

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*  
**202833Orig1s000**

**PHARMACOLOGY REVIEW(S)**

## Pharmacology/Toxicology Supervisory Memorandum

**NDA number:** 202833  
**Sequence number/date/type of submission:** 1 / March 25, 2011/ New NDA  
**Applicant:** LEO Pharma AS  
**Supervisor name:** Barbara Hill  
**Division name:** Division of Dermatology and Dental Products  
**Date:** November 1, 2011  
**Drug:** PICATO (Ingenol Mebutate) Gel, 0.015% and 0.5%  
**Drug class:** Cell death inducer  
**Indication:** Actinic keratosis

### General comments:

- I concur with the conclusions contained in Dr. Jiaqin Yao's Pharmacology/Toxicology review for this drug product.
- I concur that there are no nonclinical approval issues for this drug product and that this NDA is approvable from a Pharmacology/Toxicology perspective.
- I concur with the suggested nonclinical labeling changes proposed by Dr. Yao for this drug product contained in section 1.3.3 of his review including that the appropriate Pregnancy Category is C.

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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BARBARA A HILL  
11/01/2011

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

**PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION**

Application number: 202833  
Supporting document/s: 1  
Applicant's letter date: March 25, 2011  
CDER stamp date: March 25, 2011  
Product: PICATO (Ingenol Mebutate) Gel, 0.015% and  
0.05%  
Indication: Actinic Keratosis (AK)  
Applicant: LEO Pharma A/S  
Review Division: Dermatology and Dental Products  
Reviewer: Jiaqin Yao, PhD  
Supervisor/Team Leader: Barbara Hill, PhD  
Division Director: Susan Walker, MD  
Project Manager: Paul Phillips, MS

*Template Version: September 1, 2010*

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# 1 Executive Summary

## 1.1 Introduction

Ingenol mebutate (PEP005), an (b) (4) derivative of ingenol, was extracted and purified from *Euphorbia peplus* and developed for the topical treatment of actinic keratosis (AK).

## 1.2 Brief Discussion of Nonclinical Findings

The toxicity of ingenol mebutate and/or ingenol mebutate gel has been tested in rats (intravenous and topical for up to 6 months), rabbits, and minipigs (intravenous for up to 28 days and topical for up to 9 months). Only local irritation was noted in rats or minipigs topically treated with ingenol mebutate gel. The severity of local irritation increased with increased dose and/or treatment duration. Ingenol mebutate was negative in the Ames test, in vitro mouse lymphoma assay, and in vivo rat micronucleus test, but positive in the Syrian hamster embryo (SHE) cell transformation assay. Animal studies to evaluate the carcinogenic potential of ingenol mebutate have not been conducted. The need for carcinogenicity studies was waived due to the conditions of clinical use (i.e., up to a 3 day treatment regimen). A few tumors were noted in the intravenous 6 month rat toxicity study conducted with ingenol mebutate. It is not clear that the tumors noted in this study are treatment related because the increase in tumor incidence was very small and not statistically significant. The tumors noted in this 6 month intravenous rat toxicity study are not clinically relevant since the clinical condition of use is topical administration of the drug product and minimal systemic exposure was noted under maximal clinical conditions of use for this topical drug product. No reproductive effects were noted in an intravenous embryofetal development study conducted in pregnant rats with ingenol mebutate. An increased incidence of embryofetal mortality and a few fetal visceral and skeletal variations were noted in an intravenous embryofetal development study conducted in pregnant rabbits with ingenol mebutate. The effects noted in this study are not clinically relevant since minimal systemic exposure was noted under maximal clinical conditions of use for the drug product. No fertility and early embryonic developmental toxicology study and pre- and post-natal developmental toxicology study have been performed with ingenol mebutate. The need for fertility and pre- and post-natal developmental toxicology studies with ingenol mebutate were waived due to minimal detectable systemic exposure after topical administration under maximal clinical use conditions.

## 1.3 Recommendations

### 1.3.1 Approvability

This NDA is approvable from a pharmacology/toxicology perspective.

### 1.3.2 Additional Non Clinical Recommendations

None

### 1.3.3 Labeling

The following wording is recommended for the nonclinical sections of the label.

**INDICATIONS AND USAGE** in the Highlights of Prescribing Information portion of the label:

PICATO Gel is indicated for the topical treatment of actinic keratosis on the face and scalp and on the trunk and extremities.

*Note: Team meetings concluded that the Pharmacologic Class could be either “cell death inducer” or without designation.*

### 8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies of PICATO Gel in pregnant women. PICATO Gel should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Systemic embryofetal development studies were conducted with ingenol mebutate in rats and rabbits. Intravenous doses of 1.5, 3, and 5 µg/kg/day (9, 18, and 30 µg/m<sup>2</sup>/day) ingenol mebutate were administered during the period of organogenesis (gestational days 6 – 16) to pregnant female rats. No treatment related effects on embryofetal toxicity or teratogenicity were noted at doses up to 5 µg/kg/day (30 µg/m<sup>2</sup>/day). Intravenous doses of 1, 2, and 4 µg/kg/day (12, 24, and 48 µg/m<sup>2</sup>/day) ingenol mebutate were administered during the period of organogenesis (gestational days 6 – 18) to pregnant female rabbits. An increase in embryo-fetal mortality was noted at 4 µg/kg/day (48 µg/m<sup>2</sup>/day). An increased incidence of fetal visceral and skeletal variations was noted in all three ingenol mebutate dose groups. The clinical relevance of these findings is unclear since systemic exposure of ingenol mebutate was not detected in subjects with actinic keratosis treated with PICATO Gel, 0.05% applied to a 100 cm<sup>2</sup> treatment area. [see *Clinical Pharmacology* ([12.3](#))]

## 12 CLINICAL PHARMACOLOGY

### 12.1 Mechanism of Action

The mechanism of action of PICATO Gel for the treatment of actinic keratosis is unknown.

### 12.2 Pharmacodynamics

The pharmacodynamics of PICATO gel is unknown.

*Note: The sponsor proposed wording for this section does not provide appropriate information for this section of the label.*

### **13 NONCLINICAL TOXICOLOGY**

#### **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility**

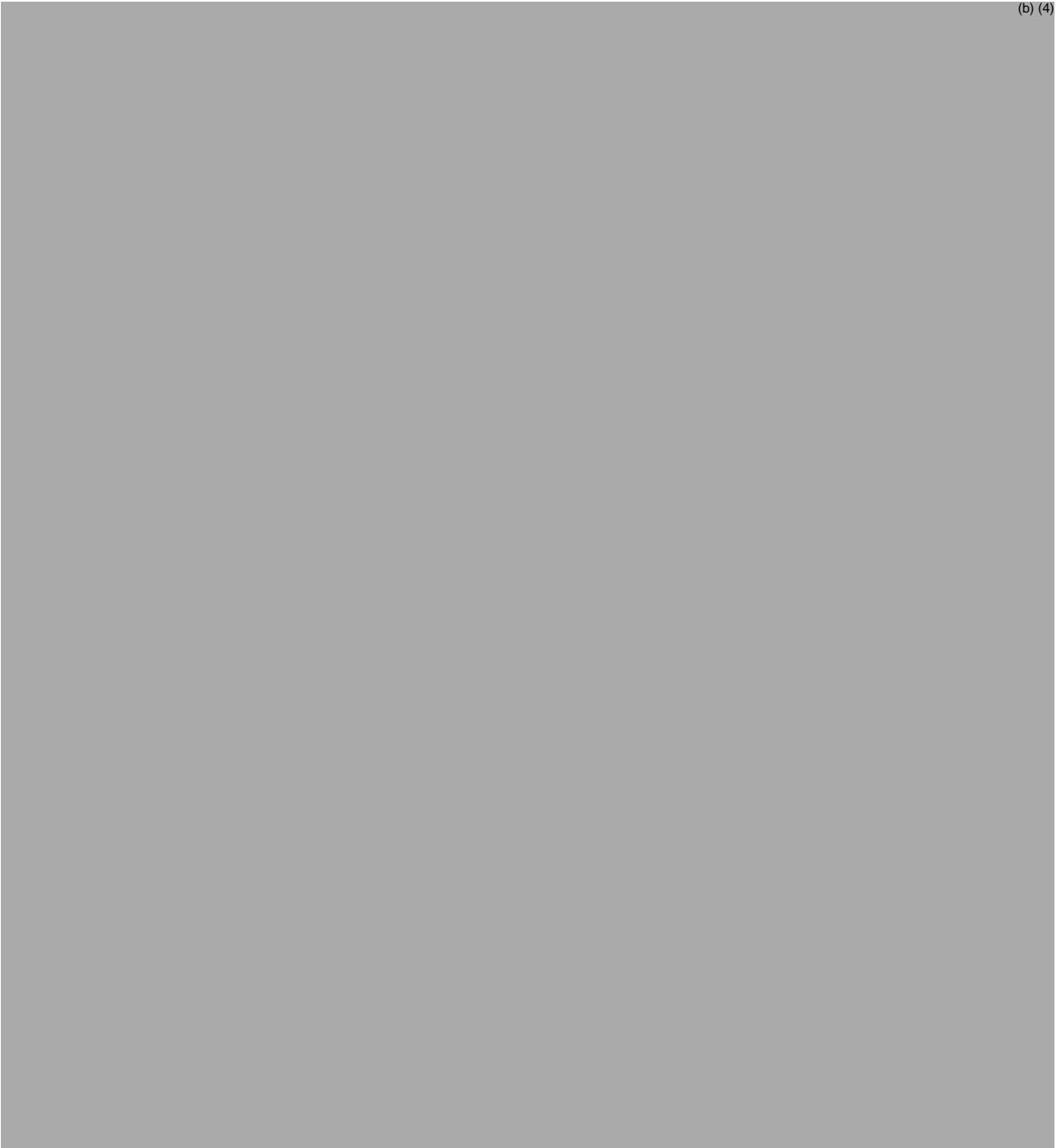
Long-term animal studies have not been performed to evaluate the carcinogenic potential of PICATO Gel or ingenol mebutate. The effects of ingenol mebutate on fertility have not been evaluated.

Ingenol mebutate was negative in the Ames test, in vitro mouse lymphoma assay, and in vivo rat micronucleus test, but positive in the Syrian hamster embryo (SHE) cell transformation assay.

The following wording for the labeling on the nonclinical information was proposed by the sponsor:



(b) (4)



## **2 Drug Information**

### **2.1 Drug**

CAS Registry Number  
75567-37-2

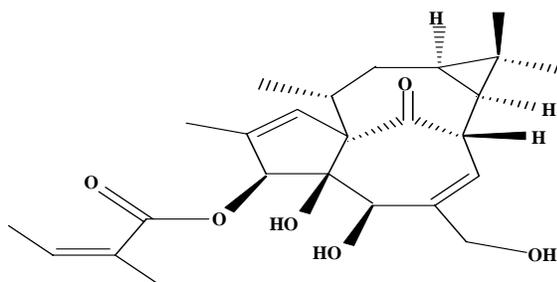
Generic Name  
Ingenol mebutate

Code Name  
AGN 204332, PEP005, (b) (4), 3-angeloyl ingenol

Chemical Name  
2-butenic acid, 2-methyl-(1aR,2S,5R,5aS,6S8aS,9R,10aR)-1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester (2Z)-(b) (4)

Molecular Formula/Molecular Weight  
C<sub>25</sub>H<sub>34</sub>O<sub>6</sub> / 430.53

Structure or Biochemical Description



Pharmacologic Class

The sponsor proposed Pharmacologic Class is (b) (4) cell death inducer (b) (4). However, Review Team meetings concluded that the Pharmacologic Class could be either “cell death inducer” or without designation.

## 2.2 Relevant INDs, NDAs, BLAs and DMFs

INDs 70,114, (b) (4), and (b) (4)

## 2.3 Drug Formulation

Name of components	% w/w	% w/w	Function	Reference to quality standard
<b>Drug substance</b> Ingenol mebutate	0.015	0.05	Drug Substance	in-house
<b>Excipients</b> Isopropyl alcohol Hydroxyethyl cellulose Benzyl alcohol Citric acid monohydrate Sodium citrate (b) (4) Purified water				(b) (4)
			USP	
			USP-NF	
			USP-NF	
			USP	
			USP	
			USP	

## 2.4 Comments on Novel Excipients

No novel excipients are used in this drug product.

## 2.5 Comments on Impurities/Degradants of Concern

There are a few impurities in the drug substance/product that are structurally closely related to ingenol mebutate (PEP005). However, due to the small percentage of each impurity in the drug product and minimal systemic absorption following topical administration of PICATO Gel under maximal clinical use conditions, there are no safety concerns for the impurities, from a Pharmacology/Toxicology perspective. For additional information on the impurities contained in this drug product and the nonclinical studies conducted to qualify these impurities, please refer to Section 10 (Special Toxicology Studies) of this review.

## 2.6 Proposed Clinical Population and Dosing Regimen

PICATO Gel is proposed to be used in patients with actinic keratosis. Actinic keratosis on the face and scalp: Apply PICATO Gel 0.015% to the affected area once daily for 3 consecutive days. Actinic keratosis on the trunk and extremities: Apply PICATO Gel 0.05% to the affected area once daily for 2 consecutive days. PICATO Gel should be applied to a defined treatment area as one contiguous area of approximately 25 cm<sup>2</sup> with 250 mg drug product.

## 2.7 Regulatory Background

The Agency had multiple Guidance meetings with the sponsor on 3/7/05, 4/10/06, and 9/16/09, End-of-Phase 2 meeting on 6/3/09, and Pre-NDA meeting on 12/15/2010.

### 3 Studies Submitted

#### 3.1 Studies Reviewed

1. Evaluation of PEP005 as an inducer of CYP1A2, CYP2C9 and CYP3A4 in fresh human hepatocytes (779157)
2. PEP005: 6-Month intravenous dose toxicity study in CrI:CD(SD) rats with a 1-month recovery period (N106162)
3. PEP005: 41 Week repeat dose/repeat treatment cycle dermal study in the mini-pig followed by an 8 week recovery period (509992)
4. Reverse mutation in five histidine-requiring strains of Salmonella typhimurium (8224378)
5. Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the MicrotitreR fluctuation technique (8224381)
6. PEP005: 28 Day intravenous (bolus) administration toxicity study in the rat (8224382)
7. PEP005: 3 Day repeat dermal toxicity study in minipigs followed by a 28 day observation period (516774)

#### 3.2 Studies Not Reviewed

The following studies were previously reviewed by Dr. Jiaqin Yao in IND 70,114.

##### Pharmacology:

1. Inhibition of cell growth by PEP005 (4.1)
2. Inhibition of cell growth: Time of exposure to PEP005 (4.4c)
3. Mechanism of PEP005 action: Timing of cell death at high dose of PEP005 (pilot study) (4b.4)
4. Cell survival study of Melanoma cells after treatment with partially purified terpene fractions from Euphorbia Peplus and TPA (P028)
5. Cell survival study of Melanoma cells after treatment with partially purified terpene fractions from Euphorbia Peplus (P030)
6. Cell survival study of DU145 (prostate carcinoma) cells after treatment with Cisplatin, Taxol, radiation, SBHA in combination with PEP005, (b) (4) (P051)
7. In vitro growth inhibition activity of PEP005 - NCI data (P093)
8. PEP005 in vivo dose response (4.3a)
9. In vivo efficacy: Best regimen (4.3b)
10. Topical treatment by 5-FU and Imiquimod (4.3c)
11. Intra-lesion dose response for PEP005 in mouse models (4b.3)
12. Mechanism of action: In vivo efficacy and granulocytes (4d.6)
13. Efficacy of topical treatment of B16 melanoma in C57 mice with Peplin compounds (P092)
14. Analysis of protein kinase screen PEP005 (4d.8)
15. Translocation of protein kinase C isoforms by Peplin compounds in HeLa cells (P090)
16. Role of apoptosis or necrosis in tumor cell killing by PEP005 (4.11)
17. Mechanism of action: PEP005-induced cell killing (apoptosis vs necrosis) (4.4b)

18. Mechanism of action: Mitochondrial disruption (4b.5)
19. Anti-cancer cytotoxic T lymphocyte induction by PEP005 (4.10)
20. Effect of PEP005 codelivered with immunomodulators on secondary tumors (4d.10 and 4b.6)
21. Respiratory burst and phagocytosis induced by Peplin compounds (P091)
22. Analysis of gene arrays with PEP005 (4d.7)
23. Inhibition of cell growth by PEP005 isomers (4d.3)
24. Cytotoxicity of PEP005 isomers (4d.4)
25. Cell survival study of different Melanoma cells after treatment with partially purified terpene fractions of Euphorbia Peplus (P031)

#### Pharmacokinetics/toxicokinetics:

1. PEP005: Pilot in vitro skin penetration study for rats, mini-pigs and human (2174/030)
2. PEP005: Rates of penetration through rat, human and mini-pig skin using a flow through in vitro system (2174-032)
3. The in vitro percutaneous absorption of 20- $^3\text{H}$ -PEP005 through mini-pig, human and rat skin (774782)
4. The in vitro percutaneous absorption of radio-labeled PEP005 in four test preparations through rat, mini-pig and human skin (779272)
5. PEP005: Single dose intravenous toxicokinetic study in the rat (2174/012)
6. In vitro binding of  $^3\text{H}$ -PEP005 to the plasma proteins of male and female rat, dog, mini-pig and human (182813)
7. The tissue distribution of total radioactivity in the rat following intravenous administration of  $^3\text{H}$ -PEP005 (183534)
8. PEP005: Metabolism in hepatocytes isolated from rat, min-pig and man and in blood from rat, rabbit, min-pig and man (2174/028)
9. PEP005: Tissue, strain and species variation in the in vitro metabolism of 20- $^3\text{H}$ -PEP005 (774824)
10. An *in vitro* investigation to assess the potential inhibition of human cytochrome P450 enzymes by PEP005 (779162)
11. Predicted (allometric scaling) human PEP005 pharmacokinetics following topical application of PEP005 Gel (PA001)

#### Safety Pharmacology:

1. Evaluation of the effects of PEP005 on hERG using HEK293 transfected cells (700517-1)
2. PEP005: Effects on general activity and behavior in rats following intravenous administration (2174/008)
3. PEP005: Cardiovascular and pulmonary evaluation in beagle dogs following intravenous administration (N106161)
4. PEP005: Cardiovascular and respiratory effects in the anaesthetized dog following intravenous administration (2174/010)

#### General toxicology:

1. PEP005: Single dose intravenous toxicity study in the rat (2174/004)

2. PEP005: Single dose intravenous toxicity study in the rabbit (2174/009)
3. PEP005: Dermal tolerance/irritation pilot study in the mini-pig (2174/025)
4. PEP005: Dermal tolerance/irritation pilot study in the mini-pig (2174/027)
5. PEP005: Dermal tolerance/irritation pilot study in the rat (2174/021)
6. PEP005 Topical Gel: 3-Day repeat dose dermal tolerance/irritation study comparing PEP005 Gel formulations of varying pH in Crl:CD (SD) rats (N106169)
7. PEP005: 3 Day repeat dermal tolerance and toxicity study in the rat followed by a 14 day observation period (2174/026)
8. PEP005: 7 Day intravenous administration dose range-finding study in the rat (2174/020)
9. PEP005: 28 Day intravenous administration toxicity study in the rat (2174/014)
10. PEP005: 13 Week dose range finding study in rats with administration by the dermal route with a 15 day recovery period (457405)
11. PEP005 Topical Gel: 6-Month dermal study in CRL:CD(SD)IGS BR rats with a 4-week recovery (N106168A)
12. PEP005: Dermal tolerance/irritation study in the rabbit (2174/011)
13. PEP005 3 Day Repeat Dermal Tolerance/Irritation Study in the Minipig Followed by a 14 Day Observation Period (507450)
14. PEP005: 3 Day repeat dermal tolerance/irritation study in the mini-pig followed by a 14 day observation period (2174/029)
15. PEP005: Maximum tolerated dose (MTD) followed by a 7 day repeated dose intravenous administration toxicity study in the mini-pig (2174/022)
16. PEP005: 28 Day intravenous administration toxicity study in the mini-pig (2174/018)
17. PEP005: 13 Week Repeat Dose Dermal Range Finding Study in the Minipig Followed by an 8 Week Recovery Period (509987)

#### Genetic toxicology:

1. PEP005: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium* (2174/001)
2. PEP005: Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the microtitre® fluctuation technique (2174/002)
3. PEP005: Induction of micronuclei in the bone marrow of treated rats (2174/007)
4. PEP005: In vitro clonal transformation assay using Syrian Golden Hamster Embryo (SHE) cells (AB34NU.310.BTL)

#### Reproductive/Developmental Toxicology:

1. PEP005: Preliminary Developmental Toxicity Study in Rats (494295)
2. Developmental Toxicity Study of Intravenously Administered PEP005 in Rats (494300)
3. PEP005: Dose Range Finding Study in Rabbits Preliminary to Developmental Toxicity Study (494316)
4. Developmental Toxicity Study of Intravenously Administered PEP005 in Rabbits (494321)

#### Special toxicology:

1. PEP005: Local lymph node assay in the mouse (individual method) (2174/031)

2. PEP005 Hemocompatibility Test (TYU001/043606)
3. Evaluation of the Effects of PEP005 on in vitro Platelet Aggregation (210706)

The following studies were not reviewed:

Primary Pharmacodynamics:

1. In Vitro Evaluation of the Novel Compound (b) (4) for Anti-cancer Activity in a Panel of Human Tumor Cell Lines in a Monolayer Assay (P115A)
2. MGG Anti-Cancer Screen - 1 (4i-2)
3. In vitro Growth Inhibitory Activity of PEP005 - Multiple Dosing II (5-002)
4. In vitro evaluation of (b) (4) for inhibition of colony formation of tumor stem cells and hematopoietic stem cells by using a clonogenic assay (P115B)
5. Initial Cell Survival Study of MM96L after treatment with Crude, and partially purified terpene fractions from Euphorbia Peplus (P001)
6. Initial Cell Survival Study of MM96L, MM329, DU145 and NFF after treatment with Crude, and partially purified terpene fractions from Euphorbia Peplus and SBHA (Suberic Bis Hydroxamic acid) (P002)
7. Initial Cell Survival Study of SK-Mel-28 after treatment with Crude, and partially purified terpenes from E. Peplus (P007)
8. Initial Cell Survival Study of MM480 &KJD cells treated with Crude Sap, and partially purified terpenes from E. Peplus (P008)
9. Initial Cell Survival Study of keratinocyte cells after treatment with Crude, and partially purified terpene fractions from Euphorbia Peplus and TPA (P011)
10. Initial Cell Survival Study of Transformed Keratinocyte, Melanoma, Ovarian and Transformed Melanocyte cells after treatment with Crude, and partially purified terpene fractions from Euphorbia Peplus, and SBHA (Suberic Sis Hydroxamic acid) (P012)
11. Initial Cell Survival Study of Squamous Cell Carcinoma cells after treatment with Crude, and partially purified terpene fractions from Euphorbia Peplus, and SBHA (Suberic Bis Hydroxamic acid) (P013)
12. Initial Cell Survival Study of Mouse Squamous Cell Carcinomas, Malignant Melanoma and Normal Fibroblast cells after treatment with Crude (MPA) from Euphorbia Peplus and TPA (P018)
13. Initial Cell Survival Study of Malignant Melanoma and Fibroblast cells after treatment with Crude (MPA) from Euphorbia Peplus and TPA (P019)
14. Initial Cell Survival Study of Malignant Melanoma and Fibroblast cells after treatment with Crude (MPA) from Euphorbia Peplus and TPA (P022)
15. Initial Cell Survival Study of Mouse Squamous Cell Carcinoma (SCC) cells after treatment with Crude Sap (MPA) from Euphorbia Peplus and TPA (P023)
16. Cell Survival Study of Melanoma cells after treatment with Crude (MPA) from Euphorbia Peplus, TPA, Synadenium and AN-1 (P025)
17. Cell Survival Study of breast, lymph, colorectal and squamous carcinoma cells after treatment with partially purified terpene fractions from Euphorbia Peplus (P032)
18. Cell Survival Study of MM96L cells after treatment with Crude Sap (b) (4) from Euphorbia Peplus, Bistratene A and TPA (P034)

19. Cell Survival Study of Malignant Melanoma (D04) cells treated with PKC activators and Crude Sap (b) (4) of E. Peplus and TPA (P049)
20. Cell Survival Study of Malignant Melanoma (MM170, HT144), Transformed Kidney (293) and Cervical Cancer (Hela Fos) cells treated with (b) (4) and partially purified terpenes of E. Peplus and TPA (P050)
21. Initial Cell Survival Study of PC-3, Lncap, MCF7, MM418C5 cells after treatment with Crude, and partially purified terpene from E. Peplus (P003)
22. Initial Cell Survival of Lung Cancer, Malignant Melanoma and Normal Fibroblast cells after treatment with Crude, and partially purified terpene fractions from Euphorbia Peplus (P014)
23. Synergism between PEP005 and PKC modulators (4d-5)
24. Cell Survival Study of MM96L and NFF cells after treatment with PKC activators (P048)
25. PEP005 / Glycine gated death channel (4g-12)
26. Study BHAMUK-001. Determination of the mechanism of induction of cell death of topical PEP005 - Interim Report (BHAMUK-001)
27. Mechanism of cell death induction by PEP005 in MCF-7 cells (PC042)
28. PEP005 potentiates the immune response (PC043)
29. In Vitro Evaluation of Effects of (b) (4) on Signal Transduction Proteins in the Cell Lines MEXF514L, GXF 251 Land LECL HL60 (P115E)
30. Basis of the bipolar assay for PKC activators (4i-5)
31. PKC Activation Kinase Activity of PKC (PQ8017-003)
32. PEP005 and the MAP Kinase Pathway (P094)
33. PEP005 / Monocyte / Macrophage Gene Array (4d-9)
34. In Vitro Evaluation of the Immunostimulatory Effects of (b) (4) on Peripheral Blood mononuclear Cells (PBMCs) (P115D)
35. In Vitro Evaluation of the Immunostimulatory Effects of (b) (4) on Peripheral Blood mononuclear Cells (PBMCs) (P164C)
36. Investigation into the effect of (b) (4) on (i) the susceptibility of melanoma cells to specific cytotoxic T lymphocytes (CTL); (ii) the ability of melanoma cells to stimulate specific CTL (PQ8017-019)
37. Enhancement of Tumour Cell Antigen Presentation to CTL; Investigation into the effect of (b) (4) on the ability of melanoma cells to stimulate NK activity (PQ8017-016)
38. Enhancement of Tumour Cell Antigen Presentation to CTL Investigation into the effect of ingenanes on (i) the susceptibility of melanoma cells to specific CTL mediated killing; (ii) the ability of melanoma cells to stimulate specific CTL (PQ8017-005)
39. Project 1: PEP005 potentiates the immune response (PC044)
40. Upregulation of stress receptors MICANKG2D ligand (PQ8017-017)
41. A Study On The Ability Of PEP005 To Stimulate Human Wound; Healing Responses In Vivo And In Vitro (PC052)
42. PEP005 Mediation of Extracellular Matrix Synthesis/Remodelling, in Relation to Dermal Fibrosis and Cosmesis (PC053)
43. Mechanism of action of topical treatment of basal cell and squamous cell carcinomas with ingenol mebutate (QIMR-07-030)

