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RESEARCH**

APPLICATION NUMBER:

125553Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Date: January 30, 2015

From: Haw-Jyh Chiu, PhD
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Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)

To: File for 351(k) BLA 125553 for ZARXIO (EP2006)

Re: Approvability for Pharmacology and Toxicology

Sandoz Inc. submitted this Biologic Licensing Application (BLA) under section 351(k) of the Public Health Service Act (PHS Act) for EP2006 (Zarxio), a proposed biosimilar product to the reference product US-licensed Neupogen. Sandoz is seeking licensure of Zarxio for the same indications currently approved for US-licensed Neupogen.

US-licensed Neupogen product contains recombinant methionyl granulocyte colony stimulating factor (met-G-CSF). G-CSF is a lineage-specific colony stimulating factor. Binding of G-CSF to the G-CSF receptor on myeloid progenitor cells and mature neutrophils initiates transduction signals that lead to the proliferation and differentiation of neutrophil committed progenitor cells, increase of mature neutrophils in the blood, enhanced neutrophil function, and mobilization of CD34-positive hematopoietic stem cells. G-CSF is not species-specific and therefore the use of the rat in nonclinical studies to support this BLA was appropriate.

Sandoz conducted animal studies to evaluate the pharmacodynamics, toxicity, toxicokinetics, and local tolerance of EP2006 when compared to EU-approved Neupogen. In a pharmacology study, administration of EP2006 or EU-approved Neupogen to normal or neutropenic rats resulted in similar dose-related increases in absolute neutrophil counts. In 28-day repeat-dose toxicology studies in rats, same target organs of toxicity were identified (i.e. spleen, bone marrow, and liver) in animals treated with either EP2006 or EU-approved Neupogen. There were also no significant differences in toxicokinetic parameters. Of note, the two repeat-dose toxicology studies tested two different formulations of EP2006 – one containing L-glutamic acid and the other containing acetic acid, the buffering agents for the to be marketed product from Sandoz and EU-approved Neupogen, respectively. No significant differences in pharmacodynamics, toxicity, and toxicokinetic parameters were noted in rats treated with EP2006 in either formulation. In a local tolerance study in rabbits, no test article-related changes in erythema, edema, hematomas, pain reactions, gross pathology, or histopathology were noted. Although direct comparison of EP2006 and US-licensed Neupogen was not conducted in animals studies, analytical bridging studies comparing EP2006, US-licensed Neupogen, and EU-approved Neupogen established that all three products are similar at the

physiochemical level. Therefore, from the perspective of the nonclinical pharmacology and toxicology discipline, the data provided in the 351(k) BLA demonstrated that there are no residual uncertainties regarding the similarity of EP2006 to the US-licensed Neupogen.

The nonclinical studies were reviewed by Dr. Christopher Sheth. The nonclinical findings are summarized in the “Executive Summary” section of the BLA review. Based on the determination of similarity of Zarxio to US-licensed Neupogen, the nonclinical sections of the labeling should be comparable to those in the labeling for US-licensed Neupogen.

Recommendation: I concur with Dr. Sheth’s conclusion that the submitted pharmacology and toxicology data support the similarity of Zarxio to US-licensed Neupogen and approval of BLA 125553 for Zarxio. From the perspective of the nonclinical discipline, there are no outstanding nonclinical issues that would preclude the approval of Zarxio for the proposed indications.

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/s/

HAW-JYH CHIU
01/30/2015

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

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: BLA 125553
Supporting document/s: 1
Applicant's letter date: May 23, 2014
CDER stamp date: May 23, 2014
Product: ZARXIO (EP2006)
Indication: To decrease the incidence of infection in patients receiving myelosuppressive anti-cancer drugs
Applicant: Sandoz Inc.
Review Division: Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
Reviewer: Christopher Sheth, PhD
Supervisor/Team Leader: Haw-Jyh Chiu, PhD (Acting)
Division Director: John Leighton, PhD, DABT
Ann Farrell, MD (DHP)
Project Manager: Jessica Boehmer, MBA

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125553 are owned by Sandoz Inc. or are data for which Sandoz Inc. has obtained a written right of reference. Any information or data necessary for approval of BLA 125553 that Sandoz Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 125553.

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1 Executive Summary

1.1 Introduction

Sandoz Inc. is requesting marketing approval for EP2006, as a proposed biosimilar product to the recombinant human granulocyte-colony stimulating factor (rhG-CSF) reference product, US-licensed Neupogen (also referred in this review as Neupogen). Neupogen was approved in the US in 1991 and the current label includes indications and usage information for: cancer patients receiving myelosuppressive chemotherapy; patients with acute myeloid leukemia receiving induction or consolidation chemotherapy; cancer patients receiving bone marrow transplant; patients undergoing peripheral blood progenitor cell collection and therapy; and patients with severe chronic neutropenia.

Colony-stimulating factors are glycoproteins which act on hematopoietic cells by binding to specific cell surface receptors and stimulating proliferation, differentiation commitment, and some end-cell functional activation. According to the approved Neupogen labeling, endogenous G-CSF is a lineage specific colony-stimulating factor which is produced by monocytes, fibroblasts, and endothelial cells. G-CSF regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigens). G-CSF is not species-specific and has been shown to have minimal direct in vivo or in vitro effects on the production of hematopoietic cell types other than the neutrophil lineage.

