conclusion:

The most rostral (first) nasal section is normally lined by squamous, transitional, and mucociliary respiratory epithelium, whereas the second section is predominantly lined by mucociliary respiratory epithelium. Nasal responses to inhaled irritants have been well studied in rats, monkeys, and mice. In all of these species, commonly reported findings after irritant exposure are transitional epithelial hyperplasia, mucus cell hyperplasia, and variable acute inflammation, with a lower incidence and severity of squamous epithelial hyperplasia. These findings characteristically exhibit a distinct anterior-posterior gradient (Harkema, 1990; 2006; Buckley, et al., 1984).

Based on the similarity between nasal findings commonly reported after exposure to irritants in the published literature and findings observed in the most rostral sections of the nasal cavity in this study, all CPD-related nasal turbinate findings were interpreted as irritant responses. None of these findings were considered dose-limiting, based on the limited severity and absence of correlative clinical observations. No CPD-related findings were present in the nares, the two most caudal sections of nasal turbinates, the nasal sinuses, nasopharynx, tongue, larynx, trachea, or lungs.

Dosing Solution Analysis

All formulations (test article and emulsion control) were provided as ready-to-use formulations in dose-actuating devices by the sponsor; or acquired commercially (saline control) and filled in dose-actuating devices provided by the sponsor. Dose analysis was not conducted.

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Study title: A 26-Week Intranasal Chronic Toxicity Study in Rats Evaluating Cyclopentadecalactone in Bland Emulsions For Nasal, Oral, Pulmonary Effects with a 4-Week Recovery Phase

Study no.:	[redacted] (b) (4) Study Number 8297079 Sponsor Reference Number SPC-CPD-Tox-201402
Study report location:	NDA 201656 SDN 1 Module 4.2.3.2 February 4, 2016
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	July 22, 2014
GLP compliance:	Yes FDA GLP Title 21 CFR Part 58 OECD GLP ENV/MC/CHEM(98)17
QA statement:	Yes
Drug, lot #, and % purity:	Vehicle in the sponsor's formulation is the test article for this study. Cyclopentadecalactone [redacted] (b) (4) % Lot # 400222; Purity 104% Cyclopentadecalactone [redacted] (b) (4) % Lot # 400626; Purity 104%

Key Study Findings

The purpose of this study was to evaluate potential nasal, oral, and pulmonary effects of cyclopentadecalactone (CPD) in comparison with those of saline and a cottonseed oil-containing emulsion when administered daily via intranasal instillation in rats for at least 26 weeks, and to assess the reversibility of any CPD-induced effects after a recovery of at least 4 weeks.

Based on nasal surface area, doses of [redacted] (b) (4) mg/animal translate to dose multiples of 458, 1525, 4574, 9136 times the maximum proposed clinical dose of 1.5 µg (see Section 11).

There were no remarkable findings in CPD or emulsion treated animals.

Methods:

Doses:	(b) (4) mg/animal (b) (4) mg/cm^2)
Frequency of dosing:	Daily
Route of administration:	Intranasal Instillation using a plastic-tipped pipet
Dose volume:	10 µL per nostril
Formulation/Vehicle:	Group 1 (Emulsion Control – cottonseed oil, polysorbate, and sorbitan in aqueous sodium citrate buffer at pH 5.5). Concentrations of each component are equivalent to final concentrations in the CPD containing formulations)] Group 6 – Saline [0.9% Sodium Chloride for Injection, USP (sterile saline) without preservatives]
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	10/sex/group (Recovery 5/sex/group)
Age:	8-9 weeks
Weight:	253 to 346 g for males and 181 to 272 g for females
Satellite groups:	Recovery Period for Groups 1, 4, and 6
Unique study design:	Study was conducted in two Phases. Concentrations in Phase II were higher than in Phase I
Deviation from study protocol:	Cited deviations are included. None impacted overall study results or data interpretation

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Table 5: Experimental Study Design (excerpted from the sponsor's submission)

Group	No. of Animals[a] Male	Female	CPD Dose Level (mg/animal)	CPD Concentration (mg/mL)
Phase I[b]				
1 (Emulsion Control)[c]	15	15		
2 (Low CPD)	10	10		
3 (Mid CPD)	10	10		
4 (High CPD)	15	15		
5 (Untreated Control)[e]	10	10		
6 (Saline Control)[f]	15	15		
Phase II[d]				
1 (Emulsion Control)[c]	15	15		
2 (Low CPD)	10	10		
3 (Mid CPD)	10	10		
4 (High CPD)	15	15		
5 (Untreated Control)[e]	10	10		
6 (Saline Control)[f]	15	15		

(b) (4)

CPD = Cyclopentadecalactone; NA = Not applicable.

a Animals designated for the recovery euthanasia (up to five animals/sex/group in Groups 1, 4, and 6) underwent 4 weeks of recovery following the dosing phase (Phase I and Phase II).

b For Days 1 through 35 of the dosing phase (Phase I), doses (10 µL/nostril) were administered to Groups 1, 2, 3, 4, and 6 in one nostril per day (alternating each day). Animals were placed on a dosing holiday for Days 36 through 42 of the dosing phase (Phase I).

c Group 1 received emulsion control only.

d Beginning on Study Day 43 (Day 1 of the dosing phase for Phase II), doses for Groups 2 and 3 were administered to the left nostril each day, and doses for Groups 1, 4, and 6 were administered as 10 µL to each nostril (total dose volume of 20 µL/animal).

e Group 5 was not dosed (untreated control is synonymous with undosed control).

f Group 6 received saline control only.

Reviewer Comments:

Dose: The (b) (4) % CPD formulations were stored at room temperature.
The (b) (4) % CPD formulations were stored in a refrigerator, set to maintain 2 to 8°C, until removed for dosing, and were used within 4 hours of removal from refrigerated storage.
See Reviewer Comment under Section 2.3 for an explanation on differences in storage conditions for (b) (4) % CPD.

Dose Volume: The dose volume of 10 µL used in this nonclinical study was close to the volume of drug administered (13 µL) to obtain the same volume per surface area as in man (Gizurarson, 1990).

Drug Delivery Device: The dose was administered by intranasal instillation using an adjustable plastic-tipped micropipette.

Dose Administration: The animal was held in an upright position resulting in the head tilted back during the administration, and the animal remained in that position for approximately five seconds after the completion of dosing.
Care was taken to avoid potential tissue damage resulting from contact of intranasal tissues with the plastic-tipped micropipette tip.

Observations and Results

Mortality

One Group 4 female (Phase II) given (b) (4) mg/animal (high dose CPD) was found dead shortly after dosing on Day 170. Based on histopathology findings [slight acute hemorrhage and minimal pigment (hemosiderin suggestive of past hemorrhage)] present in the aortic adventitia at the heart base, and timing of death (after dosing procedure), the cause of death was attributed to physical handling procedure.

Clinical Signs

No CPD related adverse events were noted. Clinical observations were similar between treated and all control groups.

Body Weights

No persistent differences in body weight or body weight change were attributed to CPD when compared to the emulsion, saline, or undosed controls, respectively.

Feed Consumption

No differences in food consumption were attributed to CPD when compared to the emulsion, saline, or undosed controls, respectively.

Ophthalmoscopy

No ophthalmic abnormalities were attributed to CPD at ≤(b) (4) mg/dose.

ECG

Not done

Hematology

No CPD-related hematology and coagulation effects were present during the dosing or recovery phase compared with animals given emulsion control or saline control or during the dosing phase compared with the untreated controls.

Clinical Chemistry

No CPD-related clinical chemistry effects were present during the dosing or recovery phase compared with animals given emulsion control or saline control or during the dosing phase compared with the untreated controls.

Urinalysis

No CPD-related urinalysis effects were present during the dosing or recovery phase compared with animals given emulsion control or saline control or during the dosing phase compared with the untreated controls.

Gross Pathology

No CPD-related macroscopic findings were noted at the terminal or recovery sacrifice.

Organ Weights

No CPD-related organ weight effects were noted at the terminal or recovery sacrifice.

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Histopathology

Adequate Battery
Yes

The focus on select tissues (larynx, lung, nasal turbinates) was based on concurrence with FDA (Type C Meeting Minutes dated March 9, 2014 under IND 76667). However, unlike the 39-week dog study reviewed above, histopathology included complete evaluation of the routine battery of tissues at terminal sacrifice. Evaluation of tissues following recovery sacrifice was contingent on findings at the terminal sacrifice.

For larynx, three step sections were prepared. For the lung with large bronchi, one cross-section was prepared from each of the four major lobes. For nasal turbinates, four sections were prepared. Nasal turbinates and larynx were processed to slide according to study-specific procedures.

Peer Review
Yes

Histological Findings
No CPD-related microscopic findings were present at the terminal sacrifice. Accordingly, tissues were not examined from recovery sacrifice animals.

Toxicokinetics

Not conducted

Dosing Solution Analysis

Test article and emulsion control formulations were provided by the sponsor, and dispensed for dosing at least monthly by (b) (4) according to the mixing procedure. Each bottle was dispensed for use on a single day according to the dispensing procedure, as directed by the Protocol. Dose analysis was not conducted.

7 Genetic Toxicology

Desmopressin: No genetic toxicity studies have been performed.

CPD: Genetic toxicity studies were conducted by (b) (4), and reviewed under IND (b) (4) by Dr.Herman Rhee (dated October 17, 2006) and for which the sponsor has obtained a letter of authorization to reference. These studies included the mouse lymphoma forward mutation assay, an in vivo mouse micronucleus assay, and the Salmonella-E.Coli/Mammalian-Microsome Reverse Mutation Assay (Ames Assay). All tests were negative for genetic toxicity.

8 Carcinogenicity

Desmopressin:

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There have been no long-term studies in animals to assess the carcinogenic or mutagenic potential of Noctiva™ nasal spray.

CPD:
Carcinogenicity studies with CPD were not conducted based on negative genetic toxicology data, limited systemic exposure, absence of accumulation based on nonclinical and clinical pharmacokinetic data, and negative histopathology data from the two chronic nonclinical toxicology studies. A carcinogenicity waiver request was submitted by the sponsor under IND 76667. Following review of the toxicology studies, the Agency concurs that carcinogenicity studies are not required at this time.

9 Reproductive and Developmental Toxicology

Desmopressin:
There have been no long-term studies in animals to assess the impairment of fertility potential of Noctiva™ nasal spray.

CPD:
Male fertility tests in rats were conducted and reviewed under IND (b) (4) by Dr. Herman Rhee (dated October 17, 2006). No remarkable CPD-related effects were noted in sperm evaluation (cauda weight, sperm motility, progressive motility, and velocity), reproductive organ weights (epididymides, prostate, seminal vesicles and testes), and pregnancy performance.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

This 505(b)(2) application submitted by Serenity Pharmaceuticals, LLC., seeks to market their drug product SER120 containing desmopressin as Noctiva™. Noctiva™ is a low dose intranasal reformulation of an FDA approved desmopressin drug product, namely, DDAVP® Nasal Spray. The proposed new clinical indication for the use of Noctiva™ is the treatment of adult patients (age not specified) with nocturia. The active ingredient in both Noctiva™ and the reference listed drug (RLD), namely, DDAVP® Nasal Spray, is desmopressin acetate.

Desmopressin is the synthetic peptide analogue of the human anti-diuretic hormone (ADH) vasopressin. The mechanism of action is based on selective V2 agonist activity stimulating reabsorption of water from the lumen of renal collecting ducts.

DDAVP® Nasal Spray, was approved in 1978 (NDA 17922) for the treatment of central cranial diabetes insipidus, and for the management of temporary polyuria and polydipsia following head trauma or surgery in the pituitary region. DDAVP® Nasal Spray was also approved for nocturnal enuresis in children aged 6-17 years old, but this indication was revoked by the Agency in December 2007 due an association with hyponatremia.

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DDAVP® Solution/Injection was later also approved for the treatment of hemophilia A and Von Willebrand's disease in 1984 (NDA 18938). Other FDA approved desmopressin products include Stimate® (NDA 020355 approved in 1994) for the treatment of Hemophilia A and Von Willebrand's disease; Concentraid (NDA 019776 approved in 1990) for the renal concentration capacity test, and Minirin® (NDA 021795 approved in 2008) for central diabetes insipidus, primary nocturnal enuresis, and renal concentration capacity test in adults. The indication for primary nocturnal enuresis was removed by the Agency in 2013 due to an association with hyponatremia.

The sponsor's drug, Noctiva™, is a relatively lower dose (0.75 and 1.5 µg per dose spray of 100 µL, compared to 10 µg per dose spray of 100 µL of DDAVP® Nasal Spray) reformulation of desmopressin acetate. The proposed dosing regimen of Noctiva™ is an intranasal administration of a 100 µL dose via a metered spray once daily approximately 30 minutes prior to bedtime. There are there are no major concerns regarding the use of the active ingredient (desmopressin) in this reformulation based on the proposed dose that is approximately 6 to 13 times lower than the clinical dose of the RLD, and the long history of safe use in humans with multiple FDA approved marketed products.

However, the sponsor's reformulation with Noctiva™ contains an excipient, CPD, not previously used in products administered by the nasal route. CPD is also known as CPE-215 or pentadecalactone. CPD has been included in the sponsor's formulation to facilitate absorption of desmopressin through the nasal mucosa to result in higher bioavailability. However, no specific nonclinical studies were submitted to this NDA to demonstrate increased bioavailability of desmopressin with Noctiva™ compared to the RLD (DDAVP® Nasal Spray).

Although CPD is used in other formulations and via different routes of administration (for example, CPD is an inactive ingredient in a transdermal product Testim®), Noctiva™ is the first proposed use of CPD in an *intranasal* formulation. Moreover, regulatory background includes concerns based on histopathological findings (squamous metaplasia in the nasal cavity) in chronic nonclinical toxicology studies conducted by (b) (4), (under IND (b) (4)) where CPD was used in an intranasal formulation for an insulin spray. Hence, the nonclinical evaluation includes a focus on the safety assessment of CPD via the intranasal route of administration.

Three nonclinical toxicology studies were submitted with this NDA. These included a 28-day bridging toxicology study comparing Noctiva™ with the RLD, and two chronic nonclinical toxicology studies evaluating CPD in the rat (26-weeks) and dog (39 weeks). All three toxicology studies included an emphasis on evaluation of nasal, oral, and pulmonary tissues based on above-mentioned histopathological lesions noted with non-sponsor conducted (b) (4)., under IND (b) (4)) chronic nonclinical toxicology studies with CPD.

CPD-related findings were limited to histopathology in the nose. These included minimal to slight hyperplasia of the nasal epithelium and mixed cell inflammation consistent with an irritant response that were not considered dose limiting. In the 39-week chronic

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nonclinical toxicology study in the dog, doses of (b) (4) mg/dog translate to dose multiples of 970, 2889, and 5789 times the proposed maximum clinical dose of 1.5 µg based on nasal surface area,

In the 26-week chronic nonclinical toxicology study in the rat, doses of (b) (4) mg/rat translate to dose multiples of 458, 1525, 4574, 9136 times the proposed maximum clinical dose of 1.5 µg based on nasal surface area. There were no CPD-related findings in this study.

The 28-day bridging toxicology study in the rat comparing Noctiva™ with the marketed desmopressin product (DDAVP® Nasal Spray) at a dose multiple approximately equivalent to the proposed clinical dose (1.5 mcg) did not reveal any remarkable findings.

A calculation of dose multiples based on the nasal surface area comparing doses between species used in the nonclinical toxicology studies and the proposed maximum clinical dose in humans is summarized in Table 6 below:

Table 6: Dose Multiples for the Chronic Toxicology Studies

Study Type	Species	*Nasal Surface Area	Drug	Dose: mg/animal	Dose: mg/sq cm	~Dose Multiple
28-day Study	Rat	14 sq cm	Desmopressin	0.00015	0.00001071	1.14
39-week Study	Dog	221 sq cm	CPD	(b) (4)		970
						2889
						5789
26-week Study	Rat	14 sq cm	CPD			458
						1525
						4574
						9136

*Nasal Surface Area is based on information from Table 2 in Gizurarson, 1990

~Dose Multiples are based on the proposed clinical dose of 1.5µg or 0.0015 mg and a human adult nasal surface area of 160 sq cm, resulting in dose of 0.00000938 mg/sq cm

Systemic exposure to CPD could not be confirmed with bioanalytical methods (see Section 5). Pharmacokinetic analyses showed high variability and low sporadic measurements for CPD. Studies conducted support the sponsor's conclusion that CPD undergoes rapid hydrolysis by endogenous esterases following exposure to CPD and does not accumulate.

The concern of CPD as a novel excipient (not previously used by the nasal route) was allayed given the high dose multiples observed in the chronic nonclinical studies (Table 6 above), the use of the MFD with regard to dose formulation and species specific limits on dosing volume, the lack of adverse histopathological findings at an approximate maximum clinical dose in the 28-day rat study compared to the studies conducted by (b) (4) and no remarkable findings in any of the other end-points evaluated. The studies evaluating the excipient CPD conducted by (b) (4) included dose volumes that exceeded the MFD and included higher frequency of dosing compared to the sponsor-conducted studies for Noctiva™.

One limitation of the 28-day bridging study in rats is that it was conducted at only an equivalent dose multiple of proposed clinical dose. However, given that there were no adverse findings at the dose multiple tested, that the dose of the active ingredient is lower than the RLD, and that the other ingredients in the formulation were included as control groups in the chronic nonclinical toxicology studies in dogs and rats, the nonclinical concerns for Noctiva™ formulation appears reasonably safe.

The sponsor-conducted 28-day bridging nonclinical toxicology study with Noctiva™ did not include a measurement of electrolytes in the clinical chemistry panel. Hence, potential imbalances in Na electrolyte impacting hyponatremia could not be evaluated. In light of the concern for clinical hyponatremia with desmopressin products, close clinical monitoring for hyponatremia is recommended with the use of Noctiva™ in patients.

Carcinogenicity studies with CPD were not conducted based on negative genetic toxicology data, limited systemic exposure, absence of accumulation based on nonclinical and clinical pharmacokinetic data, and negative histopathology data from the two chronic toxicology studies. A carcinogenicity waiver request was submitted by the sponsor. Following review of the toxicology studies, we concur that carcinogenicity studies are not required at this time.

The safety of the novel excipient CPD not previously used by the nasal route is adequately addressed and from the nonclinical perspective, Noctiva™ appears to be reasonably safe for approval.

12 Appendix/Attachments

References:

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Reference ID: 3996414

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Harkema, J.R., “Comparative pathology of the nasal mucosa in laboratory animals exposed to inhaled irritants,” Environmental Health Perspectives, 85:231-238 (1990).

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Reference ID: 3996414

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/s/

DEEPA B RAO

10/07/2016

This is a revised version of the primary nonclinical review following tertiary review by Dr.Abigail Jacobs. Changes are primarily editorial [page numbers added and minor terminology (novel excipient) changes] and do not alter the overall approval from the nonclinical team.

MUKESH SUMMAN

10/07/2016

Nonclinical supports approval

Comments on N201656 Noctiva

From A. Jacobs, AD

Date: 9/7/16

1. I concur that there are no pharm/tox approval issues.

2. The content of (b) (4) labeling should be removed.

I have conveyed other comments to the reviewer and they will be addressed as appropriate.

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

/s/

ABIGAIL C JACOBS
09/16/2016

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 201656
Supporting document/s:	Supporting Document Number 1
Applicant's letter date:	02/04/2016
CDER stamp date:	02/04/2016
Product:	Noctiva™
Indication:	Adult Nocturia
Applicant:	Serenity Pharmaceuticals, LLC
Review Division:	Division of Bone, Reproductive, and Urology Products
Reviewer:	Deepa B. Rao, D.V.M., Ph.D., D.A.B.T., D.A.C.V.P
Supervisor/Team Leader:	Mukesh Summan, Ph.D., D.A.B.T
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Disclaimer

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Any information or data necessary for approval of NDA 201656 that Serenity Pharmaceuticals Inc., does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 201656.

List of Abbreviations

ADH	Anti-Diuretic Hormone
CPD	Cyclopentadecanolide
CPE-215	Cyclopentadecalactone
DDAVP	Desamino-8-D-Arginine Vasopressin
NE	Nocturnal Enuresis
RLD	Reference Listed Drug

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1 Executive Summary

1.1 Introduction

This 505(b)(2) application submitted by Serenity Pharmaceuticals, LLC., seeks to market their drug product SER120 under the trade name Noctiva™. Noctiva™ is a low dose intranasal reformulation of an FDA approved drug - DDAVP® Nasal Spray . The proposed new indication for Noctiva™ is the treatment of adult patients (age not specified) with nocturia. The active pharmaceutical ingredient in both Noctiva™ and the reference listed drug (RLD) - DDAVP® Nasal Spray is desmopressin. Desmopressin is the synthetic peptide analogue of the human anti-diuretic hormone (ADH) vasopressin. The mechanism of action is based on selective V2 agonist activity stimulating reabsorption of water from the lumen of renal collecting ducts.

The reference listed drug, DDAVP® Nasal Spray (desmopressin acetate), was approved in 1978 (NDA 17922) for the treatment of central cranial diabetes insipidus, and for the management of temporary polyuria and polydipsia following head trauma or surgery in the pituitary region. DDAVP® Nasal Spray was also approved for nocturnal enuresis in children aged 6-17 years old, but this indication was revoked by the Agency in December 2007 due an association with hyponatremia. The sponsor's drug, Noctiva™, is a lower dose reformulation of the approved desmopressin acetate (0.75 and 1.5 µg per dose spray of 100 µL, compared to 10 µg per dose spray of 100 µL of DDAVP® Nasal Spray). The proposed dosing regimen of Noctiva™ is an intranasal administration of a 100 µL dose via a metered spray once daily approximately 30 minutes prior to bedtime. There are no major concerns regarding the use of the active ingredient (desmopressin) in this reformulation based on long history of safe use in humans.

However, the sponsor's reformulation with Noctiva™ contains a novel excipient, cyclopentadecanolide (CPD), also known as CPE-215 or pentadecalactone. CPD has been included in the sponsor's formulation to facilitate absorption of desmopressin through the nasal mucosa to result in higher bioavailability. Although CPD is used in another FDA-approved drug, it is via different route of administration (transdermal product Testim®). Noctiva™ is the first proposed use of CPD in an *intranasal* formulation. Moreover, non-sponsor conducted chronic nonclinical toxicology studies by (b) (4) using intranasal CPD (under IND (b) (4)) revealed histopathology lesions (squamous metaplasia in the nasal cavity) of concern. Hence, the nonclinical evaluation includes a focus on the safety assessment of CPD via the intranasal route of administration, for which the sponsor has obtained a letter of authorization (IND (b) (4)).

1.2 Brief Discussion of Nonclinical Findings

Based on the long history of clinical use of intranasal desmopressin, and a proposed clinical dose for Noctiva™ that is 6 to 13 times lower than the RLD (DDAVP® Nasal Spray), there are no specific concerns regarding the use of the active pharmaceutical ingredient, desmopressin, in Noctiva™.

However, Noctiva™ contains a novel excipient cyclopentadecanolide (CPD) and Noctiva™ is the first proposed use of CPD in an intranasal formulation. Nonclinical safety evaluation is primarily focused on the safety profile of this novel excipient, CPD.

CPD-related findings in a 39-week chronic nonclinical toxicology study in the dog were limited to histopathology lesions in the nose. These included minimal to slight hyperplasia of the nasal epithelium and mixed cell inflammation. Both lesions are consistent with an irritant response, and were therefore not considered to be adverse. Based on nasal surface area, doses of (b) (4) mg/dog translate to dose multiples of 970, 2889, and 5789 times the proposed maximum clinical dose of 1.5 µg (see Section 11 for dose multiple calculations).

There were no CPD-related findings in the 26-week chronic nonclinical toxicology study in the rat. Based on nasal surface area, doses of (b) (4) mg/rat translate to dose multiples of 458, 1525, 4574, 9136 times the proposed maximum clinical dose of 1.5 µg (see Section 11 for the dose multiple calculation).

A 28-day rat bridging toxicology study comparing Noctiva™ with the marketed desmopressin product (DDAVP® Nasal Spray) did not reveal any remarkable findings. Based on nasal surface area, the dose of 150 ng/rat translates to a dose multiple that is approximately equivalent to the proposed maximum 1.5 µg clinical dose.

All three toxicology studies included an emphasis on evaluation of nasal, oral, and pulmonary tissues based on concerns with non-sponsor conducted chronic nonclinical toxicology studies with CPD.

In light of the concern for clinical hyponatremia with desmopressin products, potential imbalances in Na electrolyte impacting hyponatremia could not be evaluated since the 28-day bridging nonclinical toxicology study with Noctiva™ in the rat, conducted by the sponsor, did not include a measurement of electrolytes in the clinical chemistry panel.

Carcinogenicity studies with CPD were not conducted based on negative genetic toxicology data, limited systemic exposure, absence of accumulation based on nonclinical and clinical pharmacokinetic data, and negative histopathology data from the two chronic toxicology studies. A carcinogenicity waiver request was submitted by the sponsor. Following review of the toxicology studies, we concur that carcinogenicity studies are not required at this time.

There have been no long-term studies in animals to assess the impairment of fertility potential of Noctiva™ nasal spray.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical perspective, Noctiva™ appears to be reasonably safe for approval.

1.3.2 Additional Non Clinical Recommendations

Based on lack of data regarding changes in Na electrolyte balances in the 28-day nonclinical bridging toxicology study in rats, close monitoring for hyponatremia is recommended.

1.3.3 Labeling

Sections 8 and 13 from the sponsor provided annotated label has been excerpted and tracked with the proposed changes as shown below.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk summary

(b) (4)

Animal Data

Teratology studies in rats and rabbits at doses from 0.05 to 10 mcg/kg/day (b) (4)

8.2 Lactation

(b) (4)

(b) (4)

The development and health benefits of breastfeeding should be considered along with the mother's clinical need for **NOCTIVA** and any potential adverse effects on the breastfed infant from **NOCTIVA** or from the underlying maternal condition.

8.4 Pediatric Use

(b) (4)

8.5 Geriatric Use

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

There have been no long-term studies in animals to assess the carcinogenic, mutagenic or impairment of fertility potential of **NOCTIVA** nasal spray. (b) (4)

(b) (4)

Reference

2 Drug Information

2.1 Drug

CAS Registry Number
62357-86-2 (trihydrate)

Generic Name
Desmopressin acetate

Code Name
SER120

Chemical Name
1-(3-mercaptopropanoic acid)-8-d-arginine-vasopressin monoacetate
Trihydrate

Molecular Formula: (b) (4)
Molecular Mass: (b) (4)

Structure or Biochemical Description

Pharmacologic Class
Anti-diuretic Agent

2.2 Relevant INDs, NDAs, BLAs and DMFs

1) IND 76667
Product Name: Desmopressin (SER120)
Sponsor: Serenity Pharmaceuticals, LLC.
Indication: Adult Nocturia
Status: This is the corresponding IND for the sponsor's submitted NDA
Relevance: The three nonclinical toxicology studies were conducted under this IND.

2) NDA 17922
Product Name: DDAVP®
Sponsor: Ferring Pharmaceuticals, Inc.
Indication: Treatment of central cranial diabetes insipidus and for the management of temporary polyuria and polydipsia following head trauma or surgery in the pituitary region
Status: Active
Relevance: Reference Listed Drug (RLD)

3) IND (b) (4)

(b) (4)

2.3 Drug Formulation

Table 1: Composition of Desmopressin Nasal Spray (SER120) – extracted from sponsor's report

Name of Ingredients/Quality	Composition (mg)		Function
	Strength: 7.5 µg/mL	Strength: 15 µg/mL	
	Per 100 µl Dose	Per 100 µl Dose	
Desmopressin acetate[a]/Manufacturer's specification	7.5 (b) (4)	15 (b) (4)	Active ingredient
Cyclopentadecanolide (CPD)/Manufacturer specification	(b) (4)		Permeation Enhancer
Cottonseed oil/NF			(b) (4)
Polysorbate 20/NF			
Sorbitan monolaurate/NF			
Citric acid, anhydrous/USP			
Sodium citrate Dihydrate/USP			
(b) (			
Water for Injection (WFI)/USP/EP			

[a] Composition refers to desmopressin free base; meets USP requirements

Reviewer Comment:

For nonclinical toxicology studies conducted with CPD (Section 6), concentrations of CPD included (b) (4) %. Based on the information from the sponsor, formulations with CPD concentrations above (b) (4) % do not share the same performance properties as described for lower concentrations. The sponsor states that "the formulation with (b) (4) % CPD appeared to be a suspension with particle sizes considerably larger than particle sizes in the (b) (4) % CPD formulations, and at refrigerated temperature formed precipitates that condensed into a solid". At room temperature, the (b) (4) % formulation appeared to remain a suspension and could be expressed through the metered dose nasal spray

pump. Hence, the storage conditions for CPD concentrations at (b) (4) % in the nonclinical toxicology studies were stored at room temperature (see Section 6).

2.4 Comments on Novel Excipients

All excipients in Noctiva™ formulation are listed in FDA's Inactive Ingredient Database and are present in other FDA-approved products with various routes of administration. However, three novel excipients (not present in formulations intended for intranasal administration) include CPD (b) (4), cottonseed oil (b) (4), and sorbitan monolaurate (b) (4).

The sponsor has conducted chronic nonclinical toxicology studies (see Section 6.2) in two species to demonstrate the safety of the above three ingredients via intranasal administration. Specifically, CPD has been evaluated as the test article, and a separate control group includes an emulsion with cottonseed oil and sorbitan monolaurate. There were no adverse effects with any of the above excipients from the toxicology studies in either species.

2.5 Comments on Impurities/Degradants of Concern

Concentration of individual impurities in drug substance (DS) is well below the limit of detection using a HPLC method. No peaks (HPLC method) were observed during forced degradation (acidic conditions) of the drug product in stability samples stored under long term or accelerated conditions. Contribution from residual solvents from any of the process components is well below the ICH limits. Refer to Quality Assessment Review for complete details.

2.6 Proposed Clinical Population and Dosing Regimen

Adults (age not specified) with nocturia (awakening 2 or more times to void).

The proposed dosing regimen of Noctiva™ is an intranasal administration of a 100 µL dose via a metered spray once daily approximately 30 minutes prior to bedtime.

Reviewer Comment:
The age of the patient population is not clearly specified. Refer to Clinical Review for complete details.

2.7 Regulatory Background

At the meeting with the sponsor in 2010 (July 30, 2010, Type B Meeting), the FDA recommended that a 1 month bridging study be conducted to evaluate and compare the local and systemic effects using a standard toxicology tissue battery for histopathology evaluation between SER120 product with the RLD (DDAVP® Nasal Spray). Olfactory pathway related neuroanatomical areas were specifically requested based on the intranasal route of administration.

It was confirmed in a written response dated June 12, 2012, that if systemic exposure of CPD could not be demonstrated, then chronic nonclinical toxicology studies would be required to support the safety of CPD in SER120.

In light of the evidence of histopathology lesions of concern documented in chronic nonclinical toxicology studies conducted by (b) (4) FDA reiterated the need for chronic nonclinical toxicology studies with CPD focused on local effects in the nasal and respiratory tracts in a Type C meeting in March 2015 (see Meeting Minutes dated April 9th, 2014).

3 Studies Submitted

3.1 Studies Reviewed

Toxicology:
Study 8297078: A 39-Week Intranasal Chronic Toxicity Study in Dogs Evaluating Cyclopentadecalactone in Bland Emulsions For Nasal, Oral, and Pulmonary Effects with an 8-Week Recovery Phase

Study 8297079: A 26-Week Intranasal Chronic Toxicity Study in Rats Evaluating Cyclopentadecalactone in Bland Emulsions For Nasal, Oral, and Pulmonary Effects with a 4-Week Recovery Phase

Study 8279849: A 28-Day Intranasal Toxicity and Toxicokinetic Study in Rats Evaluating SER120 Nasal Spray Compared to Commercial Desmopressin Nasal Spray Formulation with a 4-Week Recovery Phase

Pharmacokinetics:
Study vca11189: Validation of an LC-MS/MS Method for the Determination of Desmopressin in Rat Plasma (K_3EDTA)

Study 030517: Method Development for Low Level CPD in Plasma

Study 031109: Bioanalytical Method Feasibility of LC-MS/MS Assays for the Determination of CPD and 15-OH-Pentadecanoic Acid in Rat Plasma and Investigation of Stability of CPD and 15-OH-Pentadecanoic Acid in Plasma

Study 031331: In Vitro Stability of Cyclopentadecanolide (CPD) and 15-Hydroxyl-pentadecanoic Acid in Rat and Human Whole Blood

Study 031522: Bioanalytical Method Development and Qualification of LC-MS/MS Assays for the Determination of CPD and 15-OH-Pentadecanoic Acid in Rat Blood and in Human Blood

3.2 Studies Not Reviewed

Additional non-sponsor conducted supporting nonclinical toxicology studies on CPD were submitted with this NDA along with letters of authorization from respective sponsors (b) (4)

(b) (4). These studies were not reviewed in detail, since the sponsor-conducted chronic nonclinical toxicology studies with CPD in two species with appropriate control groups and variables (pH, dose volume and dosing frequency) relevant to Noctiva™'s formulation were considered sufficient to evaluate nonclinical safety.