44. Establishing the SKH1 model of UV-induced p53+ mutant patches at QIMR (QIMR-07-085)
45. Recovery of PEP005 from Topically treated B16 Tumours (4-5b)
46. Recovery of PEP005 from Blood of Topically Treated Mice (4-6)
47. Efficacy of PEP005 Formulation In Vivo (4e-3)
48. Efficacy of PEP005 Formulation in Vivo II (4f-9)
49. In vivo Anti-Cancer Activity of PEP005 Cream Formulations (5-005)
50. Testing of partially purified Ingenane 8, Ingenane 9 and An-1 of E. Peplus on C57 BL/6J B16 tumour model mice to determine best pure/dose (P033)
51. To determine the effect of topical application of plant material (water extract) and partially purified Peplin Compounds from E. Peplus and Bisindolymaleimide on B16 tumours in nude mice (P036)
52. To determine the effects of pre-treatment of skin with Crude Sap on the growth of subcutaneously implanted B16 tumours in nude mice (P037)
53. To determine the effect of intralesional treatment of Synadenium and TPA and topical application via a cream vehicle of Ingenane Mix on B16 tumours in nude mice (P038)
54. In Vivo - PEP005 Isomers (4f-6)
55. Treatment of nude mice B16 tumour model with Intralesional injection of (b) (4) PEP005 and (b) (4) of E. Peplus (P047)
56. Treatment of C57 BL/6J mice B16 tumour model with Intralesional inoculation of (b) (4) (ING060201) of E. Peplus in Captisol vehicle (P046)
57. Mode of action: B16 growth in vitro following PEP005 in vivo (4-5a)
58. Topical Efficacy of PEP005 in the Hypomorphic Mouse (4g-3)
59. The Role of the Innate Immune Response in the Treatment of Murine Squamous Cell Carcinomas with PEP005: The Role of Macrophages (4h-1iv)
60. The Role of the Innate Immune Response in the Treatment of Murine Squamous Cell Carcinomas with PEP005: The Role of NK and B Cells (4h-1ii)
61. The Role of the Innate Immune Response in the Treatment of Murine Squamous Cell Carcinomas with PEP005 (4f-10vi)
62. Neutrophil infiltration activity (PQ8017-007)
63. Induction of tumour specific CTL by Peplin compounds (PQ8017-002)
64. Examination of the role of anti-cancer immunity in the ingenol mebutate-mediated inflammatory response (QIMR-2009-14-SEPT)

#### Secondary Pharmacodynamics:

1. Project 2: The anti-leukaemic actions of PEP005 (PC038)
2. Lord - PEP005 and AML (no official title) (PC040)
3. Anti-leukaemic effect of PEP005 (PC041)
4. Preclinical evaluation of PEP005, a novel ingenol angelate, in human cancer cell lines (PC036)
5. An investigation into the chemotherapeutic efficacy of PEP005 on bladder cancer (PC032)
6. Initial Cell Survival Study of Lymphoblastoid cell lines after treatment with Crude from E. Peplus and TPA (P004)

7. Initial Cell Survival Study of Leukaemia Cells after treatment with Crude from E. Peplus, and TPA (P005)
8. Initial Cell Survival Study of Burkitt's lymphoma with after treatment with Crude from E. Peplus and TPA (P006)
9. Initial Cell Survival Study of Leukaemia & Burkitt's lymphoma cells after treatment with crude, and partially purified terpene fractions from Euphorbia Peplus, and SBHA (Suberic Bis Hydroxamic acid) (P009)
10. Initial Cell Survival Study of lymphoblastoid cells after treatment with Crude, and partially purified terpene fractions from Euphorbia Peplus and SBHA (Suberic Bis Hydroxamic acid) (P010)
11. Initial Cell Survival of Pancreatic cells after treatment with Crude, and partially purified terpene fractions from Euphorbia Peplus (P015)
12. Initial Cell Survival Study of Cockayne's Syndrome cells (Jamie Lee Williams) after treatment with Crude Sap (MPA) from Euphorbia Peplus and TPA (P016)
13. Initial Cell Survival of Acute Promyelocytic Leukaemia cells (HL 60) after treatment with Crude Sap (MPA) from Euphorbia Peplus and TPA (P017)
14. Initial Cell Survival Study of Lymphoblast and Cockayne Syndrome cells after treatment with Crude (MPA) from Euphorbia Peplus and TPA (P020)
15. Initial Cell Survival Study of Human Fibrosarcoma Cells after treatment with Crude Sap (MPA) from Euphorbia Peplus, TPA and Synad (P021)
16. Initial Cell Survival Study of Lymphoblast and Cockayne Syndrome cells after treatment with Crude (MPA) from Euphorbia Peplus and TPA (P024)
17. Cell Survival Study of cells after ovarian cancer treatment with Crude from Euphorbia Peplus, and various other saps (P026)
18. Cell Survival Study of Breast Cancer Cells (MCF7) after treatment with partially purified terpene fractions from Euphorbia Peplus and TPA (P027)
19. Cell Survival Study of Ovarian, Mouse Melanoma, Human Fibroblast and Squamous cell carcinomas cells after treatment with partially purified terpene fractions from Euphorbia Peplus and TPA (P029)
20. Induction of senescence by (b) (4) (5-006)
21. In Vitro Pharmacology: Safety Profile - Study of PEP005 (13753)
22. Inhibition of HIV infection of PBMC (4d-12)
23. Activation of latent HIV from U1 cells (4d-13)
24. HIV Mechanism of Action (4f-1)
25. Anti-HIV Activity (PQ8017-009)
26. Down regulation of HIV receptors (4d-11)
27. Effect of Peplin compounds on CD4 and CXCR4 expression in activated PBMC (PQ8017-018)
28. Effect of Peplin compounds on CD4 and CXCR4 expression in Jurkat cells (PQ8017-020)
29. Modulation of the induction of EA in the EBV producing Raji cell line by the Peplin compounds (4-9-1)
30. Anti-vaccinia virus activity of Peplin compound activated leucocytes (PQ8017-021)
31. Antibacterial Activity of PEP005 (QIMR-07-074)
32. Protection against infections in vivo and in vitro - In vitro activity against salmonella (PQ8017-013)

33. Anti-Escherichia coli activity of Peplin compound activated leucocytes (PQ8017-015)
34. Anti-parasite activity of Peplin compounds in vitro (PQ8017-014)  
Promoter Activation as a Means of Therapy (PQ8017-010)
35. Evaluation of the tolerability and antitumor activity of PEP005 in NMRI *nu/nu* mice bearing 3 cell line - derived hematopoietic malignancy xenografts (P164)
36. To determine the effects of topical E. Peplus on Nude Mice treated with Subcutaneous Prostate Cancer cell lines (P039)
37. Topical treatment with Ingenane mix from E. Peplus on Prostate Cancer (PC-3) tumours administered subcutaneously to nude mice (P040)
38. To determine the growth of pancreatic cancer cells and mouse squamous cell carcinoma cells on nude mice treated with Crude Sap (MPA) and partially purified terpene (b) (4) of E. Peplus (P041)
39. Subcutaneous Injection of PC-3 Prostate Cancer on nude mice treated (topically and intra-lesionally) with (b) (4) (MEE071200) and (b) (4) (#080101) of E. Peplus (P042)
40. Topical treatment with Ingenane mix from E. Peplus on LKUT MN clone cell line tumour administered subcutaneously to nude mice (P043)
41. Topical and Intralesional treatment with Ingenane mix from E. Peplus on Prostate cancer cells administered subcutaneously to nude mice (P044)
42. Topical treatment with Diterpenes from E. Peplus on Adenocarcinoma cells (HeLa) administered subcutaneously to nude mice (P045)
43. Evaluation of a Rabbit Papillomavirus (CRPV) model of cutaneous warts (TFS00011)
44. Efficacy study of PEP005 topical gel in a rabbit papillomavirus (CRPV) model of cutaneous warts (TFS00012)
45. Systemic antiviral activity of Peplin compounds (PQ8017-008)
46. Protection against Intra-Peritoneal Streptococcal Infection Effect of Peplin Compounds on systemic group A streptococcal infection in mice (PQ8017-011)
47. Protection against infections in vivo and in vitro; Prevention or cure of malaria (PQ8017-012)

#### Analytical Methods and Validation:

1. Validation for the determination of (b) (4) (b) (4) in dosing formulations using high performance liquid chromatography (HPLC) (2174-037)
2. Validation for the determination of PEP005 in intravenous dosing formulations using high performance liquid chromatography (HPLC) and for the determination formulation homogeneity and stability (2174-100)
3. Validation of an analytical procedure for the determination of (b) (4) (b) (4) and (b) (4) in rat whole blood (EDTA) using liquid/liquid extraction and liquid chromatography with tandem mass spectrometric detection (2174-005)
4. Partial validation and investigation into the storage stability of PEP005, (b) (4) and (b) (4) in rat whole blood using liquid/liquid extraction and liquid chromatography with tandem mass spectrometric detection (2174-062)

5. Partial validation of an analytical procedure for the determination of (b) (4) and (b) (4) in whole rabbit blood using liquid/liquid extraction and liquid chromatography with tandem mass spectrometric detection (2174-039)
6. Partial validation and investigation into the storage stability of PEP005, (b) (4) and (b) (4) in rabbit whole blood using liquid/liquid extraction and liquid chromatography with tandem mass spectrometric detection (2174-063)
7. Partial validation of an analytical procedure for the determination of (b) (4) and (b) (4) in dog whole blood (EDTA) using liquid/liquid extraction and liquid chromatography with tandem mass spectrometric detection (2174-013)
8. Partial validation and investigation into the storage stability of PEP005, (b) (4) and (b) (4) in dog whole blood using liquid/liquid extraction with liquid chromatography with tandem mass spectrometric detection (2174-064)
9. Partial validation of an analytical procedure for the determination of (b) (4) and (b) (4) in mini pig whole blood (EDTA) using liquid/liquid extraction and liquid chromatography with tandem mass spectrometric detection (2174-006)
10. Partial validation of PEP005, (b) (4) and (b) (4) in mini-pig whole blood using liquid/liquid extraction and liquid chromatography with tandem mass spectrometric detection (2174-0800)
11. Validation of an analytical procedure for the determination of (b) (4) and (b) (4) in human whole blood using liquid/liquid extraction and liquid chromatography with tandem mass spectrometric detection (2174-016)
12. Partial validation for the determination of PEP005, (b) (4) in human whole blood using liquid/liquid extraction and liquid chromatography with tandem mass spectrometric detection (2174-066)
13. Partial validation of an analytical procedure for the determination of (b) (4) and (b) (4) in receptor fluid using liquid/liquid extraction and liquid chromatography with tandem mass spectrometric detection (2174-033)
14. Investigation into the storage stability of (b) (4), (b) (4) and (b) (4) in rat whole blood using liquid/liquid extraction and liquid chromatography with tandem mass spectrometric detection (2174-041)
15. Investigation into the storage stability of (b) (4), (b) (4) and (b) (4) in mini pig whole blood using liquid/liquid extraction and liquid chromatography with tandem mass spectrometric detection (2174-042)
16. Investigation into the storage stability of PEP005, (b) (4) and (b) (4) in human blood using liquid/liquid extraction with tandem mass spectrometric detection (2174-034)
17. Investigation into the Radiochemical Stability of 20-[3H] PEP005 (209211)

#### Pharmacokinetics/Toxicokinetics:

1. PEP005: Single intravenous dose toxicokinetic study in CRL:CD (SD) rats and Wistar Han rats (N106162TK)
2. PEP005: Single intravenous dose toxicokinetic study in CRL:CD (SD) rats (N106167, Non-GLP)
3. Investigation into the metabolic stability of the 20-hydroxyl group in PEP005 (25877)

#### Toxicology:

1. PEP005: Single dose escalation urinary bladder instillation toxicity study in female Sprague-Dawley rats (24689)
2. PEP005: Dose range finding intravenous (3 h) infusion toxicity study in rats (24595)
3. PEP005: 7-Day intravenous infusion tolerance and toxicokinetics study in Crl:CD(SD) rats (N106165, Non-GLP)
4. PEP005: Pilot intravenous (3 h) infusion toxicity study in Beagle dogs (24606)
5. PEP005: 7-Day intravenous infusion toxicity study followed by 14-day recovery in Beagle dogs (N106163A)
6. PEP005: 7 day intravenous (bolus) administration toxicity study in the rat (8224379)

### 3.3 Previous Reviews Referenced

Reviews in DARRTS under IND 70,114 written by Dr. Jiaqin Yao

## 4 Pharmacology

### 4.1 Primary Pharmacology

Ingenol mebutate (PEP005), a purified active compound from the sap of the *Euphorbia peplus* plant, was developed for the topical treatment of actinic keratosis (AK). There is no disease model for AK. However, the sponsor stated that “In vitro and in vivo study results suggest that ingenol mebutate is a pleiotropic effector that inhibits tumor cell growth or induces tumor cell death via multiple mechanisms, which are influenced by concentration and cell type.”

The following studies were previously reviewed by Dr. Jiaqin Yao in IND 70114:

1. Inhibition of cell growth by PEP005 (4.1)
2. Inhibition of cell growth: Time of exposure to PEP005 (4.4c)
3. Mechanism of PEP005 action: Timing of cell death at high dose of PEP005 (pilot study) (4b.4)
4. Cell survival study of Melanoma cells after treatment with partially purified terpene fractions from Euphorbia Peplus and TPA (P028)
5. Cell survival study of Melanoma cells after treatment with partially purified terpene fractions from Euphorbia Peplus (P030)
6. Cell survival study of DU145 (prostate carcinoma) cells after treatment with Cisplatin, Taxol, radiation, SBHA in combination with PEP005, (b) (4) (P051)
7. In vitro growth inhibition activity of PEP005 - NCI data (P093)
8. PEP005 in vivo dose response (4.3a)
9. In vivo efficacy: Best regimen (4.3b)
10. Topical treatment by 5-FU and Imiquimod (4.3c)
11. Intra-lesion dose response for PEP005 in mouse models (4b.3)
12. Mechanism of action: In vivo efficacy and granulocytes (4d.6)
13. Efficacy of topical treatment of B16 melanoma in C57 mice with Peplin compounds (P092)
14. Analysis of protein kinase screen PEP005 (4d.8)

15. Translocation of protein kinase C isoforms by Peplin compounds in HeLa cells (P090)
16. Role of apoptosis or necrosis in tumor cell killing by PEP005 (4.11)
17. Mechanism of action: PEP005-induced cell killing (apoptosis vs necrosis) (4.4b)
18. Mechanism of action: Mitochondrial disruption (4b.5)
19. Anti-cancer cytotoxic T lymphocyte induction by PEP005 (4.10)
20. Effect of PEP005 codelivered with immunomodulators on secondary tumors (4d.10 and 4b.6)
21. Respiratory burst and phagocytosis induced by Peplin compounds (P091)
22. Analysis of gene arrays with PEP005 (4d.7)
23. Inhibition of cell growth by PEP005 isomers (4d.3)
24. Cytotoxicity of PEP005 isomers (4d.4)
25. Cell survival study of different Melanoma cells after treatment with partially purified terpene fractions of Euphorbia Peplus (P031)

PEP005 (ingenol mebutate) caused cell growth inhibition of several tumor cell lines (including human melanoma cell lines) in vitro (4.1, 4.4c, 4b.4, P028, P030, P051, P093). The anti-tumor activity of PEP005 was also shown in vivo in mouse models (4.3a, 4.3b, 4.3c, 4b.3, 4d.6, P092). The activity of PEP005 may involve activation of selected PKC isoforms (4d.8, P090), induction of necrosis (4.11, 4.4b, 4b.5), modulation of immune responses (4.10, 4d.10 and 4b.6, P091), and/or activation of other signal transduction pathways (4d.6, 4d.7). PEP005 and its acyl isomers, (b) (4) and (b) (4) (structures shown in the next Figure) appeared to have equivalent growth inhibition activity in a number of tumor cells in vitro (4d.3, 4d.4). Normal fibroblasts (Neonatal Forskin fibroblasts, NFF cells) were resistant to PEP005 in vitro, but it caused proliferation of the NFF cells at over 20 nM doses (P031). The sponsor also cited literature to state that PEP005 had distinct patterns of PKC isoform modulation and did not appear to have the same tumor promoting potential as other members of phorbol esters.

Figure 2: Chemical Structures of Ingenol Mebutate, (b) (4)



Other nonclinical studies on primary pharmacology, including those using in vivo murine xenograft models, have been submitted within the original submission, but not reviewed.

(b) (4)

## 4.2 Secondary Pharmacology

The submitted nonclinical studies on Secondary Pharmacology were not relevant to the indication and therefore were not reviewed.

## 4.3 Safety Pharmacology

**4.3.1. Evaluation of the effects of PEP005 on hERG using HEK293 transfected cells (700517-1):** Human Embryonic Kidney cells (HEK293 cells) stably transfected with hERG (human ether-a-go-go related gene) were exposed to PEP005 and currents were recorded at the completion of a 5-minute exposure equilibration period. There was no statistically significant inhibition of hERG tail current density for  $I_{+15}$  ( $n = 7$ ) following sequential exposure to PEP005 at concentrations of 0.5, 1.0, 2.5, and 5.0  $\mu\text{g/mL}$ . No  $\text{IC}_{50}$  value could be calculated. In this study, the positive control, E-4031 (a potent and selective  $I_{Kr}$  inhibitor) induced a significant decrease (71.6%) in average tail current for  $I_{+15}$  compared to the baseline current.

**4.3.2. PEP005: Effects on general activity and behavior in rats following intravenous administration (2174/008):** The effects of PEP005 on general activity and behavior were evaluated in groups of 4 male and 4 female rats intravenously administered doses of 1, 3, or 10  $\mu\text{g/kg}$ . No significant behavior or physiological changes were observed. However, there was a slight, transient increase in respiration rate in 3 male and 2 female rats dosed at 10  $\mu\text{g/kg}$  and discoloration of the tail in 2 male and 2 female rats dosed at 3  $\mu\text{g/kg}$  and 3 male and 4 female rats dosed at 10  $\mu\text{g/kg}$ . No further signs were observed during a 7-day post-dose observation period.

**4.3.3. PEP005: Cardiovascular and respiratory effects in the anaesthetized dog following intravenous administration (2174/010):** PEP005 was administered as escalating doses of 0.3, 1, 3, and 10  $\mu\text{g/kg}$  by intravenous infusion in groups of 4 male and 4 female anaesthetized dogs. Hemodynamic and respiratory parameters were measured at 0, 2, 10, 20, and 30 minutes following the end of each dose of vehicle or PEP005. Administration of PEP005 at 0.3, 1, and 3  $\mu\text{g/kg}$  did not have any noticeable effects on arterial blood pressure, heart rate, or ECG waveform, and respiratory parameters. A dose-related negative inotropic effects ( $dP/dt_{\text{max}}$ ) was observed following administration of 1 to 3  $\mu\text{g/kg}$  PEP005. However, no further decrease in  $dP/dt_{\text{max}}$  was noted at 10  $\mu\text{g/kg}$ . Administration of 3  $\mu\text{g/kg}$  PEP005 elicited a transient decrease (37%) in femoral blood flow. A 18% increase in mean arterial blood pressure, a 42% increase in diastolic pressure, a 49% increase in heart rate accompanied increases in the RR and the QT intervals (QTc was not affected), a sustained (44%) decrease in femoral blood flow, and a sustained 68% increase in PIF suggesting possible bronchodilation were observed at 10  $\mu\text{g/kg}$ . The blood PEP005  $C_{\text{max}}$  (0.097, 0.341, 1.10, and 3.32  $\text{ng/mL}$  in males and 0.106, 0.434, 1.48, and 4.29  $\text{ng/mL}$  in females, respectively) increased with increased dose from 0.3, 1, 3 to 10  $\mu\text{g/kg}$  in both male and female dogs.

**4.3.4. PEP005: Cardiovascular and pulmonary evaluation in beagle dogs following intravenous administration (N106161):** Four male and four female beagle dogs received 0, 1.5, 7.5, and 15 µg/kg PEP005 in 20% PEG 400/0.9% saline intravenously on four separate days with at least 48 hours between doses using a balanced Latin–Square crossover design. Dose-related body temperature decreases were associated with the mid and high dose animals at approximately 1 hour post-dosing. These body temperature decreases averaged approximately 1.4 °C and 0.6 °C in the 15 µg/kg and 7.5 µg/kg dose groups, respectively, and 0.3 °C in the vehicle group. Salivation, soft/mucoid feces, and panting were seen in both genders at ≥7.5 µg/kg and emesis was observed at all doses being more prevalent at ≥7.5 µg/kg.

PEP005 produced non-dose-dependent increases in diastolic (7 to 35%) and mean (9 to 29%) blood pressures in both sexes from immediately post-dosing to approximately 1.5 hours in all dose groups. The diastolic and mean pressure increases were greater in females than males. A slight decrease (up to 6%) in systolic pressure was observed only in females given 15 µg/kg. Dose- and duration-related increases in heart rate (20% to 64%) were observed in the males and females given ≥7.5 µg/kg. Increases were observed immediately post-dosing and lasted from 30 minutes (females) to 1 hour (males). The magnitude of the increase in heart rate was greater in males than females. There were no ECG alterations in rhythm or morphology or changes in ECG interval (PR, RR, QRS, QT and QTc) measurements that could be attributed to treatment of PEP005.

PEP005 caused respiratory rates to increase in males given ≥7.5 µg/kg and in females given 15 µg/kg immediately post-dosing continuing for up to 1.5 hours with some of the increases being statistically significant. The significant rate increases in males for the 7.5 and 15 µg/kg groups ranged from 19 to 28 breaths/minute or 68% to 140%. No significant persistent alterations in tidal volume were noted. The minute volumes of male dogs given ≥7.5 µg/kg and of females given 15 µg/kg were increased from immediately post-dosing to up to approximately 1 hour.

## 5 Pharmacokinetics/ADME/Toxicokinetics

**5.1. The in vitro percutaneous absorption of 20-[<sup>3</sup>H]-PEP005 through mini-pig, human and rat skin (774782):** [<sup>3</sup>H]-PEP005 was applied in a gel formulation (approximately 0.05%, w/w) at an application volume of 10 µL/cm<sup>2</sup> to human, mini-pig, and rat split-thickness skin membranes mounted into flow-through diffusion cells in vitro. Absorption was assessed by collecting receptor fluid in hourly fractions from 0-6 hr post-dose and then 2-hourly fractions from 6-24 hr post dose. As seen from the following table, the absorbed doses of [<sup>3</sup>H]-PEP005 through human, mini-pig, SD rat, and WI rat skin in vitro were 0.21% (0.01 µg equiv./cm<sup>2</sup>), 0.15% (0.01 µg equiv./cm<sup>2</sup>), 1.03% (0.05 µg equiv./cm<sup>2</sup>) and 2.89% (0.12 µg equiv./cm<sup>2</sup>) respectively.

	Human	Mini-Pig	Sprague Dawley Rat	Wistar Rat
Target PEP005 Concentration (% w/w)	0.05	0.05	0.05	0.05
PEP005 Concentration by Radioactivity (% w/w)	0.05	0.05	0.05	0.05
Application Level of PEP005 by Radioactivity ( $\mu\text{g equiv./cm}^2$ )	4.34	4.34	4.42	4.26
Dislodgeable Dose (% Applied Dose)	77.22	73.29	85.11	73.67
Unabsorbed Dose (% Applied Dose)	101.09	93.35	94.40	90.31
Absorbed Dose (% Applied Dose)	0.21	0.15	1.03	2.89
Dermal Delivery (% Applied Dose)	0.91	7.17	4.66	8.39
Mass Balance (% Applied Dose)	102.00	100.52	99.05	98.70
Dislodgeable Dose ( $\mu\text{g equiv./cm}^2$ )	3.35	3.17	3.76	3.14
Unabsorbed Dose ( $\mu\text{g equiv./cm}^2$ )	4.39	4.05	4.17	3.85
Absorbed Dose ( $\mu\text{g equiv./cm}^2$ )	0.01	0.01	0.05	0.12
Dermal Delivery ( $\mu\text{g equiv./cm}^2$ )	0.04	0.31	0.21	0.36
Mass Balance ( $\mu\text{g equiv./cm}^2$ )	4.43	4.36	4.38	4.21
Lag Time (h)	1	NO	2	3
Steady State Flux ( $\text{ng equiv./cm}^2/\text{h}$ )	0.39	NO	2.01	5.87
Period of Steady State Flux (h)	4-24	NO	6-24	8-24

NO = not observed

**5.2. In vitro binding of [ $^3\text{H}$ ]-PEP005 to the plasma proteins of male and female rat, dog, mini-pig and human (182813):** The in vitro plasma protein binding of [ $^3\text{H}$ ]-PEP005 to the plasma proteins of male and female Sprague Dawley and Han Wistar rats, beagle dogs, mini-pigs, and human volunteers was investigated by equilibrium dialysis at target concentrations of 0.5, 2, 5, and 20 ng/mL, following incubation at 37 °C for 2 hr. Plasma protein binding of total radioactivity was very high (>99%) in all samples tested and no apparent differences were observed between species, rat strains or across genders. Over the concentration range investigated, no dependence on concentration was observed.

Stability investigations in plasma and buffer incubates showed that 2 components were present in addition to [ $^3\text{H}$ ]-PEP005. These were proposed as [ $^3\text{H}$ ]-[REDACTED] and [ $^3\text{H}$ ]-[REDACTED]<sup>(b) (4)</sup> which are isomers of [ $^3\text{H}$ ]-PEP005. The isomerization of [ $^3\text{H}$ ]-PEP005 occurred to different degrees in the different species investigated but the ratio of components remained relatively stable in each species over the 3-hr incubation period. [ $^3\text{H}$ ]-PEP005 accounted for approximately 80% of the radioactivity in rat plasma and approximately 50% of the radioactivity in dog, mini-pig and human plasma. In buffer incubates however, [ $^3\text{H}$ ]-PEP005 accounted for only approximately 15% of the radioactivity.

**5.3. The tissue distribution of total radioactivity in the rat following intravenous administration of [ $^3\text{H}$ ]-PEP005 (183534):** A single intravenous injection of 3  $\mu\text{g/kg}$  [ $^3\text{H}$ ]-PEP005 was administered at a constant volume of 5 mL/kg to 21 male and 6 female CRL:CD (SD) rats and 18 male CRL:Lister Hooded (pigmented) rats. Following [ $^3\text{H}$ ]-PEP005 administration, urine and feces were collected from selected animals for analysis of excreted radioactivity. Three male SD rats were sacrificed at 10 min, 1, 4, 8, 24, 48, and 72 hours post-dose and 3 female SD rats were sacrificed at 1 and 24 hours post-dose. Three male Lister rats were sacrificed at 10 min, 2, 8, 24, 48, and 72 hours post-dose.

Drug-related radioactivity was well distributed in the tissues. Levels of radioactivity were higher in most tissues than in whole blood throughout the study suggesting that [<sup>3</sup>H]-PEP005 freely crosses cell membranes. Radioactivity was also measured in the brain, suggesting that the drug related radioactivity also crosses the blood:brain barrier.

Tissue distribution of [<sup>3</sup>H]-PEP005 was initially similar in both male and female SD rats. Elimination was faster in the female animals, with radioactive concentrations generally lower than males at 24 hours post-dose. The observed lower levels of radioactivity in females were most evident in kidney and liver, where mean measurements were 3-4 times less than males. Concentrations of radioactivity were initially high in the lungs, and remained elevated through 8 hours post-dose. High levels of radioactivity were measured in the liver and kidneys were high at the start of the study and remained relatively high throughout the course of the study, indicating elements of both urinary and biliary excretion. Elevated levels were also present in the adrenals, spleen and thyroid in the first few hours post-dose. By 24 hours post-dose, drug related radioactivity had declined extensively in most tissues with the exception of the liver and brown fat. In male SD rats, elimination in was mainly via the feces accounting for 78% of the dose, indicating biliary elimination. Urinary excretion accounted for 24% of the dose. Excretion of total radioactivity was rapid, with a mean of 90.5% of the radioactivity excreted by the first 24 hours post-dose.

Concentrations of radioactivity in pigmented (Lister) and non-pigmented (SD) tissues were similar, suggesting that melanin binding of [<sup>3</sup>H]-PEP005 did not occur to any great extent.