As part of Sandoz's global development strategy for EP2006, EP2006 was compared head-to-head with EU-approved Neupogen as the comparator product in five animal studies assessing the pharmacodynamics (PD), toxicity, toxicokinetics (TK) and local tolerance of the product. A graphical representation of the development program is presented in Figure 1. The nonclinical development of EP2006 involved selecting dose levels and use of the subcutaneous (SC) route of administration to maximize the sensitivity to detect potential differences between EP2006 and EU-approved Neupogen. Analytical bridging studies (see CMC review) comparing EP2006, EU-approved Neupogen, and US-licensed Neupogen established that all three products are similar at the physiochemical level. From the perspective of nonclinical pharmacology and toxicology, there are no residual uncertainties regarding the similarity of EP2006 to the reference product.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical data submitted to the BLA demonstrate the similarity (i.e., similar PD characteristics and similar safety) of EP2006 and EU-approved Neupogen.

The PD study (EP06-004) examined the hematological response in normal and neutropenic rats for 12 days following administration of either EP2006 or Neupogen on

Days 1 to 4. In normal rats, EP2006 and EU-approved Neupogen produced similar relatively sustained dose-related increases in ANC from Days 2 to 5. Both EP2006 and EU-approved Neupogen produced similar biphasic increases in ANC in neutropenic rats with two peak responses occurring on Days 2 and 5 with lower values on Days 3 and 4.

Local tolerance study (EP06-003) was conducted to assess for potential erythema, edema, hematomas, pain reactions, gross pathology, and histopathology in rabbits administered undiluted EP2006 or EU-approved Neupogen via the SC, intravenous (IV), perivascular (PV), intraarterial (IA) and intramuscular (IM) routes. No definitive drug-related changes occurred in the local tolerance study.

The 14-day TK study (EP06-002) showed that the toxicokinetics of EP2006 and EU-approved Neupogen at levels between 20 and 500 µg/kg are relatively similar in rats following single (on Day 0) and repeated (on Day 13) subcutaneous dosing.

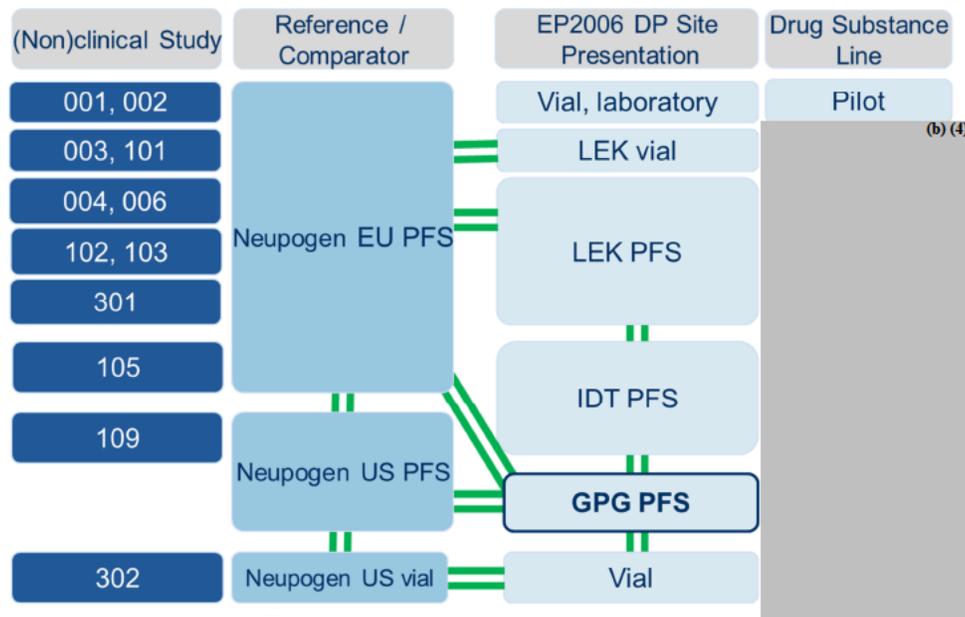
Two 28-day toxicity/TK studies (EP06-001 and EP06-006) with 6-week recovery periods were conducted in rats. Both studies were essentially identical in design, however the EP06-006 study was the most informative (pivotal) as it was conducted with the formulation of EP2006 (to be marketed in the US) containing L-glutamic acid as the buffering agent. The EP06-001 study is supportive as it was conducted with EP2006 formulated in acetic acid which is the same buffer system used in the EU-approved Neupogen comparator. It is of note that changes in the buffer systems/dilution solutions as outlined in Table 1 and Table 2 did not meaningfully affect the PD or safety signals of EP2006.

No drug-related deaths occurred in either 28-day study. Dose- and time-related increases in white blood cells, notably neutrophils, were observed in males and females treated with ≥ 20 µg/kg EP2006 or EU-approved Neupogen on Days 3, 14 and 28. Other noteworthy findings were similar between EP2006 and EU-approved Neupogen in both studies and included: swollen joints and paralysis and/or some hind leg dysfunction at 500 µg/kg; dose-related increases in alkaline phosphatase up to 9-fold greater than controls; dose-related increases in spleen weights; hyperplasia of myeloid cells, increased hematopoietic cells in bone marrow and spleen, myeloid hyperplasia in the liver (EP06-006) and riddled compacta or myelofibrosis in the femur bone (EP06-001). Exposures to rhG-CSF were similar in animals receiving equivalent dose levels of EP2006 or EU-approved Neupogen during the 28-day studies.