3.3 Previous Reviews Referenced

Dr.Herman Rhee's nonclinical review for IND (b) (4) dated October 17, 2006 (DARRTS) – for genotoxicity and male fertility studies with CPD. The sponsor has obtained a letter of authorization to reference IND (b) (4)

4 Pharmacology

4.1 Primary Pharmacology

No nonclinical studies were submitted for Noctiva™.
The pharmacological activity of desmopressin is well understood. Desmopressin selectively binds to the vasopressin V_2 receptor which is expressed at high concentrations in the cells of the renal collecting ducts. By interacting with the V_2 receptor, desmopressin, like ADH, increases water permeability of the kidney allowing for the reabsorption of water into the body.

4.2 Secondary Pharmacology

A well-known side-effect of desmopressin is hyponatremia.
Desmopressin has minimal to no binding affinity for the V_1 receptors.
In light of the concern for clinical hyponatremia with desmopressin products, the sponsor-conducted 28-day bridging nonclinical toxicology study with Noctiva™ did not include a measurement of electrolytes in the clinical chemistry panel. Hence, potential imbalances in Na electrolyte impacting hyponatremia could not be evaluated.

4.3 Safety Pharmacology

No safety pharmacology studies were submitted for Noctiva™.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Reviewer Comment:
No specific nonclinical studies were submitted to this NDA to demonstrate increased bioavailability of desmopressin with Noctiva™ compared to the RLD (DDAVP® Nasal Spray).

Sponsor-submitted pharmacokinetic nonclinical studies are summarized below:

Desmopressin:
Study vca11189: Validation of an LC-MS/MS Method for the Determination of Desmopressin in Rat Plasma (K_3EDTA)
Summary: This bioanalytical method showed an acceptable bioanalytical performance for the determination of desmopressin in rat plasma (K_3EDTA) over the

calibration range of 4.00 to 250 pg/mL using an assay volume of 500 µL.

CPD:
Study 030517: Method Development for Low Level CPD in Plasma
Summary: In this study, an attempt was made to develop a gas chromatography-mass spectrometry (GC-MS) method for the analysis of the low level cyclopentadecanolide (CPD) in rat plasma with the ranges of (b) (4) µg/mL in plasma. Although GC-MS conditions to analyze CPD and internal standard at the limit of quantitation (LOQ) of (b) (4) ng/mL in a neat solvent was successful, extraction and analysis of the CPD from rat plasma was unsuccessful.

Study 031109: Bioanalytical Method Feasibility of LC-MS/MS Assays for the Determination of CPD and 15-OH-Pentadecanoic Acid in Rat Plasma and Investigation of Stability of CPD and 15-OH-Pentadecanoic Acid in Plasma
Summary: In this study, LC-MS/MS analysis conditions, as well as stability of cyclopentadecanolide (CPD) and its hydrolysis product, 15-OH-pentadecanoic acid were investigated in the development of bioanalytical assays for in rat plasma. Results indicated that:

1) The quantitation range for CPD with NaF treated plasma was linear only between (b) (4) ng/mL. CPD was unstable in untreated rat plasma since it was rapidly hydrolyzed by esterases. Recommendations for improving sensitivity and linearity included the use of NaF/ascorbic acid to better inhibit esterase hydrolysis of CPD and modification of chromatography conditions to reduce coelution of materials causing suppression.
2) The single product of esterase hydrolysis of CPD in rat plasma was 15-OH-Pentadecanoic acid (15-OH-PDA) that could serve as a potential surrogate for CPD absorption. The quantitation range for 15-OH-PDA was linear from (b) (4) ng/mL with no apparent issues that would prevent validation. 15-OH-PDA was stable in untreated rat plasma for up to 1 hour at 37 °C.

Study 031331: In Vitro Stability of Cyclopentadecanolide (CPD) and 15-Hydroxyl-pentadecanoic Acid in Rat and Human Whole Blood
Summary: In this study, the stability of cyclopentadecanolide (CPD) and 15-OH-pentadecanoic acid incubated in fresh rat and human whole blood was evaluated. whole blood at 37°C. 15-OH-pentadecanoic acid is stable in rat and human
Results showed that CPD is unstable in rat whole blood and marginally stable in human whole blood.

Study 031522: Bioanalytical Method Development and Qualification of LC-MS/MS Assays for the Determination of CPD and 15-OH-Pentadecanoic Acid in Rat Blood and in Human Blood
Summary: The objective of this study was to develop and qualify bioanalytical methods for the analysis of CPD and its hydrolysis product 15-OH-pentadecanoic acid in rat blood. The biological matrix for assay was changed from blood or plasma to acetonitrile precipitated blood (APB) to stop esterase hydrolysis of CPD present at blood sample collection, and to eliminate LC-MS/MS ionization suppression. The method for CPD in

acetonitrile precipitated blood was linear from (b) (4) ng/mL (equivalent to (b) (4) ng/mL in blood).
The method for 15-OH-pentadecanoic acid was changed from that reported for Study 031109 to one based on (b) (4) in the negative ionization mode to improve sensitivity. Two quantitation ranges were evaluated to help compensate for the narrow linear dynamic range. The low quantitation range was linear from (b) (4) ng/mL (equivalent to (b) (4) ng/mL in blood), and the high quantitation range was linear from (b) (4) ng/mL (equivalent to (b) (4) ng/mL in blood). Stability in matrix was not of concern as 15-OH-PDA was the hydrolysis product of CPD.
During the course of this work, examination of control rat and control human plasma for the presence of 15-hydroxyl-pentadecanoic acid as an endogenous component was added to the objective. Analysis of control rat and human plasma for endogenous 15-OH-PDA in did not show any 15-OH-PDA greater than the response for (b) (4) ng/mL in plasma.

6 General Toxicology

All toxicology studies reviewed in this section focus on the toxicity of the novel excipient in Noctiva™, namely, CPD.

6.1 Single-Dose Toxicity

None submitted

6.2 Repeat-Dose Toxicity

Study title: A 28-Day Intranasal Toxicity and Toxicokinetic Study in Rats Evaluating SER120 Nasal Spray Compared to Commercial Desmopressin Nasal Spray Formulation with a 4-Week Recovery Phase

Study no.:	(b) (4) Study Number 8279849 Sponsor Reference Number SPC-SER120-Tox-201301
Study report location:	NDA 201656 SDN 1 Module 4.2.3.2 February 4, 2016
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 5, 2013
GLP compliance:	Yes FDA GLP Title 21 CFR Part 58 OECD GLP ENV/MC/CHEM(98)17
QA statement:	Yes
Drug, lot #, and % purity:	SER120 Lot No. 008007; Purity 105% Commercial desmopressin nasal spray (comparator arm) Batch No. JKL2390A Cyclopentadecalactone (placebo control) Lot No. 008005 (b) (4) %); Purity 98%

Reviewer Comment:

The Lot No. for SER120 in the (b) (4) Study Report is noted as 008007 whereas the Lot No. for the same in the Toxicology Tabulated Summary (Module 2.6.7) is noted as 00807. This was confirmed to be a typographical error based on communication with the sponsor dated August 12, 2016. The lot number used in Study number 8279849 was 008007.

Key Study Findings

Given the intranasal route of administration, the study design included special evaluation of nasal turbinates and neuroanatomical areas specific to olfactory pathways in the brain.

No remarkable findings were noted in this study based on a dose multiple (approximately 1.14 times) equivalent to the proposed clinical dose. Dose multiples are calculated based on interspecies dose comparisons of nasal cavity surface areas (see Section 11).

In light of the concern for clinical hyponatremia with desmopressin products, the sponsor-conducted 28-day nonclinical toxicology study with Noctiva™ did not include a measurement of electrolytes in the clinical chemistry panel. Hence, potential changes in Na electrolyte balances impacting hyponatremia could not be evaluated.

Methods

Doses:	SER120: 150 ng/dose Desmopressin: 1000 ng/dose
Frequency of dosing:	Once daily
Route of administration:	Intranasal instillation
Dose volume:	10 µL
Formulation/Vehicle:	Placebo control (CPD (b) (4) % at pH 5.5±0.5) Saline control (0.9% sodium chloride)
Species/Strain:	Crl:CD(SD) Rats
Number/Sex/Group:	See experimental study design table below
Age:	7-8 weeks old
Weight:	195-297 g for males 173-243 g for females
Satellite groups:	Toxicokinetics
Unique study design:	No
Deviation from study protocol:	Cited deviations are included. None impacted overall study results or data interpretation

Table 2: Experimental Study Design (excerpted from the sponsor's submission)

Group	Subgroup[a]	No. of Animals[b] Male	No. of Animals[b] Female	Desmopressin Dose Level (ng/dose)	Dose Volume (µL/dose)
1 (Placebo Control)[c]	1 (Toxicity)	15	15	0	10
	2 (Toxicokinetic)	20	20	0	10
2 (Saline Control)[d]	1 (Toxicity)	15	15	0	10
	2 (Toxicokinetic)	3	3	0	10
3 (Untreated Control)[e]	1 (Toxicity)	15	15	NA	NA
4 (Desmopressin)	1 (Toxicity)	15	15	1000	10
	2 (Toxicokinetic)	20	20	1000	10
5 (SER120)	1 (Toxicity)	15	15	150	10
	2 (Toxicokinetic)	20	20	150	10

NA = Not applicable.

a For Groups 1, 4, and 5, toxicokinetic subgroups (Subgroup 2) included two extra animals/sex as possible replacement animals.

b Toxicity animals designated for recovery sacrifice (five animals/sex/group) underwent at least 4 weeks of recovery following dose administration. Group 3 animals followed the same schedule as the other groups.

c Group 1 received placebo control article (cyclopentadecalactone (b) (4) %) only.

d Group 2 received saline control article (sterile saline) only.

e Group 3 was not dosed.

Reviewer Comments:

Dose: The doses used in this nonclinical study was just equivalent to the proposed maximum clinical dose (1.5 mcg) where the dose multiple was calculated based on nasal surface area (see Section 11).

Dose Volume: The dose volume of 10 µL used in this nonclinical study was close to the volume of drug administered (13 µL) to obtain the same volume per surface area as in man (Gizurarson, 1990).

Drug Delivery Device: The dose was administered by using an adjustable micropipette, and a new pipette tip was used for each animal.

Dose Administration: Animals were held upright for a period of time (approximately 15 seconds (Day 1 of the dosing phase) or approximately 5 seconds (Days 2 through 15 of the dosing phase).

Note: *Protocol deviation was noted from Day 1-15 of the dosing phase where holding each animal upright following dose administration was not documented.*

Observations and Results

Mortality

All animals survived to the respectively scheduled terminal and recovery sacrifice.

Clinical Signs
Clinical signs noted were non-specific and/or sporadic changes not considered to be adverse or related to the administration of CPD.

Body Weights
No differences in body weight or body weight change were noted among the treatment groups during the dosing or recovery phase.

Feed Consumption
No differences in food consumption were noted among the treatment groups during the dosing or recovery phase.

Ophthalmoscopy

Vitreal hemorrhage in one male given the comparator drug, desmopressin (Group 4) was considered an unrelated

ECG

Not conducted.

Hematology

No SER120-related changes were present on Day 29 of the dosing phase or Day 29 of the recovery phase in hematology and coagulation test results of animals given 150 ng/dose compared with placebo, saline, or untreated controls and desmopressin-treated animals.

Clinical Chemistry

No SER120-related changes were present on Day 29 of the dosing phase or Day 29 of the recovery phase in clinical chemistry test results of animals given 150 ng/dose compared with placebo, saline, or untreated controls and desmopressin-treated animals.

Urinalysis

No SER120-related changes were present on Day 29 of the dosing phase or Day 29 of the recovery phase in clinical chemistry test results of animals given 150 ng/dose compared with placebo, saline, or untreated controls and desmopressin-treated animals.

Gross Pathology

No macroscopic observations were attributed to administration of SER120 (150 ng desmopressin/dose) or desmopressin (1000 ng/dose).

Organ Weights

No organ weight differences were attributed to administration of SER120 (150 ng desmopressin/dose) or desmopressin (1000 ng/dose) at the terminal or recovery sacrifice.

Histopathology

Adequate Battery: Yes.
Protocol specified brain structures included olfactory bulbs, piriform cortex, amygdala, and entorhinal cortex.

Peer Review: No

Histological Findings
No microscopic observations were attributed to administration of SER120 (150 ng desmopressin/dose) or desmopressin (1000 ng/dose). In particular, no findings were noted in the nasal turbinates (site of administration) or protocol-specified brain structures involved in olfaction (olfactory bulbs, piriform cortex, amygdala, and entorhinal cortex).

Toxicokinetics

Samples from Group 1 (Placebo control containing (b) (4) % CPD at pH 5.5±0.5) were analyzed for CPD and its metabolite (15-hydroxyl-pentadecanoic acid - 15-OH-PDA) using liquid chromatography-tandem mass spectrometry (LC/MS-MS) assays. Neither CPD nor 15-OH- PDA was observed with a response sufficiently above the control plasma response from which to calculate a concentration or to make estimates of analyte concentrations. As a result, toxicokinetic values could not be calculated. Samples from Group 2, 4, and 5 were analyzed for systemic desmopressin content using a validated liquid chromatography/mass spectrometry assay. The analyses revealed very low concentrations of desmopressin in sporadic animals from Groups 4 and 5. In animals treated with commercial desmopressin (Group 4), a total of 9 of 36 samples had desmopressin ranging between 4.10 to13.1 pg/mL. In animals treated with SER120, a total of 3 of 36 samples had desmopressin ranging between 4.23 to 6.77 pg/mL. Desmopressin was not detected in Group 2 animals. Given the intermittent nature of the systemic levels, toxicokinetic values could not be calculated.
Group 3 was an untreated control group.

Dosing Solution Analysis

All formulations were administered as provided by the sponsor or acquired commercially. Dose analysis was not conducted.

Study title: A 39-Week Intranasal Chronic Toxicity Study in Dogs Evaluating Cyclopentadecalactone in Bland Emulsions For Nasal, Oral, and Pulmonary Effects with an 8-Week Recovery Phase

Study no.:	(b) (4) Study Number 8297078 Sponsor Reference Number SPC-CPD-Tox-201401
Study report location:	NDA 201656 SDN 1 Module 4.2.3.2 February 4, 2016
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 12, 2014
GLP compliance:	Yes FDA GLP Title 21 CFR Part 58 OECD GLP ENV/MC/CHEM(98)17
QA statement:	Yes
Drug, lot #, and % purity:	Vehicle in the sponsor's formulation is the test article for this study. Cyclopentadecalactone (b) (4) % Lot # 400222; Purity 104% Cyclopentadecalactone (b) (4) % Lot # 400626; Purity 104%

Key Study Findings

The purpose of this study was to evaluate potential nasal, oral, and pulmonary effects of cyclopentadecalactone (CPD) in comparison with those of saline and a cottonseed oil-containing emulsion when administered daily via intranasal spray to dogs for at least 39 weeks, and to assess the reversibility of any CPD-induced effects after a recovery of at least 8 weeks.

Based on nasal surface area, doses of (b) (4) mg/animal translate to dose multiples of 970, 2889, and 5789 times the proposed maximum clinical dose of 1.5 µg (see Section 11).

CPD-related findings were limited to histopathology in the nose. These included minimal to slight hyperplasia of the nasal epithelium and mixed cell inflammation consistent with an irritant response, and therefore were not considered to be adverse.

No CPD-related adverse effects were noted in animals treated with the emulsion control.

Methods:

Doses:	0, (b) (4) mg/animal ((b) (4)
	See Reviewer Comments on Dose, Dose Volume, and Drug Delivery device below
Frequency of dosing:	Once daily
Route of administration:	Intranasal Spray
Dose volume:	100 µL in each nostril/day– Groups 1, 4, and 6 100 µL in a single nostril/day (alternate nostril per dose when possible) – Groups 2 and 3
Formulation/Vehicle:	Group 5 – Untreated Control [cottonseed oil, polysorbate, and sorbitan in aqueous sodium citrate buffer at pH 5.5 (concentrations of each component equivalent to final concentrations in the CPD containing formulations)] Group 6 – Saline [0.9% Sodium Chloride for Injection, USP (sterile saline) without preservatives]
Species/Strain:	Purebred Beagle Dogs
Number/Sex/Group:	6/sex – Groups 1, 4, and 6 4/sex – Groups 2, 3, and 5
Age:	8-10 months old
Weight:	Males: 7.6-11 kg Females: 5.8-8.9 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Cited deviations are included. None impacted overall study results or data interpretation

Table 3: Experimental Study Design (excerpted from the sponsor's submission)

Group	No. of Animals[a]		CPD Dose Level (mg/animal)	CPD Concentration (mg/mL)
	Male	Female		
1 (Emulsion Control)[b]	6	6	(b) (4)	
2 (Low CPD)[c]	4	4		
3 (Mid CPD)[c]	4	4		
4 (High CPD)[b]	6	6		
5 (Untreated Control)[d]	4	4		
6 (Saline Control)[b]	6	6		

CPD = Cyclopentadecalactone; NA = Not applicable.

a Animals designated for the recovery sacrifice (two animals/sex/group in Groups 1, 4, and 6) underwent 8 weeks of recovery following the dosing phase.

b Animals in Groups 1, 4, and 6 were dosed at a fixed dose volume of 100 µL/nostril in both nostrils once daily.

c Animals in Groups 2 and 3 were dosed at a fixed volume of 100 µL in one nostril once/day

d Group 5 was not dosed.

Reviewer Comments:
Dose: The (b) (4) % CPD formulations were stored at room temperature.
The (b) (4) % CPD formulations were stored in a refrigerator, set to maintain 2 to 8°C, until removed for dosing, and were used within 4 hours of removal from refrigerated storage. See Reviewer Comment under Section 2.3 for an explanation on differences in storage conditions for (b) (4) % CPD.

Dose Volume: The dose volume used in this nonclinical study reached the maximum dose volume for the dog of 100 µL per nostril in the dog (Gad et al, 2006). The dose volume in Groups 1, 4, and 6 is equivalent to the volume of drug (Group 4) administered to obtain the same volume per surface area as in man (Gizurarson, 1990).

Drug Delivery Device: As stated in the Nonclinical Overview (Module 2.6.1), the dose actuating device used in this nonclinical study for test article delivery via intranasal administration was confirmed to be the clinical delivery device, namely, the (b) (4) Nasal Spray Pump the clinical delivery device). Each device delivered 100 µL of spray/actuation cycle.

Dose Administration: For each nostril dosed, the head of the animal was held downward during the dose and for at least 30 seconds postdose.

Results:

Mortality

All animals survived to the respective scheduled terminal and recovery sacrifice.

Clinical Signs

Clinical signs noted were non-specific and/or sporadic changes not considered to be adverse or related to the administration of CPD.

Body Weights

Statistical differences for treated groups was limited to changes in the high dose males (Group 4) treated with CPD ((b) (4) mg/animal) compared to the saline control group (Group 6).This is considered non-adverse based the marginally lower weights of the saline control groups relative to other groups, and on the unremarkable differences between the high dose males and the emulsion control group (Group 1). All other statistical differences were limited to changes between the control groups (Group 1, 5, and 6).

Feed Consumption

Mean food consumption of animals given ≤ (b) (4) mg/animal CPD were similar to those of emulsion control during the dosing and recovery phases.

Ophthalmoscopy

Abnormalities were limited to a single incidence of epiphora (watery eye) in one saline control female and one high dose female. These findings were considered incidental and not related to the administration of CPD.

Hematology and Coagulation

No CPD-related hematology and coagulation effects were present during the dosing or recovery phase in animals given CPD compared with those given emulsion or saline control.

Clinical Chemistry

No CPD-related clinical chemistry effects were present during the dosing or recovery phase in animals given CPD compared with those given emulsion or saline control.

Urinalysis

No CPD-related urinalysis effects were present during the dosing or recovery phase in animals given CPD compared with those given emulsion or saline control.

Toxicokinetics

Plasma analyses revealed sporadic, low levels of desmopressin in the animals treated with desmopressin or SER120. Neither CPD nor 15-OH- PDA (metabolite of CPD) was observed with a response sufficiently above the control plasma response from which to calculate a concentration or to make estimates of analyte concentrations. As a result, no toxicokinetic parameters could be calculated from these data for any analyte.

Organ Weights

No CPD-related organ weight effects were noted at the terminal or recovery sacrifice.

Gross Pathology

No CPD-related macroscopic findings were noted at the terminal or recovery sacrifice.

Histopathology

Adequate Battery

Histopathology was limited to nasal, oral, and pulmonary tissues. The focus on select tissues was based on concurrence with FDA (Type C Meeting Minutes dated March 9, 2014 under IND 76667).

At the terminal sacrifice, the following tissues were processed to histologic slides and examined: the larynx (three sections), lung with large bronchi (one cross-section from each of the four major lobes), nares, nasal turbinates (including nasal sinuses), nasopharynx, tongue, trachea, and macroscopic lesions.

At the recovery sacrifice, only the nasal turbinates of females were examined microscopically (since CPD-related microscopic findings were limited to the nasal turbinates of terminal sacrifice females).

For chronic systemic toxicity evaluation, the full battery of routine tissues for histopathology was agreed to be unnecessary based on the sponsor's right of reference for IND (b) (4) (which includes chronic rat and dog studies with complete histopathology).

Peer Review
Yes

Histological Findings
CPD related findings were limited to two rostral nasal sections. Histopathological lesions included minimal to slight hyperplasia of the transitional epithelium (three high dose females; Animal Nos. H05917, H05919, and H05921), and minimal hyperplasia of the squamous epithelium (one high dose female; Animal No. H05921) in the most rostral (first) section. These were characterized by increased epithelial thickness and number of cellular layers. Inflammatory infiltrates were often collocated with areas of hyperplastic epithelium. Although foci of minimal mixed cell or neutrophilic inflammation were present in the rostral nasal turbinates of many animals, including one or more of the control groups, as a background finding, slight nasal turbinate mixed cell inflammation was noted in one high dose female (Animal No. H05919) given 12 mg/animal and was collocated with hyperplastic transitional epithelium; therefore, the increased severity was considered CPD-related. Slight mucus cell hyperplasia (two high dose females; Animal Nos. H05919 and H05921) was characterized by a more diffuse increase in the apparent number of mucus (goblet) cells in the mucociliary respiratory epithelium, within the most rostral (first) section and particularly in the ventral aspect of the second section, and was not restricted to foci of inflammation.

Table 4: Incidence and Severity of Test Article-Related Microscopic Findings in Nasal Turbinate – Terminal Sacrifice (excerpted from the sponsor's report)

	CPD											
Sex	Males					Females						
Dose Level (mg/animal)												(b) (4)
Number Examined	4	4	4	4	4	4	4	4	4	4	4	4
Nasal Turbinates												
Hyperplasia, transitional epithelium												
Minimal	0	0	0	0	0	0	0	0	0	1	0	0
Slight	0	0	0	0	0	0	0	0	0	2	0	0
Hyperplasia, squamous epithelium												
Minimal	0	0	0	0	0	0	0	0	0	1	0	0
Hyperplasia, mucus cell												
Slight	0	0	0	0	0	0	0	0	0	2	0	0
Inflammation, mixed cell												
Minimal	0	1	1	1	1	1	0	2	1	1	0	1
Slight	0	0	0	0	0	0	0	0	0	1	0	0

CPD – Cyclopentadecalactone; NA – Not applicable.
a Emulsion control.
b Undosed control.
c Saline control.

At the recovery sacrifice, in females given (b) (4) mg/animal complete resolution was exhibited from CPD-related nasal turbinate microscopic findings present in the terminal sacrifice females at this dose.

Reviewer Concurrence with sponsor's conclusion:
The most rostral (first) nasal section is normally lined by squamous, transitional, and mucociliary respiratory epithelium, whereas the second section is predominantly lined

by mucociliary respiratory epithelium. Nasal responses to inhaled irritants have been well studied in rats, monkeys, and mice. In all of these species, commonly reported findings after irritant exposure are transitional epithelial hyperplasia, mucus cell hyperplasia, and variable acute inflammation, with a lower incidence and severity of squamous epithelial hyperplasia. These findings characteristically exhibit a distinct anterior-posterior gradient (Harkema, 1990; 2006; Buckley, et al., 1984).
Based on the similarity between nasal findings commonly reported after exposure to irritants in the published literature and findings observed in the most rostral sections of the nasal cavity in this study, all CPD-related nasal turbinate findings were interpreted as irritant responses. None of these findings were considered adverse, based on the limited severity and absence of correlative clinical observations. No CPD-related findings were present in the nares, the two most caudal sections of nasal turbinates, the nasal sinuses, nasopharynx, tongue, larynx, trachea, or lungs.

Dosing Solution Analysis

All formulations (test article and emulsion control) were provided as ready-to-use formulations in dose-actuating devices by the sponsor; or acquired commercially (saline control) and filled in dose-actuating devices provided by the sponsor. Dose analysis was not conducted.

Study title: A 26-Week Intranasal Chronic Toxicity Study in Rats Evaluating Cyclopentadecalactone in Bland Emulsions For Nasal, Oral, Pulmonary Effects with a 4-Week Recovery Phase

Study no.:	(b) (4) Study Number 8297079 Sponsor Reference Number SPC-CPD-Tox-201402
Study report location:	NDA 201656 SDN 1 Module 4.2.3.2 February 4, 2016
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 22, 2014
GLP compliance:	Yes FDA GLP Title 21 CFR Part 58 OECD GLP ENV/MC/CHEM(98)17
QA statement:	Yes
Drug, lot #, and % purity:	Vehicle in the sponsor's formulation is the test article for this study. Cyclopentadecalactone (b) (4) % Lot # 400222; Purity 104% Cyclopentadecalactone (b) (4) % Lot # 400626; Purity 104%

Key Study Findings

The purpose of this study was to evaluate potential nasal, oral, and pulmonary effects of cyclopentadecalactone (CPD) in comparison with those of saline and a cottonseed oil-containing emulsion when administered daily via intranasal instillation in rats for at least 26 weeks, and to assess the reversibility of any CPD-induced effects after a recovery of at least 4 weeks.

Based on nasal surface area, doses of (b) (4) mg/animal translate to dose multiples of 458, 1525, 4574, 9136 times the maximum proposed clinical dose of 1.5 µg (see Section 11).

There were no remarkable findings in CPD or emulsion treated animals.

Methods:

Doses:	(b) (4)
Frequency of dosing:	Daily
Route of administration:	Intranasal Instillation using a plastic-tipped pipet
Dose volume:	10 µL per nostril
Formulation/Vehicle:	Group 1 (Emulsion Control – cottonseed oil, polysorbate, and sorbitan in aqueous sodium citrate buffer at pH 5.5). Concentrations of each component are equivalent to final concentrations in the CPD containing formulations)] Group 6 – Saline [0.9% Sodium Chloride for Injection, USP (sterile saline) without preservatives]
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	10/sex/group (Recovery 5/sex/group)
Age:	8-9 weeks
Weight:	253 to 346 g for males and 181 to 272 g for females
Satellite groups:	Recovery Period for Groups 1, 4, and 6
Unique study design:	Study was conducted in two Phases. Concentrations in Phase II were higher than in Phase I
Deviation from study protocol:	Cited deviations are included. None impacted overall study results or data interpretation

Table 5: Experimental Study Design (excerpted from the sponsor's submission)

Group	No. of Animals[a] Male	Female	CPD Dose Level (mg/animal)	CPD Concentration (mg/mL)
Phase I[b]				
1 (Emulsion Control)[c]	15	15		(b) (4)
2 (Low CPD)	10	10		
3 (Mid CPD)	10	10		
4 (High CPD)	15	15		
5 (Untreated Control)[e]	10	10		
6 (Saline Control)[f]	15	15		
Phase II[d]				
1 (Emulsion Control)[c]	15	15		
2 (Low CPD)	10	10		
3 (Mid CPD)	10	10		
4 (High CPD)	15	15		
5 (Untreated Control)[e]	10	10		
6 (Saline Control)[f]	15	15		

CPD = Cyclopentadecalactone; NA = Not applicable.

a Animals designated for the recovery euthanasia (up to five animals/sex/group in Groups 1, 4, and 6) underwent 4 weeks of recovery following the dosing phase (Phase I and Phase II).

b For Days 1 through 35 of the dosing phase (Phase I), doses (10 µL/nostril) were administered to Groups 1, 2, 3, 4, and 6 in one nostril per day (alternating each day). Animals were placed on a dosing holiday for Days 36 through 42 of the dosing phase (Phase I).

c Group 1 received emulsion control only.

d Beginning on Study Day 43 (Day 1 of the dosing phase for Phase II), doses for Groups 2 and 3 were administered to the left nostril each day, and doses for Groups 1, 4, and 6 were administered as 10 µL to each nostril (total dose volume of 20 µL/animal).

e Group 5 was not dosed (untreated control is synonymous with undosed control).

f Group 6 received saline control only.

Reviewer Comments:

Dose: The (b) (4) % CPD formulations were stored at room temperature.
The (b) (4) % CPD formulations were stored in a refrigerator, set to maintain 2 to 8°C, until removed for dosing, and were used within 4 hours of removal from refrigerated storage.
See Reviewer Comment under Section 2.3 for an explanation on differences in storage conditions for (b) (4) % CPD.

Dose Volume: The dose volume of 10 µL used in this nonclinical study was close to the volume of drug administered (13 µL) to obtain the same volume per surface area as in man (Gizurarson, 1990).

Drug Delivery Device: The dose was administered by intranasal instillation using an adjustable plastic-tipped micropipette.

Dose Administration: The animal was held in an upright position resulting in the head tilted back during the administration, and the animal remained in that position for approximately five seconds after the completion of dosing.
Care was taken to avoid potential tissue damage resulting from contact of intranasal tissues with the plastic-tipped micropipette tip.

Observations and Results

Mortality

One Group 4 female (Phase II) given (b) (4) mg/animal (high dose CPD) was found dead shortly after dosing on Day 170. Based on histopathology findings [slight acute hemorrhage and minimal pigment (hemosiderin suggestive of past hemorrhage)] present in the aortic adventitia at the heart base, and timing of death (after dosing procedure), the cause of death was attributed to physical handling procedure.

Clinical Signs

No CPD related adverse events were noted. Clinical observations were similar between treated and all control groups.

Body Weights

No persistent differences in body weight or body weight change were attributed to CPD when compared to the emulsion, saline, or undosed controls, respectively.

Feed Consumption

No differences in food consumption were attributed to CPD when compared to the emulsion, saline, or undosed controls, respectively.

Ophthalmoscopy

No ophthalmic abnormalities were attributed to CPD at ≤(b) (4) mg/dose.

ECG

Not done

Hematology

No CPD-related hematology and coagulation effects were present during the dosing or recovery phase compared with animals given emulsion control or saline control or during the dosing phase compared with the untreated controls.

Clinical Chemistry

No CPD-related clinical chemistry effects were present during the dosing or recovery phase compared with animals given emulsion control or saline control or during the dosing phase compared with the untreated controls.

Urinalysis

No CPD-related urinalysis effects were present during the dosing or recovery phase compared with animals given emulsion control or saline control or during the dosing phase compared with the untreated controls.

Gross Pathology

No CPD-related macroscopic findings were noted at the terminal or recovery sacrifice.

Organ Weights

No CPD-related organ weight effects were noted at the terminal or recovery sacrifice.

Histopathology

Adequate Battery
Yes

The focus on select tissues (larynx, lung, nasal turbinates) was based on concurrence with FDA (Type C Meeting Minutes dated March 9, 2014 under IND 76667). However, unlike the 39-week dog study reviewed above, histopathology included complete evaluation of the routine battery of tissues at terminal sacrifice. Evaluation of tissues following recovery sacrifice was contingent on findings at the terminal sacrifice.

For larynx, three step sections were prepared. For the lung with large bronchi, one cross-section was prepared from each of the four major lobes. For nasal turbinates, four sections were prepared. Nasal turbinates and larynx were processed to slide according to study-specific procedures.

Peer Review
Yes

Histological Findings
No CPD-related microscopic findings were present at the terminal sacrifice. Accordingly, tissues were not examined from recovery sacrifice animals.

Toxicokinetics

Not conducted

Dosing Solution Analysis

Test article and emulsion control formulations were provided by the sponsor, and dispensed for dosing at least monthly by (b) (4) according to the mixing procedure. Each bottle was dispensed for use on a single day according to the dispensing procedure, as directed by the Protocol. Dose analysis was not conducted.

7 Genetic Toxicology

Desmopressin: No genetic toxicity studies have been performed.

CPD: Genetic toxicity studies were conducted by (b) (4) and reviewed under IND (b) (4) by Dr.Herman Rhee (dated October 17, 2006) and for which the sponsor has obtained a letter of authorization to reference. These studies included the mouse lymphoma forward mutation assay, an in vivo mouse micronucleus assay, and the Salmonella-E.Coli/Mammalian-Microsome Reverse Mutation Assay (Ames Assay). All tests were negative for genetic toxicity.

8 Carcinogenicity

Desmopressin:

There have been no long-term studies in animals to assess the carcinogenic or mutagenic potential of Noctiva™ nasal spray.

CPD:
Carcinogenicity studies with CPD were not conducted based on negative genetic toxicology data, limited systemic exposure, absence of accumulation based on nonclinical and clinical pharmacokinetic data, and negative histopathology data from the two chronic nonclinical toxicology studies. A carcinogenicity waiver request was submitted by the sponsor under IND 76667. Following review of the toxicology studies, the Agency concurs that carcinogenicity studies are not required at this time.

9 Reproductive and Developmental Toxicology

Desmopressin:
There have been no long-term studies in animals to assess the impairment of fertility potential of Noctiva™ nasal spray.