**5.4. PEP005: Tissue, strain and species variation in the in vitro metabolism of 20-<sup>3</sup>H-PEP005 (774824):** [<sup>3</sup>H]-PEP005 (1 or 10 μM) was incubated in vitro with whole blood, skin (skin homogenates), or liver (cryopreserved hepatocytes) from CrI:CD(SD)IGSBR (Sprague Dawley; SD) and CrI:WI(HAN)IGSBR (Wistar; WI) rats, beagle dogs, mini-pigs, and humans for up to 180 minutes. With the exception of human skin, tissue from both males and females were investigated. The metabolite profiles were assessed via HPLC with on-line radiodetection and HPLC-MS(MS).

[<sup>3</sup>H]-PEP005 was found to be relatively metabolically stable in both whole blood and skin homogenates from all species, strains and sexes. In both skin and blood there was some hydrolysis to yield ingenol, but this generally accounted for less than 1% of the radioactivity. Significant isomerization of [<sup>3</sup>H]-PEP005 to yield (b) (4) (isomers of PEP005) was also evident in both skin and blood. In contrast to skin and blood, [<sup>3</sup>H]-PEP005 was found to undergo significant metabolism in cryopreserved hepatocytes from all species, strains and sexes. The metabolite profiles were similar across all species, strains and sexes.

**5.5. An *In vitro* Investigation to Assess the Potential Inhibition of Human Cytochrome P450 Enzymes by PEP005 (779162):** The potential of PEP005 to inhibit human hepatic cytochrome P450 (CYP) isoforms CYP 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4 was evaluated in vitro with pooled human hepatic microsomes using

selective probe substrates. Human hepatic microsomal protein was incubated with selective CYP substrates in the presence of PEP005 at concentrations of 0.0002, 0.002, 0.02, 0.1, 0.2, and 2  $\mu\text{M}$  for CYP 2B6, 2C9, 2C19, 2E1, and 3A4. Due to a calculation error, PEP005 was incubated at 0.002, 0.02, 0.2, 1, 2, and 20  $\mu\text{M}$  concentrations for CYP 1A2, 2A6, 2C8 and 2D6. In addition, PEP005 (2  $\mu\text{M}$  for CYP 2B6, 2C9, 2C19, 2E1, and 3A4; 20  $\mu\text{M}$  for CYP1A2, 2A6, 2C8, and 2D6) was also pre-incubated with microsomal protein in the presence and absence of cofactor to investigate the potential for mechanism-based inhibition of the CYP isoforms. No notable inhibition was observed for the co-incubations or pre-incubations of PEP005 at the concentrations tested with the CYP isoforms CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4.  $\text{IC}_{50}$  values were not calculated since less than 50% inhibition was observed for each of the CYP isoforms tested.

**5.6. Evaluation of PEP005 as an Inducer of CYP1A2, CYP2C9 and CYP3A4 in Fresh Human Hepatocytes (779157):** Following exposure of fresh human hepatocytes to PEP005 at concentrations of 0.0002, 0.02, or 2  $\mu\text{M}$  for 72 hours, there was no increase in the metabolism of the probe substrates, phenacetin at 20  $\mu\text{M}$  for CYP1A, [ $^{14}\text{C}$ ]-tolbutamide at 100  $\mu\text{M}$  for CYP2C9, and [ $^{14}\text{C}$ ]-testosterone at 150  $\mu\text{M}$  for CYP3A4, compared to the corresponding control article. However, there was actually a decrease in metabolism with increasing PEP005 concentration, when compared to the appropriate controls. The sponsor stated that the positive control inducers, omeprazole at 30  $\mu\text{M}$  for CYP1A2 and rifampicin at 3  $\mu\text{M}$  for CYP2C9 and CYP3A4, produced acceptable induction in the tested hepatocytes. No cytotoxicity was observed following treatment of PEP005 at up to 2  $\mu\text{M}$ , the highest concentration tested.

### **Results from the clinical pharmacokinetic study conducted under maximal clinical use conditions**

As per the Clinical Pharmacology reviewer, a Clinical Pharmacokinetic study has been conducted in patients with actinic keratosis (AK) with topical administration of PICATO (ingenol mebutate) Gel under maximal clinical use conditions (100  $\text{cm}^2$  contiguous treatment area treated with 4 unit dose tubes of PICATO Gel 0.05% once daily for 2 consecutive days). Minimal systemic exposure to ingenol mebutate and/or its isomers was detected (less than LLOQ 0.1  $\text{ng/mL}$ ). Therefore, little metabolism of ingenol mebutate will occur after topical administration of AK patients with PICATO Gel.

## **6 General Toxicology**

### **6.1 Single-Dose Toxicity**

**6.1.1. PEP005: Single dose intravenous toxicity study in the rat (2174/004):** In a preliminary study, all rats treated at 40  $\mu\text{g/kg}$  died immediately after dose administration. In the main study, four groups of 5 male and 5 female rats were given a single intravenous dose of 0, 10, 20, or 30  $\mu\text{g/kg}$  at a dose volume of 5  $\text{mL/kg}$ . Tachypnea was seen in all animals immediately post-dose. Mortality was observed at doses  $\geq 20$   $\mu\text{g/kg}$  (1/10 and 4/10 in the 20 and 30  $\mu\text{g/kg}$  groups, respectively). All deaths occurred

within half hour of dosing following severe clinical change and macroscopic examination of these animals revealed marked or severe darkening and inflation of all lobes of the lungs. Limpness was observed at doses  $\geq 10$   $\mu\text{g}/\text{kg}$ ; ataxia, proneness, piloerection, lethargy, and darkening of the tails were noted at doses  $\geq 20$   $\mu\text{g}/\text{kg}$ . Recovery of the animals was complete by Day 2. The minimum lethal intravenous dose for PEP005 in rats was 20  $\mu\text{g}/\text{kg}$ .

**6.1.2. PEP005: Single dose intravenous toxicity study in the rabbit (2174/009):** In a preliminary study, rabbits treated at 30  $\mu\text{g}/\text{kg}$  showed tachypnea, ataxia, proneness, salivation, and lacrimation immediately following dosing and recovery was complete within 48 hours. In the main study, four groups of 3 male and 3 female rabbits were given a single intravenous dose of 0, 5, 10, or 20  $\mu\text{g}/\text{kg}$ . No deaths occurred during the study. Bruising and erythema at the site of test article administration were even noted in 3 of the 6 animals treated at 5  $\mu\text{g}/\text{kg}$ . Tachypnea and miosis were observed at doses  $\geq 10$   $\mu\text{g}/\text{kg}$ ; proneness, salivation, lacrimation, lethargy, ataxic, and rale were noted at 20  $\mu\text{g}/\text{kg}$ . Recovery of the animals was complete within 48 hours and all animals gained weight through the study. There were no macroscopic findings observed at necropsy on Day 15. The minimum lethal intravenous dose for PEP005 in rabbits was greater than 20  $\mu\text{g}/\text{kg}$ .

**6.1.3. PEP005: Dermal tolerance/irritation pilot study in the mini-pig (2174/025):** A single semi-occluded application of 0.01% PEP005 (local dose 0.083  $\mu\text{g}/\text{mm}^2$ ) to intact mini-pig skin for 4-hours elicited slight local erythema/eschar and very slight edema. A single topical application of 0.1% PEP005 (local dose 0.83  $\mu\text{g}/\text{mm}^2$ ) produced moderate local erythema/eschar and slight edema. Erythema/eschar was clearly dose-related and persisted throughout the study (6 days). Skin score was noted at some PEP005-treated sites (0.01% and 0.1%). Acanthosis and acute necrotizing dermatitis were dose-related. Subdued behavior was observed in the mini-pigs on Day 2.

**6.1.4. PEP005: Dermal tolerance/irritation pilot study in the mini-pig (2174/027):** A single unoccluded application of PEP005 was administered to eight 20  $\times$  30 mm locations on the cleaned dorsum of two male and two female mini-pigs. The eight locations consisted of a combination of 3 concentrations (0.01%, 0.05%, and 0.1%) and two volumes (300 and 500  $\mu\text{L}$ ) and an additional 100  $\mu\text{L}$  of 0.05% and 0.1% (30 - 500  $\mu\text{g}$ , 0.05 to 0.83  $\mu\text{g}/\text{mm}^2$ ). A wide range of dermal responses was noted from very slight to moderate erythema and moderate edema. The responses increased in severity with dose/unit area. The severity of local irritation (inflammatory cell foci, dermal fibroblasts, acanthosis, scab formation, and parakeratosis) increased with higher doses, especially over 100  $\mu\text{g}$  PEP005. A NOAEL of 30  $\mu\text{g}$  or 0.05  $\mu\text{g}/\text{mm}^2$  was established for a single dose. In another study, mini-pigs were administered with 20, 100, or 200  $\mu\text{g}/\text{day}$  (0.05, 0.25, or 0.5  $\mu\text{g}/\text{mm}^2/\text{day}$ ) for up to 5 days. All PEP005 treated sites showed a greater response than the control sites. There was no evidence of systemic toxicity and a NOAEL was not established. Only a few blood samples contained quantifiable PEP005, which were only marginally above the lower limit of quantification, following a total topical dose up to 640  $\mu\text{g}$  PEP005.

## 6.2 Repeat-Dose Toxicity

**6.2.1. PEP005: Dermal tolerance/irritation pilot study in the rat (2174/021):** Semi-occluded application of 0.01% PEP005 (local dose 0.33  $\mu\text{g}/\text{mm}^2$ ) to intact rat skin for 4-hours for 1 or 3 days elicited very slight local erythema and edema and eschar formation. Topical application of 0.1% PEP005 (local dose 3.3  $\mu\text{g}/\text{mm}^2$ ) for 1 or 3 days produced moderate to moderately severe local erythema and edema and eschar formation. Acanthosis, scab formation, fasciitis/fibrosis, acute necrotizing dermatitis, myositis/myopathy were noted. The severity of irritation was related to the frequency of dosing and to the strength of concentration. There was no evidence of reversal over the course of the study (Day 8). There were no signs of systemic toxicity following topical application.

**6.2.2. PEP005 Topical Gel: 3-Day repeat dose dermal tolerance/irritation study comparing PEP005 Gel formulations of varying pH in Crl:CD (SD) rats (N106169):** Four groups of 12 male rats were topically treated with 0.01% PEP005 gel at a target pH of 3.5, 4.0, 4.5, or 5.0 on a 600  $\text{mm}^2$  skin area once daily for 3 consecutive days. The applied daily dose was 0.025  $\mu\text{g}/\text{mm}^2/\text{day}$  (approximately 50  $\mu\text{g}/\text{kg}/\text{day}$ ). Animals were sacrificed approximately 24 hours after the last dosing. There were no deaths and no treatment-related effects on systemic clinical signs and body weight. There were no significant differences between groups in drying time of the PEP005 gel on shaved skin; the average drying time ranged from 48 to 60 minutes following dosing. Erythema was observed at the treatment site in all groups. The incidence and severity of erythema increased with repeat dosing and the highest incidence and severity was noted approximately 24 hours following the last dose administration. The severity of erythema was statistically significantly greater for Group 1 (pH 3.5) compared to Groups 3 (pH 4.5) and 4 (pH 5.0) at 6 hours, and Group 3 at 24 hours following the first dose administration. However, there were no statistically significant differences in the incidence or severity of erythema between the dose groups at any other time point. No edema was observed in any dose group at any time point. In addition, exfoliation/desquamation, discoloration, nodules, and encrustation were noted in all groups. Necrotizing epidermal inflammation, parakeratosis, epidermal hyperplasia, and infiltration of the dermis and subcutis by mononuclear inflammatory cells were observed in all groups, with a slight increase in the incidence and/or severity of findings with decreasing gel pH. The sponsor concluded that only minor differences in treatment related dermal effects were noted both in-life and histopathologically with 3-day repeat administration of 0.01% PEP005 Gel formulations at pH's ranging from 3.5 to 5.0.

**6.2.3. PEP005: 3 Day repeat dermal tolerance and toxicity study in the rat followed by a 14 day observation period**

**Key study findings:** Dose-related erythema and edema were observed with the rapidity of onset, numbers of animals affected, and the degree of response. The females consistently showed more instance, greater severity and longer duration of dermal reactions than males at 0.01% and 0.1%. Dose-related necrotizing dermatitis, fasciitis/fibrosis, myositis, acanthosis, ulceration/erosion were also observed. There

were no signs of systemic toxicity. Local dermal irritation was noted even at the lowest dose tested, 0.025 µg/mm<sup>2</sup>/day (0.01%) and no NOAEL was established.

**Study no.:** 2174/026

**Conducting laboratory and location:** [REDACTED]

(b) (4)

(b) (4)

**Date of study initiation:** 10-3-2003

**GLP compliance:** Yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, and % purity:** PEP005, batch number 3195B, (0.105 - 0.116)% Gel

### Methods

Doses: 150 µl of 0.01% (15 µg), 0.05% (75 µg), 0.1% (150 µg) concentration

Species/strain: CrI:CD (SD)IGSBR rats

Number/sex/group or time point (main study): 5/sex/group

Route, formulation, volume, and infusion rate: Topical, 150 µl to 20 × 30 mm area

Satellite groups used for toxicokinetics or recovery: 15/sex/group

Age: 6.5 to 7.5 weeks

Weight: males, 202.9 - 272.1 g; females, 149.9 - 184.4 g

Sampling times: Blood samples were taken on Days 1 and 3, at 5, 10, and 30 min and 1, 2, 4, 8, 12, 16, and 24 hr after dosing from the satellite toxicokinetic animals.

Unique study design or methodology: A single concentration (0.01%, 0.05%, or 0.1%) of test article was administered on three occasions (24 hr apart) to one marked 20 × 30 mm dorsum skin area of each rat. Another same size location received vehicle and a third one was untreated. All test sites were left unoccluded. Because of experimental mis-dosing, the results from animals treated with 0.05% gel were not considered in the study findings.

### Results:

Mortality: No deaths occurred.

Clinical signs: No signs of systemic toxicity. The majority of the animals displayed soreness on the dorsum. The vehicle gave transient and mild dermal reactions (erythema) of no toxicological significance. Dose-related erythema and edema were observed with the rapidity of onset, numbers of animals affected, and the degree of response. The females consistently showed more instance, greater severity and longer duration of dermal reactions than males at 0.01% and 0.1%. Sites receiving PEP005 still showed more severe lesions than those receiving vehicle and the control sites at the end of study (Day 17).

Body weights: There was no effect on the mean terminal weight.

Food consumption: NA.

Ophthalmoscopy: NA

EKG: NA

Hematology: NA

Clinical chemistry: NA

Urinalysis: NA

Gross pathology: Skin sores were noted at a number of PEP005-treated skin sites, fewer vehicle-treated sites, and one untreated site.

Organ weights: NA

Histopathology: Dose-related necrotizing dermatitis, fasciitis/fibrosis, myositis, acanthosis, ulceration/erosion were observed at the treatment site.

Toxicokinetics: Both  $C_{max}$  and AUC values of PEP005 increased with increasing dose on Days 1 and 3 (see the next table). Drug accumulation over the 3-day dosing period was noted. Toxicokinetic parameters for (b) (4) were obtained only for 0.1% concentration on Day 3 ( $C_{max}$ , 0.0421 and 0.0535 ng/mL,  $AUC_{(0-24h)}$ , 0.578 and 0.866 ng·h/mL, and  $T_{max}$ , 1 and 4 hr, for males and females, respectively). (b) (4) was not quantifiable in any of the samples.

Sex	Concentration (%)	PEP005 (µg)	$T_{max}$ (hr)		$C_{max}$ (ng/mL)		$AUC_{(0-24h)}$ (ng·h/mL)	
			Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
M	0.01	15	-	1	-	0.076	-	-
	0.05	75	2	1	0.079	0.394	0.944	2.37
	0.1	150	0.5	2	0.162	0.438	2.88	5.12
F	0.01	15	4	2	0.102	0.206	0.606	1.38
	0.05	75	0.5	4	0.208	0.347	1.74	3.43
	0.1	150	2	2	0.441	0.623	5.92	6.28

**6.2.4. PEP005: 7 day intravenous administration dose range-finding study in the rat (2174/020)**: Five groups of 5 male and 5 female rats received doses of 0, 1, 5, 10, or 15 µg/kg/day PEP005 for 7 days. There were no effects attributable to PEP005 on mortality, clinical signs, or macroscopic findings. There was an increase in the incidence and severity of cardiomyopathy in the heart of males given 10 µg/kg/day and males and females given 15 µg/kg/day, when compared to the controls. The mean blood PEP005  $C_{max}$  increased with increasing dose on Days 1 and 7. No apparent drug accumulation over the 7-day dosing period and no apparent gender difference were observed.

**6.2.5. PEP005: 28 Day intravenous administration toxicity study in the rat**

**Key study findings:** Rats treated at 15 µg/kg/day PEP005 showed subdued behavior, tachypnea, ataxia, and hunched posture. The mean terminal weight of males treated at 15 µg/kg/day was 13% less than that of the control group, resulting in a lower (35%) body weight gain. Both  $C_{max}$  and AUC values of PEP005 increased with increasing dose on Days 1 and 28. Only slight drug accumulation over the 28-day dosing period was noted and no apparent gender difference was observed. The NOAEL was 7.5 µg/kg/day.

**Study no.:** 2174/014

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

(b) (4)

**Date of study initiation:** 8-15-2003

**GLP compliance:** Yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, and % purity:** PEP005, batch number 070303, 99.6%

### Methods

Doses: 0, 1.5, 7.5, and 15 µg/kg/day

Species/strain: CrI:WI(GLX/BRL/han)IGSBR rats

Number/sex/group or time point (main study): 10/sex/group

Route, formulation, volume, and infusion rate: Intravenous, 20% PEG 400 in 0.9% saline, volume 5 mL/kg, infusion rate 3 mL/min.

Satellite groups used for toxicokinetics or recovery: 15/sex/group in the 3 PEP005-treated groups and 5/sex in the control group for toxicokinetics

Age: 6 weeks

Weight: males, 135.9 - 184.9 g; females, 120.2 - 164.4 g

Sampling times: Blood samples were taken on Days 1 and 28, at 0, 1, 2, 5, 15, and 30 min and 1, 2, 4, and 8 hr after dosing from the satellite toxicokinetic animals.

Unique study design or methodology: NA

### Results:

**Mortality:** Only one female treated at 7.5 µg/kg/day died on Day 22.

**Clinical signs:** Rats treated at 15 µg/kg/day showed subdued behavior, tachypnea, ataxia, and hunched posture post-dose. Clinical signs at weekly observation were limited to thinning fur, hair loss, and fur staining.

**Body weights:** The mean terminal weight of males treated at 15 µg/kg/day was 13% less than that of the control group, resulting in a lower (35%) body weight gain.

**Food consumption:** A slight decrease (8%) in the food consumption was noted in the males treated at 15 µg/kg/day at the end of study.

**Ophthalmoscopy:** There were no effects of the treatment in Week 4.

**EKG:** NA

Hematology: There were no significant treatment-related changes in Week 4.

Clinical chemistry: There were no significant treatment-related changes in Week 4.

Urinalysis: There were no significant treatment-related changes in Week 4.

Gross pathology: There were no treatment-related changes in macroscopic findings at the end of the study.

Organ weights: Only the mean brain weight in the males treated at 15 µg/kg/day was significantly lower at the end of study.

Histopathology: There were no treatment-related changes in microscopic findings at the end of the study.

Toxicokinetics: Both  $C_{max}$  and AUC values of PEP005 increased with increasing dose on Day 1 and 28. Only slight drug accumulation over the 28-day dosing period was noted and no apparent gender difference was observed. Quantifiable whole blood concentrations of (b) (4) were obtained only for 15 µg/kg/day group. (b) (4) was not quantifiable in any of the samples

Sex	Dose (µg/kg/day)	$T_{max}$ (min)		$C_{max}$ (ng/mL)		AUC <sub>(0-8h)</sub> (ng·h/mL)	
		Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
M	1.5	1	1	0.769	0.737	0.233	0.38
	7.5	1	1	4.62	7.60	1.57	1.98
	15	1	0	8.57	9.95	2.91	4.62
F	1.5	1	1	0.838	0.793	0.299	0.352
	7.5	0	2	3.47	3.48	1.74	1.52
	15	0	2	6.01	9.03	2.95	3.97

#### 6.2.6. PEP005: 6-Month intravenous dose toxicity study in Crl:CD(SD) rats with a 1-month recovery period

Study no.: N106162  
 Study report location: (b) (4)  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: May 18, 2005  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: PEP005, Lot PEP0405b, purity 98.1%

#### Key Study Findings

There were no treatment-related effects on hematology, clinical chemistry, urinalysis, and organ weights. There were a total of 30 males and 12 females died prior to the end

of dosing period. A high mortality incidence was observed in rats treated with PEP005 at 7.5 or 15 µg/kg/day (2x/week) or 15 µg/kg/day (1x/week). Dose-dependent abnormal clinical signs, including lethargy, labored/rapid respiration, ataxia, disorientation, and head tilt, were intermittently observed in PEP005-treated animals, but not in vehicle-treated animals. Slight lower body weights and food consumption were seen in males treated with PEP005 15 µg/kg/day (2x/week). Renal tubular adenoma and hyperplasia were seen in one out of 4 males and one out of 6 females receiving 15 µg/kg/day (2x/week) at the end of dosing period. Pituitary adenoma was also present in the female with the renal adenoma. Thyroid follicular cell carcinoma was observed in one out of 3 males receiving 15 µg/kg/day (2x/week) at the end of the recovery period. PEP005 was detected in samples from all male and female rats following intravenous administration of PEP005. (b) (4) was detected only in a few samples from rats treated at 7.5 or 15 µg/kg/day PEP005 (up to 0.315 or 0.640 ng/mL, respectively). However, (b) (4) was not detected in any of the samples.

It is not clear that the tumors noted in this study are treatment related because the increase in tumor incidence was very small and not statistically significant. The tumors noted in this 6 month intravenous rat toxicity study are not clinically relevant since the clinical condition of use is topical administration of the drug product and minimal systemic exposure was noted under maximal clinical conditions of use for this topical drug product.

#### Methods

Doses:	0 (vehicle), 1.5, 7.5, or 15 µg/kg/day PEP005 twice weekly, or 15 µg/kg/day once weekly
Frequency of dosing:	Twice or once weekly
Route of administration:	Intravenously
Dose volume:	5 mL/kg
Formulation/Vehicle:	20% PEG 400 in 0.9% saline for injection
Species/Strain:	Crl:CD (SD) rats
Number/Sex/Group:	10/sex/group
Age:	10 - 11 weeks
Weight:	Males 313 - 410 g; females 175 - 272 g
Satellite groups:	Three groups of 9 rats/sex/group were treated 1.5, 7.5, or 15 µg/kg/day PEP005 twice weekly for toxicokinetic analysis.
Unique study design:	Main groups were treated for 6 months and necropsied approximately 24 hours after the final dosing. The recovery rats (5/sex) in each main group were necropsied 28 days after the final dosing (recovery group).
Deviation from study protocol:	Several TK rats were reassigned to main groups to provide sufficient sample sizes at necropsy due to early deaths in the main groups.

#### Observations and Results

## Mortality

Observed twice daily. There were a total of 30 males and 12 females died prior to the end of dosing on Day 182 (see the next table). A high mortality incidence was observed in main study rats treated with PEP005 at 7.5 or 15 µg/kg/day (2x/week) or 15 (1x/week) (5/10, 6/10, and 5/10 males, and 2/10, 4/10, and 1/10 females, respectively). Similar mortality patterns were observed in the satellite toxicokinetic groups (7/9 and 6/9 males and 1/9 and 4/9 females in the 7.5 and 15 µg/kg/day twice weekly groups, respectively). For the unscheduled deaths (with the exception of one rat), disseminated thrombi were found in vessels of the lungs, which were considered to be the cause of mortality.

Table 1. Summary of Mortality

Dose Group (µg/kg/dose)	Starting Number of Animals per Group (Day 1)	Study Days Deaths Occurred (number of animals >1)	Remaining Number of Animals per Group on Day 182	Number of Animals to Necropsy on Day 183 / 211
<b>Males</b>				
0	10	--	10	6 / 4
1.5	10	143	9	6 / 3
7.5	10	4, 10, 38, 59, 136	5	5 / 0
15 (2x)	10	4, 7, 38, 45, 70, 161	4	4 / 0
15 (1x)	10	7, 21, 49, 133(2)	5	5 / 0
1.5 TK	9	--	9	0 <sup>a</sup> / 0
7.5 TK	9	35, 45, 59, 70, 98, 115, 154	2	0 <sup>b</sup> / 2*
15 TK	9	14, 35, 36, 56, 87, 169	3	0 <sup>b</sup> / 3*
<b>Females</b>				
0	10	--	10	6 / 4
1.5	10	--	10	6 / 4
7.5	10	10, 52	8	5 / 3
15 (2x)	10	31, 122, 126, 129	6	6 / 0
15 (1x)	10	182	9	5 / 4
1.5 TK	9	--	9	0 <sup>a</sup> / 0
7.5 TK	9	24	8	0 <sup>c</sup> / 0
15 TK	9	38, 98, 101, 143 <sup>d</sup>	5	0 <sup>d</sup> / 3*

\* Animals in TK groups realigned to recovery toxicology groups based on dose received.

a. 9 animals used for TK on Day 182

b. 0 animals used for TK on Day 182

c. 8 animals used for TK on Day 182

d. 2 animals used for TK on Day 182

e. Euthanized due to compromised tail circulation effecting both animal health and dose delivery.

## Clinical Signs

On dosing days, animals were observed immediately following dosing and during the last 10-minutes of the first hour post-dosing. Any observations pertaining to suspected systemic toxicological effects were monitored until 4 to 6 hours post-dosing. Abnormal clinical signs including lethargy, labored/rapid respiration, ataxia, disorientation, and head tilt were intermittently observed in all groups treated with PEP005 but not in vehicle-treated animals. In rats treated at 1.5 µg/kg/day 2x/weekly, lethargy was noted in 2 males and 2 females and labored respiration was observed in 1 male and 2 females at 10 minutes post-dosing; these observations occurred only once for each animal. Abnormal clinical signs were dose dependent with the highest incidence noted in rats treated with 15 µg/kg/day PEP005 2x/week.

## Body Weights

Recorded weekly. Group mean body weights of males at 15 µg/kg/day (2x/week) were lower compared to all other groups beginning on Day 77 and continuing throughout the remainder of the dosing and recovery periods. In males treated with PEP005 at 7.5

µg/kg/day (2x/week) or 15 µg/kg/day (1x/week), mean body weights were slightly lower, compared to vehicle-treated males, from Days 98 to 182, and 98 to 133, respectively. Females treated with 7.5 µg/kg/day PEP005 (2x/week), body weights were lower than all other groups through the most of the dosing period. These differences were not statistically significant.

### **Feed Consumption**

Recorded weekly. Mean food consumption of males treated with PEP005 15 µg/kg/day (2x/week) and females treated with 7.5 µg/kg/day (2x/week) were lower than other PEP005-treated groups and the vehicle-treated animals for the majority of the dosing period, but not statistically significantly.

### **Ophthalmoscopy**

NA

### **ECG**

NA

### **Hematology**

Blood samples were collected during Week -1 and on Day 183 prior to necropsy, and all recovery animals on Day 211 prior to necropsy. There were no treatment-related effects.

### **Clinical Chemistry**

Blood samples were collected during Week -1 and on Day 183 prior to necropsy, and all recovery animals on Day 211 prior to necropsy. There were no treatment-related effects.

### **Urinalysis**

Urine samples were collected during Week -1 and on Day 183 prior to necropsy, and all recovery animals on Day 211 prior to necropsy. There were no treatment-related effects.

### **Gross Pathology**

At terminal necropsy, a kidney nodule and enlarged pituitary were noted in one out of 6 females receiving 15 µg/kg/day (2x/week). At the end of recovery, an enlarged thyroid gland was observed in one out of 3 males receiving 15 µg/kg/day (2x/week). Renal tubular adenoma, pituitary adenoma, and thyroid follicular cell carcinoma were microscopically noted in the rats, respectively.