Figure 1 Overview of development program and comparability links

Overview development program

adapted from 3.2.R Overview analytical data to include non-clinical studies



(Excerpted from the submission)

Table 1 Drug product buffer composition of EP2006 and EU-approved Neupogen in nonclinical studies

	EP2006		EU-approved Neupogen®
Buffer	10 mM acetate	10 mM glutamate	10 mM acetate
Tonicity agent	50 mg/ml D-sorbitol	50 mg/ml D-sorbitol	50 mg/ml D-sorbitol
Surfactant	0.004% polysorbate 80	0.004% polysorbate 80	0.004% polysorbate 80
pH	4.0	4.4	4.0
EP06-001	X		X
EP06-002	X		X
EP06-003	X	X	X
EP06-004		X	X
EP06-006		X	X

(Excerpted from the submission)

Table 2 Overview on buffer systems of EP2006, EU-approved Neupogen, dilution solutions for both products and placebo controls

	EP06-001	EP06-002	EP06-003	EP06-004	EP06-006
EP2006 buffer	acetate	acetate	acetate & glutamate	glutamate	glutamate
EP2006 dilution	acetate	acetate	na	glutamate	glutamate
Neupogen [®] buffer	acetate	acetate	acetate	acetate	acetate
Neupogen [®] dilution	acetate	acetate	na	glutamate	acetate
Placebo	acetate	na	0.9 % saline	glutamate	glutamate

na = not applicable

(Excerpted from the submission)

1.3 Recommendations

1.3.1 Approvability

From the Pharmacology/Toxicology perspective EP2006 may be approved for the proposed indications.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The nonclinical sections of the label will be comparable to the label of the reference product Neupogen.

2 Drug Information

2.1 Drug

CAS Registry Number	121181-53-1
Proper Name	To be determined
Code Names used by Sandoz	EP2006, rhG-CSF, r-metHuG-CSF, "filgrastim Sandoz"
Chemical Name	N-(L-Methionyl) granulocyte colony-stimulating factor; recombinant human Granulocyte-Colony Stimulating Factor

2.1 Drug

Molecular Formula	Met-Thr-Pro-Leu-Gly-Pro-Ala-Ser-Ser-Leu- 10 Pro-Gln-Ser-Phe-Leu-Leu-Lys-Cys-Leu-Glu- 20 Gln-Val-Arg-Lys-Ile-Gln-Gly-Asp-Gly-Ala- 30 Ala-Leu-Gln-Glu-Lys-Leu-Cys-Ala-Thr-Tyr- 40 Lys-Leu-Cys-His-Pro-Glu-Glu-Leu-Val-Leu- 50 Leu-Gly-His-Ser-Leu-Gly-Ile-Pro-Trp-Ala- 60 Pro-Leu-Ser-Ser-Cys-Pro-Ser-Gln-Ala-Leu- 70 Gln-Leu-Ala-Gly-Cys-Leu-Ser-Gln-Leu-His- 80 Ser-Gly-Leu-Phe-Leu-Tyr-Gln-Gly-Leu-Leu- 90 Gln-Ala-Leu-Glu-Gly-Ile-Ser-Pro-Glu-Leu- 100 Gly-Pro-Thr-Leu-Asp-Thr-Leu-Gln-Leu-Asp- 110 Val-Ala-Asp-Phe-Ala-Thr-Thr-Ile-Trp-Gln- 120 Gln-Met-Glu-Glu-Leu-Gly-Met-Ala-Pro-Ala- 130 Leu-Gln-Pro-Thr-Gln-Gly-Ala-Met-Pro-Ala- 140 Phe-Ala-Ser-Ala-Phe-Gln-Arg-Arg-Ala-Gly- 150 Gly-Val-Leu-Val-Ala-Ser-His-Leu-Gln-Ser- 160 Phe-Leu-Glu-Val-Ser-Tyr-Arg-Val-Leu-Arg- 170 His-Leu-Ala-Gln-Pro- 175
Molecular Weight	18796.8-18800.4 (MALDI-TOF MS)
Biochemical Description	EP2006 is an <i>E.coli</i> -derived rhG-CSF with an additional N-terminal methionine and lacks an O-glycosylation at Thr133 as compared to the native human form or a cell culture-derived form. EP2006 is a non-glycosylated protein composed of 175 amino acids. The protein molecule contains five cysteines and 2 intramolecular disulfide bonds between positions Cys37-Cys43 and Cys65-Cys75.
Pharmacologic Class	Recombinant granulocyte colony-stimulating factor (G-CSF)

2.2 Relevant INDs, NDAs, BLAs and DMFs

PIND 109197 (EP2006); BLA 103353 (US-licensed Neupogen)

2.3 Drug Formulation

EP2006 will be supplied as pre-filled syringes at 300 µg/0.5 mL or 480 µg/0.8 mL; containing sterile ready for use solutions for injection intended for single applications. There are no accompanying reconstitution diluents. See tables below for composition of EP2006 300 µg/0.5 mL (Table 3) and EP2006 480 µg/0.8 mL (Table 4) solutions for injection. Sandoz compared the composition of their products side-by-side with EU-approved Neupogen (Table 5).