CPD:
Male fertility tests in rats were conducted and reviewed under IND (b) (4) by Dr. Herman Rhee (dated October 17, 2006). No remarkable CPD-related effects were noted in sperm evaluation (cauda weight, sperm motility, progressive motility, and velocity), reproductive organ weights (epididymides, prostate, seminal vesicles and testes), and pregnancy performance.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

This 505(b)(2) application submitted by Serenity Pharmaceuticals, LLC., seeks to market their drug product SER120 containing desmopressin as Noctiva™. Noctiva™ is a low dose intranasal reformulation of an FDA approved desmopressin drug product, namely, DDAVP® Nasal Spray. The proposed new clinical indication for the use of Noctiva™ is the treatment of adult patients (age not specified) with nocturia. The active ingredient in both Noctiva™ and the reference listed drug (RLD), namely, DDAVP® Nasal Spray, is desmopressin acetate.

Desmopressin is the synthetic peptide analogue of the human anti-diuretic hormone (ADH) vasopressin. The mechanism of action is based on selective V2 agonist activity stimulating reabsorption of water from the lumen of renal collecting ducts.

DDAVP® Nasal Spray, was approved in 1978 (NDA 17922) for the treatment of central cranial diabetes insipidus, and for the management of temporary polyuria and polydipsia following head trauma or surgery in the pituitary region. DDAVP® Nasal Spray was also approved for nocturnal enuresis in children aged 6-17 years old, but this indication was revoked by the Agency in December 2007 due an association with hyponatremia.

DDAVP® Solution/Injection was later also approved for the treatment of hemophilia A and Von Willebrand's disease in 1984 (NDA 18938). Other FDA approved desmopressin products include Stimate® (NDA 020355 approved in 1994) for the treatment of Hemophilia A and Von Willebrand's disease; Concentraid (NDA 019776 approved in 1990) for the renal concentration capacity test, and Minirin® (NDA 021795 approved in 2008) for central diabetes insipidus, primary nocturnal enuresis, and renal concentration capacity test in adults. The indication for primary nocturnal enuresis was removed by the Agency in 2013 due to an association with hyponatremia.

The sponsor's drug, Noctiva™, is a relatively lower dose (0.75 and 1.5 µg per dose spray of 100 µL, compared to 10 µg per dose spray of 100 µL of DDAVP® Nasal Spray) reformulation of desmopressin acetate. The proposed dosing regimen of Noctiva™ is an intranasal administration of a 100 µL dose via a metered spray once daily approximately 30 minutes prior to bedtime. There are there are no major concerns regarding the use of the active ingredient (desmopressin) in this reformulation based on the proposed dose that is approximately 6 to 13 times lower than the clinical dose of the RLD, and the long history of safe use in humans with multiple FDA approved marketed products.

However, the sponsor's reformulation with Noctiva™ contains a novel excipient CPD. CPD is also known as CPE-215 or pentadecalactone. CPD has been included in the sponsor's formulation to facilitate absorption of desmopressin through the nasal mucosa to result in higher bioavailability. However, no specific nonclinical studies were submitted to this NDA to demonstrate increased bioavailability of desmopressin with Noctiva™ compared to the RLD (DDAVP® Nasal Spray).

Although CPD is used in other formulations and via different routes of administration (for example, CPD is an inactive ingredient in a transdermal product Testim®), Noctiva™ is the first proposed use of CPD in an *intranasal* formulation. Moreover, regulatory background includes concerns based on histopathological findings (squamous metaplasia in the nasal cavity) in chronic nonclinical toxicology studies conducted by (b) (4) (under IND (b) (4)) where CPD was used in an intranasal formulation for an insulin spray. Hence, the nonclinical evaluation includes a focus on the safety assessment of CPD via the intranasal route of administration.

Three nonclinical toxicology studies were submitted with this NDA. These included a 28-day bridging toxicology study comparing Noctiva™ with the RLD, and two chronic nonclinical toxicology studies evaluating CPD in the rat (26-weeks) and dog (39 weeks). All three toxicology studies included an emphasis on evaluation of nasal, oral, and pulmonary tissues based on above-mentioned histopathological lesions noted with non-sponsor conducted ((b) (4) under IND (b) (4)) chronic nonclinical toxicology studies with CPD.

CPD-related findings were limited to histopathology in the nose. These included minimal to slight hyperplasia of the nasal epithelium and mixed cell inflammation consistent with an irritant response, and therefore were not considered to be adverse. In the 39-week chronic nonclinical toxicology study in the dog, doses of (b) (4) mg/dog translate to

dose multiples of 970, 2889, and 5789 times the proposed maximum clinical dose of 1.5 µg based on nasal surface area,

In the 26-week chronic nonclinical toxicology study in the rat, doses of (b) (4) mg/rat translate to dose multiples of 458, 1525, 4574, 9136 times the proposed maximum clinical dose of 1.5 µg based on nasal surface area. There were no CPD-related findings in this study.

The 28-day bridging toxicology study in the rat comparing Noctiva™ with the marketed desmopressin product (DDAVP® Nasal Spray) at a dose multiple approximately equivalent to the proposed clinical dose (1.5 mcg) did not reveal any remarkable findings.

A calculation of dose multiples based on the nasal surface area comparing doses between species used in the nonclinical toxicology studies and the proposed maximum clinical dose in humans is summarized in Table 6 below:

Table 6: Dose Multiples for the Chronic Toxicology Studies

Study Type	Species	*Nasal Surface Area	Drug	Dose: mg/animal	Dose: mg/sq cm	~Dose Multiple
28-day Study	Rat	14 sq cm	Desmopressin	0.00015	0.00001071	1.14
39-week Study	Dog	221 sq cm	CPD	(b) (4)		970
						2889
						5789
26-week Study	Rat	14 sq cm	CPD			458
						1525
						4574
						9136

*Nasal Surface Area is based on information from Table 2 in Gizurarson, 1990

~Dose Multiples are based on the proposed clinical dose of 1.5µg or 0.0015 mg and a human adult nasal surface area of 160 sq cm, resulting in dose of 0.00000938 mg/sq cm

Systemic exposure to CPD could not be confirmed with bioanalytical methods (see Section 5). Pharmacokinetic analyses showed high variability and low sporadic measurements for CPD. Studies conducted support the sponsor's conclusion that CPD undergoes rapid hydrolysis by endogenous esterases following exposure to CPD and does not accumulate.

The concern of CPD as a novel excipient was allayed given the high dose multiples observed in the chronic nonclinical studies (Table 6 above), the use of the MFD with regard to dose formulation and species specific limits on dosing volume, the lack of adverse histopathological findings at an approximate maximum clinical dose in the 28-day rat study compared to the studies conducted by (b) (4) and no remarkable findings in any of the other end-points evaluated. The studies evaluating the excipient CPD conducted by (b) (4) included dose volumes that exceeded the MFD and included higher frequency of dosing compared to the sponsor-conducted studies for Noctiva™.

One limitation of the 28-day bridging study in rats is that it was conducted at only an equivalent dose multiple of proposed clinical dose. However, given that there were no adverse findings at the dose multiple tested, that the dose of the active ingredient is lower than the RLD, and that the other ingredients in the formulation were included as control groups in the chronic nonclinical toxicology studies in dogs and rats, the nonclinical concerns for Noctiva™ formulation appears reasonably safe.

The sponsor-conducted 28-day bridging nonclinical toxicology study with Noctiva™ did not include a measurement of electrolytes in the clinical chemistry panel. Hence, potential imbalances in Na electrolyte impacting hyponatremia could not be evaluated. In light of the concern for clinical hyponatremia with desmopressin products, close clinical monitoring for hyponatremia is recommended with the use of Noctiva™ in patients.

Carcinogenicity studies with CPD were not conducted based on negative genetic toxicology data, limited systemic exposure, absence of accumulation based on nonclinical and clinical pharmacokinetic data, and negative histopathology data from the two chronic toxicology studies. A carcinogenicity waiver request was submitted by the sponsor. Following review of the toxicology studies, we concur that carcinogenicity studies are not required at this time.

The safety of the novel excipient CPD is adequately addressed and from the nonclinical perspective, Noctiva™ appears to be reasonably safe for approval.

12 Appendix/Attachments

References:

Buckley LA, Jiang XZ, James RA et al. Respiratory tract lesions induced by sensory irritants at the RD50 concentration. Toxicol Appl Pharmacol 1984; 74:417-429.

Gad, S.C., Cassidy, C.D., Aubert, N., Spainhour, B., and Robbe, H. Nonclinical vehicle use in studies by multiple routes in multiple species. Int. J. Toxicol. 25:499-521 (2006).

Gizurarson S., “Animal models for intranasal drug delivery studies,” Acta Pharm.Nord 2(2): 105-122 (1990).

Harkema, J.R., “Comparative pathology of the nasal mucosa in laboratory animals exposed to inhaled irritants,” Environmental Health Perspectives, 85:231-238 (1990).

Harkema, J.R., Carey, S.A., and Wagner, J.G. The Nose Revisited: A brief review of the comparative structure, function, and toxicologic pathology of the nasal epithelium. Tox. Pathol. 34(3), 252-269 (2006)

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/s/

DEEPA B RAO
08/31/2016

MUKESH SUMMAN
08/31/2016
Nonclinical Supports AP

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 201656 **Applicant: Serenity Pharmaceuticals** **Stamp Date: February 4, 2016**

Drug Name: Desmopressin, Noctiva™ **NDA Type: 505(b)(2)**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required and requested IND studies in accord with 505 (b)(1) and (b)(2) including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		A request for carcinogenicity waiver was submitted. *General Comment: Clarification on whether the sponsor is relying of any published literature for specific nonclinical requirements is requested.*
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?		X	Needs to include human dose multiples based on nasal cavity surface area
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		New excipient is cyclopentadecalactone (CPD). One- month sponsor-conducted bridging nonclinical toxicology study with reference drug (DDAVP) is included. Sponsor has also conducted two chronic toxicology studies with CPD (rat and dog). In addition, a Letter of Authorization (LOA) to CPD studies conducted under IND (b) (4) ((b) (4)) is included. Moreover, multiple toxicology studies with CPD are included in Module 4.2.3.7.7. It is unclear if these studies are sponsor-owned or whether a LOA was available for each of these studies. Clarification is needed.
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			NA
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?	X		

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___Yes_____

Compared to the reference drug (DDAVP®), SER120 is being proposed at a lower dose for the indication of adult nocturia. Nonclinical issues have been focused on the presence of a new excipient – cyclopentadecalactone or CPD.

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

1) Please clarify if you intend to rely on any published literature to satisfy nonclinical requirements. If yes, please provide a list tabulating the published literature with the corresponding specific nonclinical requirement.
2) Labeling sections addressing dose should include human dose multiples that are based on nasal cavity surface area.
3) Two toxicology studies with CPD are included in Module 4.2.3.7.7. (the 6 month rat study conducted by (b) (4), and the 28 day rat nasal study conducted by (b) (4)) for which a letter of authorization appears not to be available. Please clarify a right of reference for these studies and submit a letter of authorization.

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/s/

DEEPA B RAO
03/28/2016

LYNNDA L REID
03/28/2016

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: (b) (4)
Review number: 01
Sequence number/date/type of submission: 000/Aug.4, 2006/Commercial
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: (b) (4)
Manufacturer for drug substance: (b) (4)

Reviewer name: Herman M. Rhee, Ph.D. Pharmacologist
Division name: Division of Metabolism and Endocrinology Products (DMEP)
HFD #: HFD#510
Review completion date: 8/30/2006

Drug:

Trade name: NA
Generic name: (b) (4)
Other name: (b) (4)
Code name: NA
Chemical name: Recombinant human insulin
CAS registry number: NA
Molecular formula/molecular weight: (b) (4)
Structure:

(b) (4)

Relevant INDs/NDAs/DMFs: (b) (4)

Drug class: Insulin
Intended clinical population: Type 1 Diabetes

Clinical formulation:
The drug product is an oil-in-water emulsion intended for intranasal insulin delivery of (b) (4)% w/w. It contains non-ionic surfactant esters as emulsifiers. It is a white, opaque, low viscosity emulsion. The clinical drug product is an emulsion spray containing the active pharmaceutical ingredient and the following excipients listed below in bulk. (b) (4) The sponsor plans to use (b) (4)% pentadecalactone (exaltolide) as a mixture of rh-Insulin nasal spray as shown below. The pentadecalactone (Mol.Wt= (b) (4)) is insoluble in water. It has many other names such as Angelica lactone, 15-HPAEE (15-hydroxypentadecanoic acid), Muskalactone, or CPE-215, which was used interchangeable in this review.

(b) (4)

(b) (4)

The formulation that was used for 28-Day toxicity study in rat is shown below.

Formulation Descriptions from 28 Day Toxicity Study

Test Articles	Group Designation	Formulation Components
Formulation A	Group 4 and 6	(b)(4)% Insulin, (b)(4)% CPE-215 treatment pH 3.5 ± 0.5
Formulation D	Group 5	(b)(4)% Insulin, (b)(4)% CPE-215 treatment formulation at pH 3.5 ±0.5 (with picolinic acid)
Control Articles		
Formulation C	Group 1	(b)(4)% Insulin at pH 3.5±0.5
Formulation B	Group 2	% CPE-215 at unadjusted pH 5.3±0.5
Formulation F	Group 3	% CPE-215 at pH 3.5±0.5

Route of administration: Intranasal spray. However, the sponsor used instillation for all toxicology studies.

Proposed clinical protocol:
The sponsor is planning [redacted] (b) (4) The overall purpose of the study is to evaluate and characterize the efficacy and safety of [redacted] (b) (4) intranasal formulation. This is an exploratory 4-way crossover pharmacokinetic/pharmacodynamic study with the objective being to determine the difference in pharmacokinetic and pharmacodynamic profiles when given 4 regimens: Humulin R sc, Humalog sc, Saline Nasal Spray and [redacted] (b) (4) Intranasal Insulin Spray. The preparation day and treatment period together will last 5 days. The follow up visit will occur between day 5 and 11.

Previous clinical experience:
[redacted] (b) (4) studies done in [redacted] (b) (4) with both normal subjects and Type I diabetic patients demonstrated rapid absorption from the nasal spray with the expected glucose response. A [redacted] (b) (4) PK study performed in [redacted] (b) (4) has not been fully reported yet. [redacted] (b) (4) The [redacted] (b) (4) studies used regular fast-acting human recombinant insulin dissolved in sterile water in combination with [redacted] (b) (4) proprietary excipient CPE-215. A human exploratory study (Study [redacted] (b) (4)) in healthy volunteers demonstrated safety in use and a rapid absorption of insulin [redacted] (b) (4) with an accompanying glucodynamic effect. The [redacted] (b) (4) human study (Study [redacted] (b) (4)) confirmed these findings in diabetic patients. This was evidenced by a rise in serum insulin levels and accompanying decrease in plasma glucose at doses of [redacted] (b) (4) and above. Peak insulin levels generally were attained [redacted] (b) (4), remaining elevated [redacted] (b) (4) post-dosing. Calculated relative bioavailability against subcutaneous insulin as a comparator was in the 15-20% range. The [redacted] (b) (4) intranasal insulin formulation was generally well tolerated and absorbed as demonstrated by a rapid rise in serum insulin level and concomitant reduction of plasma glucose levels.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

4-Week Intranasal Instillation Toxicity Study with Insulin Formulations A and D in Rats (Study#7639-100)

13-Week Intranasal Instillation Toxicity Study with Insulin Rats with 4-Week Recovery (Study#7639-102)

13-Week Intranasal Instillation Toxicity Study with Insulin in Dogs (Study#7639-103)

90-Day Repeated Dose Dermal Toxicity Study in Rats with 15-PPAEE (Study#014938)

Genotoxicity studies with pentadecalactone (exaltolide):

1) L5178Y TK $^{+/-}$ Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay with Exaltolide (Pentadecalactone) (Study#22572-0-431 ICH)
2) In Vivo Mouse Micronucleus Assay with Exaltolide (Pentadecalactone) (Study#22572-0-455OECD)
3) Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with Exaltolide (Pentadecalactone)(22572-0-409 OECD)

Effects of Pentadecalactone (Exaltolide) on Male Fertility in Rats ([redacted] (b) (4) #493050, Report#20650)

Studies not reviewed within this submission:
Acute pharmacology and toxicology studies and 28-day rat ([redacted] (b) (4)).

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

In early studies subcutaneous insulin was used as positive control and was consistent as a reference medication. Subsequent formulations were applied intranasally using an established intranasal positive control for comparisons. Intranasal formulations were dispensed from intranasal atomizers commercially available for human use. An extension to the actuator of the atomizer was used to deliver formulation to the absorptive surface of the vestibule and labyrinthine turbinate region which had to be accessed through the nares, recessed within the pig snout. In these studies, blood glucose and insulin values were obtained. By comparing the pharmacokinetic and pharmacodynamic effectiveness of different insulin formulations, the current formulation can deliver intranasal insulin doses to the Yucatan pigs. Intranasal doses were delivered significantly faster than via the subcutaneous route and produced a rapid subsequent decrease in plasma glucose: the maximal insulin concentration was realized in the blood within 10 minutes after administration; the maximal blood glucose reduction was reached within 20 minutes after the insulin nasal spray is given, and the glucose reduction effect lasted for 90 to 120 minutes. The intranasal formulation in which insulin is in an acidic medium efficiently permeated the nasal mucosa; with insulin in a neutral medium, it was less efficiently delivered (Study BP-006-03).

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

Increasing the amount of cottonseed oil in the lipid phase (b) (4) (b) (4) (b) (4) Increasing the insulin concentration from (b) (4) % in the formulation gave an increase in plasma insulin commensurate with dose as shown (Fig. 1). Emulsion droplet size does not impact insulin delivery within the average size boundaries studied.

(b) (4)

Drug activity related to proposed indication:

The data of one puff administration of insulin showed increased variance, due to the occasional animal's differential responses. However, when using two puffs, one in each nostril, as done in previous studies, the variance was less due to the lower probability of delivering a null dose, missing both nares. (b) (4)% is the minimum effective level of CPE-215 for optimal delivery, and the variation of CPE-215 concentration from (b) (4)% does not affect insulin delivery and subsequent glucose response. For a manufactured batch of insulin with (b) (4)% CPE-215, a large variance of (b) (4)% of the theoretical CPE-215 value is tolerable in the formulation while still maintaining effective delivery. It was demonstrated that insulin-dose dependent increases in serum insulin levels after different doses of insulin preparations as shown (Fig. 2). It is also documented that the optimal concentration of CPE-215 was (b) (4)% as shown below. However, the differences between CPE-215 concentrations up to (b) (4)% did not contribute to the plasma levels of insulin absorption (Fig.3). Osmolarity is one of factors that affect insulin absorption being 100 mM ((b) (4)%) the optimal (Fig. 4). CPE-215 was the best enhancer because isopropyl palmitate did not work (Fig. 5). It appears that the sponsor developed finally a working intranasal delivery system for insulin as demonstrated in Fig. 6. There was a clear increase in serum insulin levels with corresponding decreases in serum glucose concentrations.

(b) (4)

Table 1. CPE-215 concentration vs. Insulin Peak Height

[CPE-215]	Insulin Peak Height (uU/mL)

(b) (4)

(b) (4)

(b) (4)

2.6.2.3 Secondary pharmacodynamics
The sponsor did not provide relevant data.

2.6.2.4 Safety pharmacology
The sponsor did not provide relevant data.

2.6.2.5 Pharmacodynamic drug interactions
The sponsor did not provide relevant data.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

The sponsor's PK/TK data are largely based on several their ADME studies in pigs. The pig's jugular veins were cannulated surgically for blood samplings 4 to 6 days before the study. At the time of dosing of nasal insulin, the pigs were restrained in a cloth sling. The pigs were afterwards free to move about their respective individual pens and were only temporarily restrained in close quarters at the front of the pen with a movable wooden gate at the time of blood sampling. Each pig was dosed twice with each of the four different formulations over a two week period with at least 18 hours between treatments. The intranasal dosing entailed dispensing 100 µL of a (b)(4)% insulin emulsion through an aerosol doser (human type intranasal actuator), once per each nostril (Total dose 50 IU), or subcutaneous dosing of insulin for the comparison purpose as presented below.

2.6.4.1 Brief summary

The results showed that there were acceptable reductions in blood glucose values, as measured in blood by the hexokinase enzymatic method, which is consistent with findings after subcutaneous insulin administration. For intranasal Formulation B, glucodynamic reduction was seen with a more rapid onset, faster to trough, but of shorter
duration (90-120 min.) than SQ (180 min.). Formulation C had a similar rapid onset to B, but of less magnitude. Formulation D, the historic negative control, was devoid of appreciable glucodynamic activity. The reproducibility, both intradose and day to day, was good (no variances of any treatment differed significantly) as shown below (Figure 2 from Study BP006-03). Formulation B is equivalent to the clinical formulation. The glucodynamic effect of formulation B is equivalent to sc formulation A up to 60 minutes post-dose iv, but this effect was no sustained.

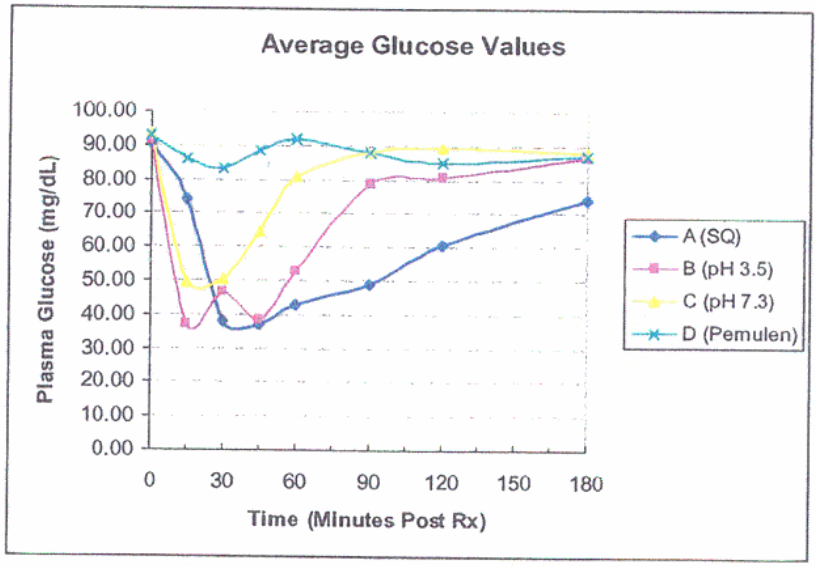


Figure 2. Average Glucose Values (n=8)

2.6.4.2 Methods and Analysis

The sponsor had at least 6 different insulin formulations which have different enhancers, osmolalities, additives, surfactants tested in mini-pigs to optimize the formulation as shown below. All formulations have (b) (4) % insulin, at pH 3.5. In order to quantitate the insulin absorption with subsequent reduction in blood glucose, the sponsor administered the Insulin Instillation Spray (IIS) to four pigs in Latin Quarter design as shown below.

The method is semi-quantitative, able to differentiate positive and negative controls, as well as discriminating effectiveness of formulation composition. The intranasal formulation in which insulin is the acidic form efficiently permeates the nasal mucosa; with insulin in a neutral form, it is less efficiently delivered. Heparinized plasma was analyzed for insulin concentration using a commercial RIA assay for insulin (b) (4) K). Insulin was reported in micro International Units/milliliter of plasma (µU/mL). Glucose was measured at the time of collection using a Glucometer (Lifescan) and was expressed in mg/dL. The figure below shows the formulations that were tested in pigs according to Latin quarter crossover design.

(b) (4)

Components of each Formulations and the Final pH that were tested in Pigs		
Formulation FFF	(b) (4)% insulin	(b) (4)% CPE-215, pH 3.5
Formulation GGG	% insulin	% CPE-215, 20 mM NaCl, pH 3.5
Formulation HHH	% insulin	% CPE-215, 0.6% NaCl, 2X tween20
Formulation JJJ	% insulin	% Isopropyl Palmitate, pH 3.5
Formulation KKK	% insulin	% CPE-215, (b) (4) pH 3.5
Formulation NNN	% insulin	% CPE-215, 20 mM picolinic acid, pH 3.5
Formulation PPP	% insulin	% CPE-215, 100 mM NaCl, pH 3.5

2.6.4.3 Absorption

Mean insulin absorption after different formulation is shown below (Fig.4). It appears that all formulations were not absorbed at the same extent, although Tmax appears to be 10 to 15 minutes as shown (Fig. 4). The higher the osmolarity, the worse is the performance of the formulation. The only difference between Formulation FFF, GGG and PPP is the sodium chloride concentration. Formulation FFF has the least sodium chloride concentration; Formulation GGG only has around (b) (4) % sodium chloride, but it is already less effective than Formulation FFF in blood glucose reduction. Formulation PPP has the highest sodium chloride concentration (still hypotonic), but has the least glucose reduction effect of all formulations. The addition of picolinic acid did not improve the glucose reduction (compare FFF and NNN). A previous study (pig Study 05-01) shows that the addition of picolinic acid may improve the performance slightly as the mean is considered, but the differences were not significant in that study among the comparators. Combining the two studies together, it can be seen that adding picolinic acid or not does not significantly change the performance of the basic formulation.

It appears that isopropyl palmitate is inferior to the CPE-215 in this study. Surfactants (b) (4) improved the absorption of insulin as shown (KKK group). As a direct result of insulin absorption after its instillation, the levels of plasma glucose were decreased (Fig. 3).

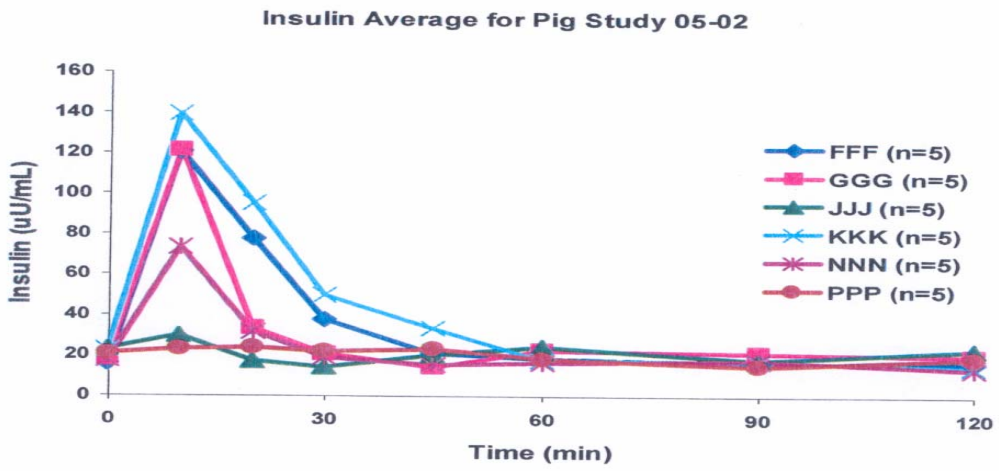


Figure 4. Study 05-02 Average Insulin Values

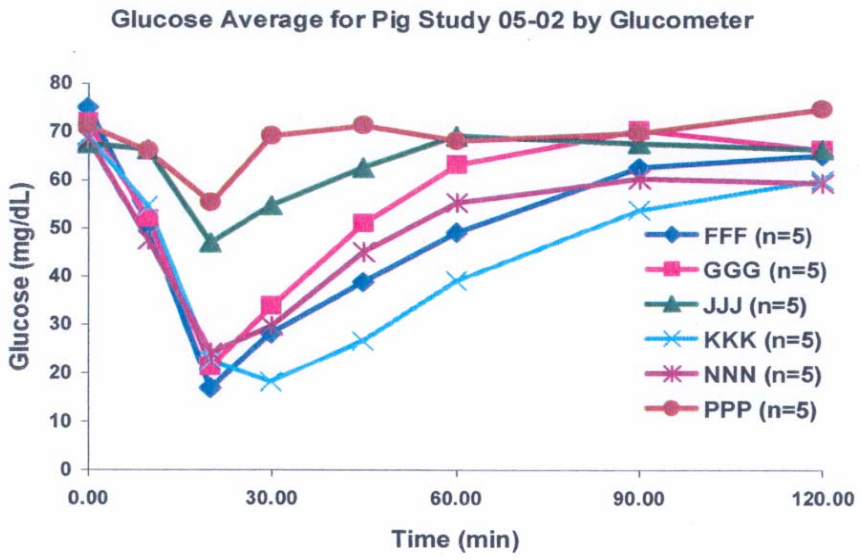


Figure 3. Study 05-02 Average Glucose Values (The value for HHH is not shown).

OTHER PHARMACOKINETIC STUDIES: NONE.

2.6.4.9 Discussion and Conclusions: **The sponsor provided relevant findings on drug absorption as summarized above. However, there were no data on distribution, metabolism, excretion and pharmacokinetic drug interactions, although the information with insulin is documented extensively.**

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

The preclinical safety profile of intranasal recombinant human insulin was assessed from 1) pilot toxicity studies conducted in normal male and female rats and dogs and 2) repeated dose toxicity studies for up to 90 days in normal rats and dogs. The intranasal recombinant human insulin [rh insulin] was administered to rats and dogs by nose only instillation as an emulsion formulation. The preclinical safety profile of recombinant human insulin is well defined, essentially related to exaggerated pharmacological manifestations associated with hypoglycemia. The toxicology testing program was conducted using purebred rats and dogs, and was intended to assess the potential for local and systemic adverse effects associated with intranasal instillation of a (b)(4)% rh insulin (b)(4)% CPE-215 formulation. Other non-clinical toxicology studies to support the safety of CPE-215 as a pharmaceutical excipient have been completed and include general toxicity and genotoxic studies. These studies are summarized below

<u>General toxicology</u>:
Please see repeated dose studies presented below.

<u>Genetic toxicology</u>:
The sponsor submitted the following three genetic toxicology studies: in vitro Ames, mouse lymphoma, in vivo mouse micronucleus tests, which are all negative. The genetic toxicity of rh-insulin is well characterized and it has used successfully so many years. The sponsor submitted the genetic toxicology data in this IND with pentadecalactone, an excipient. The three genotoxicity studies are reviewed under relevant sub-heading.

(b) (4)

Carcinogenicity:
NA

Reproductive toxicology:

The sponsor submitted the following one reproductive toxicology, which was reviewed under relevant section below.

(b) (4)

Special toxicology: NA

2.6.6.2 Single-dose toxicity

The sponsor performed the following single dose toxicity studies, which will not be reviewed because their repeated dose toxicity studies address the main toxicity issues.

Table 2: Single Dose (Pilot) Toxicity Studies with Intranasal Instillation of (b)(4)% rh Insulin with (b)(4)% CPE-215®, pH 3.5

Species	N/sex per group	rh Insulin Formulation (IU insulin/kg/dose/nostril)	Dosing Duration (Days)	MTD/MFD (IU insulin/cm²)		GLP	Report Number
				M	F		
Rat	3	2.7, 8.64, 17.28, 34.56, 51.84	1	1.85	1.85	No	7639-101
Dog	2	0.2, 0.4, 0.8, 1.6, 3.2, 4.9, 6.5	1	1.76	1.76	No	7639-104

rh insulin formulation = (b)(4)% human recombinant insulin with (b)(4)% CPE-215®, pH 3.5; doses are based on the volume administered per nostril
M = Male; F=Female
MTD/MFD = Maximum Tolerated Dose/Maximum Feasible Dose based on nasal area per species

2.6.6.3 Repeat-dose toxicity

The sponsor performed the following repeated dose toxicity studies in rats and dogs, which are reviewed below under each study number.

Table 3: Repeated Dose Toxicity Studies with Intranasal Instillation of (b)(4)% rh Insulin with (b)(4)% CPE-215®, pH 3.5

Species	N/sex per group	rh Insulin Formulation (IU/kg/dose/ nostril/day)	Dosing Duration (Days)	NOAEL (IU insulin/cm²/day)		MTD/MFD (IU insulin/cm²/day)		GLP	Report Number
				M	F	M	F		
Rat	3	51.84	3 (*t.i.d.*)	5.56	5.56	5.56	5.56	No	7639-101
Rat	10	Iletin- 25*	28 (*qd*)	1.78	1.78	1.78	1.78	No	(b) (4) CPE/Insulin
Rat	10	17.28, 34.56	28 (*t.i.d.*)	3.7	3.7	3.7	3.7	Yes	7639-100
Rat	10 - 15	17.28, 34.56	90 (*t.i.d.*)	3.7	3.7	3.7	3.7	Yes	7639-102
Dog	3 - 5	4.9, 6.5	90 (*t.i.d.*)	1.76	1.76	1.76	1.76	Yes	7639-103

rh insulin formulation = (b)(4)% human recombinant insulin with (b)(4)% CPE-215®, pH 3.5; doses are based on the volume administered per nostril given 3x daily (*t.i.d.*) or 1x daily (*qd*)
M = Male; F=Female
MTD/MFD = Maximum Tolerated Dose/Maximum Feasible Dose based on nasal area per species
NOAEL = No Observed Adverse Effect Level
* Study done with non-human/beef insulin

Study Title: 28-Day Intranasal Instillation Toxicity Study with Insulin Formulations A and D in Rats

Key findings:

One rats from group 5 died, which was due to accidental cage injury, according to the sponsor. Clinical findings were not remarkable. The test article had no effects on food consumption, body weight, ophthalmic and urine examinations. Treatment-related decreases in glucose were observed in male and female animals, which hypoglycemic effects were shortening on Day 23, compared those on Day one for some reasons. It is not known that the reduced effects were due to the potential reduction of insulin bioavailability due to histopathological changes in nasal passages such as thickening of nasal turbinates. The test article increased in ALT levels. Repetitive stimulation on nasal passages may increase eosinophils in epithelium as seen in gross histopathology examination, which was confirmed in microscopic histopathologic demonstration of eosinophils, alveolar macrophage, and sinus globlets. NOAEL = 1.85 IU insulin/cm^2 /day.