## Organ Weights

The following organs were weighed at scheduled necropsies: adrenal, brain, heart, kidney, liver, spleen, ovaries, epididymides, testes, thyroid, and pituitary (post-fixation). There were no treatment-related effects.

## Histopathology

A battery of tissues were collected from all main group rats. Each slide was examined microscopically by the study pathologist and an internal peer review was performed. Renal tubular adenoma and hyperplasia were seen in one out of 4 males and one out of 6 females receiving 15 µg/kg/day (2x/week) at the end of dosing (Day 183). Pituitary adenoma was also present in the female with the renal adenoma. Thyroid follicular cell carcinoma was observed in one out of 3 males receiving 15 µg/kg/day (2x/week) at the end of the recovery period (Day 211). The occurrence of tumors was unusual for a study of 6-months duration in rats. However, it is not clear if any of the tumor incidences are treatment-related.

## Toxicokinetics

Blood samples were collected from toxicokinetic group rats prior to dosing, and at approximately 1, 2, 5, 15, 30, 60, 120, 240, and 480 minutes after dosing on Days 1 and 182. Three samples/sex/time point were collected on Day 1 and 0 to 3 samples/sex/time point collected on Day 182, due to reassignment.

PEP005, (b) (4) were not detected in any samples from vehicle-treated animals (LLOQ 0.1 ng/mL). PEP005 was detected in samples from all male and female rats following intravenous administration of PEP005. As seen from the next table, the  $AUC_{(0-t)}$  increased in a supra-proportional manner for both males and females over the dose range of 1.5 to 15 µg/kg/day on Day 1, and in females on Day 182. Increases in  $C_{max}$  were approximately dose proportional over the dose range in male and female rats on Day 1 and in females on Day 182. The  $AUC_{(0-t)}$  and  $C_{max}$  were higher in females when compared to those in males at all dose levels on Day 1. The  $AUC_{(0-t)}$  and  $C_{max}$  were lower in females than in males at the 1.5 µg/kg/day on Day 182.

Summary of Toxicokinetic Parameters				
Sex	Dose ( $\mu\text{g}/\text{kg}/\text{day}$ )	Mean (standard deviation)		
		$C_{\text{max}}$ ( $\text{ng}/\text{mL}$ )	$T_{\text{max}}$ (min)	$\text{AUC}_{(0-t)}$ ( $\text{ng}\cdot\text{h}/\text{mL}$ )
<b>Day 1</b>				
Male	1.5	0.92	2	0.14
	7.5	3.03	1	1.38
	15	12.9	1	3.69
Female	1.5	1.24	2	0.18
	7.5	5.97	1	2.11
	15	13.8	2	4.33
<b>Day 182</b>				
Male	1.5	2.33	1	0.51
	7.5	NS	NS	NS
	15	NS	NS	NS
Female	1.5	1.44	1	0.33
	7.5	5.59	1	2.20
	15*	17.5	1	5.91

NC= not calculated, NS= no samples for analysis

\* n=1

(b) (4) was detected only in a few samples from rats treated at 7.5 or 15  $\mu\text{g}/\text{kg}/\text{day}$  PEP005 (up to 0.315 or 0.640  $\text{ng}/\text{mL}$ , respectively). However, (b) (4) was not detected in any of the samples.

### Dosing Solution Analysis

The achieved concentrations of the stock solutions were within the range of 103% to 119%; the dosing formulations were in the range of 78% to 107% of nominal. PEP005 was not found in the control samples.

*(Reviewer comments: This study report was finalized in June 2007, but submitted to FDA in November 2009 within the IND 70144)*

**6.2.7. PEP005: 13 Week Dose Range Finding Study in Rats with Administration by the Dermal Route with a 15 Day Recovery Period (457405):** Six groups of rats were treated topically as shown in the following table. Due to the severity of local dermal clinical signs seen in the Group 4 (500  $\mu\text{g}/\text{mL}$ ) and 5 (1000  $\mu\text{g}/\text{mL}$ ) animals, dosing for these animals was ceased after Day 2 and a further group (Group 6) of 3 male and 3 female animals was assigned to the Main study, dosed at a level of 100  $\mu\text{g}/\text{mL}$ , but at a different frequency to those in Group 3. The scheduled termination of Main study animals (3/sex/group) in Groups 1, 2, 3 took place after completion of 13 weeks of treatment and approximately 48 h after the final dose application. Group 6 animals (3/sex/group) were terminated during Week 13 of treatment and approximately 24 h after the final dose application. All animals (6/sex/group) in Groups 4 and 5 were terminated during Week 14 of the study. The remaining Recovery study male and female animals (3 animals per sex/group) from Groups 1, 2 and 3 were terminated 15 days after the final dose application.

Group	Dosage Concentration <sup>a</sup> (%)	Local Exposure ( $\mu\text{g}/\text{mm}^2/\text{day}$ )	Dosage Administration Regimen <sup>a</sup>
1	Placebo Gel/Untreated Control	0	Dosed once daily for 6 days followed by a 15 day recovery (shoulder – placebo gel), repeated for 13 weeks. Untreated Control (rump).
2	50 $\mu\text{g}/\text{mL}$ (0.005%)	0.0125	Dosed once daily for 6 days to two sites (shoulder and rump) followed by a 15 day recovery period, repeated for 13 weeks. Dosing completed at the shoulder and suspended at the rump at Week 7.
3	100 $\mu\text{g}/\text{mL}$ (0.01%)	0.025	Dosed alternately on two dose sites (shoulder and rump) for 6 days followed by a 15 day recovery period, repeated for 13 weeks. Dosing completed at all sites.
4	500 $\mu\text{g}/\text{mL}$ (0.05%)	0.125	Dosed once only to two sites (shoulder and rump) followed by a 13 week recovery.
5	1000 $\mu\text{g}/\text{mL}$ (0.1%)	0.25	Dosed only once at the shoulder followed by a 13 week recovery.
6	100 $\mu\text{g}/\text{mL}$ (0.01%)	0.025	Dosed once daily for 2 days to two sites (shoulder and rump) followed by a 5 day recovery period, repeated for 13 weeks. Dosing completed at the shoulder and suspended at the rump at Week 3.

<sup>a</sup> Constant dose volume of 150  $\mu\text{L}$  (0.15 mL) was applied to each site as specified in the table

Topical treatment of PEP005 in a repeat dose/recovery cycle for 13 consecutive weeks was associated with mild to severe skin reactions of erythema, skin thickening, encrustations, desquamation, and skin necrosis in all PEP005-treated groups. The reactions were more severe at the higher dose levels, as well as at the rump dose site. Female animals in Group 3 were generally noted to have a higher incidence of erythema than the equivalent male animals. Recovery was noted to a small degree during the recovery periods; however, there was also some evidence that the dermal clinical signs were becoming more severe as the number of cycles increased.

Animals in Group 2 completed dosing at the shoulder site for the full 13 weeks, although dosing at the rump site had to be suspended during Week 7, due to the intensity of the dermal response. Animals in Group 3 completed dosing at both the shoulder and rump for the full 13 weeks. Dermal responses in Group 4 and 5 were so intense as to preclude further dosing beyond Day 2 of the study. Animals in Group 6 completed dosing at the shoulder for the full 13 weeks, although dosing at the rump site had to be suspended after 2 weeks due to the intensity of the dermal response.

Body weight gain was noted to be slightly lower in all male treated groups and females in Group 6. Food consumption was also noted to be slightly lower in all male groups treated with PEP005, when compared to the Controls.

Findings at the treated sites included scabbing, raw areas, thickened and/or reddened areas in the Main study in all animals from Group 3 and some animals from Groups 2 and 6. In the Recovery study animals, 1/3 females from Group 3 had scabs on the rump. Microscopically, ulcerative hyperplastic dermatitis was present in all animals from Group 3; and in some animals from Groups 2 and 6, with a reduced incidence and severity. Following the Recovery period, hyperplastic dermatitis was found in one female from each of Groups 2 and 3, with a further Group 3 female noted with ulcerative hyperplastic dermatitis.

### 6.2.8. PEP005 Topical Gel: 6-Month dermal study in CRL:CD(SD)IGS BR rats with a 4-week recovery

**Key study findings:** There were no treatment-related effects on mortality, systemic clinical signs, body weights, food consumption, coagulation, clinical chemistry, and organ weights. There were dose-related increases in the incidence and severity of erythema and edema following each dosing cycle. There was evidence of alterations in dermal tolerance to PEP005 administration over the course of treatment (7 cycles). Additional treatment-related dermal observations included skin exfoliation/desquamation, minor abrasions, and encrustations. Following the 4-week recovery period, erythema and edema resolved in all dose groups. In rats treated with 0.02% PEP005 gel, an increase in neutrophils consistent with dermal inflammation was noted. Microscopically, there was a dose-related increase in incidence and severity of epidermal necrosis, epidermal hyperplasia, dermal inflammation, and the presence of serocellular crust formation in all PEP005-treated groups. Following a 4-week recovery period, microscopic dermal observations in all rats were resolved (vehicle and 0.005% PEP005) or nearly resolved ( $\geq 0.01\%$  PEP005). Blood levels of PEP005 were not quantifiable in a majority of samples at an LLOQ of 0.1 ng/mL.

**Study no.:** N106168A

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

**Date of study initiation:** 10-6-2006

**GLP compliance:** Yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, and % purity:** Vehicle Gel, 7037/001, 7041/001, 7041/002, and 7041/003; 0.005% Gel, 7036/001, 7040/001, 7040/003, and 7040/004; 0.01% Gel, 7035/001, 7039/001, 7039/002, and 7039/003; 0.02% Gel, 7034/001, 7038/001, 7038/002, and 7038/003. The achieved concentrations were within the range of 89 to 121 percent of target.

#### **Methods**

Doses: Untreated, vehicle, 0.0125, 0.025, and 0.05  $\mu\text{g}/\text{mm}^2/\text{day}$  (approximately 25, 50, and 100  $\mu\text{g}/\text{kg}/\text{day}$  PEP005)

Species/strain: CRL:CD(SD)IGS BR rats

Number/sex/group or time point (main study): 15/sex/group

Route, formulation, volume, and infusion rate: Topical, 150  $\mu\text{l}$  to a 20 mm  $\times$  30 mm area

Satellite groups used for toxicokinetics or recovery: 6/sex/group, 4 groups (0, 25, 50, and 100  $\mu\text{g}/\text{kg}/\text{day}$  as the main study)

Age: 8 weeks

Weight: 176.4 - 216.9 mg

Sampling times: Ten rats/sex/group were terminated following the final treatment (Week 25), and 5/sex/group were terminated at the completion of a 4-week recovery period (Week 29).

Unique study design or methodology: Four groups were topically treated with 150 µL of vehicle or PEP005 gel at concentrations of 0.005% (50 µg/mL), 0.01% (100 µg/mL), or 0.02% (200 µg/mL) to a shaved 600 mm<sup>2</sup> skin site on the dorsal shoulder. Administered topical doses were 0.0125, 0.025, or 0.05 µg/mm<sup>2</sup>/day (approximately 25, 50, and 100 µg/kg/day, respectively) for 3 consecutive days followed by a 4-week non-dosing period (designated as a dosing cycle), and repeated monthly for six months (7 cycles). One group animals were untreated. In addition, 4 groups of 6 male and female rats were assigned as satellite toxicokinetic animals and were similarly treated.

**Results:**

Mortality: Observed twice daily. There were no treatment-related deaths.

Clinical signs: Observed once daily on each day of dosing and once weekly during non-dosing weeks. There were no signs of systemic toxicity.

There were minor abrasions and/or very slight erythema occasionally observed in the untreated and vehicle groups. PEP005-related dermal observations of erythema and edema were noted at the treatment site following topical application. Statistically significant and dose-related increases in incidence and severity of erythema were noted in all of the PEP005-treated groups following each dosing cycle compared to vehicle-treated group. Observations ranged from very slight to well defined erythema (0.005%; 0.01% males) to moderate to severe erythema (0.01% females; 0.02%). PEP005-related edema was observed at the treatment site following the first treatment cycle in all treated groups, with sporadic incidences observed in the 0.01% and 0.02% groups following treatment cycles two through seven. There were no apparent gender differences in the incidence and severity of erythema or edema within the same dose groups. The highest incidence and severity of erythema and edema occurred approximately 1 day following each dose administration cycle. However, no indication of alterations in dermal tolerance to dosing was observed in either gender across treatment cycles. Additional treatment-related dermal observations included skin exfoliation/desquamation, minor abrasions, and encrustations. Following the 4-week recovery period, the dermal observations of erythema and edema resolved in all dose groups.

Body weights: Recorded weekly. There were no treatment-related body weight changes.

Food consumption: Recorded weekly. There were no treatment-related effects on food consumption.

Ophthalmoscopy: NA

Hematology and Coagulation: Blood samples were collected during Week 25 (terminal; one day after the final treatment) and Week 29 (recovery; 28 days after the final treatment). There were no effects on coagulation. However, statistically significant increases (86% and 110% for males and females, respectively) in mean neutrophil

values, compared with untreated control animals, were observed in both genders in the 0.02% PEP005 dose group at Week 25 (terminal necropsy). Additionally, females treated with 0.02% PEP005 gel showed a statistically significant increase (57%) in monocyte counts compared to untreated control females. Both observations might be consistent with PEP005-treatment related dermal inflammation/necrosis.

Clinical chemistry: Blood samples were collected as above. A slight decrease in total protein (5%) and albumin levels (9%) were seen in the 0.02% females on Day 172.

Urinalysis: NA

Gross pathology: There were no gross lesions, with the exception of PEP005-treated skin site observations (discoloration and crust formation), noted at terminal necropsy during Week 25.

Organ weights: There were no treatment-related organ weight changes.

Histopathology: There was a dose-related increase in incidence and severity of epidermal necrosis, epidermal hyperplasia, dermal inflammation, and the presence of serocellular crust formation in all PEP005-treated groups. A low incidence of very minimal epidermal hyperplasia and dermal inflammation was also noted in the vehicle-treated animals.

**Text Table 9. Incidence and Severity of Treatment-Related Microscopic Findings: 6 months, PEP005 Gel**

Group	Males					Females				
	1	2	3	4	5	1	2	3	4	5
ORGAN										
Dosage (µg/mL):	0	0	50	100	200	0	0	50	100	200
SKIN-SOA										
Total # Examined:	10	10	10	10	10	10	10	10	10	10
Diagnosis										
Necrosis, epidermis (average severity)	0 (0.0) <sup>a</sup>	0 (0.0)	4 (0.8)	8 (1.6)	10 (3.0)	0 (0.0)	0 (0.0)	4 (0.7)	2 (0.5)	9 (2.7)
Hyperplasia, epidermis (average severity)	0 (0.0)	5 (0.5)	10 (1.7)	10 (2.5)	10 (2.8)	0 (0.0)	0 (0.0)	10 (1.6)	10 (2.0)	10 (2.6)
Inflammation, Dermis (average severity)	0 (0.0)	1 (0.1)	10 (2.0)	10 (2.4)	10 (2.9)	0 (0.0)	0 (0.0)	9 (1.2)	10 (2.3)	10 (3.0)
Serocellular crust (average severity)	0 (0.0)	0 (0.0)	4 (0.7)	8 (1.9)	10 (3.0)	0 (0.0)	0 (0.0)	4 (0.5)	5 (0.7)	9 (2.8)
Fibrosis, Dermis (average severity)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

<sup>a</sup> Average severity was calculated as the sum of scores of all affected animals in a group divided by the number of animals in the group.

At the recovery necropsy, the majority of the lesions observed at the completion of the treatment period were resolved. Remaining microscopic observations consisted of a low incidence and mild severity of dermal inflammation (0.01% females; 0.02% both genders), epidermal hyperplasia (0.02 % females), and dermal fibrosis (0.01% and 0.02%).

**Toxicokinetics:** Blood samples were collected from satellite animals at pre-dose and at 30, 60, 120, 240, and 480 minutes after the third dose administration of the dosing cycle on Weeks 1 and 25. PEP005 was not quantifiable (LLOQ = 0.1 ng/mL) in any samples from rats treated with vehicle and in the majority of samples of rats treated with PEP005 gel. Some samples from PEP005-treated groups had concentration of PEP005 marginally above the LLOQ, but less than 0.2 ng/mL, with the exception of one sample collected at 60 minutes on Day 3, from one male treated with 0.02% Gel (100 µg/kg/day) which had concentration of 0.268 ng/mL. [REDACTED] (b) (4) were not quantifiable in any of the samples.

**6.2.9. PEP005: Dermal tolerance/irritation study in the rabbit (2174/011):** Semi-occluded application of 0.01% or 0.1% PEP005 (local dose 0.08 or 0.8 µg/mm<sup>2</sup>) to intact rabbit skin for 4 hours elicited dermal reactions including erythema, edema, discolouring of the test site and desquamation. Two consecutive exposure of 0.01% PEP005 (local dose 0.08 µg/mm<sup>2</sup> per day) produced minimal to moderate skin irritation, local erythema and edema, acanthosis and scab formation. Slight to moderate full thickness acute necrotizing dermatitis, dermal fasciitis/fibrosis and moderate dermal fibrosis were observed. Two consecutive exposures of 0.1% PEP005 (local dose 0.8 µg/mm<sup>2</sup>) elicited moderate to moderately severe skin irritation, local erythema and edema. Acanthosis and scab formation, moderate and severe acute necrotizing dermatitis, moderate erosion/ulceration, epidermal necrosis, and dermal fibrosis were observed. It seemed that the severity of dermal signs was affected by the frequency of dosing and by the strength of concentration. All effects showed evidence of reversal and no systemic toxicity was observed.

**6.2.10. PEP005: 3 Day repeat dermal tolerance/irritation study in the minipig followed by a 14 day observation period**

**Key study findings:** There were no deaths, systemic clinical signs, or body weight changes related to treatment. Treatment with 0.01% PEP005 gel resulted in wide variation in dermal reactions, including very slight to severe erythema and scab formation which limited the number of doses (only 1 instead of 3 in one male). The dermal responses increased in severity with increasing dose and the intensity of these dermal responses was greater in males than females. Compared with the vehicle control, the erythema scores at 24 hours after final application were significantly higher in males receiving 0.0025% or more and in females receiving 0.005% or more. Minimal to marked erosion/ulcer formation and minimal to mild acanthosis were present at the treatment sites treated with PEP005 at all concentrations in males and at concentrations of 0.005% and 0.01% in females at 24 hours post-treatment. The severity of these findings and the number of treatment sites affected increased with increasing concentrations. Although the sponsor stated that the NOAEL following 3-day repeat dosing was considered to be a concentration of 0.001% for males and 0.0025% for females, no NOAEL was established for males from this 3-day repeated dermal study in the mini-pig, due to the histopathological findings.

**Study no.:** 507450

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

**Date of study initiation:** 6-24-2004

**GLP compliance:** Yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, and % purity:** PEP005, batch number 0160X, 96%

### **Methods**

**Doses:** Each animal received four 150 µl (total 600 µl) of concentration 0.001%, 0.0025%, 0.005%, or 0.01% PEP005 gel (0.4, 1, 2, or 4 µg/kg/day; 2.5, 6.25, 12.5, or 25 ng/mm<sup>2</sup>/day, respectively)

**Species/strain:** Gottingen ApS mini-pig

**Number/sex/group or time point (main study):** 5/sex/group

**Route, formulation, volume, and infusion rate:** Topical, 150 µl to 20 × 30 mm area, 4 PEP005 dosing sites + 1 vehicle site + 1 untreated site per animal

**Satellite groups used for toxicokinetics or recovery:** None

**Age:** 5 to 10 months

**Weight:** 9.4 - 17.1 kg

**Sampling times:** Animals were killed 24 hours (3/sex/group) or 14 days (2/sex/group) after the final topical treatment.

**Unique study design or methodology:** Four groups of 5 mini-pigs per sex topically received 0.001%, 0.0025%, 0.005%, or 0.01% of PEP005 gel at a volume of 150 µl per site to four 20 × 30 mm sites per animal (total 600 µl at the same concentration) and vehicle gel at the same volume at a fifth site for 3 consecutive days. Each animal had a sixth site that was untreated and served as control. Animals were killed 24 hours (3/sex/group) or 14 days (2/sex/group) after the final treatment.

### **Results:**

**Mortality:** No deaths occurred.

**Clinical signs:** No signs of systemic toxicity. There were no dermal responses at the vehicle or untreated sites. Very slight erythema was observed at the treatment sites after applications of PEP005 0.001% gel. Treatment with 0.01% PEP005 gel resulted in wide variation in dermal reactions, including very slight to severe erythema and scab formation which limited the number of doses (only 1 instead of 3 in one male). The dermal responses increased in severity with increasing dose and the intensity of these dermal responses was greater in males than females. The erythema scores at 24 hours after final treatment, when compared with the vehicle control, revealed significantly higher scores for males receiving 0.0025% or more and for females receiving 0.005% or more.

**Body weights:** There were no treatment-related body weight changes.

**Food consumption:** NA.

**Ophthalmoscopy:** NA

Hematology: NA

Clinical chemistry: NA

Urinalysis: NA

Gross pathology: Scabs were present at the treatment sites treated with PEP005 at concentration of 0.0025% or above in the males and 0.005% or above in females at 24 hours post-treatment.

Organ weights: NA

Histopathology: Minimal to marked erosion/ulcer formation and minimal to mild acanthosis were present at the treatment sites treated with PEP005 at all concentrations in males and at concentrations of 0.005% and 0.01% in females at 24 hours post-treatment. The severity of these findings and the number of treatment sites affected increased with increasing concentrations. There was also an increase in the incidence and severity of dermal fibroplasia, scab formation, dermatitis, and parakeratosis at the treatment sites treated with PEP005 in comparison with untreated or vehicle treated sites, at 24 hours or 14 days post-treatment. Although the sponsor stated that the NOAEL following 3-day repeat dosing was considered to be a concentration of 0.001% for males and 0.0025% for females, no NOAEL was established for males from this 3-day repeated dermal study in the mini-pig, due to the histopathological findings.

Toxicokinetics: NA

#### **6.2.11. PEP005: 3 Day repeat dermal tolerance/irritation study in the mini-pig followed by a 14 day observation period**

**Key study findings**: No signs of systemic toxicity were noted. Application of 0.01% gel produced very slight to well-defined erythema with very slight edema. Application of 0.05% gel produced very slight to moderate erythema with very slight to slight edema. Application of 0.1% gel produced moderate to severe erythema with moderate edema. The intensity of the irritation response in some animals that received 0.05% or 0.1% gel limited the number of doses. Dose-related dermatitis, acanthosis, parakeratosis, scab formation, and ulceration/erosion were observed. The intensity of these dermal responses was greater in males than females. The overall level of these changes at treatment sites that received PEP005 on Day 17 was reduced when compared with Day 4. No NOAEL was established.

**Study no.:** 2174/029

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

(b) (4)  
**Date of study initiation:** 10-17-2003

**GLP compliance:** Yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, and % purity:** PEP005, batch number 3195B, 0.126% gel

### **Methods**

Doses: 150 µl of concentration 0.01% (60 µg/day or 6 µg/kg/day), 0.05% (300 µg/day or 30 µg/kg/day), or 0.1% (600 µg/day or 60 µg/kg/day)

Species/strain: Gottingen ApS mini-pig

Number/sex/group or time point (main study): 5/sex/group

Route, formulation, volume, and infusion rate: Topical, 150 µl to 20 × 30 mm area

Satellite groups used for toxicokinetics or recovery: None

Age: 3.5 to 6 months

Weight: males, 5.5 - 15.0 kg; females, 7.5 - 15.5 kg

Sampling times: Blood samples were taken at 5, 10, and 30 min and 1, 2, 4, 8, 12, 16, and 24 hr post-dosing on Days 1 and 3.

Unique study design or methodology: Three groups of 5 mini-pigs per sex topically received 0.01%, 0.05%, or 0.1% of PEP005 gel at a volume of 150 µl per site to four 20 × 30 mm site per animal. Another same-size site received vehicle and a sixth one was untreated. All treatment sites were left unoccluded. Treatment was on 3 consecutive days and the condition of the skin was monitored for either 24 hours (Day 4) or 14 days (Day 17) after the last treatment. The local topical dose was 0.025, 0.125, or 0.25 µg/mm<sup>2</sup>/day/site.

### **Results:**

**Mortality:** No deaths occurred.

**Clinical signs:** No signs of systemic toxicity. Application of 0.01% gel produced very slight to well-defined erythema with very slight edema. Application of 0.05% gel produced very slight to moderate erythema with very slight to slight edema. Application of 0.1% gel produced moderate to severe erythema with moderate edema. The intensity of the irritation response in some animals that received 0.05% or 0.1% gel limited the number of doses (only 2 instead of 3). The dermal responses increased in severity with increasing dose and the intensity of these dermal responses was greater in males than females.

**Body weights:** There was no effect on the mean terminal weight.

**Food consumption:** NA.

**Ophthalmoscopy:** NA

**EKG:** NA

**Hematology:** NA

Clinical chemistry: NA

Urinalysis: NA

Gross pathology: Skin sores were noted at almost all animals receiving PEP005.

Organ weights: NA

Histopathology: Dose-related dermatitis, acanthosis, parakeratosis, scab formation, and ulceration/erosion were observed. The overall level of these changes at sites that received PEP005 on Day 17 was reduced when compared with Day 4.

Toxicokinetics: The blood levels of [REDACTED] (b) (4) were not quantifiable in any of the samples. Only a few blood samples at various sampling time points were quantifiable for PEP005 ( $C_{max}$  was  $0.0727 \pm 0.0836$  ng/ml on Day 1 in males receiving 60  $\mu\text{g}/\text{kg}/\text{day}$  and was  $0.0380 \pm 0.0481$  ng/ml on Day 3 in females receiving 60  $\mu\text{g}/\text{kg}/\text{day}$ ).

**6.2.12. PEP005: Maximum tolerated dose (MTD) followed by a 7 day repeated dose intravenous administration toxicity study in the mini-pig (2174/022)**: Two male and two female mini-pigs were given four daily IV doses of vehicle or 5  $\mu\text{g}/\text{kg}/\text{day}$  PEP005. Transient subdued behavior, lack of foraging, salivation, and emesis were observed in mini-pigs receiving 5  $\mu\text{g}/\text{kg}/\text{day}$ . There was no effect on body weights. Intravenous administration of PEP005 to mini-pigs for 7 days at a dose of 2.5  $\mu\text{g}/\text{kg}/\text{day}$  did not show any toxicologically significant effects on clinical signs, clinical pathology endpoints, organ weights, or macroscopic findings. The mean blood PEP005  $C_{max}$  was 4.88 and 2.91 ng/mL for males and females, respectively on Day 1 and was 1.55 and 1.80 ng/mL for males and females, respectively on Day 7. The  $AUC_{(0-t)}$  was 3.96 and 1.82 ng·h/mL for males and females, respectively on Day 1 and was 1.15 and 1.40 ng·h/mL for males and females, respectively on Day 7.

**6.2.13. PEP005: 28 Day intravenous administration toxicity study in the mini-pig**

**Key study findings**: There were no treatment-related changes in body weights, clinical signs, macroscopic or microscopic findings. Both  $C_{max}$  and AUC values of PEP005 increased with increasing dose on Days 1 and 28. No apparent drug accumulation over the 28-day dosing period and no apparent gender difference were observed. The NOAEL was 3  $\mu\text{g}/\text{kg}/\text{day}$ .