Table 3 Composition of EP2006 300 µg/0.5 mL solution of injection

Component	Nominal Amount per syringe (0.5 mL)	Amount per syringe (b) (4)	Function	Reference to quality standards
Active Ingredient				
Recombinant Filgrastim ¹⁾	0.30 mg ²⁾		Active substance	In house
Other Ingredients				
Glutamic acid ³⁾	0.736 mg		Excipient, buffer	Ph. Eur./USP
Sorbitol ⁴⁾	25.00 mg		Tonicity agent	Ph. Eur./USP
Polysorbate 80	0.020 mg		Surfactant	Ph. Eur./USP
Sodium hydroxide ⁵⁾	q.s.		pH adjustment	Ph. Eur./USP
Water for injections	ad 0.5 mL		Solvent	Ph. Eur./USP

¹⁾ Recombinant Filgrastim is supplied as EP2006 bulk solution containing (b) (4) rh G-CSF, 10 mM Glutamic acid, (b) (4) Sorbitol, adjusted to pH 4.4 with sodium hydroxide

²⁾ Related to a specific biological activity of 100 MU per 1 mg Filgrastim.

(b) (4)

⁵⁾ (b) (4)

⁶⁾ Including an overfill of (b) (4) to permit the withdrawal of the nominal volume of 0.5 mL.

(Excerpted from the submission)

Table 4 Composition of EP2006 480 µg/0.8 mL solution of injection

Component	Nominal amount per syringe (0.8 mL)	Amount per syringe (b) (4)	Function	Reference to quality standards
Active Ingredient				
Recombinant Filgrastim ¹⁾	0.48 mg ²⁾		Active substance	In house
Other Ingredients				
Glutamic acid ³⁾	1.178 mg		Excipient, buffer	Ph. Eur./USP
Sorbitol ⁴⁾	40.00 mg		Tonicity agent	Ph. Eur./USP
Polysorbate 80	0.032 mg		Surfactant	Ph. Eur./USP
Sodium hydroxide ⁵⁾	q.s.		pH adjustment	Ph. Eur./USP
Water for injections	ad 0.8 mL		Solvent	Ph. Eur./USP

¹⁾ Recombinant Filgrastim is supplied as EP2006 bulk solution containing (b) (4) rh G-CSF, 10 mM Glutamic acid, (b) (4) Sorbitol, adjusted to pH 4.4 with sodium hydroxide

²⁾ Related to a specific biological activity of 100 MU per 1 mg Filgrastim.

(b) (4)

⁵⁾ (b) (4)

⁶⁾ Including an overfill of (b) (4) to permit the withdrawal of the nominal volume of 0.8 mL.

(Excerpted from the submission)

Table 5 Side-by-side comparison of EP2006 and EU-approved Neupogen

Component	Amount in mg per syringe ¹⁾		Amount in mg per syringe ¹⁾		Function	Quality ²⁾
	EP2006 300 mcg/0.5 mL (EU & US strength)	Neupogen ^o 300 mcg/0.5 mL (EU comparator)	EP2006 480 mcg/0.5 mL (EU comparator)	Neupogen ^o 480 mcg/0.5 mL (EU comparator)		
Filgrastim	0.30	0.30	0.48	0.48	Active ingredient	In house
Polysorbate 80	0.02	0.02	0.02	0.02	Surfactant	Ph. Eur. /JSP ⁴⁾
Glutamic acid	0.736	-	0.736	-	Excipient, buffer	Ph. Eur. /JSP ⁴⁾
Acetate	-	0.295	-	0.295	Buffer	-
Sorbitol	25.0	25.0	25.0	25.0	Tonicity agent	Ph. Eur. /JSP ⁴⁾
Sodium hydroxide	q.s.	q.s.	q.s.	q.s.	pH adjustment	Ph. Eur. /JSP ⁴⁾
Water for injection	(b) (4)	ad 0.5 mL	(b) (4)	ad 0.5 mL	Solvent	Ph. Eur. /JSP ⁴⁾
Total	(b) (4)	-	(b) (4)	-		
Total [mL]	0.5mL ²⁾	0.5 mL	0.5mL ²⁾	0.5 mL		

¹⁾ Does not include the overfill of (b) (4)

²⁾ Density of EP 2006 300 mcg/0.5 mL and 480 mcg/0.8 mL solution for injection: (b) (4)

³⁾ Related to EP2006 products

⁴⁾ USP relevant for US strength EP2006 drug product (300 mcg/0.5 mL)

(Excerpted from the submission)

2.4 Comments on Novel Excipients

None; the excipients used are all compendial.

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

Sandoz proposes clinical populations and dosing regimens that are consistent with current US-licensed Neupogen labeling. See approved US-licensed Neupogen label for more detailed information.

2.7 Regulatory Background

BLA 125553 was submitted on May 8, 2014 for the biologic product EP2006 under Section 351(k) of the Public Health Service Act (42 U.S.C. 262(k)). A pre-IND meeting was held on September 28, 2010 to discuss the U.S. development program for EP2006. Sandoz is seeking approval for EP2006 as a proposed biosimilar product to the single reference biologic product US-licensed Neupogen. To fulfill the nonclinical requirements for a biosimilar BLA, Sandoz submitted an in vivo PD study and four in vivo toxicology studies (a local tolerance study, a 14-day TK study, and two 28-day toxicity/TK studies with 6-week recovery periods) that included assessment of the similarity of EP2006 to EU-approved Neupogen in side-by-side comparisons in the rat.