Study No: (b) (4) 7639-100
Amendment # 000, Vol. #6, and page # 1-406
Conducting laboratory and location: (b) (4)
Date of study initiation: June 6, 2005
Sponsor: (b) (4)
GLP compliance: Yes
QA- Report: Yes (x) No ()

METHODS:
Species/strain: Crl: CD (SD)
#/sex/group or time point: 10 rats /sex/group
Age: 53-59 days old
Weight: 220-230 g
Dosage groups in administered units: The animals were assigned to one of 12 groups. Animals in Groups 1-6 were dosed three times daily (at least 4 hours between each daily dose) for 28 days. Treatment continued through the day prior to scheduled sacrifice. For the rats in Groups 7-12 were dosed once on Day 1 of the dosing phase. Day 1 of the dosing phase for males and females was a staggered start (males 1 day and females the next).

Group	Treatment	No. of Animals Male	No. of Animals Female	Dose Volume (µL/nostril)	Dose Level mg/kg/dose/nostril (IU/kg/dose/nostril)	Nominal Dose Level mg/rat/day (IU/rat/day)	Nominal Dose Level mg/cm²/day (IU/cm²/day)
1	Formulation C - Control Article 1	10	10	32	1.28 (34.56)	1.92 (51.84)	0.137 (3.70)
2	Formulation B - Control Article 2	10	10	32	0	0	0
3	Formulation F - Control Article 3	10	10	32	0	0	0
4	Test Formulation A	10	10	32	1.28 (34.56)	1.92 (51.84)	0.137 (3.70)
5	Test Formulation D	10	10	32	1.28 (34.56)	1.92 (51.84)	0.137 (3.70)
6	Test Formulation A	10	10	16	0.64 (17.28)	0.96 (25.92)	0.069 (1.85)
7	Formulation C - Control Article 1	10	10	32	1.28 (34.56)	1.92 (51.84)	0.137 (3.70)
8	Formulation B - Control Article 2	10	10	32	0	0	0
9	Formulation F - Control Article 3	10	10	32	0	0	0
10	Test Formulation A	10	10	32	1.28 (34.56)	1.92 (51.84)	0.137 (3.70)
11	Test Formulation D	10	10	32	1.28 (34.56)	1.92 (51.84)	0.137 (3.70)
12	Test Formulation A	10	10	16	0.64 (17.28)	0.96 (25.92)	0.069 (1.85)

Note: All animals (except Group 12) were dosed 32 µL in each nostril. The dose volume of 32 µL was administered as 16-µL installments alternating between the right and left nostrils to achieve the target dose volume in each nostril based on dose time of the first animal dosed. Dosing represented a single repeat dose of Day 1. Dose selection calculations were based on the assumption that the average weight of each rat was 250 g. Dose Selection calculations were based on the rat nasal surface area of 14 cm² for a 0.25-kg rat (Schreider, 1983) and insulin concentration of 10 mg/mL or 270 IU/mL.

Route, form, volume, and infusion rate: Intranasal instillation
Drug, lot#, radiolabel, and % purity: Formulation A=1110252, Formulation D=BP-16-30B; 100-102%
Formulation/vehicle: Please tables under "Clinical formulation" on page 3.

OBSERVATIONS AND TIMES:
Clinical signs: Twice daily (AM and PM)
Body weights: Before the start of dosing in all groups and once during Week 2 (Groups 1 through 6).

Food consumption: Twice daily, beginning on Day 1 of the dosing phase (Groups 1 through 12)
Ophthalmoscopy: Ophthalmologic examination was performed before the start of dosing in all groups and once during Week 4 (Groups 1 through 6).

Hematology:
Glucose levels were determined prior to dosing on the first day of treatment. All measurements were taken prior to the daily dose (predose) and approximately 30, 60, 90, 120, 180, and 240 minutes postdose.

Clinical chemistry:
Glucose levels were determined on the first day of treatment (prior to dosing) and approximately 30, 60, 90, 120, and 240 minutes postdose-and on Days 23

(males) and 24 (females) of the dosing phase prior to the first daily dose (predose) and approximately 30, 60, 90,120,180, and 240 minutes postdose.
Organ weight: At necropsy in groups 1-6, not 7-12.
Gross histopathology: At necropsy in groups 1-6

Microscopic histopathology:
Protocol-specified tissues from the rat that died at an unscheduled interval and from animals sacrificed at the scheduled sacrifice were embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined microscopically. At necropsy, animals in groups 1-6 were examined. Remaining preserved tissues were held for possible future processing and examination.

Statistical Evaluation:
Levene's test was done to test for variance homogeneity. In the case of heterogeneity of variance at $p<0.05$, rank transformation was used to stabilize the variance. Comparison tests took variance heterogeneity into consideration. One-way analysis of variance was used to analyze continuous clinical pathology values, organ weight data, food consumption, and body weight data. If the ANCOVA was significant ($p<0.05$), covariate-adjusted means was used for control versus tested group comparisons. Dunnett's t-test was used for control versus treated group comparisons.

RESULTS:
Mortality: There were no the test article-related deaths. One female in group 5 (#B91059) was found dead in the cage on Day 21 of the dosing phase. The sponsor reported that the death was due to accidental cage injury.

Clinical signs: Clinical findings were limited to several male rats [Animal# B90995 (Group 5), B91009 (Group 6), and B95313 (Group 8)]. Group 8 was control animals. Other findings included skin and pelage abnormalities(See table 1). One female [Animal# B91065 (Group 6)] was observed with a swollen right hind paw during the last couple of days prior to the scheduled necropsy. These findings appear to be incidental.

Table 1
Summary of Clinical Signs - Groups 1 to 6

Category / Sign	Sex:	Males		
	Group:	1	2	3
	Dose Level:	3.70	0.00	0.00
	Dose Units:	mg/kg	mg/kg	mg/kg
	Number in Group:	10	10	10
		N	N	N
Appearance				
Swollen, Right Hind Paw		0	0	0
Behavior				
Sensitive To Touch		0	0	0
Skin & Pelage				
Sore/Scab, Front Paws		1	0	1
Sore/Scab, Left Front Paw		0	0	1
Sore/Scab, Right Hind Paw		0	0	0

N = Number of animals with observed sign

Body weights:
All animals showed a normal overall body weight gain. Initial body weight gains following dosing appeared slightly less than body weight gains in the following weeks (Table 6). In addition, body weight losses were noted in the last few days of the study. These changes were observed across all groups and considered stress-related activities such as blood glucose measurements or ophthalmic examinations. Hence, body weight losses were not considered test article-related. Body weights of Groups 7 through 12 males had similar patterns.

Table 6
Mean Body Weight Data - Groups 1 to 6

Test Article	Form. C	Form. B	Form. F	Form. A	Form. D	Form. A
Group	1	2	3	4	5	6
Level (IU/cm2/day)	3.70	0	0	3.70	3.70	1.85
Volume (uL/nostril)	32	32	32	32	32	16

		Mean body weights (g) for Group:					
Day		1M	2M	3M	4M	5M	6M
DSNG 1	Mean	282	283	279	283	280	280
	SD	14.0	13.6	17.0	12.8	14.5	15.7
	N	10	10	10	10	10	10
DSNG 4	Mean	295	292	285	291	289	292
	SD	15.6	12.3	17.9	12.3	15.3	16.8
	N	10	10	10	10	10	10
DSNG 8	Mean	317	311	301	310	311	314
	SD	18.2	13.3	23.2	13.7	18.6	19.7
	N	10	10	10	10	10	10
DSNG 11	Mean	326	324	315	321	325	330
	SD	19.8	16.6	23.9	17.1	21.2	21.6
	N	10	10	10	10	10	10
DSNG 15	Mean	341	337	327	337	341	348
	SD	22.7	17.0	26.3	17.0	23.8	23.1
	N	10	10	10	10	10	10
DSNG 18	Mean	351	348	335	347	352	358
	SD	24.7	18.1	24.7	20.9	26.2	24.5
	N	10	10	10	10	10	10
DSNG 22	Mean	365	363	352	362	367	375
	SD	25.5	21.0	28.1	22.1	28.0	27.6
	N	10	10	10	10	10	10
DSNG 25	Mean	372	368	360	365	372	383
	SD	25.3	23.2	26.3	20.9	30.6	26.6
	N	10	10	10	10	10	10
DSNG 29	Mean	350	349	338	344	352	361
	SD	24.5	24.1	26.3	21.3	28.5	25.1
	N	10	10	10	10	10	10

Table 6
Mean Body Weight Data
Groups 1 to 6

Test Article	Form. C	Form. B	Form. F	Form. A	Form. D	Form. A
Group	1	2	3	4	5	6
Level (IU/cm2/day)	3.70	0	0	3.70	3.70	1.85
Volume (uL/nostril)	32	32	32	32	32	16

		Mean body weights (g) for Group:					
Day		1F	2F	3F	4F	5F	6F
DSNG 1	Mean	200	197	198	201	198	199
	SD	12.4	12.3	14.5	14.0	11.9	11.4
	N	10	10	10	10	10	10
DSNG 4	Mean	207	201	203	206	205	206
	SD	12.7	13.7	11.1	13.6	13.0	11.8
	N	10	10	10	10	10	10
DSNG 8	Mean	217	213	214	219	216	220
	SD	13.9	15.2	13.3	15.0	13.0	12.6
	N	10	10	10	10	10	10
DSNG 11	Mean	226	218	223	227	225	230
	SD	10.8	12.0	14.6	16.4	12.9	16.3
	N	10	10	10	10	10	10
DSNG 15	Mean	235	228	231	235	236	238
	SD	11.4	12.1	15.3	19.5	11.7	19.4
	N	10	10	10	10	10	10
DSNG 18	Mean	239	229	233	239	238	244
	SD	10.7	12.4	14.9	19.6	12.8	22.0
	N	10	10	10	10	10	10
DSNG 22	Mean	246	238	240	246	245	250
	SD	11.9	11.4	16.5	21.7	11.1	19.8
	N	10	10	10	10	9	10
DSNG 25	Mean	248	237	241	245	247	249
	SD	12.4	12.5	17.2	20.2	14.0	18.7
	N	10	10	10	10	9	10
DSNG 29	Mean	229	221	226	232	230	234
	SD	9.0	14.5	16.8	19.2	14.2	15.8
	N	10	10	10	10	9	10

Food consumption: There were no remarkable differences between the control and treated groups.
Ophthalmology: There were no treatment-related changes in the parameters.
Urinalysis: There were no significant changes in urine volumes, specific gravity or urine pH after the treatment.
Hematology:
There were no remarkable hematological effects in control and treated groups in both males and females. An exception would be eosinophils (EOS) levels that were elevated in male groups 4 and 6, which appears to be treatment-related because the rats in Groups 4 and 6 received insulin at doses of 3.7 and 1.95 IU per square cm per day. However, it is difficult to establish the causal relationship between the treatment and the observation because they are not dose-related.

Blood glucose:
On Day 1 glucose levels in groups 7-12 were evaluated in 30, 60, 90 , 120, 180 and 240 minutes after treatments (fig. 1 and 2). Formulations A (Groups 10 and 12) and D (Group 11) markedly lowered blood glucose levels in both sexes. The peak effects were observed in 60 to 90 minutes after the administration in both sexes. As expected, there were no glucose lowering effects in control groups (8 and 9). The sponsor repeated the same procedures on Day 23 in groups 1-6 in males and females. Similar decreases in glucose were noted in all treated groups except two control groups (2 and 3) as shown (fig. 3 and

4). However, it appears that the hypoglycemic effects were slightly less and the duration of insulin action was shortened in both males and females.

Figure 1
Mean (± SD) Blood Glucose Levels (mg/dL) - Males - Day 1 Repeat

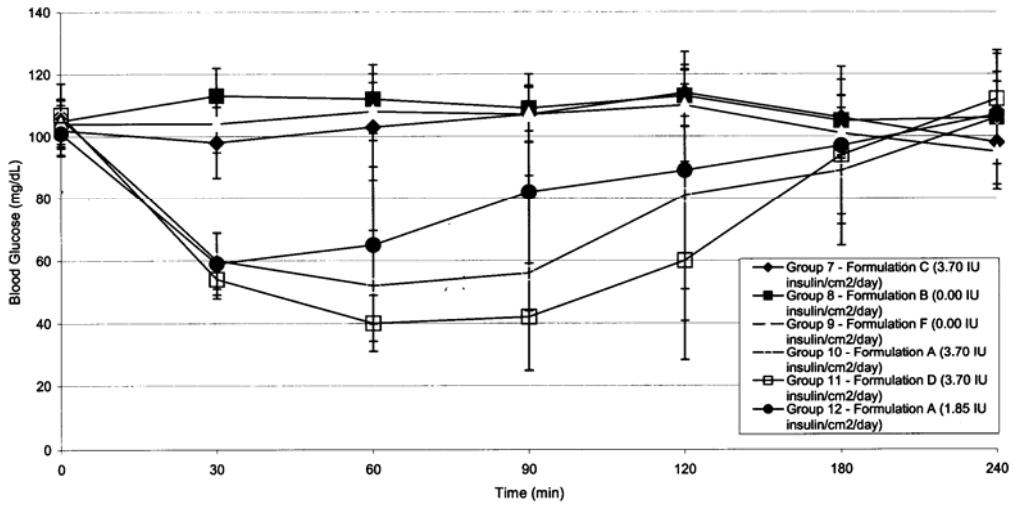


Figure 2
Mean (± SD) Blood Glucose Levels (mg/dL) - Females - Day 1 Repeat

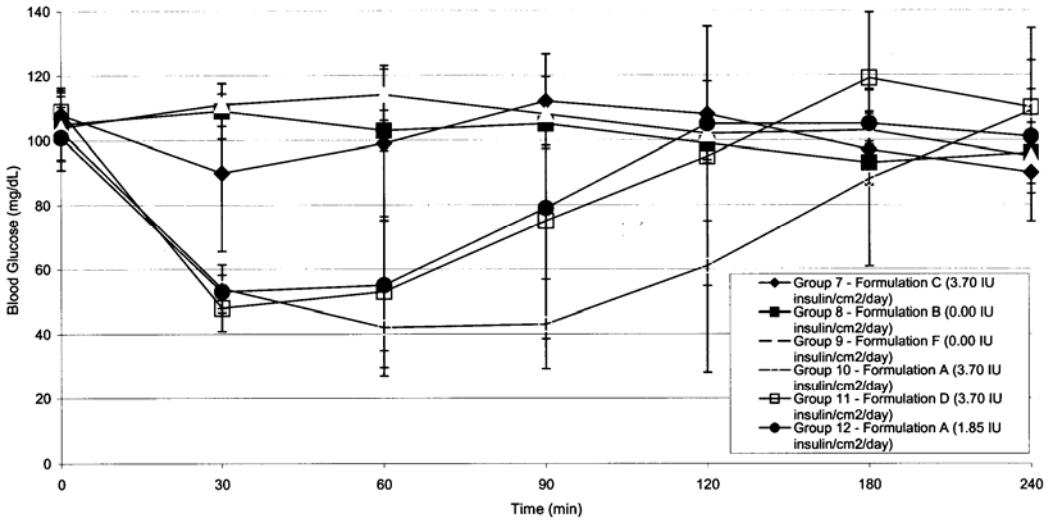


Figure 3
Mean (± SD) Blood Glucose Levels (mg/dL) - Males - Day 23

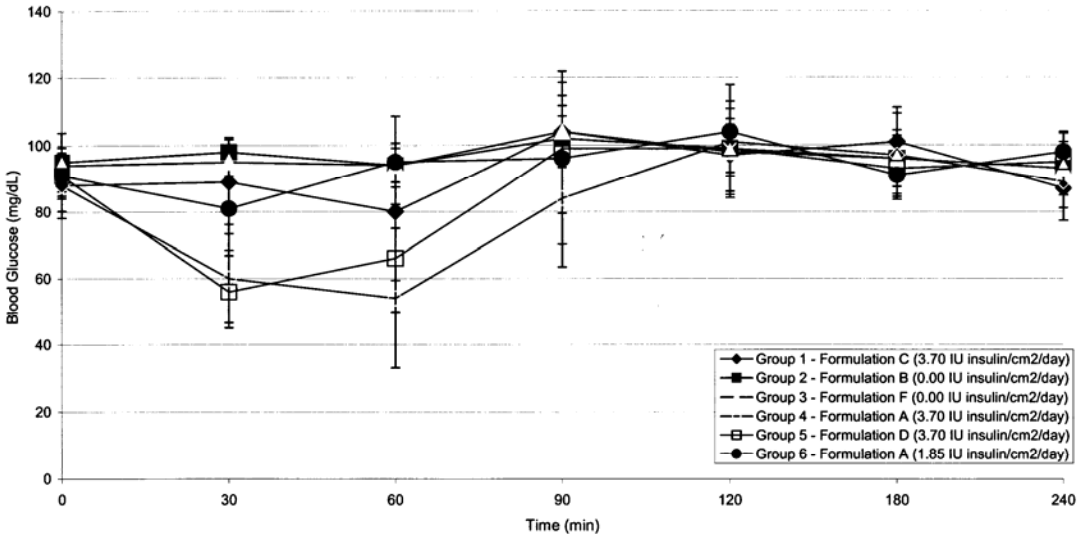
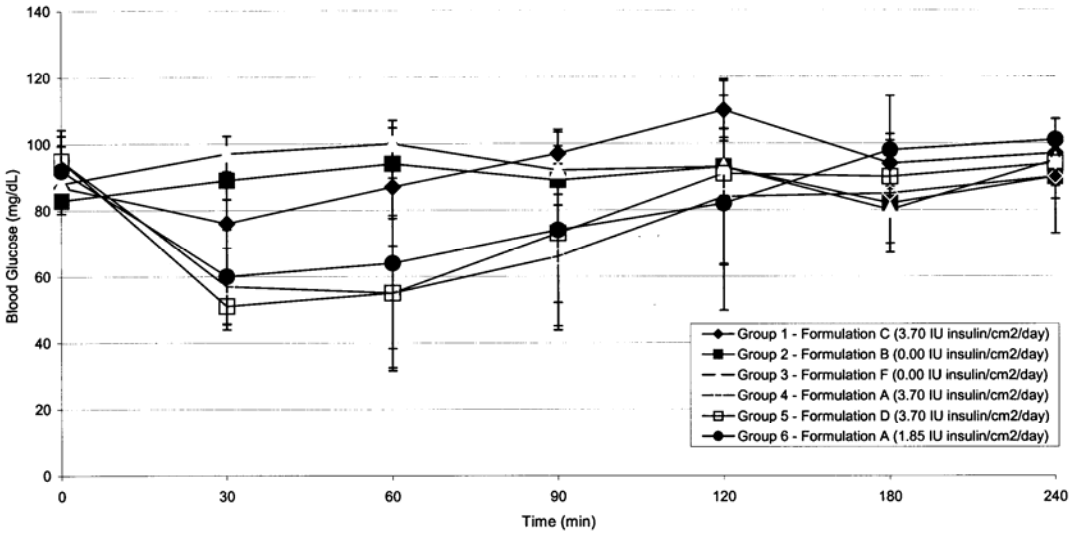


Figure 4
Mean (± SD) Blood Glucose Levels (mg/dL) - Females - Day 24



Clinical chemistry:

ALT levels were also elevated in males of groups 4, 5, and 6, which received insulin at doses of 3.7 or 1.85 IU per square cm per day (Table 11). The elevations of ALT levels have to do with the treatment because all the treated groups had the high values, although more studies need to define the mechanism. Serum potassium levels were reduced in female Group 4 rats. There were no other statistically significance changes in male and female rats after the treatment of placebo or insulin.

Table 11
Mean Clinical Chemistry Data

Test Article	Form. C	Form. B	Form. F	Form. A	Form. D	Form. A
Group	1	2	3	4	5	6
Level (IU/cm2/day)	3.70	0	0	3.70	3.70	1.85
Volume (uL/nostril)	32	32	32	32	32	16

Group/ Sex		GLU mg/dL	UN mg/dL	CREA mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	AGR
1F	Mean	94	15	0.6	7.2	5.0	2.2	2.3
	SD	6.8	2.0	0.04	0.41	0.31	0.18	0.17
	N	10	10	10	10	10	10	10
2F	Mean	96	15	0.6	7.2	5.0	2.2	2.3
	SD	4.7	2.2	0.04	0.23	0.24	0.29	0.37
	N	10	10	10	10	10	10	10
3F	Mean	95	15	0.6	7.3	5.1	2.3	2.2
	SD	12.6	1.6	0.04	0.43	0.42	0.14	0.23
	N	10	10	10	10	10	10	10
4F	Mean	94	15	0.7 ABC	7.5	5.2	2.4	2.2
	SD	12.3	1.8	0.08	0.42	0.32	0.28	0.27
	N	10	10	10	10	10	10	10
5F	Mean	93	14	0.6	7.2	5.0	2.2	2.3
	SD	18.4	1.8	0.07	0.27	0.19	0.28	0.32
	N	9	9	9	9	9	9	9
6F	Mean	96	13	0.6	7.1	4.8	2.3	2.1
	SD	7.8	2.2	0.07	0.36	0.68	0.48	0.51
	N	10	10	10	10	10	10	10

A Statistically significant from Group 1 at $p < 0.05$.
B Statistically significant from Group 2 at $p \lesssim 0.05$.
C Statistically significant from Group 3 at $p \lesssim 0.05$.

Table 11
Mean Clinical Chemistry Data

Test Article	Form. C	Form. B	Form. F	Form. A	Form. D	Form. A
Group	1	2	3	4	5	6
Level (IU/cm2/day)	3.70	0	0	3.70	3.70	1.85
Volume (uL/nostril)	32	32	32	32	32	16

Group/ Sex		PHOS mg/dL	Na mmol/L	K mmol/L	Cl mmol/L
1F	Mean	8.5	140	5.8	101
	SD	0.92	1.5	0.23	0.8
	N	10	10	10	10
2F	Mean	8.3	141	5.6	102
	SD	1.19	1.5	0.22	1.6
	N	10	10	10	10
3F	Mean	8.0	141	5.4	102
	SD	0.94	1.3	0.32	2.1
	N	10	10	10	10
4F	Mean	7.9	140	5.2 AB	100
	SD	0.96	3.0	0.48	2.0
	N	10	10	10	10
5F	Mean	8.3	141	5.6	102
	SD	0.67	2.2	0.33	2.1
	N	9	9	9	9
6F	Mean	8.3	139	5.5	101
	SD	0.92	1.7	0.42	1.9
	N	10	10	10	10

A Statistically significant from Group 1 at $p \leq 0.05$.
B Statistically significant from Group 2 at $p \leq 0.05$.

Gross pathology:

Anatomic pathology results showed treatment-related microscopic changes in the nasal turbinates, lung, and trachea. In the nasal turbinates, changes were largely restricted to the anterior portions of the nasal cavity; most of the changes were present in Level I, with a few changes in Level II. The most common change was an increase in the number of goblet cells. All of the changes in the nasal turbinates are indicative of minimal irritation to the nasal epithelium. In the trachea, minimal eosinophilic droplets were observed in the epithelium. Two male rats in group 6 had discolored lung, which had insulin. One female in group 1 had also discolored lung while another female rat in group 6 had a large foot pad (See table 13).

Table 13
Summary of Macroscopic Observations - Dosing Phase Final Phase Sacrifice

	-- Males --						-- Females --					
Group:	1	2	3	4	5	6	1	2	3	4	5	6
Number in group:	10	10	10	10	10	10	10	10	10	10	10	10
Examined/No remarkable findings ...	10	10	10	10	10	8	9	10	10	10	10	9
Spleen												
Discolored	0	0	0	0	0	0	0	0	0	0	0	1
Total:	0	0	0	0	0	0	0	0	0	0	0	1
Lung												
Discolored	0	0	0	0	0	2	1	0	0	0	0	0
Total:	0	0	0	0	0	2	1	0	0	0	0	0
Foot/Foot Pad												
Large	0	0	0	0	0	0	0	0	0	0	0	1
Total:	0	0	0	0	0	0	0	0	0	0	0	1

Histopathology:

Epithelial eosinophilic droplets were observed in treated males while the same droplets were also observed in untreated female rats (Groups 2 and 3). Similarly pulmonary alveolar macrophages were seen in treated as well as untreated while they were observed in only untreated female groups (Table 16). Increases in sinus goblet cells were significantly higher in the treated groups in males, which were also observed in untreated control in females. Thus, certain microscopic observations were common in treated male groups that may be sex specific. However, the incidences were not frequent and their dose dependency is difficult to establish due to the fact that only two concentrations of insulin were used.

Table 16
Summary of Microscopic Observations - Dosing Phase - Final Phase Sacrifice

Controls from group(s): 1

Tissues With Diagnoses	Animal sex:	-- Animals Affected -- Males --						-- Females --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
	No. in group:	10	10	10	10	10	10	10	10	10	10	10	10
Trachea	Number examined:	10	10	10	10	10	10	10	10	10	10	10	10
	Unremarkable:	10	10	10	6	10	8	10	9	9	8	10	10
Eosinophilic Droplets, Epithelium		0	0	0	4	0	2	0	1	1	2	0	0
Esophagus	Number examined:	10	10	10	10	10	10	10	10	10	10	10	10
	Unremarkable:	10	10	10	10	10	10	10	10	10	10	10	10
Lung	Number examined:	10	10	10	10	10	10	10	10	10	10	10	10
	Unremarkable:	9	9	10	10	9	7	8	8	6	8	10	10
Alveolar Macrophages		1	1	0	0	1	2	1	1	1	0	0	0
Alveolitis, Subacute		0	0	0	0	0	2	1	2	3	2	0	0
Hemorrhage, Alveolar		0	0	0	0	0	2	0	0	0	0	0	0
Edema, Alveolar		0	0	0	0	0	2	0	0	0	0	0	0
Congestion, Alveolar		0	0	0	0	0	1	0	0	0	0	0	0
Infiltration, Eosinophils, Perivascular		0	0	0	0	0	0	1	0	0	0	0	0
Nerve, Optic	Number examined:	10	10	10	10	10	10	10	10	10	10	10	9
	Unremarkable:	10	10	10	10	10	10	10	10	10	10	10	9
Eye	Number examined:	10	10	10	10	10	10	10	10	10	10	10	10
	Unremarkable:	9	10	10	9	10	10	10	10	10	10	10	10
Retinal Rosette		1	0	0	1	0	0	0	0	0	0	0	0
Nasal Turb/Sinus	Number examined:	10	10	10	10	10	10	10	10	10	10	10	10
	Unremarkable:	10	10	10	9	3	7	10	8	5	5	4	3
Level I: Increased Goblet Cells		0	0	0	1	7	3	0	2	5	5	5	7
Level II: Increased Goblet Cells		0	0	0	0	1	0	0	1	1	0	1	4
Level I: Infiltrate, Neutrophils, Lumen		0	0	0	0	1	0	0	0	0	0	0	0
Death Comment	Number examined:	10	10	10	10	10	10	10	10	10	10	10	10
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
Scheduled Sacrifice		10	10	10	10	10	10	10	10	10	10	9	10
Cage Accident		0	0	0	0	0	0	0	0	0	0	1	0
Foot/Foot Pad	Number examined:	0	0	0	0	0	0	0	0	0	0	0	1
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
Inflammation, Acute with Serous Exudate		0	0	0	0	0	0	0	0	0	0	0	1
Inflammation, Chronic Active		0	0	0	0	0	0	0	0	0	0	0	1

All Diagnoses; Phases: All; Death types: All; Date of death range: 04.Jul.05 To 12.Jul.05

Organ Weights:

Increased lung weights in males given Formulation A at 1.85 IU insulin per square cm per day (Group 6) are likely associated with the macroscopic and microscopic lesions noted in the lungs in this group (Table 14). Two Group 6 males (Animal# B91005 and B91006) had discolored lungs. Microscopic changes in these two animals consisted of minimal to slight alveolar macrophages, minimal subacute alveolitis, slight alveolar hemorrhage, and slight alveolar edema. These changes are likely to be associated with dosing procedures since neither of these two animals had changes in the nasal turbinates or trachea. The ratios (%) of parathyroid were significantly reduced in males of group 5 and 6, which were treated insulin at doses of 3.7 and 1.85 IU per square cm per day (Table 14).

Table 14
Mean Organ Weight and Organ/Terminal Body Weight Data

Test Article		Form. C	Form. B	Form. F	Form. A	Form. D	Form. A
Group		1	2	3	4	5	6
Level (IU/cm2/day)		3.70	0	0	3.70	3.70	1.85
Volume (uL/nostril)		32	32	32	32	32	16
Group/ Sex		Body weight (g)	Kidney Unadjusted (g)	Kidney Ratio (%)	Lung Unadjusted (g)	Lung Ratio (%)	Spleen Unadjusted (g)	Spleen Ratio (%)
1M	Mean	345.9200	2.6481	0.7662	1.4914	0.4322	0.7838	0.2262
	SD	25.11608	0.24511	0.05568	0.10836	0.03231	0.10729	0.02219
	N	10	10	10	10	10	10	10
2M	Mean	345.5800	2.6581	0.7703	1.4898	0.4321	0.7581	0.2189
	SD	24.17813	0.24542	0.06598	0.06449	0.02044	0.10956	0.02312
	N	10	10	10	10	10	10	10
3M	Mean	333.5400	2.5979	0.7796	1.4584	0.4382	0.6693	0.2004
	SD	26.40506	0.23407	0.04792	0.11848	0.03170	0.12271	0.03012
	N	10	10	10	10	10	10	10
4M	Mean	339.7100	2.7021	0.7956	1.5048	0.4440	0.7470	0.2194
	SD	21.62768	0.24775	0.05844	0.12328	0.03887	0.10764	0.02423
	N	10	10	10	10	10	10	10
5M	Mean	348.2000	2.5963	0.7467	1.4777	0.4249	0.8086	0.2325
	SD	29.19874	0.20645	0.03686	0.11293	0.01716	0.32901	0.09651
	N	10	10	10	10	10	10	10
6M	Mean	357.4000	2.6897	0.7524	1.7040 ABC	0.4804	0.7607	0.2128
	SD	25.48564	0.25697	0.04311	0.20843	0.07989	0.12175	0.03019
	N	10	10	10	10	10	10	10

A Statistically significant from Group 1 at $p \leq 0.05$.
B Statistically significant from Group 2 at $p \leq 0.05$.
C Statistically significant from Group 3 at $p \leq 0.05$.

Table 14
Mean Organ Weight and Organ/Terminal Body Weight Data

Test Article	Form. C	Form. B	Form. F	Form. A	Form. D	Form. A
Group	1	2	3	4	5	6
Level (IU/cm2/day)	3.70	0	0	3.70	3.70	1.85
Volume (uL/nostril)	32	32	32	32	32	16

Group/ Sex		Body weight (g)	Testis Unadjusted (g)	Testis Ratio (%)	Thyroid/ Parathyr Unadjusted (g)	Thyroid/ Parathyr Ratio (%)	Gl, Mandib Salivary Unadjusted (g)	Gl, Mandib Salivary Ratio (%)
1M	Mean	345.9200	3.3469	0.9713	0.0217	0.0063	0.6570	0.1910
	SD	25.11608	0.22566	0.08851	0.00477	0.00129	0.05356	0.02205
	N	10	10	10	10	10	10	10
2M	Mean	345.5800	3.1831	0.9255	0.0240	0.0070	0.6608	0.1916
	SD	24.17813	0.14880	0.08299	0.00668	0.00200	0.05974	0.01730
	N	10	10	10	10	10	10	10
3M	Mean	333.5400	3.1658	0.9551	0.0267	0.0081	0.6048	0.1813
	SD	26.40506	0.14160	0.09260	0.00528	0.00187	0.07258	0.01485
	N	10	10	10	10	10	10	10
4M	Mean	339.7100	3.2212	0.9489	0.0240	0.0071	0.6311	0.1859
	SD	21.62768	0.32674	0.08548	0.00518	0.00168	0.06611	0.01663
	N	10	10	10	10	10	10	10
5M	Mean	348.2000	3.2735	0.9446	0.0217	0.0063 C	0.6575	0.1897
	SD	29.19874	0.21781	0.08502	0.00245	0.00095	0.08651	0.02724
	N	10	10	10	10	10	10	10
6M	Mean	357.4000	3.2841	0.9209	0.0201	0.0056 C	0.6581	0.1845
	SD	25.48564	0.17924	0.04682	0.00491	0.00123	0.07520	0.01985
	N	10	10	10	10	10	10	10

C Statistically significant from Group 3 at $p \leq 0.05$.

Toxicokinetic Study:

No TK data are available.

Summary and Conclusion:

One rats from group 5 died, which was due to accidental cage injury, according to the sponsor. Clinical findings were sensitive to touch during dosing and skin pelage. One female has a swollen right hind paw a few days before the scheduled necropsy, which appears to be incidental.

The test article had no effects on food consumption, body weight, ophthalmic and urine examinations. Treatment-related decreases in glucose were observed in male and female animals. The sponsor failed to provide PK and TK data so that it is impossible to correlate the tachyphylactic effect with reduced blood level of insulin in this study.