**Study no.**: 2174/018

**Conducting laboratory and location**: [REDACTED] (b) (4)

[REDACTED] (b) (4)  
**Date of study initiation**: 9-9-2003

**GLP compliance**: Yes

**QA report**: yes ( x ) no ( )

**Drug, lot #, and % purity**: PEP005, batch number 070303, 99.6%

**Methods**

Doses: 0, 0.25, 1.5, and 3 µg/kg/day  
Species/strain: Gottingen ApS mini-pigs  
Number/sex/group or time point (main study): 4/sex/group  
Route, formulation, volume, and infusion rate: Intravenous, 20% PEG 400 in 0.9% saline, volume 5 mL/kg, infusion rate 1 mL/min  
Satellite groups used for toxicokinetics or recovery: None  
Age: 17 - 26 weeks  
Weight: males, 8.0 - 12.0 kg; females, 8.0 - 15.0 kg  
Sampling times: Blood samples were taken at 0, 1, 2, 5, 15, 30, and 90 min and 2, 4, and 8 hr post-dosing on Days 1 and 28 from all animals.  
Unique study design or methodology: NA

**Results:**

Mortality: No deaths occurred.

Clinical signs: There were no treatment-related clinical signs.

Body weights: There were no treatment-related changes in body weights or body weight gain.

Food consumption: Not recorded

Ophthalmoscopy: NA.

EKG: NA

Hematology: There were no significant treatment-related changes in Week 4.

Clinical chemistry: There were no significant treatment-related changes in Week 4.

Urinalysis: There were no significant treatment-related changes in Week 4.

Gross pathology: There were no treatment-related changes in macroscopic findings at the end of the study.

Organ weights: There were no treatment-related changes in organ weight at the end of the study.

Histopathology: There were no treatment-related changes in microscopic findings at the end of the study. Thrombi and granuloma were seen in the lungs of many animals, including controls.

Toxicokinetics: Both  $C_{max}$  and AUC values of PEP005 increased with increasing dose on Day 1 and 28. There was no apparent drug accumulation over the 28-day dosing

period and no apparent gender difference was observed. Only 2 samples of (b) (4) and 1 sample of (b) (4) from the high-dose group were marginally above the LLOQ.

Sex	Dose ( $\mu\text{g}/\text{kg}/\text{day}$ )	$T_{\text{max}}$ (hr)		$C_{\text{max}}$ (ng/mL)		$\text{AUC}_{(0-8\text{h})}$ (ng·h/mL)	
		Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
M	0.25	0.561	0.697	0.185	0.175	0.121	0.131
	1.5	0.503	0.519	1.26	1.39	0.837	1.03
	3	0.686	0.514	2.42	2.95	1.57	1.79
F	0.25	0.428	0.869	0.258	0.266	0.140	0.209
	1.5	1.05	0.552	2.24	1.48	0.971	0.982
	3	0.961	0.579	2.79	2.46	1.80	1.62

**6.2.14. PEP005: 13 Week Repeat Dose Dermal Range Finding Study in the Minipig Followed by an 8 Week Recovery Period (509987):** Twelve male and twelve female mini-pigs received PEP005 gels at 0.01% and 0.05% applied at volumes between 60 and 150  $\mu\text{L}$  to 6 independent dosing sites (20 mm x 30 mm = 600  $\text{mm}^2$  per site) and vehicle gel at 2 further sites of the same area for 3 or 5 consecutive days once a month for 13 weeks (4 treatment cycles per site, see the next table). In addition, each animal had a ninth site that was untreated and served as control. Animals were killed 24 h (4 males/6 females) or 8 weeks (4 males/5 females) after the final application.

Dosing Frequency	0.01% x 75 $\mu\text{L}$ (7.5 $\mu\text{g}/600 \text{mm}^2$ = 0.0125 $\mu\text{g}/\text{mm}^2$ )	0.01% x 150 $\mu\text{L}$ (15 $\mu\text{g}/600 \text{mm}^2$ = 0.025 $\mu\text{g}/\text{mm}^2$ )	0.05% x 60 $\mu\text{L}$ (30 $\mu\text{g}/600 \text{mm}^2$ = 0.05 $\mu\text{g}/\text{mm}^2$ )	0.05% x 90 $\mu\text{L}$ (45 $\mu\text{g}/600 \text{mm}^2$ = 0.075 $\mu\text{g}/\text{mm}^2$ )	Placebo (0 $\mu\text{g}/\text{mm}^2$ )
Once daily x 3 days		Site 4	Site 5	Site 6	Site 7
Once daily x 5 days	Site 1	Site 2	Site 3		Site 8

Four males and one female were killed prematurely due to adverse clinical signs unrelated to treatment with PEP005. There were no PEP005 treatment-related effects on body weights, hematology, clinical chemistry, urinalysis, and clinical signs other than the local dermal responses. The dermal administration of PEP005 at concentrations of 0.01% and 0.05% (doses up to 0.05  $\mu\text{g}/\text{mm}^2$  for 5 days and 0.075  $\mu\text{g}/\text{mm}^2$  for 3 days) caused dose- and duration-related erythema and edema responses and histopathological changes, with wide intra- and inter-individual variations.

During the first treatment cycle, the incidence and severity of erythema and edema observed at 24 hours after the final application of each 3- or 5-day dosing period increased with respect to dose. Erythema in males (well-defined to severe) and in females (well-defined or moderate) and very slight to moderate edema in both genders were observed at 0.025, 0.05, and 0.075  $\mu\text{g}/\text{mm}^2$  for 3 days. Very slight to moderate erythema and very slight edema were observed in males and females at 0.0125, 0.025, and 0.05  $\mu\text{g}/\text{mm}^2$  for 5 days. The severity of response was higher in males than in females. Several males were unable to successfully complete dosing at  $\geq 0.025 \mu\text{g}/\text{mm}^2$  for 3 and 5 days. The severity of the dermal reactions decreased with subsequent treatment cycles, indicating the development of dose tolerance. At the end of the final

treatment cycle, very slight to moderate erythema and very slight or well-defined edema and scabbing were observed at some PEP005-treated sites in both genders.

At the end of the final treatment cycle, minimal to moderate acanthosis was noted at PEP005-treated sites in both genders. Although the incidence of acanthosis was similar at all PEP005 treated sites, there was a slight increase in the severity at sites treated with 0.05  $\mu\text{g}/\text{mm}^2$  for 5 days and 0.075  $\mu\text{g}/\text{mm}^2$  for 3 days. Acanthosis was not evident at the untreated control or vehicle-treated sites. Erosion or ulceration was present in some males and females treated daily for 5 days with both 0.025 and 0.05  $\mu\text{g}/\text{mm}^2$ . The incidence and severity of erosion or ulceration was greatest at the site treated with 0.05  $\mu\text{g}/\text{mm}^2$  for 5 days. There was an increased incidence and severity of scab formation, dermatitis (or inflammatory cell infiltration of the dermis), epidermal inflammation and fibroplasia at sites treated with PEP005 compared with untreated or vehicle-treated sites.

At 8 weeks post treatment, only very slight or well-defined erythema with no edema was observed in some PEP005-treated sites. There was no significant difference in the incidence or severity of histopathological changes at the sites treated with PEP005 when compared with untreated or vehicle treated sites.

Blood samples were collected at pre-dose, 5, 10, and 30 min, 1, 2, 4, 8, 12, and 24 hours post-dose on Days 1 and 85 (first dosing day of the 4th treatment cycle) to determine the blood levels of PEP005, (b) (4). However, none of them were detected in any samples; the lower limit of quantitation (LLOQ) was 0.1 ng/mL. It indicated that there was little systemic exposure following dermal administration of PEP005 at a total dose of 142.5  $\mu\text{g}/\text{day}$  PEP005 (approximately 9.5  $\mu\text{g}/\text{kg}$  based on a body weight of 15 kg).

In conclusion, the dermal administration of PEP005 in mini-pigs at concentrations of 0.01% and 0.05% (doses up to 0.05  $\mu\text{g}/\text{mm}^2$  for 5 days and 0.075  $\mu\text{g}/\text{mm}^2$  for 3 days) caused dose- and duration-related erythema, edema, acanthosis, and erosion/ulceration at the treated sites. The severity of the dermal reactions and histopathological changes was greater in males than in females. However, the dermal observations decreased in severity with repeated dosing over the 4 dosing cycles, indicating tolerance with repeat dosing. Although the sponsor stated that a dose at 0.0125  $\mu\text{g}/\text{mm}^2$  for 5 days in males and 0.025  $\mu\text{g}/\text{mm}^2$  for 3 or 5 days in females were considered the no-observed-adverse effect level (NOAEL) in this study, this reviewer believe that no NOAEL was established in this study, because of the very slight to well-defined erythema, edema, acanthosis observed in males and females at the sites treated with the lowest tested dose (0.0125  $\mu\text{g}/\text{mm}^2$ , 0.01%).

#### **6.2.15. PEP005: 41 Week Repeat Dose/Repeat Treatment Cycle Dermal Study in the Mini-pig Followed by an 8 Week Recovery Period**

**Key study findings:** One male in Group 2 treated with 0.01% PEP005 Gel was prematurely sacrificed due to morbidity on Day 43. The animal's death was preceded

by the following clinical signs: body tremors, hunched posture, excessive scratching, flapping ears and multiple skin lesions on the body surface. Subsequent histopathology examination indicated that the animal had possible porcine dermatitis-nephropathy syndrome (PDNS). There were no treatment-related effects on clinical signs, hematology, clinical chemistry, and urinalysis parameters, other than those observed at the treatment sites. There was a dose-related trend in decreased body weight gains over the course of the study period as compared with vehicle-treated control animals and statistically significant differences were observed in females in Groups 3 and 4 during the dosing period. The body weight gain in Group 3 and 4 females was only 73% and 64% of that in the vehicle-treated females at the end of dosing period, respectively. The body weight in Group 4 females was approximately 20% less than that in the vehicle-treated females.

Dermal signs during the first treatment cycle consisted of PEP005 treatment-related incidence and severity of erythema and edema. Beginning at 24 hours after the first application, erythema responses in males (very slight to severe) and in females (very slight to moderate) and edema responses (severe in males and very slight to moderate in females) were observed at the sites treated with PEP005 Gels. The response was more noticeable at sites treated with 0.02% or 0.03% PEP005 Gel than those treated with 0.01% PEP005 Gel in both genders and the severity of response was greater in males than females. Because the severity of response increased with repeat dosing, the total number of sites dosed decreased through Day 3. Only occasional incidents of very slight erythema during Cycle 1 were observed in vehicle-treated animals. In subsequent treatment cycles, the severity of the dermal reactions decreased temporally in both genders of all PEP005-treated groups. In general, the incidence and severity of PEP005 treatment-related erythema and edema decreased beginning with Cycle 5 and dosing was completed at an increased total number of sites as compared with previous dosing cycles. At the completion of the final treatment cycle (Cycle 11), only very slight to moderate erythema and edema, with incidents of severe erythema, edema and scabbing in animals treated with 0.03% PEP005 Gel, were observed at the majority of PEP005-treated sites. The severity of the response was more noticeable at sites treated with 0.03% PEP005 Gel than those treated with 0.01% or 0.02% PEP005 Gel, with the response greater in males and females; no dermal response was noted in vehicle-treated animals. Erythema and/or edema were seen only within the first 3 days during the 8 week recovery period.

At the 41 week terminal necropsy, minimal to severe focal or multifocal ulcerative dermatitis and serocellular crusts, minimal to moderate acanthosis of the epidermis with or without rete pegs formation, and minimal or mild perivascular inflammatory cell infiltrations in the dermis were seen at the treatment sites treated with PEP005 Gel. There was no clear difference in either the incidence or severity of the findings between dose groups. At the end of the 8 week recovery period, minimal focal ulcerative dermatitis was seen at a single site in one male treated with 0.03% PEP005 Gel. The sponsor stated that histopathological evaluation of treated skin sites, including epidermal and dermal thickness, inflammation, and fibroplasia revealed normal skin architecture at the end of the observation period. No no-observed-adverse-effect level

(NOAEL) was identified in this study. No PEP005, (b) (4) was detected (LLOQ 0.1 ng/mL) in any samples collected at 0, 10, and 30 min, 1, 2, 4, 8, 12, and 24 hours post-dosing on Days 3 and 283.

**Study no.:** 509992

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

**Date of study initiation:** 12-22-2006

**GLP compliance:** Yes

**QA report:** Yes ( X ) no ( )

**Drug, lot #, and % purity:** Vehicle Gel, Batch 7046/002, 7046/003, 7046/004, and 7046/005, No detectable PEP005; 0.01% PEP005 Gel, Batch 7045/001, 7045/002, 7045/003, and 7045/004, 0.01% - 0.011% PEP005; 0.02% PEP005 Gel, Batch 7044/001, 7044/002, 7044/003, and 7044/004, 0.018% - 0.020% PEP005; 0.03% PEP005 Gel, Batch 7047/001, 7047/002, 7047/003, and 7047/004, 0.027% - 0.031% PEP005.

## Methods

Doses: See the next table.

Group	Concentration (%)	Local Dose ( $\mu\text{g}/\text{mm}^2/\text{day}$ ) <sup>a</sup>	Maximum Daily Exposure ( $\mu\text{g}/\text{day}$ ) <sup>b</sup>
1	Placebo	0	0
2	0.01	0.025	60
3	0.02	0.05	120
4	0.03	0.075	180

<sup>a</sup> Dose administered at a constant volume of 150  $\mu\text{L}$  per skin treatment site

<sup>b</sup> Dose was administered to four independent skin sites of 600  $\text{mm}^2$  per animal

Species/strain: Gottingen ApS minipigs

Number/sex/group or time point (main study): 10/sex/group

Route, formulation, volume, and infusion rate: Topical, clinical formulations (pH 3.4-3.5), 150  $\mu\text{L}$  to each 600  $\text{mm}^2$  area.

Satellite groups used for toxicokinetics or recovery: None

Age: 3 - 4 months

Weight: Males 8.3 - 11.1 kg; Females 8.6 - 11.2 kg

Unique study design or methodology: Four groups of 10 male and 10 female minipigs (10/sex/group) were topically treated 150  $\mu\text{L}$  of PEP005 gel of 0% (Group 1, vehicle), 0.01%, 0.02%, or 0.03% (Groups 2-4, respectively) to each of 4 independent treatment sites (600  $\text{mm}^2$  for each) on the dorsal flanks. In addition, each animal had a fifth site that was untreated and served as control. The doses were administered for 3 consecutive days once monthly for 41 weeks (11 treatment cycles, see the following table). At the completion of dosing, animals were killed at 24 hours (5/sex/group) or after an 8 week observation period (5/sex/group) after the final treatment.

<b>Cycle 1</b>	<b>Cycle 2</b>	<b>Cycle 3</b>	<b>Cycle 4</b>	<b>Cycle 5</b>	<b>Cycle 6</b>
Days 1 to 3	Days 29 to 31	Days 57 to 59	Days 85 to 87	Days 113 to 115	Days 141 to 143
<b>Cycle 7</b>	<b>Cycle 8</b>	<b>Cycle 9</b>	<b>Cycle 10</b>	<b>Cycle 11</b>	
Days 169 to 171	Days 197 to 199	Days 225 to 227	Days 253 to 255	Days 281 to 283	

### Observations and results:

**Viability:** Checked twice daily. One male in Group 2 treated with 0.01% PEP005 Gel was prematurely sacrificed due to morbidity on Day 43. The animal's death was preceded by the following clinical signs: body tremors, hunched posture, excessive scratching, flapping ears and multiple skin lesions on the body surface. Subsequent histopathology examination indicated that the animal had possible porcine dermatitis-nephropathy syndrome (PDNS).

**Clinical signs:** Checked twice daily. There were no treatment-related clinical signs associated with PEP005 other than those observed at the treatment sites. There was no evidence of systemic toxicity following topical administration of PEP005 Gel.

**Dermal reactions:** The condition of all test sites were examined for erythema, eschar and edema before and immediately after application of PEP005 or vehicle gels to each animal, at approximately 1, 2, and 24 hours post-dosing on each treatment day, and recorded daily from 24 hours after the final dose of each cycle until termination. Due to the severity of the dermal reactions observed at some PEP005 treated sites, not all sites were treated for the full dosing period within each treatment cycle. The number of sites treated within each treatment cycle is described in the following table (Text Table 5).

**Text Table 5. Number of Dosed Sites per Treatment Cycle<sup>a</sup>**

Cycle	Study Day	Males				Females			
		Group Number/Dose ( $\mu\text{g}/\text{mm}^2/\text{day}$ )				Group Number/Dose ( $\mu\text{g}/\text{mm}^2/\text{day}$ )			
		1 (0)	2 (0.025)	3 (0.05)	4 (0.075)	1 (0)	2 (0.025)	3 (0.05)	4 (0.075)
1	1	40/40	40/40	40/40	40/40	40/40	40/40	40/40	40/40
	2	40/40	40/40	27/40	29/40	40/40	38/40	30/40	40/40
	3	40/40	32/40	2/40	13/40	40/40	39/40	15/40	19/40
2	29	40/40	40/40	40/40	40/40	40/40	40/40	40/40	40/40
	30	39/40	23/40	10/40	6/40	40/40	40/40	40/40	39/40
	31	40/40	20/40	10/40	6/40	40/40	26/40	40/40	39/40
3	57	40/40	36/36	40/40	39/40	40/40	40/40	40/40	40/40
	58	40/40	18/36	10/40	6/40	40/40	40/40	40/40	35/40
	59	40/40	13/36	12/40	9/40	40/40	38/40	38/40	20/40
4	85	40/40	36/36	40/40	40/40	40/40	40/40	40/40	40/40
	86	40/40	17/36	27/40	28/40	40/40	40/40	39/40	40/40
	87	40/40	20/36	24/40	17/40	40/40	40/40	39/40	37/40
5	113	40/40	36/36	40/40	40/40	40/40	40/40	40/40	40/40
	114	40/40	29/36	38/40	38/40	40/40	40/40	40/40	40/40
	115	40/40	35/36	38/40	36/40	40/40	40/40	40/40	40/40
6	141	40/40	36/36	40/40	40/40	40/40	40/40	40/40	40/40
	142	40/40	28/36	31/40	33/40	40/40	40/40	40/40	40/40
	143	40/40	26/36	39/40	37/40	40/40	40/40	40/40	40/40
7	169	40/40	36/36	40/40	40/40	40/40	40/40	40/40	40/40
	170	40/40	34/36	40/40	39/40	40/40	39/40	40/40	40/40
	171	40/40	31/36	37/40	37/40	40/40	40/40	40/40	40/40
8	197	40/40	36/36	40/40	40/40	40/40	40/40	40/40	40/40
	198	40/40	29/36	38/40	36/40	40/40	40/40	40/40	40/40
	199	40/40	30/36	28/40	30/40	40/40	40/40	40/40	40/40
9	225	40/40	36/36	40/40	40/40	40/40	39/40	40/40	40/40
	226	40/40	26/36	26/40	32/40	40/40	39/40	40/40	40/40
	227	40/40	27/36	35/40	30/40	40/40	39/40	40/40	38/40
10	253	40/40	36/36	40/40	40/40	40/40	40/40	40/40	40/40
	254	40/40	31/36	38/40	36/40	40/40	40/40	40/40	40/40
	255	40/40	32/36	39/40	40/40	40/40	40/40	40/40	40/40
11	281	40/40	36/36	40/40	40/40	40/40	40/40	40/40	40/40
	282	40/40	35/36	38/40	40/40	40/40	40/40	40/40	40/40
	283	40/40	33/36	34/40	31/40	40/40	40/40	40/40	40/40

<sup>a</sup> Number of sites actually treated/Number of prospective treatment sites; all animals combined within group for each dosing day

In the vehicle-treated animals, occasional incidents of very slight erythema were observed during the 3-day dosing cycles in both genders. Occasional observations of scratches and bite marks at the treatment sites were also noted. An open wound at one treatment site of one male prevented dosing on Day 30. No edema was observed in either gender during any dosing cycle. No dermal observations were noted during the 8 week recovery period.

As seen from the next two tables for the 0.01% PEP005 Gel-treated group, in males, very slight to severe erythema and very slight to severe edema were observed throughout the 11 dosing cycles; occasional scab formation or open wounds prevented the completion of dosing at all sites; the incidence and severity of PEP005 treatment-related erythema decreased beginning with Cycle 5 (Days 113-115) and dosing was completed at an increased total number of sites as compared with earlier dosing cycles. In females, dermal responses were less severe than those observed in males and the majority of sites completed dosing with each cycle; very slight or well-defined erythema was observed with occasional incidents of moderate erythema; very slight to moderate edema was observed only during Cycle 1 and thereafter only occasional incidents of very slight or slight edema were observed during the treatment period. During the 8 week recovery period, very slight erythema (Day 286) and edema (Day 285) were observed in males and very slight erythema was observed on one occasion in a single female.

**Text Table 15. Group 2 (0.025 µg/mm<sup>2</sup>/day): MALES**

**Summary of Dermal Observations by Cycle (Cumulative Score through 24 h Post-Final Dose)**

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Number of Sites Dosed/Total Number of Sites	112/120	83/120	67/108 †	73/108	100/108	90/108
<b>Erythema/Eschar</b>						
None	17	32	20	19	64	55
Very Slight	31	42	35	35	22	21
Well-Defined	29	24	46	34	19	21
Moderate	32	15	3	8	3	7
Severe or Eschar Formation	11	7	4	12	0	0
<b>Oedema</b>						
None	90	84	91	85	98	101
Very Slight	26	17	10	10	6	1
Slight	4	10	3	7	2	2
Moderate	0	7	4	2	2	0
Severe	0	2	0	4	0	0

† From cycle 3 onwards there were only a total of 108 possible dose sites as animal 18M was killed prematurely on Day 43.

	Cycle 7	Cycle 8	Cycle 9	Cycle 10	Cycle 11
Number of Sites Dosed/Total Number of Sites	101/108	95/108	89/108	99/108	104/108
<b>Erythema/Eschar</b>					
None	70	45	51	62	61
Very Slight	28	23	31	25	34
Well-Defined	4	23	16	12	12
Moderate	5	15	10	8	1
Severe or Eschar Formation	0	2	0	1	0
<b>Oedema</b>					
None	107	95	80	100	102
Very Slight	1	7	9	4	5
Slight	0	3	9	4	1
Moderate	0	2	10	0	0
Severe	0	1	0	0	0

**Text Table 16. Group 2 (0.025 µg/mm<sup>2</sup>/day): FEMALES****Summary of Dermal Observations by Cycle (Cumulative Score through 24 h Post-Final Dose)**

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Number of Sites Dosed/Total Number of Sites	117/120	106/120	118/120	120/120	120/120	120/120
<b>Erythema/Eschar</b>						
None	33	50	96	91	96	116
Very Slight	40	49	21	22	24	4
Well-Defined	27	21	1	7	0	0
Moderate	14	0	2	0	0	0
Severe or Eschar Formation	6	0	0	0	0	0
<b>Oedema</b>						
None	76	118	120	120	118	120
Very Slight	25	2	0	0	2	0
Slight	15	0	0	0	0	0
Moderate	4	0	0	0	0	0
Severe	0	0	0	0	0	0
	Cycle 7	Cycle 8	Cycle 9	Cycle 10	Cycle 11	
Number of Sites Dosed/Total Number of Sites	119/120	120/120	117/120	120/120	120/120	
<b>Erythema/Eschar</b>						
None	95	116	76	109	108	
Very Slight	21	4	27	8	8	
Well-Defined	3	0	15	1	4	
Moderate	1	0	2	2	0	
Severe or Eschar Formation	0	0	0	0	0	
<b>Oedema</b>						
None	118	120	118	119	120	
Very Slight	2	0	2	0	0	
Slight	0	0	0	1	0	
Moderate	0	0	0	0	0	
Severe	0	0	0	0	0	

As seen from the next two tables for the 0.02% PEP005 Gel-treated group, in males, very slight to moderate erythema and edema, with incidents of severe erythema or edema, were observed throughout the 11 dosing cycles. There was no clear decrease in the overall incidence of PEP005 treatment-related erythema with repeat administration. In females, dermal responses were less severe than those observed in males and the majority of sites completed dosing with each cycle. Very slight to severe erythema and edema were observed during Cycle 1 and very slight or well-defined erythema with occasional incidents of moderate or severe edema were observed during Cycles 2 and 4. Thereafter only occasional incidents of very slight or well-defined erythema and very slight to moderate edema were observed. During the 8 week recovery period, erythema was observed up to Day 285 (females) or Day 286 (males) and no edema noted at any site.

**Text Table 17. Group 3 (0.05 µg/mm<sup>2</sup>/day): MALES****Summary of Dermal Observations by Cycle (Cumulative Score through 24 h Post-Final Dose)**

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Number of Sites Dosed/Total	69/120	60/120	62/120	91/120	116/120	110/120
Number of Sites						
<b>Erythema/Eschar</b>						
None	6	5	49	6	23	9
Very Slight	25	35	50	37	52	77
Well-Defined	48	55	18	49	32	26
Moderate	33	23	3	25	13	8
Severe or Eschar Formation	8	2	0	3	0	0
<b>Oedema</b>						
None	32	36	68	83	92	100
Very Slight	40	39	30	23	15	17
Slight	29	30	16	11	11	1
Moderate	18	15	4	3	2	2
Severe	1	0	2	0	0	0
	<b>Cycle 7</b>	<b>Cycle 8</b>	<b>Cycle 9</b>	<b>Cycle 10</b>	<b>Cycle 11</b>	
Number of Sites Dosed/Total	117/120	106/120	101/120	117/120	112/120	
Number of Sites						
<b>Erythema/Eschar</b>						
None	26	50	6	37	76	
Very Slight	59	26	44	51	34	
Well-Defined	32	39	37	21	10	
Moderate	3	5	28	5	0	
Severe or Eschar Formation	0	0	5	6	0	
<b>Oedema</b>						
None	104	94	77	104	107	
Very Slight	10	10	34	13	11	
Slight	3	16	5	2	2	
Moderate	1	0	4	0	0	
Severe	0	0	0	0	0	

**Text Table 18. Group 3 (0.05 µg/mm<sup>2</sup>/day): FEMALES****Summary of Dermal Observations by Cycle (Cumulative Score through 24 h Post-Final Dose)**

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Number of Sites Dosed/Total Number of Sites	85/120	120/120	118/120	118/120	120/120	120/120
<b>Erythema/Eschar</b>						
None	5	62	52	55	53	89
Very Slight	15	43	48	47	42	28
Well-Defined	17	14	17	17	25	3
Moderate	40	1	1	1	0	0
Severe or Eschar Formation	43	0	1	0	0	0
<b>Oedema</b>						
None	41	116	120	118	108	119
Very Slight	35	3	0	2	2	1
Slight	20	1	0	0	8	0
Moderate	21	0	0	0	2	0
Severe	3	0	0	0	0	0
	Cycle 7	Cycle 8	Cycle 9	Cycle 10	Cycle 11	
Number of Sites Dosed/Total Number of Sites	120/120	120/120	120/120	120/120	120/120	
<b>Erythema/Eschar</b>						
None	95	113	103	107	96	
Very Slight	25	2	15	13	22	
Well-Defined	0	5	2	0	2	
Moderate	0	0	0	0	0	
Severe or Eschar Formation	0	0	0	0	0	
<b>Oedema</b>						
None	120	119	120	120	120	
Very Slight	0	0	0	0	0	
Slight	0	1	0	0	0	
Moderate	0	0	0	0	0	
Severe	0	0	0	0	0	

As seen from the next two tables for the 0.03% PEP005 Gel-treated group, in males, very slight to severe erythema and edema was generally observed throughout the 11 dosing cycles. Scab formation and open wounds prevented the completion of dosing of all sites in any individual male during Cycles 1 to 4. The incidence and severity of PEP005 treatment-related erythema, edema and eschar was generally decreased beginning at Cycle 5 (Days 113-115) and dosing was completed at an increased total number of sites as compared with earlier dosing cycles. In females, dermal responses were less severe than those observed in males and the majority of sites completed throughout the 11 dosing cycles. Very slight to severe erythema and edema were observed during dosing Cycle 1 at the majority of sites, with very slight or well defined erythema, with occasional incidents of moderate or severe erythema, were observed during Cycles 2 and 6. Thereafter, very slight or well-defined erythema, with occasional incidents of very slight or slight edema, were observed during the treatment period.