Sandoz asserts that bridging to US-licensed Neupogen was established at the physiochemical level, justifying the relevance of the nonclinical studies conducted with EU-approved Neupogen (Figure 1). An Application Orientation Meeting was held on June 11, 2014.

3 Studies Submitted

3.1 Studies Reviewed

Number	Title	Location
EP06-001	28-day subcutaneous toxicity study of EP2006 (filgrastim Sandoz) in the rat followed by a 6-week recovery period	4.2.3.2
EP06-002	14-day subcutaneous toxicokinetics study of EP2006 (filgrastim Sandoz) in the rat	4.2.3.2
EP06-003	Local tolerance test of two formulations of EP2006 (filgrastim Sandoz) and Neupogen (filgrastim) vs. saline 0.9% in the rabbit	4.2.3.6
EP06-004	Comparative pharmacodynamic study of Sandoz filgrastim (EP2006) and filgrastim (Neupogen, Amgen) following subcutaneous administration	4.2.1.1
EP06-006	28-day subcutaneous toxicity study of filgrastim Sandoz (EP2006) and filgrastim (Neupogen) in the rat followed by a 6-week recovery period	4.2.3.2

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

Sandoz submitted a comparative PD study of EP2006 (batch 00 0675011G) and EU-approved Neupogen (batch N4477AE) in normal and neutropenic rats (Study EP06-004). Male CD rats were randomly allocated to 19 study groups. In the normal rat study, five different doses of EP2006 and EU-approved Neupogen were used: 10, 20, 40, 80 or 160 µg/kg. In the neutropenic rat study three different doses of EP2006 and EU-approved Neupogen were used: 30, 60, and 100 µg/kg. Neutropenia was induced by chemotherapy using a single intraperitoneal dose of 50 mg/kg cyclophosphamide on Day 0. The placebo, EP2006 and EU-approved Neupogen were administered by SC injection in the back once daily on Days 1 to 4. Animals were monitored for local tolerance, systemic tolerance (behavior, external appearance, feces, and mortality), and for effects on body weights. The PD assessment was centered on the hematological response. Blood was collected from the retrobulbar venous plexus on Days 1 (neutropenic rats only), 2, 3, 4, 5, 6, 7, 8 and 12. The hematological evaluation included assessment of: differential blood counts (including lymphocytes, monocytes, granulocytes (neutrophils, eosinophils, and basophils)) and large unstained cells (LUC) (10^3 cells/µL); erythrocytes (RBC) (10^{12} /L); hematocrit value (%); hemoglobin content (g/L); and platelets (10^9 /L).

All normal animals treated with EP2006 or EU-approved Neupogen survived until study termination on Day 12. The unscheduled deaths that did occur included one control animal on Day 4 and two neutropenic animals on Day 12 (1 exposed to 60 µg/kg of EP2006 and 1 exposed to 30 µg/kg of EU-approved Neupogen). With regards to local tolerance, no injection site findings were observed in any of the animals. With regards to systemic tolerance, none of the normal or neutropenic animals treated with EP2006 or EU-approved Neupogen exhibited any changes in behavior or external appearance. Body weights of normal and neutropenic rats were unaffected by either treatment when compared with concurrent controls.

In normal rats, absolute neutrophil counts (ANC) increased dose-dependently and peak values for the high dose groups given 160 µg/kg of EP2006 or EU-approved Neupogen were observed on Day 5 (Figure 2 and Figure 3). These increases in neutrophils in conjunction with increases in eosinophils, basophils, LUCs and monocytes were primarily responsible for the dose-related increases in white blood cell (WBC) counts that were observed to reach maximal levels 2 to 6 days after administration. Dose-related increases in lymphocyte counts were also observed 2 to 6 days after administration. Erythrocyte parameters (RBC, hemoglobin, and hematocrit) in all groups exhibited slight and steady decreases over the treatment period; likely due to blood draws, while platelet parameters were relatively unaffected by EP2006 or EU-approved Neupogen in normal rats over the course of the study.

In neutropenic control rats maximal suppression of WBC and ANC parameters were observed around Days 3 to 4 and 4 to 5, respectively. ANC values recovered to baseline values around Day 8, whereas WBC recovered to baseline closer to Day 12. Neutropenic rats treated with either EP2006 or EU-approved Neupogen exhibited marked increases in ANC values around Days 2 to 3 and again around Days 5 to 6, which were above ANC values in non-neutropenic concurrent control rats. WBC values in neutropenic rats treated with EP2006 or EU-approved Neupogen also peaked on Days 2 and 5 but the levels did not exceed WBC values observed in the non-neutropenic controls. All other differential count responses were similar between the EP2006 and EU-approved Neupogen groups. Erythrocyte parameters and platelets exhibited slight but steady declines over the treatment period and began recovering on Days 8 and 6, respectively.

Overall, the results demonstrate that the PD effects of EP2006 are similar to those of EU-approved Neupogen in both normal (Figure 2, Figure 3, Table 6) and neutropenic rats (Figure 4, Figure 5, Table 7).