The test article increased in ALT levels. Repetitive stimulation on nasal passages may increase eosinophils in epithelium as seen in gross histopathology examination, which was confirmed in microscopic histopathologic demonstration of eosinophils, alveolar macrophage, and sinus globlets. NOAEL = 1.85 IU insulin/cm^2 /day. In future studies PK and TK data must be provided and histopathological changes in the nasal passages should be monitored regularly in clinic, which should be included in investigators brochures.

Study title: 13-Week Intranasal Instillation Toxicity Study with Insulin in Rats with a 4- Week Recovery

Key study findings:
The 13-week toxicology study in rats did not produce any new findings from the 4-week study in the same species. Three-times-daily intranasal administration of Test Formulation A for 13 weeks was well-tolerated and caused no remarkable toxic effects at doses up to 3.70 IU/cm^2/day. Test Formulation A (b) (4) % CPE-215) did cause an expected pharmacologic effect to briefly decrease blood glucose, but the magnitude of this effect was decreased after 13 weeks of treatment. An observation of tachyphylaxis to glucose lowering effect is seen.

Study no.: Study #7639-102
Volume #: 7, and page #:1-297
Conducting laboratory and location: (b) (4)
Date of study initiation: December, 2005
GLP compliance: Yes
QA report: yes (x) no ( ), but no signatures
Drug, lot #, and % purity: Lot#: 1110279 (the Lot # for formulation C was 1110280)

Methods

Doses: 0.96 or 1.92 mg/rat/day or 25.92 or 51.84 IU/rat/day, which were equivalent to 0.64 or 1.28 mg/kg/dose/nostril

Species/strain: Rat/Crl:CD(SD)

Number/sex/group or time point (main study): 10-15 rats/sex/group for formulation A

Route, formulation, volume, and infusion rate: Nasal instillation in a volume of 16-32 µL/nostril, TID. See table below for formulations.

Satellite groups used for toxicokinetics or recovery: 10 rats/sex/group that were used for blood samples collection before necropsy.

Age: 42-51 days old

Weight: Males: 204-275 g; Females: 166-224 g

Sampling times: NA

Unique study design or methodology (if any): Intranasal instillation

Group	Treatment[a]	No. of Animals[d] Male	Female	Dose Volume[a] (µL/nostril)	Dose Level[b,c] mg/kg/dose/nostril (IU/kg/dose/nostril)	Nominal Dose Level[c] mg/rat/day (IU/rat/day)	mg/cm²/day (IU/cm²/day)
1	Control Formulation K - Control Article 1[e]	15	15	32	0	0	0
2	Control Formulation C - Control Article 2[f]	10	10	32	1.28 (34.56)	1.92 (51.84)	0.137 (3.70)
3	Control Formulation F - Control Article 3[g]	15	15	32	0	0	0
4	Test Formulation A	15	15	32	1.28 (34.56)	1.92 (51.84)	0.137 (3.70)
5	Test Formulation A	10	10	16	0.64 (17.28)	0.96 (25.92)	0.069 (1.85)

a All animals (except Group 5) were dosed 32 µL in each nostril. The dose volume of 32 µL was administered as 16-µL installments alternating between the right and left nostrils to achieve the target dose volume in each nostril. The dose volume for animals in Group 5 was 16 µL/nostril.

b Dosing occurred three times daily (at least 4 hours between each daily dose).

c Dose calculations were based on the assumption that the average weight of each rat was 250 g. Dose calculations were based on the rat nasal surface area of 14 cm² for a 0.25-kg rat (Schreider, 1983) and insulin concentration of 10 mg/mL or 270 IU/mL.

d Animals designated for recovery phase sacrifice (five animals/sex in Groups 1, 3, and 4) underwent 4 weeks of recovery following at least 13 weeks of dose administration.

e Formulation K: Buffered water adjusted to pH 3.5 ± 0.5.

f Formulation C: (b)(4) insulin, at pH 3.5 ± 0.5.

g Formulation F: (b)(4) CPE-215 at pH 3.5 ± 0.5.

Observation and Times:
Clinical signs: Twice daily
Body weights: Once predose phase and weekly thereafter
Food consumption: weekly
Ophthalmoscopy: Once predose phase, week 13 and 17 week after recovery
EKG: NA
Hematology: Before necropsy
Clinical chemistry: Before necropsy
Urinalysis: NA
Gross pathology: Indicated organs were weighted upon necropsy as shown below.
Organ weights (specify organs weighed if not in histopath table):
Histopathology: specify groups examined, special stains, etc
Adequate Battery: yes (x), no ( )—explain
Peer review: yes (x), no ( )
Toxicokinetics: Blood samples were collected a6 10. 20, 30, 45minutes, 1, 4, and 24 hours before necropsy for estimation of plasma insulin.

Results:

Mortality:
One male given 0.96 IU/day of Test Formulation A (Animal No. B54112) was sacrificed in a moribund condition on Day 80 of the dosing phase. The cause of death could not be determined, based on the macroscopic and microscopic pathology findings. Clinical signs included audible and labored respiration on the day of sacrifice, but no other abnormal signs had been seen. The death was not considered related to treatment with Test Formulation A at this dose level. All other animals survived to scheduled sacrifice.

Clinical signs:
A few abnormal clinical signs were observed at low frequency across all the groups, so that the sponsor was considered they are not related to test article.

Table 1
Summary of Clinical Signs - Dosing Phase

Category / Sign	Sex:	Males				
	Group:	1	2	3	4	5
	Dose Level:	0.00	3.70	0.00	3.70	1.85
	Dose Units:	(IU/sq cm/day)	(IU/sq cm/day)	(IU/sq cm/day)	(IU/sq cm/day)	(IU/sq cm/day)
	Number in Group:	15	10	15	15	10
		N	N	N	N	N
Appearance						
Convulsions		0	0	0	0	0
Malocclusion		1	0	0	2	0
Missing, Teeth		1	0	0	0	0
Excretion(s)						
Nonformed Feces		0	0	0	1	1
Eye(s)						
Protruding, Eyes		0	0	0	0	0
Mass						
Mass		0	0	1	0	0
Respiration						
Audible		0	0	0	0	1
Labored		0	0	0	0	1
Sacrifice						
Sacrifice Approved By, Certified Tech		0	0	0	0	1
Skin & Pelage						
Pale, Ears		0	0	0	0	0
Rough Haircoat		0	0	0	0	0
Scaly Skin, Tail		0	0	0	0	0

N = Number of animals with observed sign

Body weights:

No statistically significant differences were noted between the mean body weights of any of the groups. The body weight change of males given 1.85 IU/cm2/day was significantly higher than that of males in all three control groups during Week 7 of the dosing phase. The body weight change of males given 3.70 IU/ cm2/day was significantly higher than that of males in Group 1 or 3 during Weeks 1 and 4 of the recovery phase as well as over the full recovery phase. The body weight change of females given 3.70 IU/ cm2/day was significantly lower than that of females in Group 1 or 3 during Week 3 of the dosing phase but significantly higher than that of females in Group 1 or 2 during Week 11 of the dosing phase. Since these significant differences occurred sporadically, were both greater and less than those of controls, and did not result in any significant difference in the mean weights of these animals, the statistically significant differences were not considered biologically meaningful.

Table 5
Mean Body Weight Data - Dosing Phase

Test Article	Control K	Control C	Control F	Test Formulation A	
Group	1	2	3	4	5
Level (IU/sq cm/day)	0	3.7	0	3.7	1.85

Week		Mean body weights (g) for Group: 1M	2M	3M	4M	5M
DSNG 1	Mean	234	236	237	236	238
	SD	16.8	18.2	16.6	16.9	18.7
	N	15	10	15	15	10
DSNG 2	Mean	287	287	286	286	292
	SD	20.9	22.1	19.6	26.6	20.3
	N	15	10	15	15	10
DSNG 3	Mean	335	332	328	332	338
	SD	28.9	24.3	25.2	34.6	26.0
	N	15	10	15	15	10
DSNG 4	Mean	373	370	365	371	384
	SD	32.5	31.4	28.9	43.0	32.8
	N	15	10	15	15	10
DSNG 5	Mean	403	402	398	404	423
	SD	35.4	34.6	35.7	49.5	35.1
	N	15	10	15	15	10
DSNG 6	Mean	436	434	428	438	457
	SD	35.9	36.6	40.3	52.9	40.1
	N	15	10	15	15	10
DSNG 7	Mean	459	456	453	459	483
	SD	37.5	36.7	41.9	56.1	43.8
	N	15	10	15	15	10
DSNG 8	Mean	480	474	474	482	512
	SD	40.6	42.6	48.5	56.8	44.9
	N	15	10	15	15	10
DSNG 9	Mean	502	496	497	502	534
	SD	38.0	47.2	48.2	61.0	45.4
	N	15	10	15	15	10

Food consumption:

The food consumption of males given 3.70 IU/rat/day of the recovery phase was significantly higher than that of males in Group I during Week I. This does correspond to a significantly larger body weight change seen in these males during that same interval, but since a similar effect was not observed in the females, and no consequent statistically significant difference in body weights was noted, this food consumption difference was not considered biologically meaningful.

Table 9
Mean Food Consumption Data - Dosing Phase

Test Article	Control K	Control C	Control F	Test Formulation A
Group	1	2	3	4	5
Level (IU/sq cm/day)	0	3.7	0	3.7	1.85

Week		Mean food consumption (g/animal/period) for Group: 1M	2M	3M	4M	5M
DSNG 1	Mean	203	204	197	199	202
	SD	16.2	17.5	15.2	19.1	18.7
	N	15	10	15	15	10
DSNG 2	Mean	207	209	203	212	212
	SD	20.3	21.6	22.8	24.6	19.5
	N	15	10	15	15	10
DSNG 3	Mean	210	213	206	215	220
	SD	22.2	24.6	24.2	25.6	26.3
	N	15	10	15	15	10
DSNG 4	Mean	210	214	212	221	227
	SD	22.1	27.6	26.2	27.2	24.1
	N	15	10	15	15	10
DSNG 5	Mean	228	231	227	234	242
	SD	17.6	23.3	25.2	25.6	23.2
	N	15	10	15	15	10
DSNG 6	Mean	222	225	222	233	245
	SD	17.4	22.4	27.2	25.5	22.2
	N	15	10	15	15	10
DSNG 7	Mean	229	235	228	233	247
	SD	20.6	26.3	28.9	25.5	23.7
	N	15	10	15	15	10
DSNG 8	Mean	224	233	225	231	243
	SD	15.6	27.6	27.9	24.9	22.6
	N	15	10	15	15	10
DSNG 9	Mean	230	237	229	232	243
	SD	18.4	21.5	28.4	26.7	26.4
	N	15	10	15	15	10

Ophthalmoscopy: Retinal degeneration was seen during the dosing phase and recovery phase examinations in a few animals in each group (including the control groups), so this ophthalmologic lesion was not considered test article-related.

Table 3
Summary of Ophthalmic Observations - Dosing Phase

Category / Sign	Sex:	Males				
	Group:	1	2	3	4	5
	Dose Level:	0.00	3.70	0.00	3.70	1.85
	Dose Units:	(IU/sq cm/day)	IU/sq cm/day)	(IU/sq cm/day)	(IU/sq cm/day)	(IU/sq cm/day)
	Number in Group:	15	10	15	15	10
		N	N	N	N	N
Retina						
Retinal Degeneration-Focal, Eye-Left		1	0	0	1	0
Retinal Degeneration-Focal, Eye-Right		0	0	0	0	1

N = Number of animals with observed sign

EKG: Not determined.

Hematology:
Animals given Test Formulation A at either 1.85 or 3.70 IU/cm2/day had lower blood glucose at 30 and 60 minutes postdose during Week 1 but returned to normal baseline levels by 90 minutes postdose. The magnitude of the decrease in blood glucose was dose-related. A similar effect of Test Formulation A to decrease blood glucose at 30 minutes postdose was observed during Week 13 of the dosing phase, but the magnitude of the decrease was lower than during Week 1 and only evident in animals given 3.70 lU/cm^2/day.

Clinical chemistry:
Treatment effects on serum chemistry were not remarkable. Blood glucose lowering effects were also not impressive, although there were brief drops in serum glucose in both male and female rats as shown below. The glucose lowing effects were completed returned toward normal after a few hours after the administration.

Figure 3
Blood Glucose Levels (mg/dL) - Males - Week 13

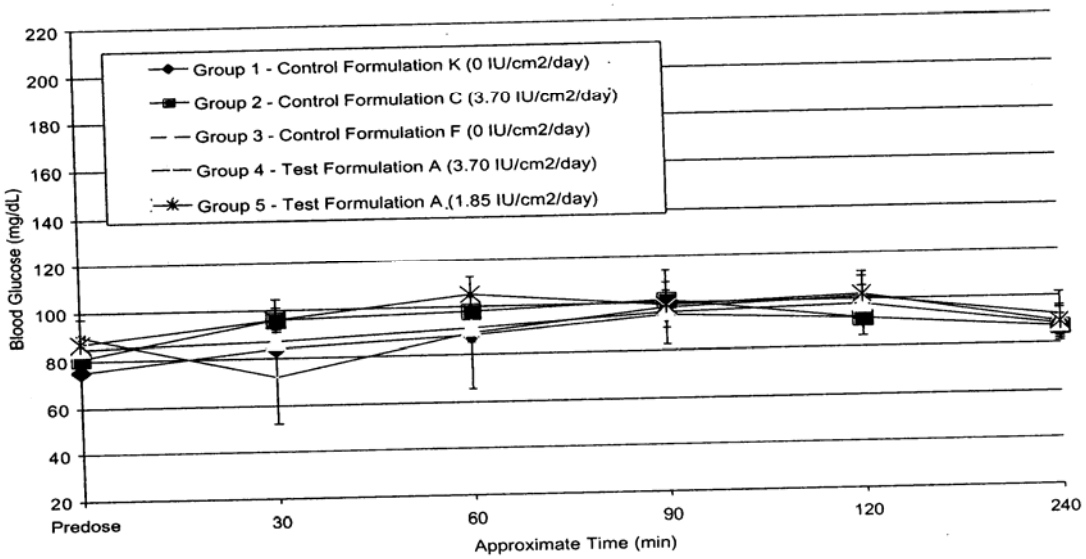
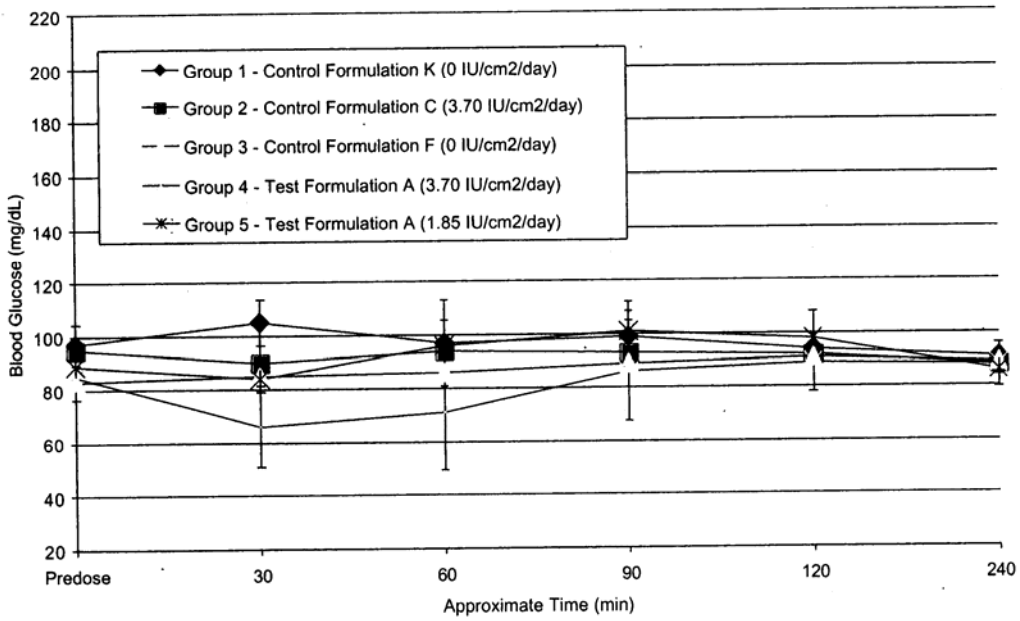


Figure 4
Blood Glucose Levels (mg/dL) - Females - Week 13



Urinalysis: NA

Gross pathology: There were no visible abnormalities noted during the pre study evaluation for any of the animals that developed retinal degeneration.

Table 15
Summary of Macroscopic Observations - Dosing Phase

	-- Males --					-- Females --				
Group:	1	2	3	4	5	1	2	3	4	5
Number in group:	10	10	10	10	10	10	10	10	10	10
Examined/No remarkable findings ...	9	10	10	8	9	9	9	10	10	10
Thymus										
Discolored	0	0	0	1	0	1	0	0	0	0
Total:	0	0	0	1	0	1	0	0	0	0
Kidney										
Large	1	0	0	0	0	0	0	0	0	0
Total:	1	0	0	0	0	0	0	0	0	0
Prostate										
Gelatinous	0	0	0	1	0	0	0	0	0	0
Large	0	0	0	1	0	0	0	0	0	0
Total:	0	0	0	2	0	0	0	0	0	0
Mammary, Female										
Confirmed mass	0	0	0	0	0	0	1	0	0	0
Total:	0	0	0	0	0	0	1	0	0	0
Cavity, Abdomin										
Mass	0	0	0	0	1	0	0	0	0	0
Total:	0	0	0	0	1	0	0	0	0	0

Organ weights (specify organs weighed if not in histopath table):
At the dosing and recovery phase final sacrifices, no test article-related terminal body or organ weight changes were seen. The few statistically significant absolute and relative organ weight changes present, along with all other weight differences seen in both sexes at the scheduled sacrifices. However, the changes were attributed to normal biological variation and not to test article administration.

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

Very few statistically significant or otherwise notable differences for clinical pathology test results were observed between the control groups (Groups I, 2, and 3) and groups receiving the test article (Groups 4 and 5). All of the differences were considered incidental because they were very small, not dose-related, and consistent with normal biological variation. Notably high aminotransferase activities for two females in the high-dose group (Group 4) were considered incidental because individual females in each of the control groups also exhibited high activities. The few microscopic findings present were considered unrelated to Test Formulation A administration.

No test article-related macroscopic or microscopic findings were seen at either scheduled sacrifice. The few gross observations present were considered spontaneous or incidental findings. Microscopically, a low incidence of inflammatory lesions was seen at various levels of the nasal turbinates and sinuses in animals from multiple dose groups, including animals that received one variety of the control article at the dosing phase final sacrifice. Because no convincing test article-related trends in the data were noted and because controls were sometimes similarly affected by most findings, all microscopic findings were considered spontaneous or treatment-related but not Test-article-related.

Table 21
Summary of Microscopic Observations - Dosing Phase

Controls from group(s): 1	Animal sex:	-- Animals Males --					Affected -- Females --				
Tissues With Diagnoses	Dosage group:	Ctls	2	3	4	5	Ctls	2	3	4	5
	No. in group:	10	10	10	10	10	10	10	10	10	10
Trachea	Number examined:	10	10	10	10	10	10	10	10	10	10
	Unremarkable:	10	10	10	10	10	10	10	9	10	10
Inflammation, Acute		0	0	0	0	0	0	0	1	0	0
Esophagus	Number examined:	10	10	10	10	10	10	10	10	10	10
	Unremarkable:	10	10	10	10	10	10	10	9	10	10
Inflammation, Acute		0	0	0	0	0	0	0	1	0	0
Lung	Number examined:	10	10	10	10	10	10	10	10	10	10
	Unremarkable:	6	5	5	5	7	6	5	9	8	10
Infiltrate, Macrophages, Alveolus		3	4	5	5	3	4	3	1	0	0
Inflammation, Subacute		1	1	0	0	0	0	0	0	2	0
Infiltrate, Macrophages, Pigmented		0	0	0	0	0	0	2	0	0	0
Hemorrhage/Hemoglobin Crystals		0	0	0	0	0	0	0	0	0	0
Eye	Number examined:	10	10	10	10	10	10	10	10	10	10
	Unremarkable:	10	9	10	10	8	10	10	10	10	10
Rosette, Retina		0	1	0	0	2	0	0	0	0	0
Atrophy, Retina		0	0	0	0	0	0	0	0	0	0
Nerve, Optic	Number examined:	10	10	10	10	10	10	10	10	9	10
	Unremarkable:	10	10	10	10	10	10	10	10	9	10
Nasal Turbinates	Number examined:	10	10	10	10	10	10	10	10	10	10
	Unremarkable:	8	9	8	6	7	9	8	5	6	7
Level I: Inflammation, Subacute		1	0	2	2	1	0	1	3	1	3
Level II: Inflammation, Subacute		1	1	0	3	2	0	1	3	2	1
Level III: Inflammation, Subacute		0	0	0	0	2	1	0	3	2	0
Level III: Exudate, Suppurative, with Associated Fungal -Elements		0	0	0	0	0	0	0	0	0	0
Nasal Sinus	Number examined:	10	10	10	10	10	10	10	10	10	10
	Unremarkable:	7	8	9	7	8	7	9	10	9	8
Inflammation, Subacute		1	1	0	0	1	2	1	0	0	1
Exudate, Serous, Lumen		2	1	1	3	1	2	1	0	1	2
Nares	Number examined:	6	8	8	5	9	8	9	9	10	9
	Unremarkable:	6	8	8	5	8	8	9	9	10	9
Exudate, Serous		0	0	0	0	1	0	0	0	0	0

All Diagnoses; Phases: P2; Death types: All; Date of death range: 26.Feb.06 To 15.Mar.06

Toxicokinetics: The sponsor did not provide data.

Study title: 13-Week Intranasal Instillation Toxicity Study with Insulin in Dogs with a 4-Week Recovery

Key study findings: Three to five beagle dogs/sex/group were given either Formulation K (buffered water), Formulation C (b)(4)% insulin/1.76 IU/cm^2/day), or (b)(4)% CPE-215 (Formulation F) as control. The test article groups were administered the test article, (b)(4)% insulin (b)(4)% CPE-215 (Formulation A), at 1.32 or 1.76 IU/cm^2/day. Doses were administered three times daily. All animals survived to their scheduled sacrifice. There were no significant differences between the control groups and treated group in clinical signs. The high dose group animals (1.76 IU/cm^2/day of Formulation A) had tremors, ataxia, hypoactivity, and lateral recumbency due to presumable insulin action, which required Karo syrup treatment for a few HD animals. No treatment-related clinical signs were observed during the recovery phase. Males given Formulation A at 1. 761U/cm2/day and females given Formulation A at 1.32 or 1.76 IU/cm2/day had significantly greater body weight gain, with more food consumption, during the first week of the dosing phase when compared with each of the three vehicle control groups. Blood glucose declined for up to 4 hours after dosing with Formulation A at 1.32 or 1.76 IU/cm^2/day. The effect lasted longer in the females. The animals given Formulation A at 1.76 IU/cm^2/day tended to have a slightly larger blood glucose depression compared with the animals given Formulation A at 1.32 IU/cm^2/day. The effect was strongest on Day 1 of the dosing phase, appeared to be slightly less pronounced at Week 6 of the dosing phase, and did not appear to cause an effect in males at Week 13 of the dosing phase, although females still showed the blood glucose depression at this interval. Administration of the test article had no remarkable effect on clinical pathology test, organ weight and mean terminal body weight after recovery phase.

Study no.: Study #7639-103
Volume #: 9 page 42-241; Vol. 10 page #:1-273
Conducting laboratory and location: (b) (4)
Date of study initiation: After 3/30/3006 before 7/12/2006
GLP compliance: Yes
QA report: yes (x) no ( ), but no signatures
Drug, lot #, and % purity: Lot#: 1110279 (b)(4)% insulin and (b)(4)% CPE-215, which is comparable to the clinical formulation. Lot # for formulation C was 1110280, which had just (b)(4)% insulin)

Methods

Doses: (b)(4)% insulin (b)(4)% CPE-215 (Formulation A) at 1.32 or 1.76 IU/cm^2/day.
Species/strain: Dog/Beagle
Number/sex/group or time point (main study): 3-5 dogs/sex/group
Route, formulation, volume, and infusion rate: Please see the two tables below.
Satellite groups used for toxicokinetics or recovery: None.
Age: Not listed
Weight: Males: 10 kg; Females: 8.6 kg
Sampling times: NA

Unique study design or methodology (if any): Intranasal instillation

Formulation A had (b)(4)% insulin and (b)(4)% CPE-215.
Formulation C had (b)(4)% insulin as an insulin control.
Formulation F had (b)(4)% CPE-215 as an enhancer control.
Formulation K had buffered water, pH=3.5.

Test Article	Lot No.	Storage	Purity	Expiration Date	Reserve (Archive) Sample
Formulation A: (b)(4)% insulin, (b)(4)% CPE-215	1110279	In a refrigerator, set to maintain 2 to 8°C	To be provided	To be provided	Collected

The control articles provided by the sponsor were Formulation C and Formulation F. The control articles were supplied by the sponsor as follows.

Control Article	Supplier	Lot/Batch No.	Storage	Purity	Expiration Date	Reserve (Archive) Sample
Formulation C: (b)(4)% insulin	Sponsor	1110280	In a refrigerator, set to maintain 2 to 8°C	To be provided	To be provided	Collected
Formulation F: % CPE-215	Sponsor	1110281	In a refrigerator, set to maintain 2 to 8°C	To be provided	01 Oct 2006	Collected

Control Article 1, Formulation K: Buffered water adjusted to pH 3.5 ± 0.5 was prepared by (b) (4) and stored in a refrigerator, set to maintain 2 to 8°C. A reserve (archive) sample was not collected for this control article.

Test System and Study Design

Male and female purebred beagles were received from [redacted] (b) (4) Animals were assigned to study groups as follows.

Group	Treatment[a]	No. of Animals[a] Male	Female	Dose Volume (µL/nostril)	Dose Level[b] mg/kg/dose/nostril (IU/kg/dose/nostril)	Nominal Dose Level[b,c] mg/dog/day (IU/dog/day)	mg/cm²/day (IU/cm²/day)
1	Formulation K - Control Article 1	5	5	240[b]	0	0	0
2	Formulation C - Control Article 2	3	3	240[b]	0.240 (6.5)	14.4 (390)	0.065 (1.76)
3	Formulation F - Control Article 3	5	5	240[b]	0	0	0
4	Formulation A - Low	3	3	180[b]	0.180 (4.9)	10.8 (292)	0.049 (1.32)
5	Formulation A - High	5	5	240[b]	0.240 (6.5)	14.4 (390)	0.065 (1.76)

Dosing occurred three times daily (at least 4 hours between each daily dose).

a Three animals/sex/group (based on survival) were sacrificed after at least 13 weeks of treatment; the remaining two animals/sex (based on survival) in Groups 1, 3, and 5 were sacrificed after at least 13 weeks of treatment and at least 4 weeks of recovery.

b Except for Group 4 animals, animals in all other groups were dosed 240 µL in each nostril. The dose volume of 240 µL was administered as 120-µL installments alternating between the right and left nostrils to achieve the target dose volume in each nostril. Group 4 animals were dosed with one installment of 120 µL followed by another installment of 60 µL in each nostril.

c Dose selection calculations were based on the assumption that the average weight of each dog is 10 kg. Dose selection calculations were based on the dog nasal surface area of 221 cm² for a 10-kg dog (Gizurarson, S., 1990) and insulin concentration of 10 mg/mL or 270 IU/mL.

Observation and Times:

Clinical signs: Twice daily

Body weights: Once predose phase and weekly thereafter

Food consumption: weekly

Ophthalmoscopy: Once predose phase, week 13 and 17 week after recovery

EKG: No statement

Hematology: Once predose phase, prior to dosing on Day 1 dosing phase, during Weeks 6 and 13, and during week 4 of the recovery phase

Clinical chemistry: Once predose phase, prior to dosing on Day 1 dosing phase, during Weeks 6 and 13 and during week 4 of the recovery phase

Urinalysis: No statement

Gross pathology: Indicated organs were weighted upon necropsy as shown below.

Organ weights (specify organs weighed if not in histopath table):

Histopathology: specify groups examined, special stains, etc

Adequate Battery: yes (x), no ( )—explain

Peer review: yes (x), no ( )

Toxicokinetics: Samples were taken on Day 92 of the dosing phase approximately 10, 20, 30, 45, and 60 minutes and 2, 4, and 24 hours postdose. Serum samples were analyzed by [redacted] (b) (4).

Results:

Mortality: It appears that there is no death.
Clinical signs:
Clinical signs such as excretion of loose stool were observed all male and female dogs except male that had (b)(4)% CPE-215(Formulation F). Skin scabs and eye discharge were also noted without clear pattern as shown below. There might be slightly increased incidences of hypoactivity, nasal snorting, face pressing and recumbent positions in the HD groups, which may be related to insulin dose as a result of insulin spray. Such responses were observed from the animals during the recovery period.

Table 1
Summary of Clinical Signs - Predose Phase

Category / Sign	Sex:	Males				
	Group:	1	2	3	4	5
	Dose Level:	0.0	1.76	0.0	1.32	1.76
	Dose Units:	IU/sq cm/day	IU/sq cm/day	IU/sq cm/day	IU/sq cm/day	IU/sq cm/day
	Number in Group:	5	3	5	3	5
		N	N	N	N	N
Appearance						
Swollen, Muzzle		0	0	0	0	1
Discharge						
Appears to be Cycling		0	0	0	0	0
Vomitus, Containing Food		0	0	1	0	1
Excretion						
Discolored Feces, Red in Color		0	0	1	0	0
Liquid Feces		0	0	1	1	0
Mucoid Feces		0	0	1	1	0
No Feces		0	0	1	0	0
Nonformed Feces		2	0	2	1	1
Eyes						
Clear Discharge, Eyes		0	0	1	0	0
Clear Discharge, Right Eye		1	0	0	0	0
Skin & Pelage						
Red Skin, Muzzle		0	0	0	0	0
Scab(s), Muzzle		0	0	1	0	0
Scab(s), Ventral Neck		1	0	0	1	0

N = Number of animals with observed sign

Table 1
Summary of Clinical Signs
Predose Phase

Category / Sign	Females				
Sex:					
Group:	1	2	3	4	5
Dose Level:	0.0	1.76	0.0	1.32	1.76
Dose Units:	IU/sq cm/day	IU/sq cm/day	IU/sq cm/day	IU/sq cm/day	IU/sq cm/day
Number in Group:	5	3	5	3	5
	N	N	N	N	N
Appearance					
Swollen, Muzzle	0	0	0	0	0
Discharge					
Appears to be Cycling	0	1	0	0	1
Vomitus, Containing Food	0	1	1	0	0
Excretion					
Discolored Feces, Red in Color	0	1	0	0	0
Liquid Feces	0	0	1	1	1
Mucoid Feces	0	1	1	0	0
No Feces	0	0	0	0	0
Nonformed Feces	2	1	4	2	2
Eyes					
Clear Discharge, Eyes	0	1	0	0	0
Clear Discharge, Right Eye	1	0	0	0	1
Skin & Pelage					
Red Skin, Muzzle	0	0	1	0	0
Scab(s), Muzzle	0	0	0	0	0
Scab(s), Ventral Neck	1	0	2	1	1

N = Number of animals with observed sign

Table 2
Summary of Clinical Signs - Dosing Phase

Category / Sign	Males				
Sex:					
Group:	1	2	3	4	5
Dose Level:	0.0	1.76	0.0	1.32	1.76
Dose Units:	IU/sq cm/day	IU/sq cm/day	IU/sq cm/day	IU/sq cm/day	IU/sq cm/day
Number in Group:	5	3	5	3	5
	N	N	N	N	N
Appearance					
Cagesore, Hind Paws	0	0	1	0	0
Cagesore, Left Front Leg	0	0	0	1	0
Cagesore, Left Hind Paw	1	0	1	0	0
Cagesore, Right Hind Paw	0	0	1	0	0
Limited Use, Right Front Leg	0	0	0	0	1
Swollen, Vulva	0	0	0	0	0
Tremors, Entire Body	0	0	0	0	0
Behavior					
Ataxic	0	0	0	0	0
Barrel Rolling	0	0	0	0	0
Face Pressing	0	0	1	1	1
Face Pressing, Less than 1 minute	0	0	0	2	1
Face Pressing, Greater than 1 minute	0	0	1	0	0
Face Pressing, Less than 2 minutes	0	0	1	0	0
Hypoactive	0	0	0	0	2
Recumbent, Lateral	0	0	0	0	0
Nasal Snorting	0	0	0	0	0
Nasal Snorting, Intermittent	0	0	0	1	0
Nasal Snorting, Less than 1 minute	0	0	3	1	1
Nasal Snorting, Less than 1 minute, All Digit(s)	0	0	0	0	0
Nasal Snorting, Greater than 1 minute	0	0	3	1	4
Nasal Snorting, Less than 2 minutes	0	0	2	1	2
Nasal Snorting, Greater than 2 minutes	0	0	2	0	0

N = Number of animals with observed sign

Table 2
Summary of Clinical Signs
Dosing Phase

Category / Sign	Sex:	Females				
	Group:	1	2	3	4	5
	Dose Level:	0.0	1.76	0.0	1.32	1.76
	Dose Units:	IU/sq cm/day	IU/sq cm/day	IU/sq cm/day	IU/sq cm/day	IU/sq cm/day
	Number in Group:	5	3	5	3	5
		N	N	N	N	N
Appearance						
Cagesore, Hind Paws		0	0	0	0	0
Cagesore, Left Front Leg		0	0	0	0	0
Cagesore, Left Hind Paw		0	0	0	0	0
Cagesore, Right Hind Paw		0	0	0	0	0
Limited Use, Right Front Leg		0	0	0	0	0
Swollen, Vulva		0	0	1	1	0
Tremors, Entire Body		0	0	0	0	1
Behavior						
Ataxic		0	0	0	0	1
Barrel Rolling		0	0	2	0	2
Face Pressing		0	0	5	0	3
Face Pressing, Less than 1 minute		0	0	1	0	1
Face Pressing, Greater than 1 minute		0	0	0	0	2
Face Pressing, Less than 2 minutes		0	0	0	0	0
Hypoactive		0	0	0	0	2
Recumbent, Lateral		0	0	0	0	1
Nasal Snorting		0	0	0	0	1
Nasal Snorting, Intermittent		0	0	0	0	0
Nasal Snorting, Less than 1 minute		0	2	4	2	3
Nasal Snorting, Less than 1 minute, All Digit(s)		0	0	1	0	0
Nasal Snorting, Greater than 1 minute		0	1	3	1	2
Nasal Snorting, Less than 2 minutes		0	0	2	1	1
Nasal Snorting, Greater than 2 minutes		0	1	1	1	0

N = Number of animals with observed sign

Ophthalmic Observations:
There were no treatment related abnormalities in ophthalmic parameters.