During the 8 week recovery period, erythema was observed up to Day 285 (females) or Day 286 (males) and no edema noted at any site.

**Text Table 19. Group 4 (0.075 µg/mm<sup>2</sup>/day): MALES**

**Summary of Dermal Observations by Cycle (Cumulative Score through 24 h Post-Final Dose)**

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Number of Sites Dosed/Total	82/120	52/120	54/120	85/120	114/120	110/120
Number of Sites						
<b>Erythema/Eschar</b>						
None	3	10	31	2	28	8
Very Slight	20	22	61	28	47	66
Well-Defined	43	46	12	43	35	36
Moderate	41	26	13	29	8	10
Severe or Eschar Formation	13	16	3	18	2	0
<b>Oedema</b>						
None	26	35	58	75	85	98
Very Slight	22	37	24	15	18	19
Slight	45	27	23	16	12	3
Moderate	16	11	8	10	4	0
Severe	11	10	7	4	1	0
	<b>Cycle 7</b>	<b>Cycle 8</b>	<b>Cycle 9</b>	<b>Cycle 10</b>	<b>Cycle 11</b>	
Number of Sites Dosed/Total	116/120	106/120	102/120	116/120	109/120	
Number of Sites						
<b>Erythema/Eschar</b>						
None	23	71	5	33	53	
Very Slight	37	25	29	57	47	
Well-Defined	42	13	37	19	18	
Moderate	16	8	30	5	1	
Severe or Eschar Formation	2	3	19	6	1	
<b>Oedema</b>						
None	80	101	72	105	90	
Very Slight	21	8	21	8	19	
Slight	13	7	9	3	6	
Moderate	5	4	16	2	5	
Severe	1	0	2	2	0	

**Text Table 20. Group 4 (0.075 µg/mm<sup>2</sup>/day): FEMALES****Summary of Dermal Observations by Cycle (Cumulative Score through 24 h Post-Final Dose)**

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Number of Sites Dosed/Total Number of Sites	99/120	118/120	95/120	117/120	120/120	120/120
<b>Erythema/Eschar</b>						
None	5	47	27	51	72	53
Very Slight	40	44	22	39	38	30
Well-Defined	34	21	34	27	10	27
Moderate	21	8	6	3	0	10
Severe or Eschar Formation	20	0	11	0	0	0
<b>Oedema</b>						
None	78	114	92	116	120	90
Very Slight	25	4	3	1	0	13
Slight	11	1	3	3	0	14
Moderate	4	1	2	0	0	1
Severe	2	0	0	0	0	2
	Cycle 7	Cycle 8	Cycle 9	Cycle 10	Cycle 11	
Number of Sites Dosed/Total Number of Sites	120/120	120/120	118/120	120/120	120/120	
<b>Erythema/Eschar</b>						
None	61	84	72	97	88	
Very Slight	47	23	29	21	31	
Well-Defined	12	13	19	2	1	
Moderate	0	0	0	0	0	
Severe or Eschar Formation	0	0	0	0	0	
<b>Oedema</b>						
None	120	117	116	120	120	
Very Slight	0	1	1	0	0	
Slight	0	2	3	0	0	
Moderate	0	0	0	0	0	
Severe	0	0	0	0	0	

**Body weights:** Recorded once in the week before the commencement of dosing, on the first day of each dosing cycle (before dose administration), once weekly thereafter during the post-dose observation period and on the day of necropsy. There was a dose-related trend in decreased body weight gains over the course of the study period as compared with vehicle-treated control animals and statistically significant differences were observed in females in Groups 3 and 4 during the dosing period. The body weight gain in Groups 3 and 4 females was only 73% and 64% of that in the vehicle-treated females at the end of dosing period, respectively. The body weight in Group 4 females was approximately 20% less than that in the vehicle-treated females. No differences in body weights or body weight gain were observed in either gender of any dose group in comparison with the vehicle-treated group at the end of the recovery period.

**Food consumption:** NA

Ophthalmoscopy: NA

EKG: NA

Hematology: Performed on all animals on one occasion during the pretrial period, in Week 41 of treatment, and in Week 8 of recovery. There were no treatment-related effects.

Clinical chemistry: Performed on all animals on one occasion during the pretrial period, in Week 41 of treatment, and in Week 8 of recovery. There were no treatment-related effects.

Urinalysis: Urine samples were collected from animals over an approximate 4 hour period on one occasion during the pretrial period, in Week 41 of treatment, and in Week 8 of recovery. There were no treatment-related effects.

Gross pathology: The necropsy procedure included a review of the history of each animal and a detailed examination of the external features and orifices, the neck and associated tissues and the cranial, thoracic, abdominal and pelvic cavities and their viscera. The external and cut surfaces of the organs and tissues were examined as appropriate. All gross lesions were recorded in descriptive terms including location(s), size (in mm), shape, color, consistency, and number. A gross assessment of the dermal test sites performed at necropsy included skin thickness, size (in three dimensions), color, documentation of lesion(s) shape (e.g. redness, raw areas/ulceration and scab formation), and presence or characterization of any lesion exudates.

Scab formation and raw areas were present at PEP005 treated sites in many animals in all dose groups (Group 2, 0.01%; Group 3, 0.02%; Group 4, 0.03%). Occasional scab formation was present at treatment sites of vehicle animals (Group 1) and untreated sites in all groups. There were no treatment-related gross dermal findings present in surviving animals at the end of recovery period.

Organ weights: NA

Histopathology: Representative samples of the tissues detailed in the table below (Text Table 12) were taken from all animals and preserved in 10% neutral buffered formalin, unless otherwise stated. There were no treatment-related effects, except at the treatment sites.

**Text Table 12. Necropsy/Pathology Tissue List**

Tissues	Examined	Comments
Abnormal Tissue	-	-
Adrenal Gland x 2	-	-
Aortic Arch	-	-
Brain	-	-
Ear Tag	-	Retained for identification purposes.
Epididymis x 2	-	-
Eye x 2	-	Both eyes with optic nerves were fixed in Davidson's fluid.
Gall Bladder	-	-
Gastro-intestinal tract:		
Stomach	-	Samples of stomach body and pylorus were obtained.
Duodenum	-	
Jejunum	-	
Ileum	-	
Caecum	-	
Colon	-	
Rectum	-	
Heart	-	-
Joint with Bone	-	Stifle joint.
Kidney x 2	-	-
Larynx	-	-
Liver	-	-
Lung	-	One lobe was perfusion fixed. Gross lesions in other lobes were immersion fixed.
Marrow Smear	-	Duplicate rib marrow smears were taken from all animals, air-dried and fixed in methanol.
Mesenteric Lymph Node	-	-
Oesophagus	-	-

**Text Table 12. Necropsy/Pathology Tissue List: continued**

Ovary x 2	-	-
Pancreas	-	-
Pituitary Gland	-	-
Prostate Gland	-	-
Sciatic Nerve	-	-
Skeletal Muscle	-	-
Skin + Mammary Gland	x	All test, Placebo and control sites were examined (see Section 7.9.3).
Spinal Cord	-	Cervical, thoracic and lumbar samples taken.
Spleen	-	-
Sternum	-	-
Submandibular Lymph Node	-	-
Submaxillary Salivary Gland	-	-
Testis x 2	-	Retained in Bouin's.
Thymus Gland	-	-
Thyroid and Parathyroid Glands	-	-
Tongue	-	-
Trachea	-	-
Urinary Bladder	-	-
Uterus with Cervix	-	-
Vagina	-	-

Skin samples from all test sites for all animals were dehydrated, embedded in paraffin wax, sectioned at approximately 4-6  $\mu\text{m}$  thickness, stained with hematoxylin and eosin (H&E) and examined. Findings were assigned one of five severity grades: minimal, mild, moderate, marked or severe. Because there were no toxicologically significant differences found between the PEP005 dosing sites within each animal by group, the histopathology findings were reported as combined for all sites treated with PEP005 (Sites 1 to 4). The untreated site (Site 5) was reported separately.

At 24 hours following the final topical treatment, minimal to severe focal to multifocal ulcerative dermatitis and serocellular crusts were present at the sites treated with 0.01%, 0.02%, or 0.03% PEP005 Gel (Groups 2, 3 and 4, respectively) in many animals. These findings correlated with the gross necropsy observations of scab formation and raw areas. There was no clear difference in either the incidence or severity of the findings between dose groups. Serocellular crusts of minimal severity were recorded at one treatment site in two vehicle-treated animals and of minimal or mild severity at the untreated site in three vehicle-treated animals. Minimal to moderate acanthosis and/or elongated rete pegs were seen in the epidermis of some animals at the sites treated with PEP005 at all concentrations. There was minimal or mild perivascular inflammatory cell infiltration in the dermis of animals at the sites treated with PEP005 at all concentrations. This finding was also seen at a single treatment site of one vehicle-treated animal. At the untreated site in all PEP005- or vehicle-treated groups, there was a low incidence and severity of most findings (ulcerative dermatitis,

acanthosis, serocellular crusts, perivascular inflammatory cell infiltration and elongated rete pegs) as seen at the PEP005- or vehicle-treated sites.

At the end of the recovery period, minimal focal ulcerative dermatitis was only observed at a single treatment site in one male treated with 0.03% PEP005 Gel. Acanthosis and elongated rete pegs previously observed in main study animals at all PEP005 concentrations were not observed in the epidermis in any recovery animal. Perivascular inflammatory cell infiltration was seen in occasional PEP005 Gel-treated animals at all concentrations without any notable increase in incidence when compared with the vehicle-treated group.

**Toxicokinetics:** Blood samples were collected from all animals in Groups 1 and 4 on Day 3 and on the final day of dosing in Week 41 (Day 283 of cycle 11) at 0, 10, and 30 min, 1, 2, 4, 8, 12, and 24 hours post-dose to determine the levels of PEP005, (b) (4) and (b) (4) in whole blood. No PEP005, (b) (4) was detected in any samples collected on Days 3 and 283 (LLOQ 0.1 ng/mL).

**Other:** The potential effects of PEP005 on remodelling of the skin structures were assessed histopathologically. The skin thickness (epidermis and dermis) was quantitatively determined using a graticule. Observations of inflammation and fibroblast proliferation were recorded and graded by the pathologist and a simple Masson Trichrome stain was used to assess any fibroplasias. There were no treatment-related effects.

## 7 Genetic Toxicology

### 7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

**Study title:** PEP005: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*

**Key findings:** No significant increases in the frequency of revertant colonies were observed in any strains, with or without metabolic activation, at any dose of PEP005, indicating that PEP005 was not mutagenic under the conditions tested.

**Study no:** 2174/001

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

**Date of study initiation:** 5-8-2002

**GLP compliance:** Yes

**QA reports:** Yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** PEP005, 270602, 99.82%; 240902, 99.26%

#### **Methods:**

**Strains/species/cell line:** *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and TA102

Doses used in definitive study: 4.096 – 5000 µg/plate

Basis of dose selection: A range finding study (up to 1000 µg/plate) was performed only in strain TA100.

Negative controls: DMSO

Positive controls: See next table

Incubation and sampling times: 3 days

## **Results**

Study validity: The first experiment with all 5 strains was conducted using up to 1000 µg/plate PEP005 in the presence or absence of S9. The second experiment in all 5 strains (See next table) included a 1-hr pre-incubation step in the presence of S9. PEP005 was tested in another repeat experiment only using TA1535 at doses of 15.81, 50, 158.1, 500, 1580, and 5000 µg/plate in the presence or absence of S9. The assay was considered valid, because the negative controls count fell within the normal ranges, all positive controls induced marked increases in the frequency of revertant colonies, both with and without metabolic activation, and no more than 5% of the plates were lost.

Study outcome: The mean number of revertant colonies and the standard deviations for PEP005, positive and vehicle controls, both with and without metabolic activation from one study are presented in the next table. The repeat experiment only in TA1535 with a high dose (5000 µg/plate) of PEP005 did not show positive results in the presence or absence of S9. In the two independent experiments using all five strains, no significant increases in the frequency of revertant colonies were observed in any strains, with or without metabolic activation, at any dose of PEP005, indicating that PEP005 was not mutagenic under the conditions tested.

PEP005 (µg/plate)	S9- mix	Number of colonies (mean ± S.D.)				
		TA98	TA100	TA1535	TA1537	TA102
0	-	25 ± 7	127 ± 12	23 ± 10	8 ± 2	478 ± 27
4.096	-	23 ± 3	122 ± 6		10 ± 3	500 ± 51
10.24	-	22 ± 4	121 ± 14	23 ± 1	9 ± 4	520 ± 25
25.6	-	21 ± 2	123 ± 24	25 ± 3	9 ± 4	485 ± 35
64	-	28 ± 7	126 ± 12	24 ± 8	11 ± 1	456 ± 8
160	-	20 ± 8	127 ± 7	31 ± 5	10 ± 4	254 ± 84
400	-	20 ± 7	103 ± 24	24 ± 6	11 ± 8	204 ± 1
1000	-	22 ± 7	109 ± 16	29 ± 3	5 ± 3	0 ± 0
2500	-			22 ± 3		
Positive control <sup>a</sup>	-	694 ± 12	463 ± 75	599 ± 85	198 ± 36	743 ± 20
0	+	32 ± 3	113 ± 13	21 ± 5	13 ± 3	354 ± 25
4.096	+	39 ± 7	100 ± 10		13 ± 6	278 ± 20
10.24	+	27 ± 8	111 ± 4	16 ± 6	19 ± 2	286 ± 4
25.6	+	29 ± 10	104 ± 5	23 ± 1	14 ± 4	327 ± 65
64	+	24 ± 4	108 ± 24	20 ± 2	11 ± 3	326 ± 13
160	+	30 ± 5	106 ± 4	20 ± 6	9 ± 1	274 ± 14
400	+	28 ± 4	99 ± 14	23 ± 9	8 ± 2	235 ± 5
1000	+	24 ± 5	86 ± 11	15 ± 4	14 ± 6	198 ± 29
2500	+			22 ± 8		
Positive control <sup>b</sup>	+	324 ± 23	925 ± 117	196 ± 16	154 ± 31	1340 ± 81

<sup>a</sup> TA98, 5 µg/plate 2-nitrofluorene; TA100, 2 µg/plate sodium azide; TA1535, 2 µg/plate sodium azide; TA1537, 50 µg/plate 9-aminoacridine; TA102, 25 µg/plate glutaraldehyde

<sup>b</sup> TA98, 10 µg/plate benzo(a)pyrene; TA100, 5 µg/plate 2-aminoanthracene; TA1535, 5 µg/plate 2-aminoanthracene; TA1537, 5 µg/plate 2-aminoanthracene; TA102, 20 µg/plate 2-aminoanthracene

## 7.2 In Vitro Assays in Mammalian Cells

**Study title:** PEP005: Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the microtitre® fluctuation technique

**Key findings:** PEP005 did not induce a statistically significant or dose-related increase in the mutant frequency. PEP005 was considered to be non-mutagenic in L5178Y cells under the conditions of the test.

**Study no:** 2174/002

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** 5-8-2002

**GLP compliance:** Yes

**QA reports:** Yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** PEP005, 270602, 99.82%; 240902, 99.26%

**Methods:**

Strains/species/cell line: L5178Y TK +/- mouse lymphoma cell line.

Doses used in definitive study: See the next table.

Basis of dose selection: A range finding study (up to 50 µg/mL) was performed for 3-hr treatment with or without S9 and 24-hr treatment without S9. Three-hr treatment with 50 µg/mL PEP005 yield 10% relative survival in the absence of S9 and 47% relative survival in the presence of S9; 24-hr treatment of 50 µg/mL PEP005 yielded 9% relative survival in the absence of S9.

Negative controls: DMSO

Positive controls: 4-Nitroquinoline 1-oxide (NQO) and benzo(a)pyrene (BP), see the next table.

Incubation and sampling times: Three-hr exposures were used both with and without metabolic activation and 24-hr exposure without activation. After 48-hr expression, cells were diluted and plated for mutant frequency in selective medium containing 3 µg/mL 5-trifluorothymidine (TFT) in 96-well microtitre plates. Cells were also diluted and plated for viability in non-selective medium. Plates were scored after 12 days incubation.

**Results**

Study validity: The vehicle controls had acceptable mutant frequency values, which were within the normal range for the L5178Y cell line at the TK locus. The positive controls, both in the absence and presence of metabolic activation, induced marked increases in the mutant frequency, indicating the satisfactory performance of the test and of the activity of the metabolizing system (see the next table).

Study outcome: PEP005 did not induce a statistically significant or dose-related increase in the mutant frequency, at any dose level, with or without metabolic activation for 3-hour treatment and without metabolic activation for 20-hour treatment (see the next table). PEP005 was considered to be non-mutagenic to L5178Y cells under the conditions of the test.

Treatment (µg/mL)	3-hour -S9		Treatment (µg/mL)	3-hour +S9	
	Relative total growth (%)	Mutation frequency (X 10 <sup>-6</sup> )		Relative total growth (%)	Mutation frequency (X 10 <sup>-6</sup> )
0	100	120.14	0	100	87.13
5	98	102.26 NS	10	63	95.50 NS
10	104	89.53 NS	20	60	111.37 NS
20	73	88.07 NS	30	54	92.69 NS
30	64	119.04 NS	40	48	114.48 NS
35	79	98.82 NS	50	47	115.62 NS
40	55	123.52 NS	60	42	107.42 NS
45	(59)	(93.00)	70	29	123.99 NS
50	11!	183.16! NS	80	16	107.85 NS
0.15 (NQO)	65	619.55	2 (BP)	31	731.86
0.2 (NQO)	65	539.99	3 (BP)	20	846.87
Treatment (µg/mL)	24-hour -S9		Treatment (µg/mL)	3-hour -S9	
	Relative total growth (%)	Mutation frequency (X 10 <sup>-6</sup> )		Relative total growth (%)	Mutation frequency (X 10 <sup>-6</sup> )
0	100	158.33	0	100	131.44
25	111	158.64 NS	30	88	114.14 NS
32.5	130	124.08 NS	40	94	121.24 NS
40	113	123.53 NS	50	83	148.13 NS
45	84	134.90 NS	60	77	155.45 NS
50	80	151.79 NS	70	78	131.86 NS
55	55	139.45 NS	80	49	136.71 NS
60	26	210.17 NS	90	14	196.65 NS
65	3	261.22 X			
0.02 (NQO)	148	391.88	2 (BP)	43	817.48
0.04 (NQO)	139	545.40	3 (BP)	16	1157.43

Positive controls: NQO, 4-nitroquinoline 1-oxide; BP, benzo(a)pyrene.

NS, not significantly different

Data in parentheses indicates marked heterogeneity observed.

! Based on one replicate only

X, Treatment excluded from final test statistics due to excessive toxicity.

### 7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

**Study title:** PEP005: Induction of micronuclei in the bone marrow of treated rats

**Key findings:** PEP005 at doses up to 20 µg/kg did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes (PCE) in rat bone marrow.

**Study no:** 2174/007

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** 1-9-2003

**GLP compliance:** Yes

**QA reports:** Yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** PEP005, 141002, 99.17%

**Methods:**

Strains/species/cell line: Crl:WI (Glx/BRL/Han) IGS BR rats, 7 males/dose group

Doses used in definitive study: 0, 5, 10, and 20 µg/kg

Basis of dose selection: A range-finding experiment showed that intravenous treatment with PEP005 once daily for two consecutive days at 20 µg/kg caused clinical signs of toxicity without deaths. No substantial inter-sex difference in toxicity was observed.

Negative controls: Vehicle, 20% PEG 400 /saline

Positive controls: A single dose of 20 mg/kg Cyclophosphamide (CPA) for 24 hours.

Incubation and sampling times: Rats received an intravenous bolus injection once daily on two consecutive days. Bone marrow sampling took place at 24 hours after the second dose.

**Results**

Study validity: The positive control induced a statistically significant increase in the frequency of micronucleated PCE; the negative and positive controls were consistent with the historical control data.

Study outcome: Abnormal breathing after treatment and piloerection after first dose only were noted in all animals treated with 10 or 20 µg/kg of PEP005. Groups of animals treated with PEP005 exhibited PCE to NCE ratios that were similar to the vehicle control. There were no statistically significant increases in micronucleated PCE in any PEP005-treated groups, compared to the vehicle control.

Treatment (µg/kg)	PCE Scored	PCE/NCE	Micronucleated PCE	Frequency of micronucleated PCE per 1000 cells
Vehicle (0)	14000	2.30	7	0.50 ± 0.58
PEP005 (5)	14000	2.84	11	0.79 ± 1.07
PEP005 (10)	14000	2.32	15	1.07 ± 0.53
PEP005 (20)	14000	2.97	2	0.14 ± 0.24
Positive control	14000	1.34	115	8.21 ± 3.31

Because of the nature of actinic keratosis (chronic condition and pre-malignancy), the activity of PEP005 to stimulate normal human fibroblast proliferation in vitro, and structure similarity to phorbol esters (tumor promoters), the sponsor was requested to address the tumor promoting potential of PEP005 on premalignant lesions. The sponsor conducted a study with PEP005 using the in vitro SHE cell assay to address this issue.

## 7.4 Other Genetic Toxicity Studies

**Study title:** PEP005: In vitro clonal transformation assay using Syrian Golden Hamster Embryo (SHE) cells

**Key findings:** PEP005 was positive in the SHE cell transformation assay when tested following a 24-hour or 7-day treatment period.

**Study no.:** AB34NU.310.BTL

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

**Date of study initiation:** 8-22-2006

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, and % purity:** PEP005, Lot Number A021568B, Purity 98.1%.

### Methods

Strains/species/cell line: Syrian golden hamster embryo (SHE) cells

Doses used in definitive study: 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 µg/mL for 24-hr exposure; 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, and 0.5 µg/mL for 7-day exposure.

Basis of dose selection: In the preliminary cytotoxicity assay, visible precipitation was present at 100 µg/mL PEP005 in medium; for 24-hr exposure, 5 µg/mL PEP005 reduced the relative plating efficiency (RPE) to 50.2%; for 7-day exposure, the RPE was reduced to 68.1% at 0.5 µg/mL.

Negative controls: DMSO

Positive controls: B(a)P 10 µg/mL for 24-hr exposure and 5.0 µg/mL for 7-day exposure

Incubation and sampling times: SHE cells were exposed to DMSO, B(a)P, or various concentrations of PEP005 at pH 6.7 for 24 hr or 7 days in the absence of a supplemental exogenous mammalian metabolic activation system. Following treatment, the cells were cultured for estimation of the cytotoxic effects of treatment and the induction of phenotypic transformation as measured by morphological transformation of the cells.

### Results

Study validity: The following criteria were established and met for assay validity: 1) Greater than 1000 colonies/group for all groups. 2) An average colony number per dish for vehicle controls between 25 and 40 colonies. 3) The transformation frequencies of a B(a)P positive control group must be statistically greater than the transformation frequency of the vehicle control group ( $p < 0.05$ ). 4) No colonies present in the dishes with feeder cells-only. The positive control [B(a)P] caused statistically significant increases in transformation frequency at a concentration of 10 µg/mL for 24-hr exposure

and at 5.0 µg/mL for 7-day exposure. The transformation frequency induced by the solvent control (DMSO) was within the historical range in this laboratory.

Study outcome: In the 24-hour dosing regimen, the RPE was reduced to 83.2% at the highest dose (5.0 µg/mL) and to 90.6% at the lowest dose (0.05 µg/mL) of PEP005 evaluated. In the 7-day dosing regimen, the PEP005 reduced the RPE to 66.5% at the highest dose (0.5 µg/mL) and increased to 105.8% at the lowest dose (0.005 µg/mL). A dose-dependent increase in toxicity was observed. Statistically significantly increased transformation frequencies were observed with PEP005 treatments at 0.1, 0.5, 1.0, 2.5, and 5.0 µg/mL in the 24-hour dosing regimen and with PEP005 treatments of 0.025, 0.05, 0.1, and 0.5 µg/mL in the 7-day dosing regimen (see the following tables).

#### SHE CELL TRANSFORMATION ASSAY SUMMARY

##### 24 Hour

Study No.: AB34NU.310.BTL.B1

Dose	Total Colonies	Cells Seeded per Dish	Mean Colonies per Dish	PE(%) <sup>1</sup>	RPE (%) <sup>2</sup>	Total Colonies Scored	MTC <sup>3</sup>	MTF <sup>4</sup>	MTF p Value (One-sided Fisher's Exact Test)
DMSO	1205	120	30.9	25.7	100	1202	5	0.42	N/A
B(a)P 10 µg/mL	1658	120	41.5	34.5	134.2	1656	21	1.27	* 0.013
<b>PEP005</b>									
0.05 µg/mL	1120	120	28.0	23.3	90.6	1112	6	0.54	0.45
0.1 µg/mL	1092	120	27.3	22.8	88.4	1079	12	1.11	* 0.047
0.25 µg/mL	1073	120	26.8	22.4	86.8	1072	11	1.03	0.070
0.5 µg/mL	1195	120	29.9	24.9	96.7	1184	17	1.44	* 0.0080
1.0 µg/mL	1684	180	42.1	23.4	90.8	1680	21	1.25	* 0.014
2.5 µg/mL	1969	240	49.2	20.5	79.7	1961	20	1.02	* 0.046
5.0 µg/mL	2056	240	51.4	21.4	83.2	2047	29	1.42	* 0.0041

\* Statistically significant (p ≤0.05)

<sup>1</sup>PE = mean plating efficiency

<sup>2</sup>RPE = relative plating efficiency

<sup>3</sup>MTC = morphologically transformed colonies

<sup>4</sup>MTF = morphologically transformed frequency

+ = Not scored due to toxicity.

## SHE CELL TRANSFORMATION ASSAY SUMMARY

7 Day

Study No.: AB34NU.310.BTL.B2

Dose	Total Colonies	Cells Seeded per Dish	Mean Colonies per Dish	PE(%) <sup>1</sup>	RPE (%) <sup>2</sup>	Total Colonies Scored	MTC <sup>3</sup>	MTF <sup>4</sup>	MTF p Value (One-sided Fisher's Exact Test)
DMSO	1323	120	33.1	27.6	100	1322	6	0.45	N/A
B(a)P 5.0 µg/mL	1542	120	38.6	32.1	116.6	1526	21	1.38	* 0.0088
<b>PEP005</b>									
0.005 µg/mL	1400	120	35.0	29.2	105.8	1391	12	0.86	0.14
0.01 µg/mL	1414	120	35.4	29.5	106.9	1403	15	1.07	0.053
0.025 µg/mL	1328	120	33.2	27.7	100.4	1322	16	1.21	* 0.027
0.05 µg/mL	1171	120	29.3	24.4	88.5	1159	16	1.38	* 0.013
0.1 µg/mL	1000	120	25.0	20.8	75.6	992	20	2.02	* 0.00048
0.25 µg/mL	878	120	22.0	18.3	66.4	+	+	+	+
0.5 µg/mL	1319	180	33.0	18.3	66.5	1299	27	2.08	* 0.00015

\* Statistically significant ( $p \leq 0.05$ )<sup>1</sup>PE = mean plating efficiency<sup>2</sup>RPE = relative plating efficiency<sup>3</sup>MTC = morphologically transformed colonies<sup>4</sup>MTF = morphologically transformed frequency

+ = Not scored due to toxicity.

## 8 Carcinogenicity

No carcinogenicity studies have been conducted with PICATO Gel or ingenol mebutate. Although actinic keratosis is considered as a chronic condition, based on current clinical management strategies for patients with actinic keratosis, it is anticipated that the total exposure duration of PICATO Gel even with retreatment may be relatively short. Therefore, the Agency determined that a carcinogenicity study was not necessary to support this short term treatment regimen (2- 3 days).

## 9 Reproductive and Developmental Toxicology

### 9.1 Fertility and Early Embryonic Development

No fertility and early embryonic development toxicity study with PICATO Gel or ingenol mebutate has been conducted. The need for a fertility and early embryonic development toxicity study was waived due to minimal systemic exposure after topical administration under maximal use clinical conditions.