Figure 2 Number of neutrophilic granulocytes in normal rats treated with EP2006

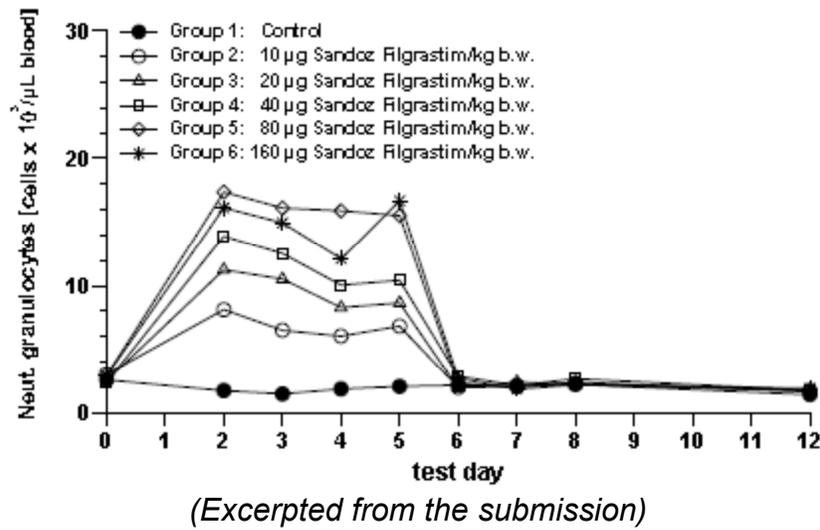


Figure 3 Number of neutrophilic granulocytes in normal rats treated with EU-approved Neupogen

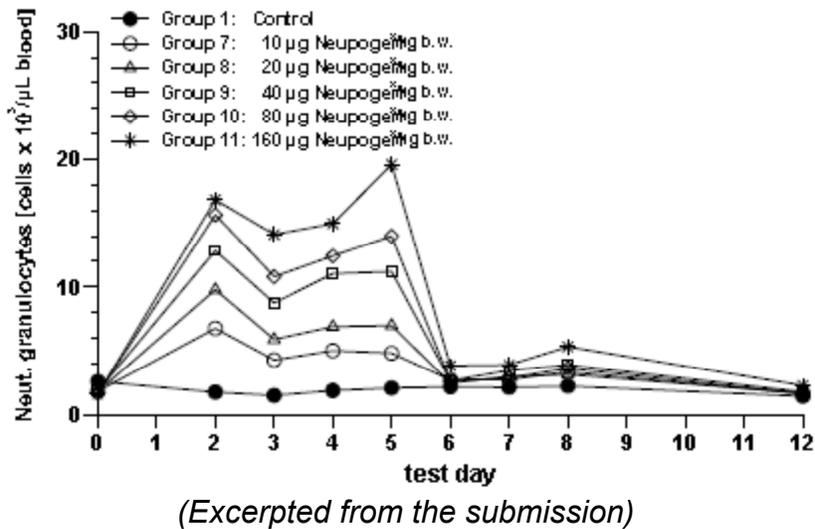


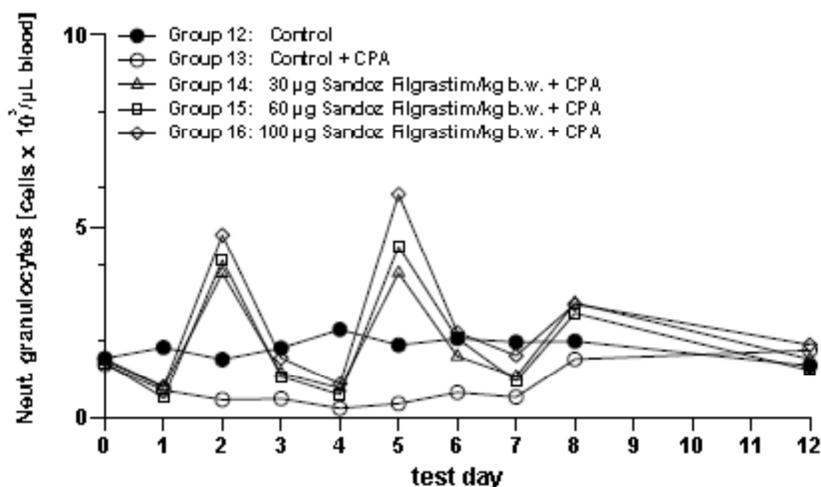
Table 6 Neutrophil count comparison between treatment groups of equal strength – normal rats

Group 1 (Test)	Group 2 (Reference)	AUEC [cells x 10 ³ / 1 * days]		E _{max} [cells x 10 ³ / l]	
		Ratio*	95% CI for ratio* of means	Ratio*	95% CI for ratio* of means
Sandoz 10 µg/kg	Neupogen [®] 10 µg/kg	1.12	0.95 - 1.33	1.28	1.08 - 1.51
Sandoz 20 µg/kg	Neupogen [®] 20 µg/kg	1.15	0.97 - 1.36	1.17	0.99 - 1.39
Sandoz 40 µg/kg	Neupogen [®] 40 µg/kg	1.02	0.86 - 1.21	1.06	0.90 - 1.25
Sandoz 80 µg/kg	Neupogen [®] 80 µg/kg	1.12	0.95 - 1.33	1.14	0.96 - 1.35
Sandoz 160 µg/kg	Neupogen [®] 160 µg/kg	0.85	0.72 - 1.01	0.94	0.80 - 1.11