Body Weights:
There were no significant differences in body weights of the three control and two treated groups in both males and females during the active treatment and after the end of recovery phase. However, mean body weight gain in the HD group (group 5 after 1.76 IU) within one week after the onset of the treatment in both male and female dogs as shown below (Tables 8).

Table 8
Mean Body Weight Change Data - Dosing Phase

Test Article	Formulation K	Formulation C	Formulation F	Formulation A	
Group	1	2	3	4	5
Level (IU/sq cm/day)	0	1.76	0	1.32	1.76

Day		Mean body weight gain (kg) for Group: 1M	2M	3M	4M	5M
DSNG 1- DSNG 8	Mean	0.5	0.4	0.3	0.5	0.8 A,B,C
	SD	0.12	0.12	0.10	0.32	0.20
	N	5	3	5	3	5
DSNG 8- DSNG 15	Mean	-0.1	0.2	0.1	0.1	0.0
	SD	0.15	0.10	0.26	0.06	0.17
	N	5	3	5	3	5
DSNG 15- DSNG 22	Mean	0.2	0.3	0.1	0.1	0.2
	SD	0.34	0.42	0.34	0.15	0.35
	N	5	3	5	3	5
DSNG 22- DSNG 29	Mean	0.3	0.1	0.1	0.2	-0.1
	SD	0.16	0.10	0.22	0.10	0.24
	N	5	3	5	3	5
DSNG 29- DSNG 36	Mean	-0.1	0.4	0.0	0.2	0.1
	SD	0.24	0.35	0.07	0.15	0.13
	N	5	3	5	3	5
DSNG 36- DSNG 43	Mean	0.1	0.0	-0.0	0.0	0.2
	SD	0.38	0.52	0.18	0.17	0.11
	N	5	3	5	3	5
DSNG 43- DSNG 50	Mean	0.1	0.1	0.2	0.0	0.0
	SD	0.10	0.26	0.29	0.10	0.26
	N	5	3	5	3	5
DSNG 50- DSNG 57	Mean	0.1	0.3	0.2	0.3	0.4
	SD	0.26	0.31	0.31	0.23	0.25
	N	5	3	5	3	5
DSNG 57- DSNG 64	Mean	0.1	0.2	0.1	-0.1	-0.1
	SD	0.18	0.12	0.17	0.21	0.13
	N	5	3	5	3	5
DSNG 64- DSNG 71	Mean	-0.2	-0.3	-0.2	-0.2	-0.1
	SD	0.08	0.15	0.18	0.12	0.13
	N	5	3	5	3	5

A Statistically significant from Group 1 at $p \leq 0.05$.
B Statistically significant from Group 2 at $p \leq 0.05$.
C Statistically significant from Group 3 at $p \leq 0.05$.

Table 8
Mean Body Weight Change Data
Dosing Phase

Test Article	Formulation K	Formulation C	Formulation F	Formulation A	
Group	1	2	3	4	5
Level (IU/sq cm/day)	0	1.76	0	1.32	1.76

Day		Mean body weight gain (kg) for Group: 1F	2F	3F	4F	5F
DSNG 1- DSNG 8	Mean	0.2	0.2	0.2	0.6 A,B,C	0.7 A,B,C
	SD	0.16	0.15	0.05	0.20	0.22
	N	5	3	5	3	5
DSNG 8- DSNG 15	Mean	-0.0	0.1	0.0	-0.0	0.1
	SD	0.38	0.15	0.16	0.06	0.19
	N	5	3	5	3	5
DSNG 15- DSNG 22	Mean	0.2	0.0	0.1	0.1	0.1
	SD	0.05	0.10	0.25	0.15	0.13
	N	5	3	5	3	5
DSNG 22- DSNG 29	Mean	-0.0	-0.0	0.0	0.0	-0.0
	SD	0.16	0.15	0.18	0.21	0.15
	N	5	3	5	3	5
DSNG 29- DSNG 36	Mean	0.2	-0.0	0.1	-0.1	0.1
	SD	0.19	0.20	0.16	0.17	0.26
	N	5	3	5	3	5
DSNG 36- DSNG 43	Mean	-0.1	0.1	0.1	0.0	0.0
	SD	0.27	0.21	0.47	0.10	0.24
	N	5	3	5	3	5
DSNG 43- DSNG 50	Mean	0.1	0.1	0.0	0.1	0.1
	SD	0.24	0.21	0.22	0.10	0.14
	N	5	3	5	3	5
DSNG 50- DSNG 57	Mean	0.3	0.1	0.2	0.2	0.1
	SD	0.04	0.23	0.11	0.35	0.27
	N	5	3	5	3	5
DSNG 57- DSNG 64	Mean	0.1	-0.1	0.1	0.0	0.2
	SD	0.18	0.06	0.25	0.10	0.27
	N	5	3	5	3	5
DSNG 64- DSNG 71	Mean	-0.1	-0.1	-0.1	-0.2	-0.0
	SD	0.16	0.15	0.22	0.12	0.17
	N	5	3	5	3	5

A Statistically significant from Group 1 at $p \leq 0.05$.
B Statistically significant from Group 2 at $p \leq 0.05$.
C Statistically significant from Group 3 at $p \leq 0.05$.

Food Consumption:

Mean food consumption did not differ significantly in both male and female dogs in pre-dosing period. The high dose insulin treatment (Group 5) increased food consumption within one week after insulin treatment as the mean body weights were increased in male dogs as shown above. In females, the mean body weight increased in low dose (1.32 IU/cm ^{2} /day-group 4) as well as the high dose group as shown (Tables 10) below.

Table 10
Mean Food Consumption Data
Dosing Phase

Test Article	Formulation K	Formulation C	Formulation F	Formulation A	
Group	1	2	3	4	5
Level (IU/sq cm/day)	0	1.76	0	1.32	1.76

Day		Mean food consumption (g/animal/period) for Group: 1F	2F	3F	4F	5F
PRED 11- PRED 17	Mean	1471	1094	1337	1374	1277
	SD	325.6	287.4	328.1	77.3	141.6
	N	5	3	5	3	5
DSNG 1- DSNG 7	Mean	1582	1272	1397	2054 B,C	2118 A,B,C
	SD	317.8	325.4	214.1	402.1	428.3
	N	5	3	5	3	5
DSNG 8- DSNG 14	Mean	1549	1306	1437	1931	1723
	SD	345.5	236.6	177.2	362.1	373.1
	N	5	3	5	3	5
DSNG 15- DSNG 21	Mean	1821	1448	1600	1740	1812
	SD	352.8	416.5	272.0	232.4	291.2
	N	5	3	5	3	5
DSNG 22- DSNG 28	Mean	1557	1124	1516	1497	1556
	SD	288.6	61.5	276.7	67.9	348.6
	N	5	2	4	3	5
DSNG 29- DSNG 35	Mean	1650	1519	1588	1509	1506
	SD	383.4	252.1	416.5	407.9	361.8
	N	5	3	5	3	5
DSNG 36- DSNG 42	Mean	1538	1356	1372	1662	1495
	SD	332.6	327.2	502.6	326.8	231.8
	N	5	3	5	3	5
DSNG 43- DSNG 49	Mean	1679	1593	1648	1785	1669
	SD	304.6	224.2	571.2	228.4	286.0
	N	5	3	4	3	5
DSNG 50- DSNG 56	Mean	1595	1389	1661	1742	1682
	SD	328.7	493.7	283.3	289.9	208.0
	N	5	3	5	3	5

A Statistically significant from Group 1 at $p \leq 0.05$.
B Statistically significant from Group 2 at $p \leq 0.05$.
C Statistically significant from Group 3 at $p \leq 0.05$.

Serum Glucose Concentrations:

The sponsor monitored serum glucose levels at 15, 30, 60, 90, 120 and 250 minutes after drug administration as shown (Table 12) below. There were significant decreases in glucose levels in the LD and HD animals (Groups 4 and 5) within 30 minutes after the treatment, which lasted approximately 90 minutes in male dogs. In females, the low levels were maintained up to 2 hours.

An interesting findings that were observed on Day 1 on the hypoglycemic effects of the treatment in groups 4 and 5 were somewhat reduced in Week 13 both in males and females. (Table 14). In other words, the degree of hypoglycemia as well as the hypoglycemic duration were compromised in Week 13, in particular, in male dogs (Please see Tables 14). Potential treatment time-dependent changes in drug nasal absorption should be correlated.

Table 12
Mean Blood Glucose Levels - Day 1

mg/dL

	Dosing phase						
Day:	1a	1b	1c	1d	1e	1f	1g
				M a l e s			
Group 1 - 0.0 IU/sq cm/day							
Mean	67	69	70	74	71	71	63
SD	4.8	4.6	7.8	8.6	8.4	6.7	3.9
N	5	5	5	5	5	5	5
Group 2 - 1.76 IU/sq cm/day							
Mean	65	54	65	67	67	69	70
SD	4.2	8.9	20.2	5.0	5.7	8.1	11.7
N	3	3	3	3	3	3	3
Group 3 - 0.0 IU/sq cm/day							
Mean	68	68	70	71	68	73	62
SD	5.8	4.8	6.2	9.5	7.7	13.5	4.3
N	5	5	5	5	5	5	5
Group 4 - 1.32 IU/sq cm/day							
Mean	70	50	30	40	54	71	74
SD	5.2	7.5	5.6	22.3	30.6	19.3	6.0
N	3	3	3	3	3	3	3
Group 5 - 1.76 IU/sq cm/day							
Mean	71	44	33	<30	38	60	77
SD	7.4	16.4	21.4	<17.3	14.8	29.8	12.8
N	5	5	5	5	5	5	5

a Predose.
b 15 minutes postdose.
c 30 minutes postdose.
d 60 minutes postdose.
e 90 minutes postdose.
f 120 minutes postdose.
g 240 minutes postdose.

Table 12
Mean Blood Glucose Levels
Day 1

mg/dL

Day:	1a	1b	1c	1d	1e	1f	1g
				Dosing phase			
				F e m a l e s			
Group 1 - 0.0 IU/sq cm/day							
Mean	61	69	66	68	67	64	61
SD	6.0	8.3	4.9	5.4	9.8	9.2	9.0
N	5	5	5	5	5	5	5
Group 2 - 1.76 IU/sq cm/day							
Mean	63	59	62	60	65	64	61
SD	7.5	9.6	9.5	10.6	11.4	15.8	7.0
N	3	3	3	3	3	3	3
Group 3 - 0.0 IU/sq cm/day							
Mean	69	75	72	70	66	67	64
SD	8.2	6.2	5.8	7.9	5.2	4.0	5.9
N	5	5	5	5	5	5	5
Group 4 - 1.32 IU/sq cm/day							
Mean	69	39	27	<30	<33	46	91
SD	7.1	12.3	2.1	<12.5	<20.8	28.7	15.0
N	3	3	3	3	3	3	3
Group 5 - 1.76 IU/sq cm/day							
Mean	62	42	<23	<24	<24	31	82
SD	2.4	5.0	<1.8	<4.1	<3.8	9.7	9.0
N	5	5	5	5	5	5	5

a Predose.
b 15 minutes postdose.
c 30 minutes postdose.
d 60 minutes postdose.
e 90 minutes postdose.
f 120 minutes postdose.
g 240 minutes postdose.

Table 14
Mean Blood Glucose Levels - Week 13

mg/dL

Day:	89a	89b	89c	89d	89e	89f	89g
				Dosing phase			
				M a l e s			
Group 1 - 0.0 IU/sq cm/day							
Mean	67	67	64	66	71	68	67
SD	9.1	3.8	10.5	4.7	9.3	8.4	7.2
N	5	5	5	5	5	5	5
Group 2 - 1.76 IU/sq cm/day							
Mean	73	70	67	68	67	69	66
SD	19.5	10.0	1.0	5.5	3.8	8.5	9.6
N	3	3	3	3	3	3	3
Group 3 - 0.0 IU/sq cm/day							
Mean	66	68	68	69	69	69	64
SD	6.9	6.2	4.6	6.2	7.4	6.2	6.4
N	5	5	5	5	5	5	5
Group 4 - 1.32 IU/sq cm/day							
Mean	72	67	69	71	70	71	61
SD	3.2	1.7	5.2	3.5	3.2	1.7	4.2
N	3	3	3	3	3	3	3
Group 5 - 1.76 IU/sq cm/day							
Mean	66	55	56	55	55	66	64
SD	8.2	17.4	19.6	20.7	17.5	5.5	5.7
N	5	5	5	5	5	5	5

a Predose.
b 15 minutes postdose.
c 30 minutes postdose.
d 60 minutes postdose.
e 90 minutes postdose.
f 120 minutes postdose.
g 240 minutes postdose.

Table 14
Mean Blood Glucose Levels
Week 13

mg/dL

Day:	89a	89b	89c	89d	89e	89f	89g
				Dosing phase			
				F e m a l e s			
Group 1 - 0.0 IU/sq cm/day							
Mean	74	70	71	65	72	72	72
SD	6.1	7.8	9.6	4.7	10.7	14.8	12.9
N	5	5	5	5	5	5	5
Group 2 - 1.76 IU/sq cm/day							
Mean	69	61	58	66	61	73	67
SD	15.6	9.6	8.7	11.1	11.1	9.8	15.9
N	3	3	3	3	3	3	3
Group 3 - 0.0 IU/sq cm/day							
Mean	66	69	69	67	63	70	61
SD	4.2	7.3	1.9	5.3	13.2	3.1	4.9
N	5	5	5	5	5	5	5
Group 4 - 1.32 IU/sq cm/day							
Mean	78	41	<24	<24	31	66	69
SD	3.2	10.6	<4.0	<4.6	9.5	10.1	4.6
N	3	3	3	3	3	3	3
Group 5 - 1.76 IU/sq cm/day							
Mean	74	39	<29	<33	<36	<44	77
SD	7.9	19.4	<19.2	<21.0	<22.6	<24.1	9.9
N	5	5	5	5	5	5	5

a Predose.
b 15 minutes postdose.
c 30 minutes postdose.
d 60 minutes postdose.
e 90 minutes postdose.
f 120 minutes postdose.
g 240 minutes postdose.

Clinical chemistry:
Urea nitrogen concentrations were decreased in group 4 males and increased in group 5 males of the treated groups on dosing Day 38, which were recovered on Day 87 and after recovery period (Table 16). In the two male groups, albumin was increased on Day 38, which was recovered on Day 87 and after recovery as shown below (Table 16). Creatine, total proteins, globulins, alkaline phosphatase, calcium, and potassium concentrations were altered in some groups on different date, which were usually recovered after recovery period. The changes were no consistent in many parameters, which appear to be sporadic observations.

Table 16
Mean Clinical Chemistry Data

Test Article	Formulation K	Formulation C	Formulation F	Formulation A	
Group	1	2	3	4	5
Level (IU/sq cm/day)	0	1.76	0	1.32	1.76

Group/ Sex		UN mg/dL PRED 8	DSNG 1	DSNG 38	DSNG 87	RECO 25
1M	Mean	14	14	17	17	15
	SD	1.9	1.3	1.5	3.3	3.5
	N	5	5	5	5	2
2M	Mean	14	15	19	16	.
	SD	2.1	1.7	1.0	3.2	.
	N	3	3	3	3	0
3M	Mean	12	13	16	18	13
	SD	1.3	2.1	1.3	3.0	3.5
	N	5	5	5	5	2
4M	Mean	13	14	15 B	16	.
	SD	2.1	2.5	3.6	3.5	.
	N	3	3	3	3	0
5M	Mean	15	16	19 AC	19	16
	SD	2.2	1.2	1.5	1.8	0.7
	N	5	5	5	5	2

A Statistically significant from Group 1 at $p \leq 0.05$.
B Statistically significant from Group 2 at $p \leq 0.05$.
C Statistically significant from Group 3 at $p \leq 0.05$.

Table 16
Mean Clinical Chemistry Data

Test Article	Formulation K	Formulation C	Formulation F	Formulation A	
Group	1	2	3	4	5
Level (IU/sq cm/day)	0	1.76	0	1.32	1.76

Group/ Sex		ALB g/dL PRED 8	DSNG 1	DSNG 38	DSNG 87	RECO 25
1M	Mean	3.3	3.4	3.1	3.7	3.8
	SD	0.11	0.18	0.18	0.20	0.14
	N	5	5	5	5	2
2M	Mean	3.2	3.3	3.1	3.6	.
	SD	0.17	0.15	0.10	0.06	.
	N	3	3	3	3	0
3M	Mean	3.2	3.3	3.3	3.7	3.8
	SD	0.13	0.11	0.14	0.30	0.35
	N	5	5	5	5	2
4M	Mean	3.3	3.5	3.7 ABC	4.0	.
	SD	0.00	0.10	0.06	0.10	.
	N	3	3	3	3	0
5M	Mean	3.2	3.4	3.5 AB	3.9	3.9
	SD	0.08	0.31	0.26	0.11	0.00
	N	5	5	5	5	2

A Statistically significant from Group 1 at $p \leq 0.05$.
B Statistically significant from Group 2 at $p \leq 0.05$.
C Statistically significant from Group 3 at $p \leq 0.05$.

Hematology:

The treated male groups (4 and 5) had increased RBC significantly on Day 38, which was not confirmed in female dogs in the groups 4 and 5 (Please see Table 15). There were no other remarkable treatment-related differences between the control groups and treated groups except WBC which was significantly elevated in the treated group in pre-dosing period and on Day 87 days in females (Tables 15). The increases in WBC in the treated female groups returned to normal values on Day 1 and 38, which was positive again on Day 87. Thus, it appears that the changes were not treatment-related (Table 15).

Table 15
Mean Hematology Data

Test Article	Formulation K	Formulation C	Formulation F	Formulation A	
Group	1	2	3	4	5
Level (IU/sq cm/day)	0	1.76	0	1.32	1.76

Group/ Sex		RBC E6/uL PRED 8	DSNG 1	DSNG 38	DSNG 87	RECO 25
1M	Mean	7.07	7.08	6.79	7.17	7.42
	SD	0.469	0.398	0.299	0.266	0.255
	N	5	5	5	5	2
2M	Mean	7.01	6.56	6.04	6.47	.
	SD	0.132	0.275	0.412	0.261	.
	N	3	3	3	3	0
3M	Mean	7.20	7.03	6.74	7.16	6.98
	SD	0.260	0.293	0.198	0.481	0.099
	N	5	5	5	5	2
4M	Mean	7.03	7.00	7.09 B	7.08	.
	SD	0.259	0.433	0.390	0.156	.
	N	3	3	3	3	0
5M	Mean	7.21	7.16	7.27 BC	7.16	7.52
	SD	0.293	0.589	0.557	0.311	0.071
	N	5	5	5	5	2

B Statistically significant from Group 2 at $p \leq 0.05$.
C Statistically significant from Group 3 at $p \leq 0.05$.

Table 15
Mean Hematology Data

Test Article	Formulation K	Formulation C	Formulation F	Formulation A	
Group	1	2	3	4	5
Level (IU/sq cm/day)	0	1.76	0	1.32	1.76

Group/ Sex		RBC E6/uL PRED 8	DSNG 1	DSNG 38	DSNG 87	RECO 25
1F	Mean	7.31	7.19	6.63	6.42	6.31
	SD	0.330	0.314	0.220	0.557	0.679
	N	5	5	5	5	2
2F	Mean	7.16	7.31	6.19	6.45	.
	SD	0.340	0.210	0.684	0.386	.
	N	3	3	3	3	0
3F	Mean	7.43	7.16	6.72	6.62	6.48
	SD	0.167	0.559	0.946	0.644	0.438
	N	5	5	5	5	2
4F	Mean	7.68	7.69	6.90	7.05	.
	SD	0.395	0.220	0.355	0.251	.
	N	3	3	3	3	0
5F	Mean	7.42	7.15	7.04	7.09	6.93
	SD	0.501	0.264	0.425	0.319	0.099
	N	5	5	5	5	2

Table 15
Mean Hematology Data

Test Article	Formulation K	Formulation C	Formulation F	Formulation A	
Group	1	2	3	4	5
Level (IU/sq cm/day)	0	1.76	0	1.32	1.76

Group/ Sex		WBC E3/uL PRED 8	DSNG 1	DSNG 38	DSNG 87	RECO 25
1F	Mean	10.06	9.11	10.19	8.27	8.24
	SD	1.168	0.887	0.861	0.693	0.750
	N	5	5	5	5	2
2F	Mean	9.22	12.80	8.44	10.21	.
	SD	1.698	6.203	1.272	2.397	.
	N	3	3	3	3	0
3F	Mean	12.46	11.09	11.68	12.90	9.13
	SD	0.910	0.643	0.511	1.997	0.396
	N	5	5	5	5	2
4F	Mean	12.20 B	10.27	10.70	11.94 A	.
	SD	0.514	1.258	1.616	3.234	.
	N	3	3	3	3	0
5F	Mean	12.46 AB	10.60	9.95	12.92 A	10.36
	SD	2.594	2.594	2.370	4.395	2.984
	N	5	5	5	5	2

A Statistically significant from Group 1 at $p \leq 0.05$.
B Statistically significant from Group 2 at $p \leq 0.05$.

Urinalysis:

Specific gravity in male treated groups was increased compared to relevant one of three control groups on dosing day of 87, which was returned to normal after 25 day of recovery period. There was no such effect in the female group. There were decreases in urine pH in the two treated group on dosing day one, which was also returned to normal on Days 38 and 87. There were no other changes.

Table 17
Mean Urinalysis Data

Test Article	Formulation K	Formulation C	Formulation F	Formulation A	
Group	1	2	3	4	5
Level (IU/sq cm/day)	0	1.76	0	1.32	1.76

Group/ Sex		SPGR PRED 8	DSNG 1	DSNG 38	DSNG 87	RECO 25
1M	Mean	1.019	1.026	1.024	1.019	1.024
	SD	0.0076	0.0025	0.0117	0.0065	0.0085
	N	5	5	5	5	2
2M	Mean	1.009	1.039	1.031	1.019	.
	SD	0.0107	0.0118	0.0240	0.0040	.
	N	3	3	3	3	0
3M	Mean	1.022	1.030	1.023	1.025	1.022
	SD	0.0136	0.0074	0.0195	0.0167	0.0120
	N	5	5	5	5	2
4M	Mean	1.029	1.047	1.022	1.047 ABC	.
	SD	0.0145	0.0114	0.0186	0.0177	.
	N	3	3	3	3	0
5M	Mean	1.026	1.029	1.034	1.037 A	1.017
	SD	0.0128	0.0208	0.0152	0.0099	0.0021
	N	5	5	5	5	2

A Statistically significant from Group 1 at $p \leq 0.05$.
B Statistically significant from Group 2 at $p \leq 0.05$.
C Statistically significant from Group 3 at $p \leq 0.05$.

EKG: Not determined.

Gross pathology: Discolored cecum and colon was observed in two low dose males (Table 18). In male control group 1, discolored lung and stomach were observed in each one dogs. One high dose male had crusted skin while one female in the low dose group had a large ovary. One control female animal had a discolored heart. There were no other visible abnormalities.

Table 18
Summary of Macroscopic Observations - Dosing Phase - Final Phase Sacrifice

	-- Males --					-- Females --				
Group:	1	2	3	4	5	1	2	3	4	5
Number in group:	3	3	3	3	3	3	3	3	3	3
Examined/No remarkable findings ...	3	3	3	2	2	2	3	3	2	3
Heart										
Discolored	0	0	0	0	0	1	0	0	0	0
Total:	0	0	0	0	0	1	0	0	0	0
Cecum										
Discolored	0	0	0	1	0	0	0	0	0	0
Total:	0	0	0	1	0	0	0	0	0	0
Colon										
Discolored	0	0	0	1	0	0	0	0	0	0
Total:	0	0	0	1	0	0	0	0	0	0
Ovary										
Large	0	0	0	0	0	0	0	0	1	0
Total:	0	0	0	0	0	0	0	0	1	0
Skin/SubQ, Other										
Crusted	0	0	0	0	1	0	0	0	0	0
Total:	0	0	0	0	1	0	0	0	0	0

Organ weights (specify organs weighed if not in histopath table):
At the dosing and recovery phase final sacrifices, no test article-related terminal body or organ weight changes were seen. The few statistically significant absolute and relative organ weight changes present. For examples, mean pituitary weight in the treated females was increased significantly compared to control group 2 or 3. Mean thymus weight of the LD female dog was decreased significantly compared to control group 3 while the ratio of thymus to brain weight was increased in the HD treated female (Table 21). The reviewer considers that the changes were attributed to normal biological variation and not to test article administration.

Table 21
Mean Organ Weight and Organ/Brain Weight Data
Dosing Phase - Final Phase Sacrifice

Test Article		Formulation K	Formulation C		Formulation F		Formulation A	
Group		1	2		3		4	5
Level (IU/sq cm/day)		0	1.76		0		1.32	1.76
Group/ Sex		Brain weight (g)	Adrenal Unadjusted (g)	Adrenal Ratio (%)	Pituitary Unadjusted (g)	Pituitary Ratio (%)	Thymus Unadjusted (g)	Thymus Ratio (%)
1F	Mean	69.425	1.237	1.777	0.060	0.087	3.856	5.535
	SD	1.9290	0.2160	0.2590	0.0035	0.0065	1.8225	2.5439
	N	3	3	3	3	3	3	3
2F	Mean	74.701	1.254	1.732	0.048	0.065	5.052	6.822
	SD	11.6656	0.1456	0.5103	0.0029	0.0095	1.8720	2.3120
	N	3	3	3	3	3	3	3
3F	Mean	70.705	1.356	1.916	0.053	0.076	11.443	16.018
	SD	5.4369	0.1426	0.0522	0.0121	0.0210	3.5730	3.9787
	N	3	3	3	3	3	3	3
4F	Mean	77.003	1.380	1.794	0.068 B,C	0.089	4.662 C	6.134 C
	SD	2.7067	0.0365	0.0901	0.0096	0.0145	2.7404	3.7157
	N	3	3	3	3	3	3	3
5F	Mean	68.576	1.067	1.554	0.066 B	0.097	7.815	11.395 A
	SD	2.3568	0.1398	0.1603	0.0067	0.0099	2.1407	3.1527
	N	3	3	3	3	3	3	3

A Statistically significant from Group 1 at $p \leq 0.05$.
B Statistically significant from Group 2 at $p \leq 0.05$.
C Statistically significant from Group 3 at $p \leq 0.05$.

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

Tables 24 and 25 summarized the microscopic observations in males and females at terminal sacrifice and after 4-week recovery period. Nares, nasal sinus, nasal turbinates, tracheas and lungs in the treated male and female animals were not different from those in one of three control groups (1, 2, or 3) as shown below. One male in the group 5 had retinale rosetta and lung inflammation after the recovery period. One female also had trachea inflammation after recovery period. It appears that such changes may be within the normal variations, rather than due to treatment-related toxicity according to reviewer's various other toxicologic evaluations.

Table 24
Summary of Microscopic Observations - Dosing Phase - Final Phase Sacrifice

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --					-- Females --				
	Dosage group:	Ctls	2	3	4	5	Ctls	2	3	4	5
Tissues With Diagnoses	No. in group:	3	3	3	3	3	3	3	3	3	3
Eye	Number examined:	3	3	3	3	3	3	3	3	3	3
	Unremarkable:	3	3	3	3	3	3	3	2	3	3
Rosette, Retina		0	0	0	0	0	0	0	1	0	0
Nerve, Optic	Number examined:	3	3	3	3	3	3	3	3	3	3
	Unremarkable:	3	3	3	3	3	3	3	3	3	3
Lung	Number examined:	3	3	3	3	3	3	3	3	3	3
	Unremarkable:	3	2	2	3	3	3	3	3	3	2
Infiltrate, Macrophages, Alveolus		0	1	0	0	0	0	0	0	0	0
Inflammation, Subacute, Focal		0	0	1	0	0	0	0	0	0	1
Trachea	Number examined:	3	3	3	3	3	3	3	3	3	3
	Unremarkable:	3	3	3	3	3	3	3	3	2	2
Inflammation, Subacute		0	0	0	0	0	0	0	0	1	1
Esophagus	Number examined:	3	3	3	3	3	3	3	3	3	3
	Unremarkable:	3	2	3	3	3	3	3	3	3	3
Inflammation, Subacute, Tunica Muscularis		0	1	0	0	0	0	0	0	0	0
Nares	Number examined:	3	3	3	3	3	3	3	3	3	3
	Unremarkable:	3	3	3	3	2	1	3	2	2	2
Inflammation, Lymphohistiocytic, Adnexal Occasionally		0	0	0	0	1	2	0	1	1	1
-Associated Intrafollicular Parasites consistent with -Demodex spp.											
Nasal Sinus	Number examined:	3	3	3	3	3	3	3	3	3	3
	Unremarkable:	3	3	3	3	3	3	3	3	3	3
Nasal Turbinates	Number examined:	3	3	3	3	3	3	3	3	3	3
	Unremarkable:	3	3	3	3	3	3	3	3	2	3
Inflammation, Subacute		0	0	0	0	0	0	0	0	1	0
Death Comment	Number examined:	3	3	3	3	3	3	3	3	3	3
	Unremarkable:	0	0	0	0	0	0	0	0	0	0
Scheduled Sacrifice		3	3	3	3	3	3	3	3	3	3
Bone, Femur	Number examined:	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0

All Diagnoses; Phases: P2; Death types: Scheduled FS; Date of death range: 18.Apr.06 To 18.Apr.06

Table 25
Summary of Microscopic Observations - Recovery Phase - Final Phase Sacrifice

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --			-- Females -		
	Dosage group:	Ctls	3	5	Ctls	3	5
Tissues With Diagnoses	No. in group:	2	2	2	2	2	2
Eye	Number examined:	2	2	2	2	2	2
	Unremarkable:	2	2	1	2	2	2
Rosette, Retina		0	0	1	0	0	0
Nerve, Optic	Number examined:	2	2	2	2	2	2
	Unremarkable:	2	2	2	2	2	2
Lung	Number examined:	2	2	2	2	2	2
	Unremarkable:	2	2	1	1	1	2
Infiltrate, Macrophages, Alveolus		0	0	1	0	0	0
Inflammation, Subacute, Focal		0	0	0	1	1	0
Trachea	Number examined:	2	2	2	2	2	2
	Unremarkable:	2	2	2	2	2	1
Inflammation, Subacute		0	0	0	0	0	1
Esophagus	Number examined:	2	2	2	2	2	2
	Unremarkable:	2	2	2	2	2	2
Inflammation, Subacute, Tunica Muscularis		0	0	0	0	0	0
Nares	Number examined:	2	2	2	2	2	2
	Unremarkable:	1	1	1	1	1	1
Inflammation, Lymphohistiocytic, Adnexal Occasionally -Associated Intrafollicular Parasites consistent with -Demodex spp.		1	1	1	1	1	1
Nasal Sinus	Number examined:	2	2	2	2	2	2
	Unremarkable:	2	2	2	2	2	2
Nasal Turbinates	Number examined:	2	2	2	2	2	2
	Unremarkable:	2	2	2	2	2	2
Inflammation, Subacute		0	0	0	0	0	0
Death Comment	Number examined:	2	2	2	2	2	2
	Unremarkable:	0	0	0	0	0	0
Scheduled Sacrifice		2	2	2	2	2	2
Bone, Femur	Number examined:	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0

All Diagnoses; Phases: P3; Death types: Scheduled FS; Date of death range: 16.May.06 To 16.May.06

Toxicokinetics: No TK data are available.

Study Title: 90-Day Repeated Dose Dermal Toxicity Study in Rats with 15-PPAEE

Key findings:
To evaluate the potential toxicity of 15-HPAEE, 15 rats/sex/group were administered 15-HPAEE topically at doses of 0, 0.96 or 9.6 mg/kg/day for 91 days. A male and a female rat receiving 0.96 mg/kg/day died on Day 86 and 42. The cause of death was not related to the treatment. Clinical signs such as skin staining, cold to touch, malocclusion, scabbed at applied area and hair loss were commonly observed clinical signs, although they were not dose-related. The treatment had no effect on body weight, food consumption, hematology and clinical chemistry. There were no treatment effect on dermatology scores, macro- and microscopic findings. Thus, it appears that 15-HPAEE is non-toxic locally at skin.