## 9.2 Embryonic Fetal Development

**9.2.1. Preliminary Developmental Toxicity Study in Rats (494295):** Four groups of 6 mated SD female rats received 0, 1, 2, or 4 µg/kg PEP005 in 20% PEG400/0.9% saline once daily by intravenous injection on Days 6 - 16 of gestation and were killed on Day 20 of gestation. There were no maternal or embryo-fetal findings in all groups that were attributable to intravenous treatment of PEP005. Based on the lack of maternal or embryo-fetal toxicity in this study at 4 µg/kg/day, and early mortality observed in a previously reported toxicity study of intravenously administered PEP005 at ≥7.5 µg/kg/day, the sponsor stated that the dose levels of 0, 1.5, 3 and 5 µg/kg/day PEP005 were selected in the subsequent definitive developmental toxicity study in rats.

### 9.2.2. Developmental Toxicity Study of Intravenously Administered PEP005 in Rats

**Key study findings:** Intravenous administration of up to 5 µg/kg/day PEP005 in pregnant rats on Days 6 - 16 of gestation did not produce adverse effects on fetal development. However, one dam died immediately after dosing on Day 12 of gestation.

**Study no.:** 494300

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** 5-30-2006

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, radiolabel, and % purity:** Batch PEP0407, 99.7% purity

**Formulation/vehicle:** 20% PEG400/0.9% NaCl (w:w)

#### Methods

Doses: 0, 1.5, 3, and 5 µg/kg/day

Species/strain: CrI CD (SD) BR rat

Number/sex/group: 20/group, mated females

Route, formulation, volume, and infusion rate: Intravenous injection, 5 mL/kg dose volume, and 3 mL/min injection rate

Satellite groups used for toxicokinetics: None

Study design: Mated female rats received 0, 1.5, 3, or 5 µg/kg PEP005 in 20% PEG400/0.9% saline once daily by intravenous injection on Days 6 - 16 of gestation and were killed on Day 20 of gestation.

Parameters and endpoints evaluated: Mortality, clinical signs, body weight, and food consumption on dams; body weight, external, soft tissue, and skeletal examinations, and sex of fetuses.

#### Results:

**Mortality (dams):** One rat at 5 µg/kg/day was found dead immediately after dosing on Day 12 of gestation. This animal had dark lung lobes at necropsy. However, the cause of death was not determined grossly or by histological examination.

Clinical signs (dams): There were no treatment-related clinical signs.

Body weight (dams): There were no treatment-related effects on body weights.

Food consumption (dams): There were no treatment-related effects.

Toxicokinetics: NA

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): There was a slight but non-statistically significant increase in early embryonic deaths in the 1.5 and 3 µg/kg/day PEP005 groups, compared with the Controls ( $0.8 \pm 1.7$ ,  $1.1 \pm 1.2$ ,  $1.5 \pm 2.1$ , and  $0.4 \pm 0.5$  per dam OR 6%, 8%, 11%, and 3% for the 0, 1.5, 3, and 5 µg/kg/day groups, respectively). This effect was most marked at 3 µg/kg/day, which was mainly due to one dam with 9 early deaths. There were no differences in fetal body weights and sex ratio.

Offspring: There were no treatment-related effects on the type and distribution of the fetal abnormalities and variants, and the incidences of the skeletal ossification parameters. The total number of fetuses with minor visceral abnormalities/variants was 12%, 11%, 17% and 13% of the total number examined, and the total number of fetuses with minor skeletal abnormalities/variants was 9%, 10%, 6% and 6% of the total number examined in the 0, 1.5, 3, and 5 µg/kg/day groups, respectively.

**9.2.3. Dose Range Finding Study in Rabbits Preliminary to Developmental Toxicity Study (494316)**: In a unmated phase, four groups of 2 unmated female rabbits received intravenous doses of 2, 4, or 5 µg/kg/day PEP005 for 12 days or 10 µg/kg/day PEP005 for 3 days due to the severity of the clinical signs including labored breathing, subdued behavior, hunched posture, salivation, staggering, discharge from nose and an unwillingness to move. At 5 µg/kg/day, both animals had an increased breathing rate; one animal also had an unwillingness to move, a body weight loss, and reduced food consumption. At 2 and 4 µg/kg/day, clinical signs included an increased breathing rate. Accordingly, dose levels of 1, 2, and 4 µg/kg/day were selected for the mated phase.

Four groups of 6 mated female rabbits received intravenous doses of 0, 1, 2, or 4 µg/kg/day PEP005 in 20% PEG400/0.9% saline on Days 6 - 18 of gestation and were killed on Day 22 of gestation. In all PEP005-treated groups, treatment caused an increased breathing rate that generally occurred immediately after dosing. In the 4 µg/kg/day group, there was also a reduction in group mean body weight gains and food consumption throughout the treatment period. However, there were no treatment-related effects on the total number of live implants, early embryonic deaths, late embryonic deaths, fetal deaths, fetal weights, and external fetal abnormalities. The sponsor selected the dose levels of 0, 1, 2 and 4 µg/kg/day for the subsequent definitive developmental toxicity study in rabbits.

In addition, blood samples for the determination of blood levels of PEP005 as well as isomers (b) (4) in mated animals were collected from four animals per group on Days 6 and 16 of gestation at pre-dose, 5 min, 30 min, 1 h, 2 h, and 4 h post-dose. PEP005 was quantifiable in samples from rabbits at 1, 2, and 4 µg/kg/day on Days 6 and 16 of gestation (see the following table). However, (b) (4) were not quantifiable in samples from any dose group (LLOQ 0.1 ng/mL).

Dose (µg/kg/day)	Day of Gestation		Plasma Concentration			
			Cmax (ng/mL)	Tmax (Hours)	AUC <sub>(0-t)</sub> (ng.h/mL)	AUC <sub>(0-∞)</sub> (ng.h/mL)
1	6	Mean	0.208	0.083	-	-
		SD (n-1)	0.0998	-	-	-
	16	Mean	0.233	0.083	-	-
		SD (n-1)	0.08279	-	-	-
2	6	Mean	0.514	0.083	0.249	-
		SD (n-1)	0.1977	-	0.07095	-
	16	Mean	0.477	0.083	0.223	-
		SD (n-1)	0.06181	-	0.01358	-
4	6	Mean	1.42	0.083	0.898	1.12
		SD (n-1)	0.2610	-	0.1142	-
	16	Mean	0.931	0.083	0.616	0.875
		SD (n-1)	0.1373	-	0.1449	-

#### 9.2.4. Developmental Toxicity Study of Intravenously Administered PEP005 in Rabbits

**Key study findings:** Intravenous administration of 1, 2, or 4 µg/kg/day PEP005 on Days 6 - 18 of gestation caused increased breathing rate in pregnant rabbits. There were no treatment-related effects on maternal body weights, maternal food consumption, fetal body weights and sex ratio. An increase in early embryonic deaths and an increase in the number of fetuses with the jugal (malar) connected/fused to the zygomatic process of the maxilla were seen in the 4 µg/kg/day group. An increase in the incidence of fetuses with incompletely ossified cervical vertebral arches was also seen in both 2 and 4 µg/kg/day groups. In addition, there was a dose-related increase in the number of fetuses with variation in the origin of arteries arising directly from the aortic arch (5%, 8%, 11%, and 13% in the 0, 1, 2, and 4 µg/kg/day, respectively). Because a higher incidence of unilateral/bilateral rib 7 costal cartilage not attached to the sternum was seen in all PEP005-treated groups (14%, 23%, 33%, and 24% in the 0, 1, 2, and 4 µg/kg/day groups, respectively), which was outside the laboratory historical control range (7% - 20%), no NOAEL was established in this study, which was in contrast to the sponsor's conclusion that the fetal NOAEL was 1 µg/kg/day PEP005.

**Study no.:** 494321

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

(b) (4)

**Date of study initiation:** 8-4-2006

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, radiolabel, and % purity:** Batch PEP0407, 99.7% purity

**Formulation/vehicle:** 20% PEG400/0.9% NaCl (w:w)

**Methods**

Doses: 0, 1, 2, and 4 µg/kg/day

Species/strain: Hsdlf:NZW rabbit

Number/sex/group: 20/group, mated females

Route, formulation, volume, and infusion rate: Intravenous injection, 2 mL/kg dose volume, and 2 mL/min injection rate

Satellite groups used for toxicokinetics: None

Study design: Mated female rats received 0, 1, 2, or 4 µg/kg PEP005 in 20% PEG400/0.9% saline once daily by intravenous injection on Days 6 - 18 of gestation and were killed on Day 29 of gestation.

Parameters and endpoints evaluated: Mortality, clinical signs, body weight, and food consumption on dams; body weight, external, soft tissue, and skeletal examinations, and sex of fetuses.

**Results:**

Mortality (dams): There was no PEP005 treatment-related mortality. One control animal was killed prematurely on Day 25 of gestation due to aborting its fetuses.

Clinical signs (dams): Increased breathing rate was seen immediately/shortly after dosing in all PEP005-treated animals.

Body weight (dams): There were no treatment-related effects on body weights.

Food consumption (dams): There were no treatment-related effects on food consumption.

Toxicokinetics: NA

Terminal and necroscopic evaluations:C-section data: There was a slight but non-statistically significant increase in early embryonic deaths in the 4 µg/kg/day PEP005 group, compared with the Control group ( $0.7 \pm 1.2$ ,  $0.5 \pm 0.7$ ,  $0.7 \pm 0.8$ , and  $1.2 \pm 2.2$  per dam OR 7%, 5%, 7%, and 13% in the 0, 1, 2, and 4 µg/kg/day groups, respectively). There were no treatment-related differences in fetal body weights and sex ratio.

Offspring: There was an increase in the incidence of fetuses with incompletely ossified cervical vertebral arches in the 2 and 4 µg/kg/day PEP005 groups (5% and 6%, respectively, outside the laboratory historical control range 0-3%), compared to the Control group (2%). There was a dose-related increase in the number of fetuses with variation in the origin of arteries arising directly from the aortic arch (8%, 11%, and 13% for 1, 2, and 4 µg/kg/day, respectively) compared to the Controls (5%); the incidence at 4 µg/kg/day PEP005 was slightly outside the laboratory historical control range (2% - 11%). At 4 µg/kg/day PEP005, there was an increase (12%) in the number of fetuses with the jugal (malar) connected/fused to the zygomatic process of the maxilla (laboratory historical control range 0.8% - 10%), compared to the Control group (5%). In addition, compared to the Control group, all PEP005-treated groups had an increased

incidence of unilateral/bilateral rib 7 costal cartilage not attached to the sternum (14%, 23%, 33%, and 24% in the 0, 1, 2, and 4 µg/kg/day groups, respectively), which was outside the laboratory historical control range (7% - 20%). However, the sponsor stated that “there was no clear dose related response and therefore this finding was considered to be incidental.”

**Table 6** *Developmental Toxicity Study of Intravenously Administered PEP005 in Rabbits*  
**Group Incidence of Minor Abnormalities and Variants**

Abnormality/Variant	Group/Dose Level (µg/kg/day)			
	1	2	3	4
	(0)	(1)	(2)	(4)
Incidence of Foetuses (Litters)				
<u>Visceral</u>				
Eye reduced (in size)	1(1)	0	0	1(1)
Eye central lenticular opacity	1(1)	0	0	0
Incisors not erupted	2(2)	1(1)	0	1(1)
Pinna folded forward	0	1(1)	0	1(1)
Cervical remnant of thymus	1(1)	0	0	1(1)
Variation in origin of arteries arising directly from aortic arch	7(5)	13(6)	14(8)	19(11)
Gall bladder reduced (in size)	0	2(2)	0	0
Gall bladder club shaped	0	1(1)	1(1)	0
Gall bladder clear cyst	0	0	0	1(1)
Spleen pale colouration	2(1)	1(1)	0	0
Stomach distended by gas	0	1(1)	0	1(1)
Ovary cyst	2(2)	0	0	1(1)
Forelimb minimal flexure	0	1(1)	0	0
Small foetus	2(2)	2(2)	0	0
Number with minor visceral abnormalities/variant	13(9)	21(12)	14(8)	23(13)
Total number examined	130(15)	168(18)	124(15)	143(18)

**Table 6**                      **Group Incidence of Minor Abnormalities and Variants**  
**(continued)**

Parameter	Group/Dose Level (µg/kg/day)			
	1	2	3	4
	(0)	(1)	(2)	(4)
Incidence of Foetuses (Litters)				
<u>Skeletal</u>				
Cranial bone(s) unossified/incompletely ossified	2(2)	3(3)	1(1)	1(1)
Cranial bone(s) linear ossification irregularity/ies	6(5)	7(6)	2(2)	1(1)
Sutural bone(s)/ Sutural deviation	0	4(3)	1(1)	1(1)
Jugal connected/fused to zygomatic process of maxilla	6(3)	12(6)	1(1)	17(6)
Greater horn of hyoid bent in/outwards	5(4)	8(6)	2(2)	8(5)
Cervical vertebra hemicentric	0	1(1)	0	0
Cervical vertebral arch(es) reduced (in size)	1(1)	0	0	0
Cervical vertebral arch(es) incompletely ossified	2(2)	2(2)	6(5)	9(3)
Cervical rib(s)	6(3)	4(3)	11(4)	4(4)
Cervical rib long with cartilage fused to thoracic rib costal cartilage	0	0	1(1)	0
Rib(s) connected	1(1)	0	0	0
Rib(s) thickened area	0	1(1)	0	0
Additional ossified centre/area cranial to/arising from sternbrae 1	1(1)	0	1(1)	1(1)
Sternebra/ae connected/fused	2(2)	7(4)	1(1)	3(3)

**Table 6** *Group Incidence of Minor Abnormalities and Variants*  
(continued)

Parameter	Group/Dose Level (µg/kg/day)			
	1	2	3	4
	(0)	(1)	(2)	(4)
Incidence of Foetuses (Litters)				
<u>Skeletal (continued)</u>				
Rib 1 costal cartilage bifurcated at point of attachment to sternum	1(1)	0	0	0
Rib costal cartilage(s) asymmetrically aligned at point of attachment to sternum	2(2)	5(4)	0	5(4)
Rib(s) costal cartilages caudally displaced	1(1)	1(1)	0	0
Unilateral/bilateral rib 7 costal cartilage not attached to sternum	18(8)	38(11)	41(13)	34(14)
Pelvic girdle displacement :				
Unilateral	6(5)	3(3)	1(1)	4(3)
Bilateral	11(6)	11(5)	9(2)	22(8)
Sacral vertebral centrum connected to sacro-iliac articulation centre	0	1(1)	0	0
Astragalus incompletely ossified/unossified	2(2)	0	0	0
12 complete ribs	68(15)	107(15)	81(15)	85(16)
Vestigial supernumerary rib(s) on 1st lumbar vertebra	12(9)	15(10)	9(6)	13(8)
Reduced supernumerary rib(s) on 1st lumbar vertebra	20(9)	14(11)	11(5)	11(7)
Complete supernumerary rib(s) on 13th thoracic vertebra	30(11)	32(11)	23(10)	34(10)
Number with minor skeletal abnormality/variant	52(15)	77(18)	62(15)	82(18)
Total number examined	130(15)	168(18)	124(15)	143(18)

Because systemic exposure to the drug substance and metabolites during clinical use under conditions of maximum exposure does not occur at a measurable level, the fertility and early embryonic development toxicity study and the prenatal and postnatal development toxicity study have not been requested.

### 9.3 Prenatal and Postnatal Development

No prenatal and postnatal development toxicity study with PICATO Gel or ingenol mebutate has been conducted. The need for a prenatal and postnatal development

toxicity study was waived due to minimal systemic exposure after topical administration under maximal use clinical conditions.

## 10 Special Toxicology Studies

### 10.1. PEP005: Local lymph node assay in the mouse (individual method)

**Key study findings:** The LLNA assay is not an appropriate assay to determine if PEP005 may have the potential to cause skin sensitization. PEP005 causes irritation and other alterations of the immune system (releases cytokines in the skin which will cause a positive response in the LLNA assay) which confounds the interpretation of this assay. Therefore, this assay is not conclusive concerning the sensitization potential of PEP005.

**Study no.:** 2174/031

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** 10-14-2003

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, and % purity:** PEP005/IPA gel, 3195B

**Formulation/vehicle:** Vehicle/IPA gel, batch number 3195A

#### Methods

**Doses:** 0%, 0.0025%, 0.005%, and 0.01% PEP005 in Vehicle gel

**Study design:** Test article (50 µl) was applied daily to the outer aspect of both auditory pinnae of each mouse (CBA/Ca mice; 5 females/dose) for 3 days. The proliferative response of the auricular lymph node (incorporation of <sup>3</sup>H-methyl thymidine) was assessed 5 days following the initial application. α-Hexylcinnamaldehyde (25% in acetone/olive) was used as positive control in the same study.

**Results:** Erythema and edema of the pinnae were noted in animals treated with PEP005 at 0.01% from Day 2. No signs of irritation were noted in 0.005% and 0.0025% groups. Because the low mean DPM value for vehicle control group, the stimulation index (SI) values of all groups were very high (See the next table). The mean DPM value obtained with the vehicle (80% acetone in olive oil) that was used for the negative control in this laboratory, based on a year's studies, was 672. When this value was used to calculate the SI for the positive control in this study, a SI of 5.89 was obtained and was comparable to the historical data (4.66 to 9.08). When 672 was used to calculate the SI for the test article groups, the SI values were 2.75, 3.72, and 5.25 for PEP005 0.0025%, 0.005%, and 0.01%, respectively. Ideally, this study should include a positive control group with the clinical vehicle. The results indicated that PEP005 may have the potential to cause skin sensitization. However, since the test article caused skin irritation, which will produce positive results in the study, the results of this study cannot be used to predict the sensitization potential of PEP005.

Treatment	DPM (mean ± SD)	SI (test/Vehicle)	SI (test/576)
Vehicle	59 ± 45	-	-
PEP005 0.0025%	1846 ± 1090	31.2	2.75
PEP005 0.005%	2497 ± 1261	42.2	3.72
PEP005 0.01%	3526 ± 1678	59.6	5.25
Positive control	3957 ± 2411	66.8	5.89

**10.2. PEP005 hemocompatibility test (TYU001/043606):** PEP005 showed no hemolytic potential when incubated with rat, rabbit, dog, or human whole blood in the concentration range of 0.1 to 100.0 ng/mL. PEP005 showed no flocculation, precipitation, or coagulation when incubated with rat, rabbit, dog, or human plasma in the concentration range of 0.1 to 109.9 ng/mL.

**10.3. Evaluation of the effects of PEP005 on in vitro platelet aggregation (210706):** PEP005 induced platelet aggregation in both platelet rich plasma and washed platelets. Effective concentrations producing 50% of the maximal effect ( $EC_{50}$ ) were 6.46 ng/mL (~15 nM) and 2.03 ng/mL (~4 nM) for platelet rich plasma and washed platelets, respectively. The approximate 3-fold higher sensitivity to platelet aggregation in washed platelets suggests that protein binding properties of PEP005 might affect/alter its biological effects. The aggregating ability of PEP005 on platelet rich plasma was paralleled by thromboxane B2 generation (stable metabolite of thromboxane A2). However, the  $EC_{50}$  of PEP005 for thromboxane B2 generation was higher ( $\geq 10$  ng/mL or 23 nM) than the  $EC_{50}$  for platelet aggregation.

There are a few impurities structurally closely related to PEP005. As seen from the next table, the proposed specifications for these impurities are higher than the recommended qualification threshold for drug substance (b) (4)

Organic impurities:	(b) (4)
(b) (4)	(b) (4)

In order to further toxicologically qualify impurities in the drug substance, a spiked batch of PEP005 (Batch PEP005~TOX-MIX27JUL10) was synthesized with as high a concentration of impurities as reasonably possible (See the next table), since the individual impurities are difficult to synthesize and/or isolate, and the following studies were conducted.

TEST	RESULTS
Description	(b) (4)
Ingenol mebutate purity by HPLC	(b) (4)
Impurities	(b) (4)

**10.4. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium***

Study no.: 8224378  
 Study report location: (b) (4)  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: August 16, 2010  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: PEP005, batch PEP005~TOX-MIX27JUL10 was spiked with impurities at known concentrations: PEP005 83.0%, (b) (4)

**Key Study Findings**

No significant increases in the frequency of revertant colonies were observed in any strains with or without metabolic activation, indicating that the test article was not mutagenic under the conditions tested.

## Methods

- Strains: *Salmonella typhimurium* TA1535, TA1537, TA98, TA100, and TA102
- Concentrations in definitive study: 1.6, 8, 40, 200, 1000, and 5000 µg/plate in the first experiment; 15.8, 50, 158, 500, 1581, and 5000 µg/plate in the second experiment.
- Basis of concentration selection: A range finding study at 1.6, 8, 40, 200, 1000, and 5000 µg/plate was performed only in strain TA100 in the absence or presence of S9. Evidence of toxicity was observed at 5000 µg/plate solely in the presence of S9.
- Negative control: DMSO
- Positive control: In the absence of S9: TA98, 5 µg/plate 2-nitrofluorene; TA100 and TA1535, 2 µg/plate sodium azide; TA1537, 50 µg/plate 9-aminoacridine; TA102, 0.2 µg/plate mitomycin C. In the presence of S9: TA98, 10 µg/plate benzo(a)pyrene; TA100, TA1535, and TA1537, 5 µg/plate 2-aminoanthracene; TA102, 20 µg/plate 2-aminoanthracene
- Formulation/Vehicle: DMSO
- Incubation & sampling time: Triplicate plates without and with S9 mix (10%) were used. The plates were incubated at 37 °C for 3 days. Following incubation, these plates were examined for evidence of toxicity to the background lawn, and where possible revertant colonies were counted electronically using a Sorcerer Colony Counter or manually. As the results of the first experiment were negative, treatments in the presence of S9 in the second experiment included a 1-hour pre-incubation step.

## Study Validity

The assay was considered valid, because the negative controls count fell within the acceptable ranges, all positive controls induced marked increases in the frequency of revertant colonies, both with and without metabolic activation.

## Results

Following these treatments, evidence of toxicity was observed in all strains at 1581 and/or 5000 µg/plate in the absence and presence of S9. Precipitation was observed

on all test plates treated at concentrations of 5000 µg/plate in the absence and presence of S9. Although a statistically significant increase in revertant numbers was observed in TA100 in the presence of S9 following treatment with 50 µg/plate in the second experiment (Dunnett's Test,  $P < 0.01$ ; see the next table), the increase was of insufficient magnitude (0.4-fold increase compared to DMSO) to be considered as evidence of mutagenic activity in this assay. The results indicated that the test article was not mutagenic under the conditions tested.

Experiment	PEP005 (µg/plate)	Number of colonies (mean ± S.D.)	
		TA100 - S9	TA100 + S9
First	DMSO	123.4 ± 13.2	135.0 ± 8.5
	1.6	116.0 ± 17.1	161.3 ± 16.1
	8	128.3 ± 7.1	153.0 ± 15.1
	40	114.3 ± 7.4	155.0 ± 8.7
	200	128.3 ± 3.5	153.0 ± 3.0
	1000	129.0 ± 6.9	140.0 ± 15.6
	5000	96.3 ± 5.5	103.0 ± 2.6
	Positive control	755.3 ± 47.4	1230.0 ± 109.3
Second	DMSO	118.6 ± 13.8	113.2 ± 16.9
	15.8	132.7 ± 19.7	126.7 ± 9.6
	50	125.3 ± 9.1	153.7 ± 26.8 **
	158	119.7 ± 9.3	115.7 ± 7.1
	500	148.7 ± 19.5	86.3 ± 6.5
	1581	126.7 ± 9.9	97.0 ± 18.2
	5000	80.3 ± 4.0	69.3 ± 7.6
	Positive control	671.0 ± 12.1	1006.0 ± 90.0

\*\* Dunnett's Test,  $P < 0.01$

#### 10.5. Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the microtitre® fluctuation technique

Study no.: 8224381  
 Study report location: (b) (4)  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: August 16, 2010  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: PEP005, batch PEP005~TOX-MIX27JUL10 was spiked with impurities at known concentrations: PEP005 83.0%, (b) (4)

#### Key Study Findings

The test article did not induce a statistically significant or dose-related increase in the mutant frequency. The test article was non-mutagenic in L5178Y cells under the conditions of the test.

## Methods

Cell line:	L5178Y TK +/- mouse lymphoma cell line.
Concentrations in definitive study:	See the next table.
Basis of concentration selection:	A range finding study (up to 100 µg/mL) was performed for 3-hr treatment with or without S9 and 24-hr treatment without S9. Complete toxicity was observed at the highest concentration with or without S9 (100 µg/mL) after 3-hr treatment. The highest concentration to give >10% RTG was 50 µg/mL with or without of S9, which gave 74% and 99% RTG, respectively; 24-hr treatment without S9 at 50 or 100 µg/mL yielded 49% or 0% RTG, respectively.
Negative control:	DMSO
Positive control:	Methyl methane sulphonate (MMS) and benzo(a)pyrene [B(a)P].
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Three-hr exposures were used both with and without metabolic activation and 24-hr exposure without activation. After 48 hr expression, cells were diluted and plated for mutant frequency in selective medium containing 3 µg/mL 5-trifluorothymidine (TFT) in 96-well microtitre plates. Cells were also diluted and plated for viability in non-selective medium. Plates were scored after 12 to 13 days incubation.

## Study Validity

The vehicle controls had acceptable mutant frequency values, which were within the normal range (50 - 170 mutants/10<sup>-6</sup> cells) for the L5178Y cell line at the TK locus. The positive controls, both in the absence and presence of metabolic activation, induced marked increases in the mutant frequency, indicating the satisfactory performance of the test and of the activity of the metabolizing system (see the next table). For the negative controls, the proportion of small colony mutants in the absence and presence of S9 ranged from 55% to 62% in Experiment 1 and from 38% to 73% in Experiment 2.

## Results

The test article did not induce a significant or dose-related increase in the mutant frequency, at any dose level, with or without metabolic activation for 3-hour treatment and without metabolic activation for 20-hour treatment (see the next two tables).

**Experiment 1 (3 hour treatment in the absence and presence of S-9)**

Treatment ( $\mu\text{g/mL}$ )	-S-9		Treatment ( $\mu\text{g/mL}$ )	+S-9	
	%RTG	MF§		%RTG	MF§
0	100	75.35	0	100	50.13
9.524	79	80.66	9.524	94	79.90
19.05	87	85.27	19.05	68	81.40
33.33	87	93.15	33.33	69	81.32
47.62	80	79.90	47.62	63	61.57
57.14	32	84.73	57.14	32	65.01
64.29	27	97.36	64.29	43	73.78
71.43	12	80.84	71.43	36	86.13
			76.19	34	85.33
			80.95	40	46.54
			85.71	42	75.96
			90.48	34	59.65
			95.24	24	98.71
Linear trend			NS		
MMS			B[a]P		
15	65	529.01	2	48	611.87
20	58	741.39	3	23	982.68

§ 5-TFT resistant mutants/ $10^6$  viable cells 2 days after treatment

%RTG % Relative total growth

NS Not significant

**Experiment 2 (3 hour treatment in the presence of S-9 and 24 hour treatment in the absence of S-9)**

Treatment (µg/mL)	-S-9		Treatment (µg/mL)	+S-9	
	%RTG	MF§		%RTG	MF§
0	100	69.01	0	100	73.88
30	85	63.50!	20	101	78.00
40	91	53.49	40	90	66.74
50	85	70.90	50	82	66.59
60	70	60.57	60	66	80.63
70	50	50.70	80	43	67.00
80	47	47.58	90	36	76.81
90	23	86.76	100	43	69.90
100	31	54.74	110	47	72.66
			120	33	80.77
			150	2	47.48
Linear trend		NS	Linear trend		NS
MMS			B[a]P		
5	36	1034.81	2	79	308.61
7.5	16	2423.91	3	60	589.76

§ 5-TFT resistant mutants/10<sup>6</sup> viable cells 2 days after treatment  
 %RTG % Relative total growth (adjusted by Day 0 factor for 24 hour treatment)  
 NS Not significant  
 ! Based on one replicate only due to a technical error

**10.6. PEP005: 28 Day intravenous (bolus) administration toxicity study in the rat**

Study no.: 8224382  
 Study report location: (b) (4)  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: July 27, 2010  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: PEP005, batch PEP005~TOX-MIX27JUL10 was spiked with impurities at known concentrations. PEP005 83.0%, (b) (4)

**Key Study Findings**

There were no treatment-related effects on body weights, food consumption, ophthalmoscopy examinations, hematology, clinical chemistry, and gross pathology.