*: ratio test / reference

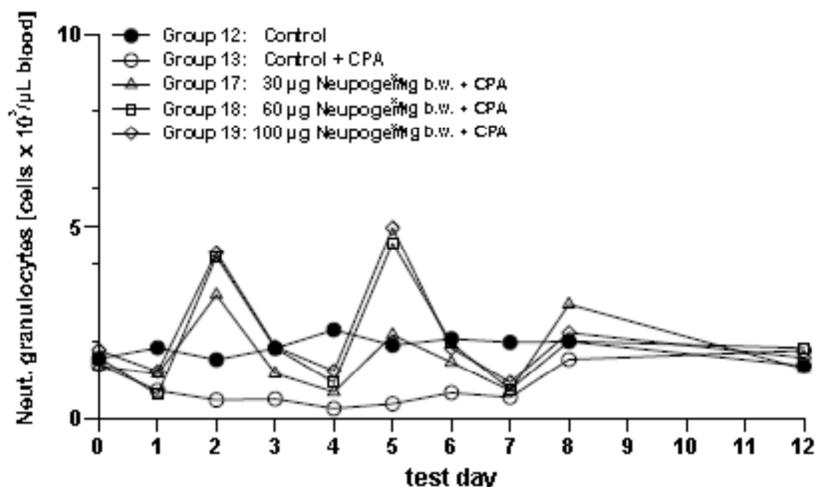
(Excerpted from the submission)

Figure 4 Number of neutrophilic granulocytes in neutropenic rats treated with EP2006



(Excerpted from the submission)

Figure 5 Number of neutrophilic granulocytes in neutropenic rats treated with EU-approved Neupogen



(Excerpted from the submission)

Table 7 Neutrophil count comparison between treatment groups of equal strength – neutropenic rats

Group 1 (Test)	Group 2 (Reference)	AUEC [cells x 10 ³ / l * days]		E _{max} [cells x 10 ³ / l]	
		Ratio*	95% CI for ratio* of means	Ratio*	95% CI for ratio* of means
Sandoz 30 μg/kg	Neupogen® 30 μg/kg	1.10	0.91 - 1.40	1.14	0.85 - 1.51
Sandoz 60 μg/kg	Neupogen® 60 μg/kg	1.00	0.80 - 1.25	0.93	0.69 - 1.24
Sandoz 100 μg/kg	Neupogen® 100 μg/kg	1.09	0.87 - 1.35	1.12	0.84 - 1.51

*: ratio test / reference

(Excerpted from the submission)

4.2 Secondary Pharmacology

No studies were submitted for review.

4.3 Safety Pharmacology

Not applicable.

5 Toxicokinetics

5.1 TK

The toxicokinetics of EP2006 were evaluated in studies EP06-001, EP06-002, and EP06-006, and are summarized below with the individual toxicology study summaries.

6 General Toxicology

6.1 Single-Dose Toxicity

The local tolerance study EP06-003 utilized single-dose administration (see Section 10).

6.2 Repeat-Dose Toxicity

Study title: 14-day subcutaneous toxicokinetics study of EP2006 (filgrastim Sandoz) in the rat

Study no.:	EP06-002
Study report location:	4.2.3.2
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	November 18, 2003
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	EP2006, batch RS21, 0.96 mg/mL EU-approved Neupogen, batch NO577AA, 0.48 mg/0.5mL

Key Study Findings

The toxicokinetics of EP2006 and EU-approved Neupogen at levels between 20 and 500 µg/kg are relatively similar in rats following single (on Day 0) and repeated (on Day 13) subcutaneous dosing.

Methods

Doses:	EP2006: 20, 100, 500 µg/kg EU-approved Neupogen: 20 and 500 µg/kg
Frequency of dosing:	Once daily for 14 days
Route of administration:	SC
Dose volume:	2 mL/kg
Formulation/Vehicle:	Acetate-formulation buffer
Species/Strain:	Wistar rat
Number/Sex/Group:	10 or 11 males/Group
Age:	Approximately 6 weeks
Weight at first dose:	132 to 169 g
Satellite groups:	None
Unique study design:	EP2006 versus EU-approved Neupogen
Deviation from study protocol:	None that affected study interpretation

Study Design

Dose groups	Number of animals	Animal numbers	Substance and concentration	Dose [ml/kg]	Dose [$\mu\text{g}/\text{kg}$ b.w.]
1	10 males	001 – 010	EP2006 / 10 $\mu\text{g}/\text{ml}$	2	20
2	10 males	011 – 020	EP2006 / 50 $\mu\text{g}/\text{ml}$	2	100
3	10 +1 males	021 – 030 +R1	EP2006 / 250 $\mu\text{g}/\text{ml}$	2	500
4	10 males	031 – 040	Neupogen [®] / 10 $\mu\text{g}/\text{ml}$	2	20
5	10 +1 males	041 – 050 +R2	Neupogen [®] / 250 $\mu\text{g}/\text{ml}$	2	500

(Excerpted from the submission)