Study No: 014938
Amendment # 000, Vol. #12, and page # 1-267
Conducting laboratory and location: (b) (4)
Date of study initiation: 8/20/2002
Sponsor: (b) (4)
GLP compliance: Yes
QA- Report: Yes () No (x)

METHODS:
Species/strain: Crl:CD(SD)
#/sex/group or time point: 15 rats /sex/group
Age: 8 weeks old
Weight: 260-320 g for males; 180-235 g for females.
Dosage groups in administered units: The animals were assigned to one of 3 groups as shown below. The test article was administered for 24 h/day via dermal application for 91 consecutive days. The first day of dosing was Day O. Because the control and 15-HPAEE-containing gels were applied to skin at 400 mg/kg of body weight, the concentration of 15-HPAEE in the gel was adjusted appropriately. The doses of the 15-HPAEE are shown in the table below.

Group	Test article/ Control	Concentration of 15-HPAEE mg/g	Dose 15-HPAEE (mg/kg BW/day)	Number of Rats per Sex
1	Control	0	0	15
2	15-HPAEE	2.4	0.96	15
3	15-HPAEE	24	9.6	15

The rats were observed for clinical signs following dosing. Clinical signs included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, as well as, evaluations of respiration, behavior and excretions. Observations were recorded at approximately 6 h post-dosing. Scores for dermal irritation at the application site were recorded pretest and once weekly. The Week 13 scoring was done on the day of necropsy. All application sites were observed for erythema and edema according to the Draize System below.

Draize Scoring System

Erythema and Eschar Formation

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

Edema

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Results:

Mortality: A female receiving 0.96 mg/kg/day died on Day 42. This animal had multiple findings at necropsy. A male receiving 0.96 mg/kg/day died on Day 86 following blood collection. The sponsor indicated that neither death was attributed to the administration of 15-HPAEE without providing the exact causes of the deaths.

Clinical Signs: A certain clinical sign was observed only male or female animals. Skin staining, cold to touch, malocclusion, scabbed at applied area and hair loss were commonly observed clinical signs. Many of those clinical signs were not dose-related. However, it appears that the incidences of scabbed and/or sores were increased in the HD animals as shown below.

Clinical Signs in 90-Day Repeated Dose Dermal Toxicity Study in Rats with 15-PPAEE@						
Clinical signs	Control		Low dose		High dose	
	Male	Female	Male	Female	Male	Female
Anogenital staining*	0/0		0/0		1/8	
Cold to touch		0/0		1/2		0/0
Malocclusion	-/-	2/19	1/35	-/-	4/148	1/6
Scabbed, sores*	-/-	0/0	1/7	0/0	2/21	3/14
Hair loss, all area*,**	5/216	4/278	2/34	2/34	3/98	4/324
Paleness		0/0		1/2		0/0
Nasal discharge*	7/9	3/6	4/10	3/5	7/13	4/6
Labored breathing*	0/0		0/0		1/1	
Decreased activity**		0/0		1/2		0/0
@All groups had 15 rats/sex at the beginning and first/second values are #rat/#incidences. * and ** indicate slight and moderate, respectively.						

Dermal Scoring:

Some males and females from all groups exhibited very slight to slight erythema or desquamation. One of pronounced desquamation (DE) is shown below (Table 23). There was no apparent dosage response for dermal scoring parameters, with the exception of desquamation in the high dose males. However, desquamation is not considered to be an adverse effect according to the sponsor.

Table 23: Dermal Irritation Scores - Males (Continued)

Study: 014938

	Pretest			Week 1			Week 2			Week 3			Week 4			Week 5			Week 6		
	ER	ED	O	ER	ED	O	ER	ED	O	ER	ED	O	ER	ED	O	ER	ED	O	ER	ED	O
Male, Group 3																					
157986	0	0	-	1	0	-	0	0	DE	0	0	-	0	0	-	0	0	-	0	0	-
157987	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
157988	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
157989	0	0	-	1	0	DE	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
157990	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
157991	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
157992	0	0	-	0	0	DE	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
157993	0	0	-	0	0	DE	0	0	DE	0	0	-	0	0	-	0	0	-	0	0	-
157994	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
157995	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
157996	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	DE	0	0	-
157997	0	0	-	1	0	DE	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
157998	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
157999	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
158000	0	0	-	2	0	DE	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-

ER = Erythema; ED - Edema; O = Other; - = No finding; DE = Desquamation

Body weight: The treatment had no effects on body weight, body weight gain.
Food consumption: There was no treatment-related in food consumption.
Ophthalmology: There were no findings attributable to the test article noted during the Day 85 ophthalmology examination.

Hematology:
In male rats hematocrit was decreased in the treated group while MCHC was increased in the HD group (Table 9). There were no other changes in the parameters in male as well as in females.

Table 9: Mean Red Blood Cell and Clotting Parameters Data – Males

Study: 014938 Hematology

	RBC M/µL Day 86	HGB g/dL Day 86	HCT % Day 86	MCV fL Day 86	MCH pg Day 86	MCHC g/dL Day 86	PLT K/µL Day 86	PT SEC Day 86
Male, Control								
Mean	9.167	16.09	48.67	53.12	17.59	33.10	1137.7	16.13
SD	0.409	0.52	2.05	2.02	0.61	0.52	119.3	0.51
N	15	15	15	15	15	15	15	15
Male, Low Dose								
Mean	8.845	15.73	46.93*	53.09	17.79	33.50	1225.2	16.43
SD	0.380	0.70	1.94	1.69	0.72	0.66	122.9	1.01
N	14	14	14	14	14	14	14	15
Male, High Dose								
Mean	8.860	15.74	46.56**	52.66	17.79	33.82**	1183.8	16.36
SD	0.540	0.64	1.85	2.43	0.74	0.61	358.8	0.89
N	15	15	15	15	15	15	15	14

Key: Experimental Unit = Animal

*Statistically significant difference from control group ($p \leq 0.05$)
**Statistically significant difference from control group ($p \leq 0.01$)

Clinical chemistry:
Chloride was increased from 104 to 107 mEq/L in the HD males. In the same group total bilirubin was decreased from 0.3 to 0.22 mg/dL on Day 86. Total protein was also decreased to 7.31 from 7.67 g/dL in the LD group. In females, only CK was reduced to 131.1 from the control 287 IU/L on Day 86. There were no other changes and the sponsor considered that the clinical chemistry changes would not have biological impact.

Macroscopic observation at necropsy:
There were no treatment-related findings.

Organ weights:
There were no treatment-related effects on mean organ weights (absolute, relative to body weight or relative to brain weight) was observed. A few statistically significant differences were not considered to be treatment related.

Histopathology:
There were no histomorphological tissue alterations attributable to the dermal administration of 15-HPAEE to rats at dose levels up to 9.6 mg/kg BW /day for 90 consecutive days.

2.6.6.4 Genetic toxicology

Study title: L5178Y TK $^{+/-}$ Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay with Exaltolide (Pentadecalactone)

Key findings:
In the initial mutation assay performed in the absence of metabolic activation, eight treatments ranging from 5.00 to 45.0 µg/mL were analyzed for mutant induction and no cytotoxicity to high cytotoxicity was induced. None of the analyzed treatments induced a mutant frequency that exceeded the minimum criteria for a positive response. In the confirmatory nonactivation mutation assay, which was performed with a 24-hour treatment period, seven treatments at doses of 5.00 to 35.0 µg/mL were analyzed and no cytotoxicity to high cytotoxicity was induced. None of the treatments induced a mutant frequency that exceeded the minimum criteria for a positive response. The test article was, therefore, evaluated as negative without metabolic activation.

In the initial mutation assay performed in the presence of S9 metabolic activation, eight treatments from 10.0 to 80.0 µg/mL were analyzed. No cytotoxicity to moderately high cytotoxicity was induced. While a desired highly cytotoxic response was not achieved, a small increase in concentration from 80.0 to 90.0 µg/mL resulted in excessive cytotoxicity. None of the analyzed treatments induced a mutant frequency that exceeded the minimum criteria for a positive response.

In the combinatory assay, eight treatments from 20.0 to 85.0 µg/mL were analyzed. and weak cytotoxicity to high cytotoxicity was induced. Treatment at 75.0 µg/mL induced a mutant frequency that just exceeded the minimum criteria for a positive response. However, two higher, more cytotoxic concentrations were negative, and there was no positive response in the initial trial. Therefore, the increase was considered spurious and the test article was evaluated as negative with metabolic activation. The test article was evaluated as negative for inducing forward mutations at the TK locus in L5178Y mouse lymphoma cells under nonactivation and activation conditions used in this assay.

Study no.: 22572-0-431 ICH
Volume #13, and page #: 1-32
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/12/2001
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: G/2 0154020

Methods

Strains/species/cell line: Mouse lymphoma L5178Y cell
Doses used in definitive study:
Assay without activation: 5, 7.5, 10, 15, 20, 35 and 40 µg/mL

Assay with activation: 20, 40, 60, 65, 70, 75, 80, 85, and 90 μg/mL

Basis of dose selection:
The measurement of the cytotoxicity of each treatment was the relative suspension growth of the cells over the 2-day expression period (for the 4-hour treatment) or the relative suspension growth over the 3-day treatment and expression period (for the 24-hour treatment) multiplied by the relative cloning efficiency at the time of selection. Although not strictly a measure of cell survival, this parameter (called relative growth or relative total growth, RTG) provides a measure of the effectiveness of treatment and was used as the basis for selecting doses for any necessary subsequent trials. The expression of RTG was the parameter that was used to assess treatment cytotoxicity and was obtained by multiplying the relative suspension growth by the relative cloning efficiency/100.

Negative controls:
DMSO (Lot#100K014) and vehicle
Positive controls:
Methyl methanesulfonate (MMS) at 6.5 to 13μg/mL (Lot# 08109BU)
Methyl cholanthrene (MCA) at 2 to 4 μg/mL (Lot#77H2515)

Incubation and sampling times:
Logarithmically growing mouse lymphoma L5178Y cells ($6x10^6$) per tube were exposed to the test article for 4 hours. Expression period of 2 days was used to allow for mutant recovery, growth and expression of $TK^{-/-}$ phenotype. If the cells in a culture failed to multiply to a density of 4×10^5 cells/mL on the first day after treatment, the culture was not subcultured. On Day 2, cell counts were again determined, and appropriate cultures were selected for cloning and mutant selection.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
Acceptable Controls:
The average absolute cloning efficiency of the vehicle controls should be between 60% and 130%. A value greater than 100% is possible because of errors in cell counts and variations in cel1 division during unavoidable delays between counting and cloning of many cultures. A minimum acceptable value for the average suspension growth of the vehicle controls for two days is an 8-fold increase over the original cell concentrations. Lower values render an assay unacceptable because of poor cel1 growth.

Acceptable High Dose:
For test articles with weak or no mutagenic activity, an assay should include applied concentrations that reduce the RTG to 10% or 20% of the average vehicle control or reach the maximum applied concentrations given in the evaluation criteria. Because mutant frequencies increase as a function of lethality, an attempt to obtain treatments in the range of 10% to 20% RTG must be made in order to consider the assay as conclusive. This requirement was waived if the concentration of the highest assayed dose was at least 75% of a higher.

Criteria for Positive Results:
The test article is evaluated as positive if dose-dependent increases of 2-fold or greater in mutant frequency are obtained over the concurrent background mutant frequency. The background mutant frequency is defined as the average mutant frequency of the vehicle control cultures. The 2-fold or greater increase is based on extensive experience which indicates such responses are repeatable in additional trials. It is desirable to obtain this relationship for at least three doses, but this goal depends on the dose steps chosen for the assay and toxicity at which mutagenic activity appears. The dose-dependent requirement is waived if a large increase in mutant frequency (4-fold or higher) is obtained for a single dose at or near the highest testable toxicity.

Dose Range Finding Assay:

The test article, Exaltolide, was tested in a preliminary dose range finding assay with a treatment period of approximately 4 hours both with and without S9 metabolic activation and a preliminary dose range finding non activation assay with a treatment period of approximately 24 hours. Ten treatments were used in each case that ranged from 1.97 to 1000 µg/mL; a vehicle control was included under each activation condition.
In the absence of rat liver S9 metabolic activity with a 4-hour treatment period (Table 1), Exaltolide was not cytotoxic to weakly cytotoxic from 1.97 to 3.13 µg/mL and excessively cytotoxic from 62.5 to 1000 µg/mL. In the presence of metabolic activation (Table 1), the test article induced no cytotoxicity to weak cytotoxicity from 1.97 to 31.3 µg/mL. Moderate cytotoxicity was observed at 62.5 µg/mL, and excessive cytotoxicity from 125 to 1000 µg/mL. In the nonactivation dose range finding assay using a 24-hour treatment period (Table 2), the test article induced no cytotoxicity to weak cytotoxicity from 1.97 to 15.7 µg/mL and excessive cytotoxicity at and above 31.3 µg/mL. The mutation assays were initiated with treatments based on these results.

TABLE 1: CYTOTOXICITY ASSAY WITH EXALTOLIDE

TEST ARTICLE: EXALTOLIDE

STUDY NUMBER: 22572-0-431 ICH

TEST DATE: 06/20/2001

VEHICLE: DMSO

COMMENTS ON TREATMENT: ~4-Hour Treatment Period

Applied Concentration µg/mL	Without S9 Activation		With S9 Activation	
	Cell Density/mL ($x10^5$)[a]	% Relative to Vehicle Control[b]	Cell Density/mL ($x10^5$)[a]	% Relative to Vehicle Control[b]
VC[c]	9.9	100.0	8.6	100.0
1.97	9.8	99.0	8.4	97.7
3.93	12.4	125.3	8.5	98.8
7.85	9.7	98.0	8.0	93.0
15.7	13.9	140.4	8.0	93.0
31.3	7.6	76.8	6.6	76.7
62.5+	0.0	0.0	3.9	45.3
125++	0.0	0.0	0.2	2.3
250++	0.0	0.0	0.0	0.0
500++	0.0	0.0	0.0	0.0
1000++	0.0	0.0	0.0	0.0

[a]Cell density determined by hemocytometer
[b]Relative to vehicle control cell density for all treatments
[c]VC = Vehicle control, 1% DMSO
+Precipitate remaining at the termination of treatment under nonactivation conditions
++Precipitate remaining at the termination of treatment under activation and nonactivation conditions

TABLE 2: CYTOTOXICITY ASSAY WITH EXALTOLIDE WITHOUT S9 ACTIVATION (24 HOUR)

TEST ARTICLE: EXALTOLIDE

STUDY NUMBER: 22572-0-431 ICH

TEST DATE: 06/20/2001

VEHICLE: DMSO

COMMENTS ON TREATMENT: ~24-Hour Treatment Period

Applied Concentration µg/mL	Cell Density/mL ($x10^5$)[a] DAY 1	DAY 2	% Relative to Vehicle Control[b]
VC[c]	8.9	15.8	100.0
1.97	8.1	17.2	99.1
3.93	9.2	16.2	106.0
7.85	7.3	17.1	88.8
15.7	6.8	12.1	58.5
31.3	0.7*	1.2	2.6
62.5+	0.0*	0.0	0.0
125+	0.0*	0.0	0.0
250+	0.0*	0.0	0.0
500+	0.0*	0.0	0.0
1000+	0.0*	0.0	0.0

[a]Cell density determined by hemocytometer
[b]Relative to vehicle control cell density for all treatments
[c]VC = Vehicle control, 1% DMSO
*Not subcultured
+Precipitate remaining at the termination of treatment

Nonactivation Mutation Assay:
In the confirmatory nonactivation assay, the treatment period was approximately 24 hours and results are shown in Table 5. Eight treatments at 5, 7.5, 10, 10, 15, 20, 35, 40, 45, 50, 55 and 60 µg/mL were used for initiation. Treatments at and above 40.0 µg/mL were terminated due to excessive cytotoxicity. The remaining seven treatments were selected for mutant analysis and induced no cytotoxicity to high cytotoxicity (122.9% to 9.9% relative growths). None of the analyzed treatments induced a mutant frequency that exceeded the minimum criterion of 142.7×10^{-6}. The test article was therefore considered negative without metabolic activation.

Activation Mutation Assay:
In the confirmatory assay with metabolic activation (Table 9), thirteen treatments at 20, 40, 60, 65, 70, 75, 80, 85, 90, 95, 100, 120 and 150 µg/mL were initiated. Treatments at and above 90 µg/mL were terminated due to excessive cytotoxicity. The remaining eight treatments induced weak cytotoxicity to high cytotoxicity (68.3% to 17.6% relative growths). Treatment at 75 µg/mL induced a mutant frequency that exceeded the minimum criteria of 267.3×10^{-6}. However, two higher, more cytotoxic concentrations were negative, and there was no positive response in the initial trial. Therefore, the test article was evaluated as negative with metabolic activation.

TABLE 5: CONFIRMATORY MUTATION ASSAY WITHOUT ACTIVATION

A. TEST ARTICLE: Exaltolide
B. GENETICS ASSAY NO.: 22572-0-431 ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL

E. TREATMENT DATE: 07/17/2001
F. CELLS ANALYZED: $3x10^6$
G. TREATMENT PERIOD: ~24 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Counts (Cell/mL, 10E5 Units)			Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2	Day 3								
Nonactivation Controls[e]					AVG VC				AVG VC		
Vehicle Control	11.3	11.1	13.4	62.3		180	561	93.5		106.1	64.2
Vehicle Control	12.8	9.4	14.3	63.7		184	517	86.2		100.2	71.3
Vehicle Control	11.0	12.2	14.5	72.1	66.0	164	417	69.5	83.0	91.3	78.5
MMS 6.5 µg/mL	8.7	8.8	12.9	36.6		1237	324	54.0		36.0	763.6[f]
MMS 6.5 µg/mL	8.1	11.2	8.9	29.9		1140	341	56.9		31.0	667.7[f]
Test Compound µg/mL				Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
5.00	9.5	13.7	14.7	107.3		176	571	114.5		122.9	61.6
7.50	10.4	12.8	13.4	100.1		205	467	93.7		93.8	87.9
10.0	9.2	11.9	19.9	122.2		156	422	84.7		103.6	73.9
10.0	9.2	10.0	18.0	92.9		148	436	87.6		81.4	68.0
15.0	9.1	11.3	13.8	79.6		214	548	109.9		87.5	78.1
20.0	9.2	10.1	15.4	80.3		201	472	94.8		76.1	85.0
35.0	1.0[g]	10.1	9.3	15.8		214	313	62.8		9.9	136.6
40.0	1.1[g]	1.4[g]	2.9				Too toxic to clone				

[a]RSG = [Treatment termination (Day 1) cell density/$3x10^5$] x [Day 2 cell density/$3x10^5$ or Day 1 density if not split back] x [Day 3 cell density/$3x10^5$ or Day 2 density if not split back]

[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100

[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100

[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4

Decimal is moved to express the frequency in units of 10E-6

[e]Vehicle Control = 1% DMSO

Positive Control: MMS = Methyl methanesulfonate

[f]Mutagenic. Exceeds Minimum Criterion of 142.7 X 10E-6

[g]Not subcultured

TABLE 9: CONFIRMATORY MUTATION ASSAY WITH ACTIVATION

A. TEST ARTICLE: Exaltolide
B. GENETICS ASSAY NO.: 22572-0-431 ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 μg/mL

E. TREATMENT DATE: 07/17/2001
F. CELLS ANALYZED: $3x10^6$
G. TREATMENT PERIOD: ~4 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Counts (Cell/mL, 10E5 Units)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2								
S9-Activation Controls[e] S9 Batch Number: 1111				AVG VC				AVG VC		
Vehicle Control	12.6	17.4	24.4		325	453	75.5		107.7	143.6
Vehicle Control	12.0	15.7	20.9		320	447	74.6		91.4	142.9
Vehicle Control	12.5	15.0	20.8	22.0	283	494	82.4	77.5	100.5	114.3
MCA 2 μg/mL	6.0	10.9	7.3		873	340	56.7		24.1	512.8[f]
MCA 4 μg/mL	6.5	8.7	6.3		922	337	56.2		20.7	546.9[f]
Test Compound μg/mL			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
20.0	9.6	13.6	65.8		448	482	103.8		68.3	186
40.0	8.9	14.7	65.9		421	418	89.9		59.3	201.6
60.0	10.1	15.1	76.9		404	399	85.9		66.0	202.2
65.0	6.5	18.4	60.3		403	380	81.7		49.2	212.1
70.0	7.5	13.4	50.7		440	413	89.0		45.1	212.7
75.0	3.9[g]	16.6	25.1		528	383	82.4		20.7	275.8[f]
80.0	2.8[g]	15.9	24.0		439	351	75.6		18.2	249.7
85.0	2.6[g]	12.3	18.6		523	439	94.4		17.6	238.3
90.0	1.0[g]	8.1				Too toxic to clone				

[a]RSG = (Day 1 Count/3) * (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4
Decimal is moved to express the frequency in units of 10E-6
[e]Vehicle Control = 1% DMSO
Positive Control: MCA = Methylcholanthrene
[f]Mutagenic. Exceeds Minimum Criterion of 267.3 X 10E-6
[g]Not subcultured

Comments Mutation Assay:

The average cloning efficiencies for the vehicle controls were 80.4% and 83.0% without activation and 81.6% and 77.5% with S9 metabolic activation, which demonstrated acceptable cloning conditions for the assays. In the confirmatory activation assay, the average mutant frequency of the vehicle control cultures was slightly higher than the normal range, but all other criteria were acceptable and the assay was considered acceptable for evaluation. The control cultures, MMS (nonactivation) and MCA (activation) induced large increases in mutant frequency that was greatly in excess of the minimum criteria.

CONCLUSION:

The mutation assay conditions were considered acceptable for evaluation of the test results as described above. Based on the positive criteria given above, it appears that the test article (Exa1tolide) was evaluated as negative for inducing forward mutations at the K locus in L5178Y mouse lymphoma cells under nonactivation and activation conditions used in this study.

Appendix: historical Control Data

HISTORICAL CONTROL DATA

Nonactivation Studies

Pooled negative and vehicle control mutant frequencies

Mean (± SD)	$66.7 \pm 14.1 \times 10^{-6}$
Range	43.3 to 111.9×10^{-6}
Number of experiments	50

Positive control mutant frequencies (6.5 µg/mL methyl methanesulfonate)

Mean (± SD)	$411.7 \pm 247.5 \times 10^{-6}$
Range	205.4 to 1514.1×10^{-6}
Number of experiments	50

Positive control mutant frequencies (13.0 µg/mL methyl methanesulfonate)

Mean (± SD)	$433.2 \pm 154.4 \times 10^{-6}$
Range	232.1 to 1135.7×10^{-6}
Number of experiments	50

Activation Studies

Pooled negative and vehicle control mutant frequencies

Mean (± SD)	$73.2 \pm 11.2 \times 10^{-6}$
Range	48.5 to 99.8×10^{-6}
Number of experiments	50

Positive control mutant frequencies (2.0 µg/mL 3-methylcholanthrene)

Mean (± SD)	$334.0 \pm 81.8 \times 10^{-6}$
Range	208.6 to 592.2×10^{-6}
Number of experiments	50

Positive control mutant frequencies (4.0 µg/mL 3-methylcholanthrene)

Mean (± SD)	$352.2 \pm 72.0 \times 10^{-6}$
Range	208.9 to 518.1×10^{-6}
Number of experiments	50

Study title: In Vivo Mouse Micronucleus Assay with Exaltolide (Pentadecalactone)

Key findings:
Based on the results of the dose range finding assay, the maximum tolerated dose was estimated to be 2000 mg/kg. In the micronucleus assay, six male mice/group received the test article orally (gavage) at doses of 500, 1000, and 2000 mg/kg. The test article was dissolved in corn oil. Five animals that were dosed with the positive control article were euthanized approximately 24 hours after dosing for extraction of the bone marrow. Five animals dosed with the test article at the 2000 mg/kg dose level and five animals dosed with the vehicle control article were euthanized approximately 24 and 48 hours after dosing for extraction of the bone marrow. At least 2000 PCEs per animal were analyzed for the frequency of micronuclei. Cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in at least the first 500 erythrocytes for each animal. Exaltolide induced no signs of clinical toxicity in any of the treated animals but was cytotoxic to the bone marrow (i.e., a statistically significant decrease in the PCE(PCE/ NCE ratio) at the 500 mg/kg dose level. A statistically significant increase in micronucleated PCEs was not observed at any dose level or harvest time point. The test article, Exaltolide, was evaluated as negative in the mouse bone marrow micronucleus assay under the conditions of this assay.

Study no.: (b) (4) 22572-0-455OECD

Volume #13 and page #: 33-48
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/12/2001
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: G/2 0154020

Methods

Strains/species/cell line: Six male mice/Crl:CD-1(ICR)BR per group
Doses used in definitive study: 500, 1000, and 2000 mg/kg

Basis of dose selection:
A dose range finding assay was performed using the same treatment regimen used in the micronucleus assay because no appropriate toxicity data were available (e.g., the same species, strain, same route, etc.). No doses higher than 2,000 mg/kg were tested. The daily observations of toxic signs and/or mortality data were used to estimate the highest appropriate dose level (maximum tolerated dose) for the micronucleus assay.

Micronucleus assay was used male mice because there were no differences in toxicity between the sexes, based on the dose range finding assay. The high dose, unless non-toxic, should have produced some indication of toxicity, e.g., toxic signs, death, or depression of the ratio of PCEs to normochromatic erythrocytes (NCEs). The use of a high dose, as defined above, increased the likelihood that a weak clastogen could be detected.

Assay Acceptance Criteria:
The vehicle control group had less than approximately 0.4% micronucleated PCEs and the group mean was within the historical control range. The positive control group had a statistically significantly higher ($p \leq 0.01$) number of micronucleated PCEs than the vehicle control group and was consistent with historical positive control data.

Negative controls:
Corn oil ([redacted] (b) (4), Lot#12-389)
Positive controls:
Cyclophosphamide ([redacted] (b) (4), lot# 108H0568, CAS #6055-19-2)

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

Criteria for Positive Results:
The criteria for a positive response were the detection of a statistically significant increase in micronucleated PCEs for at least one dose level, and a statistically significant dose-related response. A test article that did not induce both of these responses was considered negative. Statistical significance was not the only determinant of a positive response because the biological relevance of the results was also considered for the final evaluation.

Study outcome:
Dose Range finding assay: Dose levels of 500, 1000, and 2000 mg/kg were chosen for the Dose Range Finding assay. Eighteen animals, approximately 8 weeks old at the time of dosing, with a weight range of 31.5 - 33.5 g and 23.7 - 27.7 g, for the males and females, respectively, were used in this study. At the termination of this assay all surviving animals were euthanized by CO_2 inhalation followed by incision of the diaphragm. The treatment regimen for this assay is shown below. All animals were examined immediately after dosing, about 1 hour toxic signs and/or mortality. All animals appeared normal immediately after dosing and remained healthy until the end of the observation period. Based on these results, the maximum tolerated dose was estimated to be 2000 mg/kg.

Treatment Regimen for Dose Rangefinding Assay with Exaltolide

Target Treatment (mg/kg)	Stock Concentration (mg/mL)	Number of Animals Male	Number of Animals Female	Route of Administration	Dosing Volume (mL/kg)
500	50	3	3	oral gavage	10
1000	100	3	3	oral gavage	10
2000	200	3	3	oral gavage	10

Micronucleus Assay:
Based on results from the dose range finding assay, dose levels of 500, 1000, and 2000 mg/kg were selected for testing in mice in this assay. Only males were used in the micronucleus assay because there were no substantial differences in clinical observations between the sexes in the dose range finding assay. Forty-two animals, approximately 8 weeks old at the time of dosing, with a weight range of 27.8 - 35.2 g were used in this assay. An outline of the dosing scheme and harvest time points is in a table below. Animals were examined as described above in the dose range finding studies.

Dosing Scheme for the Micronucleus Assay with Exaltolide

Target Treatment (mg/kg)	Stock Concentration (mg/mL)	Route of Administration	Dosing Volume (mL/kg)	Males/ Harvest Timepoint 24-Hour	48-Hour
500	50	oral gavage	10	6	-
1000	100	oral gavage	10	6	-
2000	200	oral gavage	10	6	6
Vehicle Control, Corn Oil	0	oral gavage	10	6	6
Positive Control, Cyclophosphamide, 80	8	oral gavage	10	6	-

Slides prepared from the bone marrow collected from five animals per group at the designated harvest time points were scored for micronuclei and the PCE to NCE cell ratio. The micronucleus frequency (expressed as percent micronucleated cells) was determined by analyzing the number of micronucleated PCE from at least 2000 PCEs per animal. The PCE: NCE ratio was determined by-scoring the number of PCEs and NCEs observed scoring at least the first 500 erythrocytes per animal.

The historical background frequency of micronucleated cells were expressed as percent micronucleated cells based on the number of PCEs analyzed. The historical background frequency of micronuclei in the Crl:CD-1(ICR) BR strain at this laboratory is about 0.0 to 0.4%, which is within the range of the published data. Micronuclei were darkly stained and generally round, although almond- and ring-shaped micronuclei occasionally occurred. Micronuclei were sharp bordered and generally between one-twentieth and one-fifth the size of the PCEs. The unit of scoring was the micronucleated cell, not the micronucleus; thus, the occasional cell with more than one micronucleus was counted as one micronucleated PCE, not two (or more) micronuclei. The staining procedure permitted the differentiation by color of PCEs and NCEs (bluish-gray and red, respectively).

Exaltolide did not induce signs of clinical toxicity in any of the treated animals but was cytotoxic to the bone marrow (i.e., a statistically significant decrease in the PCE:NCE ratio) at the at the 2000 mg/kg dose level at the 48-hour harvest time point. A statistically significant increase in micronucleated PCEs was not observed at any dose level or harvest time point. The positive control, cyclophosphamide, induced statistical1y significant increases in micronucleated PCEs as compared to that of the vehicle controls, with a mean of 1.72% as shown (Table 1).

TABLE 1: MICRONUCLEUS DATA SUMMARY TABLE

ASSAY: 22572 TEST ARTICLE: Exaltolide

TREATMENT	DOSE	HARVEST TIME	% MICRONUCLEATED PCEs MEAN OF 2000 PER ANIMAL ± S.E.	RATIO PCE:NCE MEAN ± S.E.
CONTROLS				
VEHICLE	Corn Oil	24 hr	0.03 ± 0.02	0.56 ± 0.04
		48 hr	0.09 ± 0.03	0.75 ± 0.06
POSITIVE	CP 80 mg/kg	24 hr	1.72 ± 0.18*	0.64 ± 0.06
TEST ARTICLE	500 mg/kg	24 hr	0.05 ± 0.02	0.76 ± 0.07
	1000 mg/kg	24 hr	0.09 ± 0.02	0.52 ± 0.06
	2000 mg/kg	24 hr	0.03 ± 0.02	0.82 ± 0.11
		48 hr	0.04 ± 0.01	0.55 ± 0.02**

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
** Significantly less than the corresponding vehicle control, $p \leq 0.05$.
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

CONCLUSION

It appears that the test article, Exaltolide, is negative in the mouse bone marrow micronucleus assay under the conditions of this assay.

Study title: Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with Exaltolide (Pentadecalactone)

Key findings:
Exaltolide was evaluated in the initial mutagenicity assay at doses of 33.3, 100, 333, 1000, 3330 and 5000 µg/plate in five tester strains with S9, and in strain WP2uvrA without S9. The test article also was evaluated in all four Salmonella tester strains at doses of 1.0, 3.3, 10.0, 33.3, 66.7 and 100 µg/plate without S9. All doses of test article, as well as the concurrent vehicle and positive controls, were evaluated using three plates per dose. Revertant frequencies for all doses of Exaltolide, in all tester strains with and without S9, approximated or were less than control values. Exaltolide again was found to be incompletely soluble at doses 8330 µg/plate with and without S9, but normal growth was observed in all tester strains at all doses evaluated with and without S9. These results were confirmed in an independent experiment.

Due to a lack of toxicity in the Salmonella strains in the absence of S9, the test article was reevaluated in all four of these strains at doses of 10.0, 33.3, 100, 333, 1000, 3330 and 5000 µg/plate without S9. Exaltolide again was found to be incompletely soluble at doses 8330 µg/plate, and normal background growth was observed in all tester strains at all doses evaluated. Revertant frequencies for all doses of Exaltolide, in all tester strains, approximated control values. These results were confirmed in an independent experiment, except that the test article was insoluble at doses ≥1000 µg/plate. The results of the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay indicate that under the conditions of this study, the test article, Exaltolide did not cause a positive increase in the mean number of revertants per plate in any of the tester strains in the presence or absence of Aroclor-induced rat liver S9.

Study no.: 22572-0-409OECD
Volume #13, and page #: 49-77
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/13/2001
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Exaltolide: Lot#119417.0009

Methods

Strains/species/cell line: Salmonella typhimurium(TA98, 100, 1535 and 1537) and Escherichia coli(WP2uvrA) as shown below.