There were a few decedents treated with  $\geq 7.5$   $\mu\text{g}/\text{kg}/\text{day}$ , due to the poor conditions of the tail as well as isolated incidents of convulsions. Post-dosing observations including decreased activity, piloerection, dyspnoea, and blue coloration to the tail were observed in males and females treated at  $\geq 7.5$   $\mu\text{g}/\text{kg}/\text{day}$ . Urine volume was increased and urinary specific gravity was decreased in males and females in a dose-related manner. Prostate weight was slightly decreased (10%-12%) in PEP005-treated males and liver weight was slightly increased (10%-12%) in females treated at  $\geq 7.5$   $\mu\text{g}/\text{kg}/\text{day}$ . Microscopically there were changes at the tail vein injection site suggestive of minor irritation. The No Observed Adverse Effect Level (NOAEL) was 1.5  $\mu\text{g}/\text{kg}/\text{day}$  in this study. Blood concentrations of PEP005 and (b) (4) were quantifiable in all samples and blood sample concentrations of (b) (4) were below the limit of quantification (0.1 ng/mL) with the exception of animals treated at 15  $\mu\text{g}/\text{kg}/\text{day}$ , where concentrations of up to approximately 0.6 ng/mL were observed at the end of dosing.

## Methods

Doses:	0 (vehicle), 1.5, 7.5, or 15 $\mu\text{g}/\text{kg}/\text{day}$
Frequency of dosing:	Once daily
Route of administration:	Intravenously
Dose volume:	5 mL/kg
Formulation/Vehicle:	20% PEG 400 in 0.9% saline for injection
Species/Strain:	WI(GLX/BRL/Han)IGSBR rats
Number/Sex/Group:	10/sex/group
Age:	6 weeks
Weight:	Males 156.1 - 211.3 g; females 117.2 - 169.0 g
Satellite groups:	Three groups of 15 rats/sex/group were treated at intravenous doses of 1.5, 7.5, or 15 $\mu\text{g}/\text{kg}/\text{day}$ . One group of 3 rats/sex received vehicle.
Unique study design:	The test article was administered intravenously into the lateral caudal vein. The animals were treated once daily for 31 days (males) and 32 days (females), excluding the day of necropsy.
Deviation from study protocol:	No significant deviations affect the conclusions.

## Observations and Results

### Mortality

Observed twice daily. One male treated at 7.5  $\mu\text{g}/\text{kg}/\text{day}$  convulsed after dosing on Day 1 and was replaced with a 'spare' animal. There were 4 further decedents from the main study groups: one female treated at 7.5  $\mu\text{g}/\text{kg}/\text{day}$  and removed from the study on Day 14 due to severity of tail lesions, and one male and two females treated at 15  $\mu\text{g}/\text{kg}/\text{day}$  and removed from the study on Days 9, 5, and 16 due to convulsions, severity of tail lesions, and found dead respectively. In addition, there were two decedents from the satellite groups: one male treated at 7.5  $\mu\text{g}/\text{kg}/\text{day}$  that was found dead just after dosing on Day 16, and one male treated at 15  $\mu\text{g}/\text{kg}/\text{day}$  that was removed from the study on Day 20 due to the poor condition of the tail.

**Clinical Signs**

Observed upon return to the home cage and approximately 0.5, 1, 2, and 4 hours post-dosing; detailed physical examination conducted weekly. Post-dosing observations included decreased activity, piloerection, dyspnea, and blue coloration to the tail. Decreased activity and piloerection were observed in males and females treated at 7.5 µg/kg/day in Week 1. In males and females treated at 15 µg/kg/day, decreased activity and piloerection were also observed up to Day 10 along with dyspnea and dark coloration to the tail. These observations were most prolonged on Day 1 lasting up to 4 hours post-dosing, after which these observations were only seen up to 1 hour post-dosing.

**Body Weights**

Recorded weekly. There were no treatment-related effects.

**Feed Consumption**

Recorded weekly. There were no treatment-related effects.

**Ophthalmoscopy**

Performed on all animals pre-treatment and animals assigned to Groups 1 and 4 in Week 4. There were no treatment-related effects.

**ECG**

NA

**Hematology**

Blood samples were collected at necropsy. There were no treatment-related effects.

**Clinical Chemistry**

Blood samples were collected at necropsy. There were no treatment-related effects.

**Urinalysis**

Urine samples were collected from all animals in Week 4 over a 6-hour daytime period. Urine volume was increased and urinary specific gravity was decreased in males and females in a dose-related manner.

**Gross Pathology**

There were no treatment-related effects.

## Organ Weights

The following organs were weighed at scheduled necropsies: adrenal, brain, heart, kidney, liver, ovaries, pituitary, popliteal lymph nodes, prostate, spleen, testes and epididymides, and thyroid with parathyroids. Prostate weight was slightly decreased (10%-12%) in PEP005-treated males and liver weight was slightly increased (10%-12%) in females treated at 7.5 or 15 µg/kg/day.

## Histopathology

A battery of tissues were collected from rats in the control and high-dose group, as well as decedents in all groups, and microscopically examined. In the low- and mid-dose groups, only gross lesions were examined microscopically. There was a minor increase in incidence and/or severity of microscopic findings, including intimal proliferation, thrombosis, perivascular hemorrhage, and cellulitis, at the tail vein injection sites of the high-dose group compared with the controls.

## Toxicokinetics

Blood samples were collected from toxicokinetic group rats at the end of dosing, approximately 2, 5, 10, 15, and 30 minutes post-dosing on Days 1 and 28. As seen from the next table, blood concentrations of PEP005 and (b) (4) were quantifiable in all samples and blood sample concentrations of (b) (4) were below the limit of quantification (0.1 ng/mL) with the exception of animals treated at 15 µg/kg/day, where concentrations of up to approximately 0.6 ng/mL were observed at the end of dosing. Blood concentrations of PEP005, (b) (4) decreased steadily after the end of dosing. Generally maximum levels were observed immediately after the end of dosing on Days 1 and 28. Systemic exposure of rats to PEP005, (b) (4) was dose related. Both AUC and C<sub>max</sub> values increased with increasing dose. The increase in AUC values was greater than the increase in dose; however the increase in C<sub>max</sub> values was similar to the increase in dose. There was no evidence of accumulation of PEP005 in rat whole blood following repeat dosing and systemic exposure of rats to PEP005, (b) (4) was sex-independent.

Occasion	Analyte	Gender	PEP005 dose level (ug/kg/day)	Dose ratio	Dose Group	AUC <sub>(0-∞)</sub> (ng.min/mL)	AUC ratio	C <sub>max</sub> (ng/mL)	C <sub>max</sub> ratio	T <sub>max</sub> (h)
Day 1	PEP005	Male	1.5	1	2	0.197	1	1.36	1	0
Day 1	PEP005	Male	7.5	5	3	1.59	8	4.39	3	0
Day 1	PEP005	Male	15	10	4	4.10	21	9.39	7	0
Day 1	PEP005	Female	1.5	1	2	0.203	1	1.37	1	0
Day 1	PEP005	Female	7.5	5	3	1.57	8	4.25	3	0
Day 1	PEP005	Female	15	10	4	4.35	21	10.3	8	0
Day 28	PEP005	Male	1.5	1	2	0.0965	1	0.960	1	0
Day 28	PEP005	Male	7.5	5	3	1.12	12	1.68	2	0.03
Day 28	PEP005	Male	15	10	4	1.97	20	11.3	12	0
Day 28	PEP005	Female	1.5	1	2	0.0832	1	0.850	1	0
Day 28	PEP005	Female	7.5	5	3	0.824	10	3.66	4	0
Day 28	PEP005	Female	15	10	4	1.46	18	10.1	12	0
Day 1	(b) (4)	Male	1.5	1	2	0.0234	1	0.313	1	0
Day 1	(b) (4)	Male	7.5	5	3	0.236	10	1.46	5	0
Day 1	(b) (4)	Male	15	10	4	0.604	26	3.62	12	0
Day 1	(b) (4)	Female	1.5	1	2	0.0201	1	0.287	1	0
Day 1	(b) (4)	Female	7.5	5	3	0.214	11	1.56	5	0
Day 1	(b) (4)	Female	15	10	4	0.542	27	3.29	11	0
Occasion	Analyte	Gender	PEP005 Dose Level (ug/kg/day)	Dose ratio	Dose Group	AUC <sub>(0-∞)</sub> (ng.min/mL)	AUC ratio	C <sub>max</sub> (ng/mL)	C <sub>max</sub> ratio	T <sub>max</sub> (h)
Day 28	(b) (4)	Male	1.5	1	2	0.0489	1	0.399	1	0
Day 28	(b) (4)	Male	7.5	5	3	0.394	8	1.47	4	0
Day 28	(b) (4)	Male	15	10	4	0.899	18	4.98	12	0
Day 28	(b) (4)	Female	1.5	1	2	0.0446	1	0.375	1	0
Day 28	(b) (4)	Female	7.5	5	3	0.331	7	1.76	5	0
Day 28	(b) (4)	Female	15	10	4	0.715	16	5.18	14	0
Day 1	(b) (4)	Male	1.5	1	2	N/A	-	N/A	-	N/A
Day 1	(b) (4)	Male	7.5	5	3	N/A	-	N/A	-	N/A
Day 1	(b) (4)	Male	15	10	4	0.0216	-	0.306	-	0
Day 1	(b) (4)	Female	1.5	1	2	N/A	-	N/A	-	N/A
Day 1	(b) (4)	Female	7.5	5	3	N/A	-	N/A	-	N/A
Day 1	(b) (4)	Female	15	10	4	N/A	-	N/A	-	N/A
Day 28	(b) (4)	Male	1.5	1	2	N/A	-	N/A	-	N/A
Day 28	(b) (4)	Male	7.5	5	3	N/A	-	N/A	-	N/A
Day 28	(b) (4)	Male	15	10	4	0.0492	-	0.496	-	0
Day 28	(b) (4)	Female	1.5	1	2	N/A	-	N/A	-	N/A
Day 28	(b) (4)	Female	7.5	5	3	N/A	-	N/A	-	N/A
Day 28	(b) (4)	Female	15	10	4	0.0381	-	0.539	-	0

### Dosing Solution Analysis

Formulations prepared for dosing on Days 1, 7, 8, 15, 22, and 29 were analyzed to determine achieved concentration. Results were within the target range on Days 1 and 7 (90% to 110% of nominal). However on Day 8 the results for the low and middle dose groups were outside the target range as were all formulations on Days 15, 22 and 29. On all occasions the results were lower than expected with the lowest group mean

value being 74% of nominal concentration (Day 29 low dose group). Test article was not found in the control samples.

### 10.7. PEP005: 3 Day repeat dermal toxicity study in minipigs followed by a 28 day observation period

Study no.: 516774  
Study report location: (b) (4)  
Conducting laboratory and location: (b) (4)  
Date of study initiation: August 30, 2010  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: PEP005 Gel, Batch 7088/002; the drug substance from the batch PEP005~TOX-MIX27JUL10 was spiked with impurities at known concentrations: PEP005 83.0%, (b) (4)

### Key Study Findings

There were no treatment-related effects on systemic clinical signs, body weights, food consumption, ophthalmoscopy examinations, hematology, clinical chemistry, urinalysis, and organ weights. Very slight to moderate erythema and very slight to severe edema were observed at the treatment sites in males and females commencing 24 hours after the first application of 0.02% PEP005 Gel, with increasing incidence and severity up to Day 4 (approximately 24 hours after the final application). Dermal reactions observed were slightly more severe in females than males. During the 28 day recovery period, erythema was observed up to Day 9 (males) and edema up to Day 20 (males), with mild scabbing observed up to the end of recovery period (Day 32). Following 3-day topical treatment, minimal to marked dermatitis, often ulcerative in nature, was present at 0.02% PEP005 Gel-treated sites in all males and in almost all females. Minimal to moderate epidermal hyperplasia was also noted in almost all 0.02% PEP005 Gel-treated sites. At the end of recovery period, only minimal to mild scabs or minimal crusting was present at the PEP005 Gel-treated sites of one male and both females. No PEP005, (b) (4) was detected in any blood samples.

## Methods

Doses: Vehicle and 0.02% PEP005 Gel (0 and 500 µg/day PEP005 (b) (4))

Frequency of dosing: Once daily

Route of administration: Topical

Dose volume: 625 µL/site (25 cm<sup>2</sup>)

Formulation/Vehicle: Not provided, pH 3.5

Species/Strain: Gottingen A/S minipigs

Number/Sex/Group: 5/sex/group

Age: 5 - 6 months

Weight: Males 8.7 - 12.0 kg; females 10.6 - 12.4 kg

Satellite groups: None.

Unique study design: Two groups of 5 male and 5 females minipigs were topically administered 625 µL Vehicle Gel or 0.02% PEP005 Gel to 4 independent treatment sites (2500 mm<sup>2</sup>) on the dorsal flanks. In addition, each animal had a fifth site that was untreated and served as control. The doses, 0 and 0.05 µg/mm<sup>2</sup>/day (at a maximum exposure of 0 and 500 µg/day), were administered for 3 consecutive days. At the completion of dosing, animals were killed approximately 24 h (3 males/3 females per group) or after a 28 day observation period (2 males/2 females per group) after the final treatment.

Deviation from study protocol: No significant deviations affect the conclusions.

## Observations and Results

### Mortality

Observed twice daily. No deaths occurred.

### Clinical Signs

All animals were observed at regular intervals throughout each day for any evidence of ill health or reaction to treatment. The condition of all treatment sites were examined for erythema, eschar and edema before and at approximately 1, 2, and 24 hours after topical treatment. There were no treatment-related clinical signs associated with PEP005 other than those observed at the treatment sites. Very slight to moderate erythema and every slight to severe edema were observed at the treatment sites in males and females commencing 24 hours after the first application of 0.02% PEP005 Gel, with increasing incidence and severity up to Day 4 (approximately 24 hours after the final application). A cumulative summary of erythema and edema scores observed 24 hours after each dosing is presented in the next table. Dermal reactions observed were slightly more severe in females than males. Other observations included scabbing and red spots at the treatment sites. During the 28 day recovery period, erythema was

observed up to Day 9 (males) and edema up to Day 20 (males), with mild scabbing observed up to the end of recovery period (Day 32).

Summary of Dermal Observations over the 3 day Treatment Period  
(Cumulative Score (+24 h) through 24 h Post-Final Dose)

	Group 1 (0 µg/mm <sup>2</sup> )		Group 2 (0.05 µg/mm <sup>2</sup> )	
	Males	Females	Males	Females
<b>Number of Sites Dosed/ Total Number of Sites</b>	60/60	60/60	60/60	60/60
<b>Erythem/Eschar</b>				
None	60	60	13	11
Very Slight	0	0	29	22
Slight	0	0	13	20
Moderate	0	0	5	7
Severe or Eschar Formation	0	0	0	0
<b>Oedema</b>				
None	60	60	35	21
Very Slight	0	0	11	13
Slight	0	0	10	25
Moderate	0	0	3	0
Severe	0	0	1	1

### Body Weights

Recorded weekly. There were no treatment-related effects.

### Feed Consumption

Recorded daily. There were no treatment-related effects.

### Ophthalmoscopy

Performed on all animals pre-treatment, on Day 2 of treatment, and during Week 4 of recovery. There were no treatment-related effects.

### ECG

NA

### Hematology

Blood samples were collected pre-treatment, on Day 3 of treatment, and during Week 4 of recovery. There were no treatment-related effects.

### Clinical Chemistry

Blood samples were collected from all animals pre-treatment, on Day 3 of treatment, and during Week 4 of recovery. There were no treatment-related effects.

## Urinalysis

Urine samples were collected from all animals pre-treatment, on Day 2 of treatment, and during Week 4 of recovery. There were no treatment-related effects.

## Gross Pathology

Scab formation was present at all 0.02% PEP005 Gel-treated sites in all males and almost all females at the end of treatment period. In the recovery group animals, scab formation was present at three 0.02% PEP005 Gel-treated sites in one male and all sites of both females.

## Organ Weights

The following organs were weighed at scheduled necropsies: adrenal, brain, epididymis, heart, kidney, liver, lung, ovaries, pituitary, pancreas, prostate, spleen, testes, thyroid, thymus, and uterus. There were no treatment-related effects.

## Histopathology

A battery of tissues were collected and microscopically examined by a veterinary pathologist and an internal peer review was undertaken. At 24 hours following the final topical treatment, minimal to marked dermatitis, often ulcerative in nature, was present at 0.02% PEP005 Gel-treated sites in all males and in almost all females. Minimal to moderate epidermal hyperplasia was also noted in almost all 0.02% PEP005 Gel-treated sites. At the end of recovery period, only minimal to mild scabs or minimal crusting was present at the PEP005 Gel-treated sites of one male and both females.

## Toxicokinetics

Blood samples were collected from all animals at pre-dosing, 30 minutes, 1, 2, 4, 8, 12, and 24 hours post-dosing on Day 3. Blood concentrations of PEP005, (b) (4) (b) (4) in all animals receiving vehicle or 0.02% PEP005 Gel were below the lower limit of quantitation (LLOQ) of 0.1 ng/mL.

## Dosing Solution Analysis

The recovered concentration was within range (93% of nominal). PEP005 was not detected in the Vehicle Gel. The amount of (b) (4) found in the spiked formulation was 10.71% expressed as a percentage of the total area of (b) (4) and PEP005 peaks. PEP005 was 0.0180% - 0.0183% and (b) (4) was 0.0020% in the test article, 0.02% PEP005 Gel.

## 11 Integrated Summary and Safety Evaluation

The local activity of ingenol mebutate appears to involve induction of cell necrosis. The exact mechanism of cell necrosis induced by ingenol mebutate is unknown. Only local irritation including erythema and edema at the treatment sites was noted in rats, rabbits, or minipigs after topical treatment with ingenol mebutate gel. Treatment related dermatitis, acanthosis, parakeratosis, scab formation, and ulceration/erosion were also observed. These treatment related effects increased in severity with increased dose and duration of treatment. No significant effects on the cardiovascular system, respiratory system or central nervous system were observed when rats or dogs were treated intravenously with doses less than 7.5 µg/kg ingenol mebutate.

The pharmacokinetics of ingenol mebutate were evaluated in rats and minipigs following both intravenous and topical administration, and in dogs following intravenous administration. Systemic exposure to ingenol mebutate was minimal following topical administration in animals. The dermal bioavailability was estimated to be less than 5% in rats. Only a few blood samples contained quantifiable levels of ingenol mebutate, which were only marginally above the lower limit of quantitation (0.1 ng/mL), after topical administration of ingenol mebutate gel in minipigs. In addition, the absorbed dose of [<sup>3</sup>H]-ingenol mebutate through human, minipig, SD rat, and WI rat skin was less than 3% of applied dose in vitro. These results indicate that systemic exposure to ingenol mebutate was very limited after topical exposure to ingenol mebutate gel. In addition, minimal systemic exposure to ingenol mebutate was noted in the clinical pharmacokinetic study conducted in patients with actinic keratosis (AK) with topical administration of PICATO (ingenol mebutate) Gel under maximal clinical use conditions.

The in vitro metabolism of [<sup>3</sup>H]-ingenol mebutate was evaluated after incubation with whole blood, skin (skin homogenates), or hepatocytes from SD and WI rats, dogs, minipigs, and humans for up to 180 minutes. [<sup>3</sup>H]-ingenol mebutate was relatively metabolically stable in blood and skin homogenates while extensive metabolism of [<sup>3</sup>H]-ingenol mebutate was observed in hepatocytes. Because the systemic exposure of ingenol mebutate was minimal following topical administration, little metabolism of ingenol mebutate will occur after topical treatment of AK patients with PICATO Gel. Therefore, no further characterization of the metabolites of ingenol mebutate is needed within this NDA.

No notable inhibition was observed in vitro for the co-incubations or pre-incubations of ingenol mebutate at up to 20 µM with the CYP isoforms CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4.

The general toxicity of ingenol mebutate and/or ingenol mebutate gel has been evaluated in rats (intravenous and topical for up to 6 months), rabbits (topically), and minipigs (intravenous for up to 28 days and topical for up to 9 months). The local dermal tolerance/irritation of ingenol mebutate gel was evaluated topically using concentrations up to 0.1% ingenol mebutate gel. No signs of systemic toxicity were observed in animals treated topically with repeat doses of ingenol mebutate gel greater

than will be used clinically. A wide range of dermal responses including erythema and edema were noted at the treatment sites and the responses increased in severity with increased dose and/or treatment area. The severity of dermatitis, inflammatory cell foci, dermal fibroblasts, acanthosis, scab formation, parakeratosis, and/or ulceration/erosion increased with increasing dose and/or treatment duration and the intensity of dermal irritation responses in some animals limited the number of doses. The overall level of dermal responses at the treatment sites that received ingenol mebutate gel was either reduced in severity or completely reversible during the 4- or 8-week recovery period. Dose- and frequency-dependent dermal responses in the rat and minipig were similar.

Ingenol mebutate was negative in the Ames test, the in vitro mouse lymphoma assay, and the in vivo rat micronucleus assay. Because of the nature of actinic keratosis (AK), the activity of ingenol mebutate to stimulate normal human fibroblast proliferation in vitro, and its structural similarity to known tumor promoters (e.g., TPA), the sponsor was requested to address the tumor promoting potential of ingenol mebutate. Ingenol mebutate was positive in the in vitro SHE cell transformation assay. However, this positive in the SHE cell transformation assay is not a cause for concern for the clinical conditions of use for PICATO Gel (i.e., 2 or 3 day treatment regimen). A few tumors were noted in the 6-month intravenous rat study at the high dose of 15  $\mu\text{g}/\text{kg}/\text{day}$  ingenol mebutate administered twice weekly for 6 months. It is not clear that the tumors noted in this study are treatment related because the increase in tumor incidence was very small and not statistically significant. The tumors noted in this 6 month intravenous rat toxicity study are not clinically relevant since the clinical conditions of use is topical administration of the drug product and minimal systemic exposure was noted under maximal clinical conditions of use for this topical drug product. Although AK is considered to be a chronic indication both clinically and for regulatory purposes, the proposed clinical treatment regimen of PICATO Gel will be no more than 3 topical applications. Therefore, the need for nonclinical evaluation of carcinogenicity is not necessary at this time to support this short term (3-day) topical treatment regimen. However, the need for carcinogenicity studies would be re-evaluated in the future if the clinical conditions of use should change.

The teratogenic effects of ingenol mebutate have been evaluated in the pregnant rats and rabbits by intravenous administration to achieve high systemic exposure. Intravenous treatment of up to 5  $\mu\text{g}/\text{kg}/\text{day}$  (30  $\mu\text{g}/\text{m}^2/\text{day}$ ) ingenol mebutate in pregnant rats on Days 6 - 16 of gestation did not cause developmental toxicity. Intravenous treatment of 1, 2, or 4  $\mu\text{g}/\text{kg}/\text{day}$  (12, 24, or 48  $\mu\text{g}/\text{m}^2/\text{day}$ ) ingenol mebutate on Days 6 - 18 of gestation caused increased breathing rate in pregnant rabbits. There were no treatment-related effects on maternal body weights, maternal food consumption, fetal body weights and sex ratio. However, an increase in early embryonic deaths and an increase in the number of fetuses with the jugal (malar) connected/fused to the zygomatic process of the maxilla were seen in the 4  $\mu\text{g}/\text{kg}/\text{day}$  group. An increase in the incidence of fetuses with incompletely ossified cervical vertebral arches was also seen in both 2 and 4  $\mu\text{g}/\text{kg}/\text{day}$  groups. In addition, there was a dose-related increase in the number of fetuses with variation in the origin of arteries arising directly from the aortic arch (5%, 8%, 11%, and 13% in the 0, 1, 2, and 4  $\mu\text{g}/\text{kg}/\text{day}$ , respectively).

Because a higher incidence of unilateral/bilateral rib 7 costal cartilage not attached to the sternum was seen in all ingenol mebutate treated groups (14%, 23%, 33%, and 24% in the 0, 1, 2, and 4 µg/kg/day groups), no NOAEL was established in this study. However, these treatment findings are not clinically relevant due to the minimal systemic exposure noted in AK patients following topical administration of the drug product under maximal clinical use conditions.

Conduct of a fertility and early embryonic development toxicity study and a prenatal and postnatal development toxicity study are waived due to the minimal systemic exposure noted in AK patients following topical administration of the drug product under maximal clinical use conditions.

A mouse local lymph node assay was conducted with ingenol mebutate gel. However, this assay can not be used to determine skin sensitization potential associated with ingenol mebutate gel because this topical drug product induces irritation and releases cytokines into the skin which will confound the interpretation of this study. Ingenol mebutate does not absorb light at wavelengths  $\geq 290$  nm and no phototoxic effects were noted in humans following single and multiple topical treatments of PICATO Gel, 0.01%.

There are a few impurities in the drug substance/product that are structurally closely related to ingenol mebutate. The sponsor conducted adequate nonclinical studies to qualify these impurities. The sponsor proposed a specification limit of (b) (4) for an individual impurity in the drug substance. The recommended maximum therapeutic dose of PICATO Gel 0.05% is 250 mg/day and the corresponding maximum human exposure to an individual impurity would be (b) (4) assuming 100% absorption after topical administration. This level for the impurity is less than the acceptable level (1.5 µg/day) for genotoxic or carcinogenic impurities with lifetime oral exposure. In addition, since there is minimal systemic exposure to the active after topical administration of the drug product under conditions of maximal clinical use, then the potential systemic exposure to any impurity in the drug product at a specification level of (b) (4) would be negligible. From a Pharmacology/Toxicology perspective, there are no safety concerns for the impurities contained in the drug product. The proposed impurity specifications for PICATO Gel are acceptable from a Pharmacological/Toxicological perspective.

## 12 Appendix/Attachments

None

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/s/  
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JIAQIN YAO  
11/01/2011

BARBARA A HILL  
11/01/2011  
I concur

Comments on N202833 ingenol mebutate gel  
From Abigail Jacobs, AD  
Date: Sept. 23, 2011

1. I agree that there are no outstanding pharm/tox issues and that the pregnancy category and nonclinical portions of labeling proposed by the division are appropriate
2. I have discussed various topics with the reviewer and Supervisor and my comments have been addressed.

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/s/  
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ABIGAIL C JACOBS  
11/01/2011

## PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

**NDA Number: 202833**

**Applicant: LEO Pharma A/S**

**Stamp Date: March 25, 2011**

**Drug Name: Picato (ingenol mebutate) Gel, 0.015% and 0.05%**

**NDA Type: 505b(1)**

On initial overview of the NDA application for RTF:

	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 b1 and b2 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		
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 <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR  
NDA/BLA or Supplement**

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE?**     Yes    

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

N/A

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

N/A

Jiaqin Yao May 16, 2011  
 \_\_\_\_\_  
 Reviewing Pharmacologist Date

Barbara Hill May 16, 2011  
 \_\_\_\_\_  
 Team Leader/Supervisor Date

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/s/  
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JIAQIN YAO  
05/17/2011

BARBARA A HILL  
05/17/2011