TABLE I. TESTER STRAIN GENOTYPES				
Tester Strain	*his/trp* Mutation	Additional Mutations		Plasmid
		Repair	LPS	
TA98	*his*D3052	*uvr*B	*rfa*	pKM101
TA100	*his*G46	*uvr*B	*rfa*	pKM101
TA1535	*his*G46	*uvr*B	*rfa*	–
TA1537	*his*C3076	*uvr*B	*rfa*	–
WP2*uvr*A	*trp*	*uvr*A	–	–

Doses used in definitive study:
Assay without activation: 1, 3, 10, 33, 67 and 100 µg/mL
Assay with activation: 33, 100, 333, 1000, 3330, and 5000 µg/mL

Basis of dose selection:

A dose range finding assay was conducted on the test article using tester strains TA100 and *WP2uvrA* in the presence and absence of S9 (one plate per dose). Ten doses of test article, from 6.67 to 5000 µg/plate, were evaluated (Tables 1 and 4). Cytotoxicity, as evidenced by a reduction in the number of revertants per plate and/or a thinning of the background lawn, was observed in tester strain TA100 at doses ≥3330 µg/plate in the presence of S9 and at doses ≥66.7 µg/plate in the absence of *S9.* In addition, the test article was found to be incompletely soluble at doses ≥3300 µg/plate with and without S9 except in strain *WP2uvrA* with S9 in which no precipitate was observed. Thus, the sponsor selected the 5000 and 100 µg/plate as maximum doses in the presence and absence of S9 fractions, respectively for the mutagenicity assay.

TABLE 1 : DOSE RANGEFINDING STUDY

Test Article ID: Exaltolide

Experiment ID: 22572-A1 | Date Plated: 26-Jun-01

Vehicle: DMSO | Date Counted: 28-Jun-01

		TA100 Revertants Per Plate			
		With S9		Without S9	
µg/Plate		Revertants Per Plate	Background Lawn Evaluation[a]	Revertants Per Plate	Background Lawn Evaluation[a]
0.00 (Vehicle) (50 µL)		119	N	90	N
Test Article	6.67	98	N	95	N
	10.0	104	N	84	N
	33.3	105	N	88	N
	66.7	97	N	91	R
	100	93	N	61	R
	333	79	N	72	R
	667	102	N	51	R
	1000	82	N	46	R
	3330	68	NP	18	RP
	5000	66	NP	9	RP

[a] Background Lawn Evaluation Codes:
N = normal R = reduced A = absent
P = precipitate O = obscured by precipitate

TABLE 4 : MUTAGENICITY ASSAY RESULTS – SUMMARY

Test Article ID: Exaltolide

Experiment ID: 22572-B1

Date Plated: 05-Jul-01 | Vehicle: DMSO

Date Counted: 09-Jul-01 | Plating Aliquot: 50 µL

	Dose\Plate		Mean Revertants Per Plate with Standard Deviation								Back-ground Lawn[a]
			TA98		TA100		TA1535		TA1537		
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver											
Vehicle Control			27	2	107	7	14	2	8	3	N
Test Article	33.3	µg	21	8	81	4	11	5	6	1	N
	100	µg	23	2	96	10	12	3	9	5	N
	333	µg	22	4	85	7	11	4	9	1	N
	1000	µg	24	6	86	17	9	3	11	4	N
	3330	µg	26	5	68	6	12	2	4	1	NP
	5000	µg	24	4	83	14	9	3	5	1	NP
Positive Control[b]			367	22	823	28	119	15	126	6	N
Microsomes: None											
Vehicle Control			15	2	74	7	8	3	7	3	N
Test Article	1.00	µg	21	9	80	18	11	5	7	2	N
	3.33	µg	13	3	77	14	11	4	6	2	N
	10.0	µg	18	3	73	3	15	6	7	1	N
	33.3	µg	16	4	58	16	11	4	8	2	N
	66.7	µg	13	5	71	7	13	6	3	2	N
	100	µg	13	2	68	11	9	3	4	2	N
Positive Control[c]			242	21	872	21	650	21	879	85	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b]			[c]		
TA98	benzo[a]pyrene	2.5 µg/plate	TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate

Negative controls:
DMSO (Lot#100K014) and vehicle

Positive controls:

TABLE II. POSITIVE CONTROLS

Tester Strain	S9 Mix	Positive Control	Dose (µg/plate)
TA98	+	benzo[a]pyrene	2.5
TA98	–	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	–	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	–	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	–	ICR-191	2.0
WP2*uvr*A	+	2-aminoanthracene	25.0
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0

The sources and grades of positive control articles are as follows:

benzo[a]pyrene (CAS #50-32-8), (b) (4) purity ≥97%
2-aminoanthracene (CAS #613-13-8), (b) (4) purity ≥90%
2-nitrofluorene (CAS #607-57-8), (b) (4) purity ≥98%
sodium azide (CAS #26628-22-8), purity ≥99%
ICR-191 (CAS #17070-45-0), (b) (4) purity ≥90%
4-nitroquinoline-N-oxide (CAS #56-57-5), (b) (4) purity ≥99%.

Incubation and sampling times:

This methodology (Ames et al., 1975) has been shown to detect a wide range of classes of chemical mutagens. In the plate incorporation methodology, the test article, the tester strain, and the 89 mix (where appropriate) were combined in molten agar which was overlaid onto a minimal agar plate. Following incubation, revertant colonies were counted. All doses of the test article, the vehicle controls and the positive controls were plated in triplicate. After the required components had been added, the mixture was vortexed and overlaid onto the surface of 25 mL of minimal bottom agar contained in a 15 x 100 petri dish. After the overlay solidified, the plates were inverted and incubated for 52 ± 4 hours at 37 ± 2°C. Positive control articles were plated using a 50 µL plating aliquot.

Results:

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Tester strain culture density: The cell densities of all tester strain cultures were greater than or equal to 0.5 x 10^9 bacteria/mL, demonstrating that appropriate numbers of bacteria were plated. In addition, the optical densities of these cultures reached a target value demonstrated to produce cultures with at least 0.5 x 10^9 bacteria/mL.

Positive control values: The mean value of the positive control without S9 Mix for each tester strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain, demonstrating that the tester strains were capable of identifying a mutagen. An acceptable positive control in the presence of S9 for a specific strain was evaluated as having demonstrated the integrity of the S9 mix and the ability of the tester strain to detect a mutagen. Tester Strains TA1535 and TA1537, for a test article to be considered positive, it had to produce at least a 3-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.

Assay evaluation criteria: Once the criteria for a valid assay had been met, responses observed in the assay were evaluated. Tester Strains TA98, TA100 and *WP2uvrA*. For a test article to be considered positive, it had to produce at least a 2-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.

Acceptable high dose: In the dose range finding studies (Table 1 and 4 above) show that the test article reduced revertants per plate significantly at a dose of 5000 µg/plate in the presence or absence of S9 fraction. Cytotoxicity is detectable as a decrease in the number of revertant colonies per plate and/or by a thinning or disappearance of the bacterial background lawn. A slight thinning of the bacterial background lawn which was not accompanied by a reduction in the number of revertants per plate was not evaluated as an indication of cytotoxicity. There were half dozen doses that were non-toxic.

Criteria for Positive Results:

The mean value of the positive control in the presence of S9 Mix for each tester strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain, demonstrating that the S9 mix was capable of metabolizing a promutagen to its mutagenic form(s).

Study outcome: For dose range finding study, please see Tables 1 and 4 above.

Mutagenesis Assay:

Based upon the results of the dose range finding assay, Exaltolide was evaluated in the initial mutagenicity assay at doses of 33.3; 100, 333, 1000, 3330 and 5000

µg/plate in all five tester strains with S9, and in strain *WP2uvrA* without S9. The test article also was evaluated in all four *Salmonella* tester strains at doses of 1.00, 3.33, 10.0, 33.3, 66.7 and 100 µg/plate without *S9.* All doses of test article, as well as the concurrent vehicle and positive controls, were evaluated using three plates per dose. Revertant frequencies for all doses of ExaItoIide, in all tester strains with and without S9, approximated or were less than control values. Exaltolide was again found to be incompletely soluble at doses ≥3300 µg/plate with and without S9, but normal growth was observed in all tester strains at all doses evaluated with and without S9. These results were confirmed in several triplicated independent experiment as summarized below (Tables 5, 7, and 10). Mean revertants per plate of all strains in the study were within the historical control data as attached below.

TABLE 5 : MUTAGENICITY ASSAY RESULTS – INDIVIDUAL PLATE COUNTS AND SUMMARY

Test Article ID: Exaltolide

Experiment ID: 22572-B1

Date Plated: 05-Jul-01 Vehicle: DMSO

Date Counted: 09-Jul-01 Plating Aliquot: 50 µL

Dose\Plate			Revertants Per Plate WP2*uvr*A 1	2	3	Mean Revertants Per Plate with Standard Deviation WP2*uvr*A Mean	S.D.	Background Lawn[a]
Microsomes: Rat Liver								
Vehicle Control			16	12	25	18	7	N
Test Article	33.3	µg	18	17	24	20	4	N
	100	µg	25	11	19	18	7	N
	333	µg	19	14	11	15	4	N
	1000	µg	11	14	15	13	2	N
	3330	µg	14	19	14	16	3	NP
	5000	µg	16	9	12	12	4	NP
Positive Control[b]			418	339	435	397	51	N
Microsomes: None								
Vehicle Control			18	19	24	20	3	N
Test Article	33.3	µg	16	17	12	15	3	N
	100	µg	16	18	16	17	1	N
	333	µg	10	15	16	14	3	N
	1000	µg	21	20	15	19	3	N
	3330	µg	9	19	14	14	5	NP
	5000	µg	15	12	14	14	2	NP
Positive Control[c]			330	329	348	336	11	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] WP2*uvr*A 2-aminoanthracene 25.0 µg/plate [c] WP2*uvr*A 4-nitroquinoline-N-oxide 1.0 µg/plate

TABLE 7 : MUTAGENICITY ASSAY RESULTS – SUMMARY

Test Article ID: Exaltolide

Experiment ID: 22572-C1

Date Plated: 18-Jul-01 Vehicle: DMSO

Date Counted: 25-Jul-01 Plating Aliquot: 50 µL

	Dose/Plate		Mean Revertants Per Plate with Standard Deviation								Background Lawn[a]
			TA98		TA100		TA1535		TA1537		
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver											
Vehicle Control			27	11	103	11	15	1	12	2	N
Test Article	33.3	µg	22	5	98	3	9	4	11	5	N
	100	µg	21	5	99	9	11	6	17	2	N
	333	µg	18	5	89	7	10	2	8	2	N
	1000	µg	17	5	79	7	10	2	10	5	N
	3330	µg	17	3	61	6	10	4	6	1	NP
	5000	µg	11	3	68	5	5	3	6	1	NP
Positive Control[b]			276	14	683	47	111	11	151	12	N
Microsomes: None											
Vehicle Control			9	3	75	16	12	3	10	2	N
Test Article	1.00	µg	13	3	75	20	9	1	7	3	N
	3.33	µg	15	2	74	8	9	2	10	5	N
	10.0	µg	15	4	73	12	8	3	9	6	N
	33.3	µg	13	5	85	14	11	3	9	3	N
	66.7	µg	13	3	68	13	10	1	8	2	N
	100	µg	11	2	68	12	8	2	8	3	N
Positive Control[c]			308	36	1059	20	704	35	757	26	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA98	benzo[a]pyrene	2.5 µg/plate	[c] TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate

TABLE 10 : MUTAGENICITY ASSAY RESULTS – INDIVIDUAL PLATE COUNTS AND SUMMARY

Test Article ID: Exaltolide

Experiment ID: 22572-D2

Date Plated: 21-Aug-01 Vehicle: DMSO

Date Counted: 23-Aug-01,24-Aug-01 Plating Aliquot: 50 µL

Revertants Per Plate

Dose/Plate		TA98			TA100			TA1535			TA1537			Background Lawn[a]
		1	2	3	1	2	3	1	2	3	1	2	3	
Microsomes: None Vehicle Control		20	21	17	82	88	76	9	17	11	4	14	10	N
Test Article 10.0	µg	14	17	15	67	84	101	11	19	14	7	7	7	N
33.3	µg	17	16	11	88	87	71	12	14	14	6	1	7	N
100	µg	14	17	14	87	70	68	6	7	9	7	8	9	N
333	µg	12	8	15	93	61	59	9	7	15	3	6	7	N
1000	µg	15	19	19	33	34	27	18	10	8	5	5	1	NP
3330	µg	7	15	25	17	25	23	9	12	9	6	1	3	NP
5000	µg	17	16	16	11	37	27	7	11	14	3	3	3	NP
Positive Control[c]		273	229	270	1329	1250	1367	892	941	892	682	713	662	N

Mean Revertants Per Plate with Standard Deviation

Dose/Plate		TA98		TA100		TA1535		TA1537		Background Lawn[a]
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: None Vehicle Control		19	2	82	6	12	4	9	5	N
Test Article 10.0	µg	15	2	84	17	15	4	7	0	N
33.3	µg	15	3	82	10	13	1	5	3	N
100	µg	15	2	75	10	7	2	8	1	N
333	µg	12	4	71	19	10	4	5	2	N
1000	µg	18	2	31	4	12	5	4	2	NP
3330	µg	16	9	22	4	10	2	3	3	NP
5000	µg	16	1	25	13	11	4	3	0	NP
Positive Control[c]		257	25	1315	60	908	28	686	26	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate

Attachment: Historical Control Data:

HISTORICAL CONTROL DATA FOR BACTERIAL MUTAGENICITY STUDIES

Plate Incorporation Method - Report Period 99L

VEHICLE CONTROLS WITH S9 MIX					
Strain	TA98	TA100	TA1535	TA1537	WP2*uvr*A
Mean Revertants per Plate	26.2	109.6	13.1	9.1	18.5
Standard Deviation	6.8	18.5	4.0	4.5	6.1
Maximum	48	164	27	26	48
Minimum	7	62	4	2	6
Count	244	248	194	182	130

VEHICLE CONTROLS WITHOUT S9 MIX					
Strain	TA98	TA100	TA1535	TA1537	WP2*uvr*A
Mean Revertants per Plate	16.2	92.5	11.6	6.5	16.8
Standard Deviation	4.9	15.1	4.0	3.2	5.0
Maximum	32	145	23	21	30
Minimum	6	57	3	1	6
Count	224	213	191	176	122

POSITIVE CONTROLS WITH S9 MIX*					
Strain	TA98	TA100	TA1535	TA1537	WP2*uvr*A
Mean Revertants per Plate	411.4	796.4	139.4	144.3	374.7
Standard Deviation	61	230.5	28.8	34.4	123.3
Maximum	572	1343	252	222	669
Minimum	249	335	67	57	165
Count	170	218	182	170	129

POSITIVE CONTROLS WITHOUT S9 MIX**					
Strain	TA98	TA100	TA1535	TA1537	WP2*uvr*A
Mean Revertants per Plate	234.5	617.2	553.0	585.1	297.6
Standard Deviation	49.1	123.8	101.3	130.4	128.1
Maximum	381	1050	852	890	781
Minimum	121	354	361	338	112
Count	204	192	180	165	121

* TA98	benzo (a) pyrene	2.5 µg/plate
TA100	2-aminoanthracene	2.5µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate
WP2*uvr*A	2-aminoanthracene	25.0 µg/plate

**TA98	2-nitrofluorene	1.0 µg/plate
TA100	sodium azide	2.0 µg/plate
TA1535	sodium azide	2.0 µg/plate
TA1537	ICR-191	2.0 µg/plate
WP2*uvr*A	4-nitroquinoline-N-oxide	1.0 µg/plate

Conclusion:

The results of the *Salmonella-Escherichia coli/Mammalian-Microsome* Reverse Mutation Assay with a Confirmatory Assay indicate that the test article, Exaltolide, did not cause a positive increase in the mean number of revertants per plate in any of the tester strains in the presence or absence of Aroclor-induced rat liver S9.

2.6.6.5 Carcinogenicity

No Carcinogenicity study has been conducted.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Pentadecalactone(Exaltolide): Effects on Male Fertility in Rats

Key study findings:
Sprague-Dawley rats received pentadecalactone orally (gavage) at doses of 0, 200, 500 and 1000 mg/kg/day for 4 weeks prior to mating with untreated females, then throughout mating and continued for at least 9 weeks of dosing prior to necropsy for females. The females were killed on Day 14-16 of gestation and their reproductive tracts examined; the number of implants and their status were recorded. Male rats were monitored for clinical signs of toxicity, bodyweight and food consumption. At necropsy, the weights of the male reproductive organs were recorded; epididymal sperm motility and counts were assessed.

Treatment-related findings in the males were limited to sporadic increases in salivation immediately post dosing in all animals given 1000 mg/kg/day, 14 animals given 500 mg/kg/day and 2 animals given 200 mg/kg/day. There was no effect on body weight, food consumption and organ weights at any dose level. Mating performance and the resulting pregnancy performance were not affected by treatment. There were no effects on sperm mobility or sperm count at any dose level. Under the conditions of this study, it was concluded that 1000 mg/kg/day was the NOAEL for effects on male fertility.

Study no.: (b) (4) #493050; Report#20640
Volume #13, and page #: 79-104
Conducting laboratory and location: (b) (4)
Date of study initiation: 8/20/2001
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Exaltolide, Lot#AB13199

Methods

Doses: 0, 200, 500 and 1000 mg/kg/day
Species/strain: Sprague-Dawley rat/Crl:CD(SD) IGS BR
Number/sex/group: 24 rats/sex/group
Route, formulation, volume, and infusion rate: Oral (Gavage)
Satellite groups used for toxicokinetics/replacement: 3/sex/group
Study design:

The males were doses once daily for 4 weeks prior to mating, then throughout the mating period and until at least 9 weeks of treatment had been completed. No females were treated, which were killed on Day 14-16 of gestation to assess pregnancy performance. Vaginal lavages were taken early each morning, commencing on the day of pairing, until mating had occurred, at which point the animals were separated. The stage of estrus observed in each vaginal lavage was recorded. The day of detection of a clear positive sign of mating, a copulatory plug *in situ* and/or sperm in the lavage, was designated Day 0 of gestation.

Parameters and endpoints evaluated:

Animals' viability was check twice a day; clinical signs for the first 1-2 hours after dosing during the first week of treatment were recorded. Body weight and food consumption were recorded daily. Fertility index (male) was calculated: the number of litter divided by number of paired. The females were killed on a calculated Day 14, 15 or 16 of gestation for evaluation of their pregnancies. For females that did not show a clear positive mating sign, the day of sacrifice was 14 days after the last night of pairing. Male animals were killed after completion of at least 9 weeks of treatment.

The right epididymis from each surviving male was submitted for sperm evaluation. The cauda epididymis was separated from the caput, and weighed. The tip of the epididymis was then removed and processed for Computer Aided Semen Analysis (CASA). The remaining portion of the cauda epididymis was then weighed and used for the sperm count. The tip of the cauda epididymis was placed in 3 ml of Medium 199 which contained 0.2% BSA and HEPES and the sperm were allowed to 'swim out' into the surrounding medium. This sperm suspension was further diluted with the medium, and a 5 µl aliquot was examined using a Hamilton Thorne sperm motility analyser. At least 200 motile sperm per animal were assessed, except where it was obvious that motility for that animal was compromised, and the following parameters were assessed: Motility, Progressive mobility, Straight Line Velocity (VSL), which is defined as the mean velocity from the beginning to the end of a track, expressed in µm/second.

Results

Mortality and Clinical signs: There was no death except one control rat (#22) was killed by dosing accident. It appears that salivation was the test article-dose related (Table 1) below. There were no treatment-related remarkable clinical signs.

Table 1 ***Pentadecalactone Effects on Male Fertility in Rats Group Incidence of Clinical Observations and Necropsy Findings***

Observation/Finding	Group/Dose Level ($mg.kg^{-1}.day^{-1}$)			
	1 (0)	2 (200)	3 (500)	4 (1000)
Total number of animals	24	24	24	24
Salivation	0	2	14	24
Staining	2	6	5	16
Wet coat around mouth/ muzzle	0	0	2	5
Sparse hair/ bald area(s)	5	3	1	3
Encrusted toe(s)	3	1	1	0
Red extremities	3	1	2	1
Encrusted eyelid	1	1	2	0
Scab(s)	1	4	4	1
Soft faeces	1	0	1	0
Abnormal respiration	1	1	0	0
Subdued behaviour	1	1	0	0
Swelling	1	0	0	0
Piloerection	1	0	0	0
Tooth overgrown/ damaged	1	0	0	0
Tray paper/ cage stained red	0	4	0	0
Cut on eyelid	0	0	1	0
Unkempt coat	0	0	0	1
Liver abnormal	1	0	0	0
Lungs dark	1	1	0	0
Submaxillary salivary glands gelatinous	1	0	0	0
Creamy fluid in thoracic cavity	0	1	0	0
Testis flaccid/ epididymis small	1	0	1	0
Seminal vesicles small	0	0	0	1
Animal killed prematurely	1	0	0	0

Body weight and Food consumption: There were no differences between the control and treated groups in the two parameters.

Toxicokinetics: Not provided the data.

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

Mating/fertility index: The number of rats that did not becoming pregnant on Day 1 appears high in the MD and HD group (Table 4) below. There were no remarkable differences in all groups in male fertility index.

Table 4 ***Pentadecalactone Effects on Male Fertility in Rats Mating Performance and Fertility Indices***

Number of Nights to Positive Mating Sign	Group/Dose Level (mg Pentadecalactone.kg^{-1}.day^{-1})			
	1 (0)	2 (200)	3 (500)	4 (1000)
	Number of Animals (Number of these not becoming pregnant)			
1	5	6	10	9
2	8	11	7	11
3	6	2	2	3
4	4	4	1	1
5	0	1	2	0
No clear indication of mating with first mate	1(1)	-	2(2)	-
Mated with second female	1	-	2(1)	-
No indication of mating with second mate	-	-	1	-
Median number of nights to positive mating sign	2	2	2	2
Number passing one or more oestruses	0	0	2	0
Number of males paired	24	24	24	24
Number of siring males	24[a]	24	23[a]	24
Male Fertility Index (%)	100	100	96	100

[a] = Includes males that mated with their second female

Pregnancy performance: The number of pregnancy was comparable in control and treated groups. Per cent of pre-implantation loss were 2 to 6, which was not different in all groups. Per cent of total early embryonic deaths was highest in the control group (6%). And the per cent deaths in the treated groups were 3, 5, and 4, respectively in the LD, MD and HD groups. Mean live implants and early embryonic deaths were also not significantly different as shown below (Table 5).

Table 5 ***Pentadecalactone Effects on Male Fertility in Rats Pregnancy Performance***

	Group/Dose Level (mg Pentadecalactone.kg^{-1}.day^{-1})			
	1 (0)	2 (200)	3 (500)	4 (1000)
Number pregnant	24	24	23	24
Total corpora lutea graviditatis	320	330	315	303
Total number of implants	303	324	295	295
Pre-implantation loss as %	5	2	6	3
Total live implants (%)	286 (94)	315 (97)	277 (94)	283 (96)
Total dead implants (%)	17 (6)	9 (3)	18 (6)	12 (4)
Total early embryonic deaths (%)	17 (6)	9 (3)	16 (5)	12 (4)
Total late embryonic deaths (%)	0	0	2 (1)	0
Mean corpora lutea graviditatis	13.3 ± 1.7	13.8 ± 1.3	13.7 ± 1.7	12.6 ± 1.5
Mean implants	12.6 ± 1.7	13.5 ± 1.4	12.8 ± 1.4	12.3 ± 1.7
Mean live implants	11.9 ± 2.0	13.1 ± 1.4	12.0 ± 1.6	11.8 ± 1.7
Mean dead implants	0.7 ± 1.3	0.4 ± 0.6	0.8 ± 0.6	0.5 ± 0.6
Mean early embryonic deaths	0.7 ± 1.3	0.4 ± 0.6	0.7 ± 0.6	0.5 ± 0.6
Mean late embryonic deaths	0	0	0.1 ± 0.3	0

Means are given ± Standard Deviation

Sperm evaluation:

Sperm parameters such as cauda weight, sperm motility, progressive militia and its velocity for the forward movement were compared in the control and treated group (Table 6) below. It appears that there were no remarkable differences between the control and treated groups, which suggests that the test had no direct effects on these parameters.

Table 6 ***Pentadecalactone Effects on Male Fertility in Rats Epididymal Sperm Evaluation Group Mean Values ± Standard Deviation***

	Group/Dose Level (mg Pentadecalactone.kg^{-1}.day^{-1})			
	1 (0)	2 (200)	3 (500)	4 (1000)
Cauda Weight (g)	0.3092 ± 0.0272	0.3079 ± 0.0357	0.3107 ± 0.0272	0.3094 ± 0.0299
Count per g Cauda	410 ± 98	383 ± 139	433 ± 90	422 ± 137
Count per Cauda	127 ± 35	118 ± 44	135 ± 32	130 ± 42
Motility (%)	75.9 ± 14.4	80.2 ± 10.8	80.3 ± 12.3	79.3 ± 11.1
Progressive Motility (%)	35.1 ± 10.1	37.2 ± 9.3	36.3 ± 8	34.9 ± 8
Straight Line Velocity (µm.s^{-1})	96.7 ± 6.9	99.7 ± 10.1	100.3 ± 6.5	100.7 ± 7

Counts are expressed in millions

Male organ weights:

Mean absolute weights of epididymides, prostate, seminal vesicles, testes and male rat body weight were not different in the control from those of the treated groups, which suggests the test articles has no effect on male sex organ development as shown below (Table 7).

Table 7 ***Pentadecalactone Effects on Male Fertility in Rats Absolute Organ Weights (g) Group Mean Values: Males***

Group/Dose Level ($mg.kg^{-1}.day^{-1}$)		Body Weight (g)	Epididymides	Prostate	Seminal Vesicles	Testes
1 (0)	Number	23	22	23	23	22
	Mean	532	1.3958	0.749	2.3853	3.72
	SD	42	0.1200	0.135	0.3768	0.26
2 (200)	Number	24	24	24	24	24
	Mean	523	1.3471	0.660	2.3427	3.65
	SD	50	0.0967	0.126	0.3925	0.38
	Prob.					
3 (500)	Number	24	23	24	24	23
	Mean	533	1.3826	0.667	2.3537	3.75
	SD	49	0.1105	0.156	0.3598	0.33
	Prob.					
4 (1000)	Number	24	24	24	24	24
	Mean	514	1.3550	0.677	2.3645	3.64
	SD	47	0.1031	0.169	0.2648	0.34
	Prob.					

2.6.6.7 Local tolerance: No study was performed.

2.6.6.8 Special toxicology studies: None

2.6.7 TOXICOLOGY TABULATED SUMMARY

The sponsor submitted three summary tables for toxicology studies.

(b) (4)

(b) (4)

(b) (4)

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

The sponsor performed genotoxicity studies with the excipient (pentadecalactone, exaltolide) in addition to 13-Week toxicity studies in rats and dogs. 4-Week intranasal instillation toxicity study (Study#7639-100) was conducted in 10 rats/sex/group at insulin doses of 0.64 and 1.28 mg/kg/day, which contained 1.28 and 2.56 mg/kg/day of pentadecalactone. There were no remarkable clinical signs and toxicology parameters except an expected hypoglycemic response and a slight increase in ALT levels in the mice of treated groups. Repetitive stimulation on nasal passages may increase eosinophils in epithelium as seen in gross histopathology examination, which was confirmed in microscopic histopathologic demonstration of eosinophils, alveolar macrophage, and sinus globlets. The findings were confirmed in 13-Week Intranasal Instillation Toxicity Study with Insulin Rats with 4-Week Recovery (Study#7639-102).

In 13-Week Intranasal Instillation Toxicity Study with Insulin in Dogs (Study#7639-103), males given insulin spray at 1.761U/cm^2/day and females given at 1.32 or 1.76 IU/cm^2/day had significantly greater body weight gain, with more food consumption, during the first week of the dosing phase when compared with each of the three vehicle control groups. Administration of the test article had no remarkable effects on clinical pathology test, organ weight and mean terminal body weight after recovery phase in dogs.

Blood glucose declined for up to 4 hours after dosing with insulin spray at 1.32 or 1.76 IU/cm^2/day. The effect lasted longer in the females. The animals given insulin at 1.76 IU/cm^2/day tended to have a slightly larger blood glucose depression compared with the animals given insulin at 1.32 IU/cm^2/day. The effect was pronounced on Day 1 of the dosing phase, appeared to be slightly less pronounced at Week 6 of the dosing phase, and did not appear to cause an effect in males at Week 13 of the dosing phase, although females still showed the blood glucose depression at this interval. Treatment-related decreases in glucose were observed in 4- and 13-Week rat studies, which hypoglycemic effects were shortening on Day 23, compared those on Day 1 for some reasons.

In order to justify the use of (b) (4)% pentadecalactone intranasally (the product was approved for dermal use up to (b) (4)%), 15 rats/sex/group were administered pentadecalactone topically at doses of (b) (4) mg/kg/day for 91 days. Clinical signs such as skin staining, cold to touch, malocclusion, scabbed at applied area and hair loss were commonly observed clinical signs, although they were not dose-related. The treatment had no effect on body weight, food consumption, hematology and clinical chemistry. There were no treatment effect on dermatology scores, macro- and microscopic findings. However, a dermal study will not qualify an excipient for use intra-nasally. The 90-day rat and dog studies that used (b) (4)% CPE-215 should qualify.

The sponsor performed three genotoxicity studies with pentadecalactone: 1) L5178Y TK $^{+/-}$ Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay with pentadecalactone (Study#22572-0-431 ICH), 2) In Vivo Mouse Micronucleus Assay with pentadecalactone (Study#22572-0-455OECD) and 3) Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with pentadecalactone(22572-0-409 OECD). All the three genotoxicity studies were performed under acceptable conditions and the result was negative.

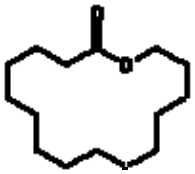
Finally the sponsor evaluated the effects of pentadecalactone on male fertility in rats (b) (4) #493050, Report#20650). SD rats received pentadecalactone orally (gavage) at doses of 0, 200, 500 and 1000 mg/kg/day for 4 weeks prior to mating with untreated females, then throughout mating and continued for at least 9 weeks of dosing prior to necropsy. The females were killed on Day 14-16 of gestation and their reproductive tracts examined; the number of implants and their status were recorded. Male rats were monitored for clinical signs of toxicity, bodyweight and food consumption. At necropsy, the weights of the male reproductive organs were recorded; epididymal sperm motility and counts were assessed.

Treatment-related findings in the males were limited to increases in salivation immediately post dosing in all treated-animals, which appears to be related to the dose. The incidences of skin staining were also dose-dependently increased. There was no effect on body weight, food consumption and organ weights at any dose level. Mating performance and the resulting pregnancy performance were not affected by treatment. There were no effects on sperm mobility or sperm count at any dose level. Under the conditions of this study, it was concluded that 1000 mg/kg/day was the NOAEL for effects on male fertility. There was no carcinogenicity study in this IND.

Rats received 1.28 mg/kg/day/nostril in both 4- and 13-Week toxicology studies, which is approximately (b) (4) mg/m^2. The dose was 5.2 times higher than the recommended human dose of 100 IU, based on body surface area comparison. In case of dog, the exposure ratio was approximately 3.5 because each dog received insulin at a dose of (b) (4) mg/kg/day/nostril, which is equivalent to (b) (4) mg of insulin/m^2.

Internal comments:

The chemical structure of pentadecalactone is shown to the right.



Pentadecalactone (other names are: Exaltolide; 15-Pentadecanolide; Angelica lactone; omega-pentadecalactone; Cyclopentadecanolide, etc) is the excipient that was approved for topical application up to (b) (4)%. The sponsor plans to use (b) (4)% in a mixture with insulin for nasal spray. The compound is negative in all three genotoxicity studies. There were no remarkable toxicology findings that are related to the test article as presented. Local toxicity study with the test article was negative. In male fertility study, there was a dose-related increase in salivation (0, 2, 14, and 24 rats out of 24 rats in control, (b) (4) mg pentadecalactone groups).

In this study both of rats and dogs, blood glucose declined rapidly for up to 4 hours after dosing with insulin spray. Treatment-related decreases in glucose were observed in 4- and 13-Week rat studies, which hypoglycemic effects were shortening on Day 23, compared those on Day 1 for some reasons. The hypoglycemic effect lasted longer in the female dogs, which appears to be related to the insulin dose. The effect was pronounced on Day 1 of the dosing phase, appeared to be slightly less pronounced at Week 6 of the dosing phase, and did not appear to cause an effect in males at Week 13 of the dosing phase, although female dogs still showed the blood glucose depression at this interval. It is not known that the reduced effects were due to the potential reduction of insulin bioavailability due to histopathological changes in nasal passages such as thickening of nasal turbinates. However, the tachyphylaxis that was observed in 4-Week to 13-Week toxicology studies in rats and 13-week toxicity study in dogs should be keep in mind.

External comments (to sponsor):

In 4- and 13-Week toxicology studies both in rats and dogs, blood glucose declined rapidly for up to 4 hours after dosing with insulin spray. However, it appears that the hypoglycemic effect was pronounced on Day 1, compared to the 13 weeks later in both rats and dogs. Please explain this tachyphylaxis, which may also occur in human.

Herman Rhee, Ph.D., Review Pharmacologist

cc: IND Arch
HFD510
HFD510/Davis-Bruno/J. Zawadzki/R.Hedin/ H.Rhee

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/s/

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