Observations and Results

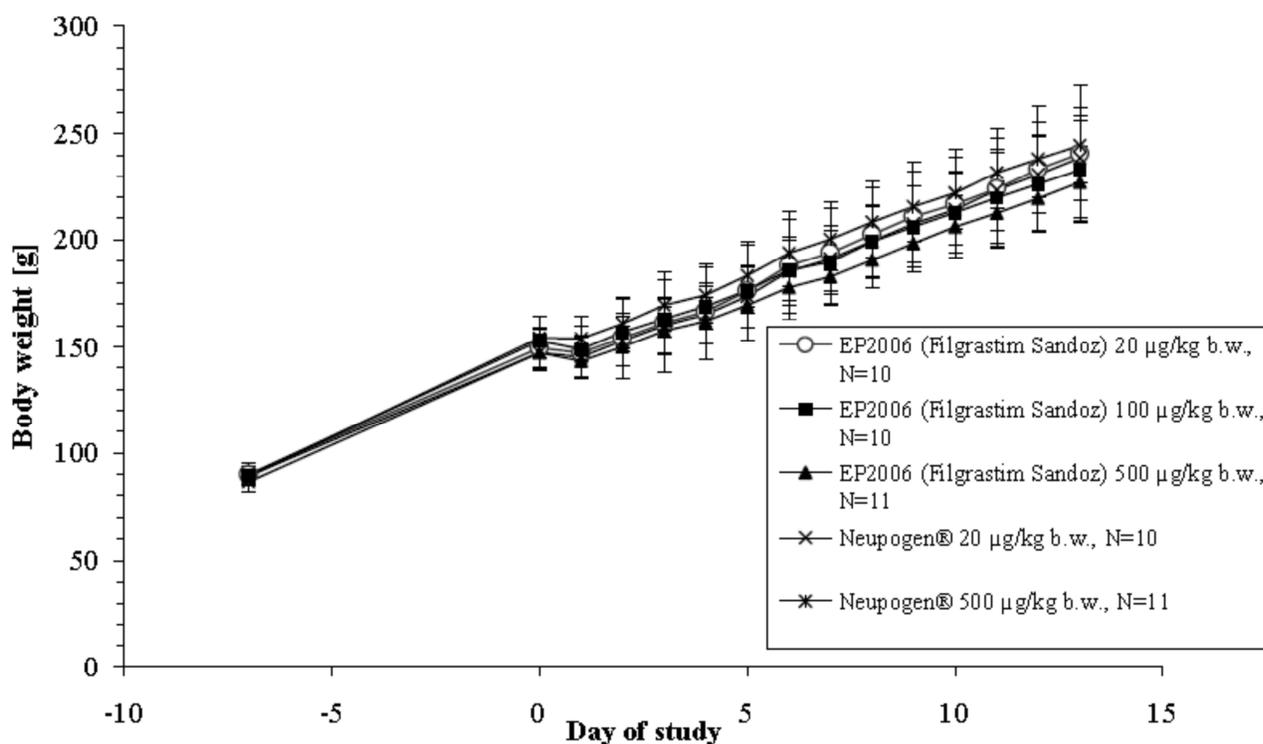
Mortality

Viability and mortality were recorded daily during the treatment period. No unscheduled deaths or signs of early mortality were observed over the treatment period.

Body Weights

Body weights were recorded once prestudy (Day -7) and daily during the treatment period (Figure 6).

Figure 6 Body weight of rats treated with SC EP2006 or EU-approved Neupogen for 14 days



(Excerpted from the submission)

Toxicokinetics

The toxicokinetics of EP2006 (20, 100 or 500 µg/kg) were compared with the toxicokinetics of EU-approved Neupogen (20 or 500 µg/kg) in male rats following single and repeated subcutaneous dosing. Blood was collected retroorbitally at 0, 0.5, 1, 3, 6, and 24 hours after the first dose (on Day 0) and 0.5, 1, 3, 6, 24, and 48 hours after the last dose (on Day 13). The concentration-time plots are shown in Figure 7 (Day 0) and Figure 8 (Day 13) and a summary of the toxicokinetic values are presented in Table 8.

- EP2006
 - C_{max}
 - Increased proportionally to increases in dose from 20 to 500 µg/kg
 - Day 13 values were -6 to 8% of Day 1 values
 - C_{max} following a single 20 µg/kg dose was 2-fold greater for EP2006 than EU-approved Neupogen
 - $AUC_{(0-t)}$
 - Increased proportionally to increases in dose from 20 to 500 µg/kg
 - Day 13 values were 6 to 20% of Day 1 values
 - $AUC_{(0-t)}$ following a single 20 µg/kg dose was 2-fold greater for EP2006 than EU-approved Neupogen
 - t_{max}
 - Increased from 1 to 3 hours following repeated dosing at 20 and 100 but not 500 µg/kg
 - $t_{1/2}$
 - Increased 9 to 41% following repeated dosing
 - Volume of distribution (Vd) and Clearance (CL)
 - Following repeated dosing, Vd increased at 20 and 500 µg/kg and decreased at 100 µg/kg
 - CL decreased slightly following repeated dosing at all dose levels
- EU-approved Neupogen
 - C_{max}
 - Increased proportionally to increase in dose from 20 to 500 µg/kg
 - Day 13 values were 15 to 82% of Day 1 values
 - $AUC_{(0-t)}$
 - Increased proportionally to increases in dose from 20 to 500 µg/kg
 - Day 13 values were 51 to 56% of Day 1 values
 - t_{max}
 - Increased from 1 to 3 hours following repeated dosing at 20 but not 500 µg/kg
 - $t_{1/2}$
 - Decreased 32% following repeated dosing at 20 µg/kg and increased 6% following repeated dosing at 500 µg/kg

- Vd and CL
 - Vd and CL values for rats receiving 20 µg/kg EU-approved Neupogen were approximately 2-fold greater than any other group

Figure 7 Mean G-CSF concentration in rat serum after a single dose of EP2006 or EU-approved Neupogen – Day 0

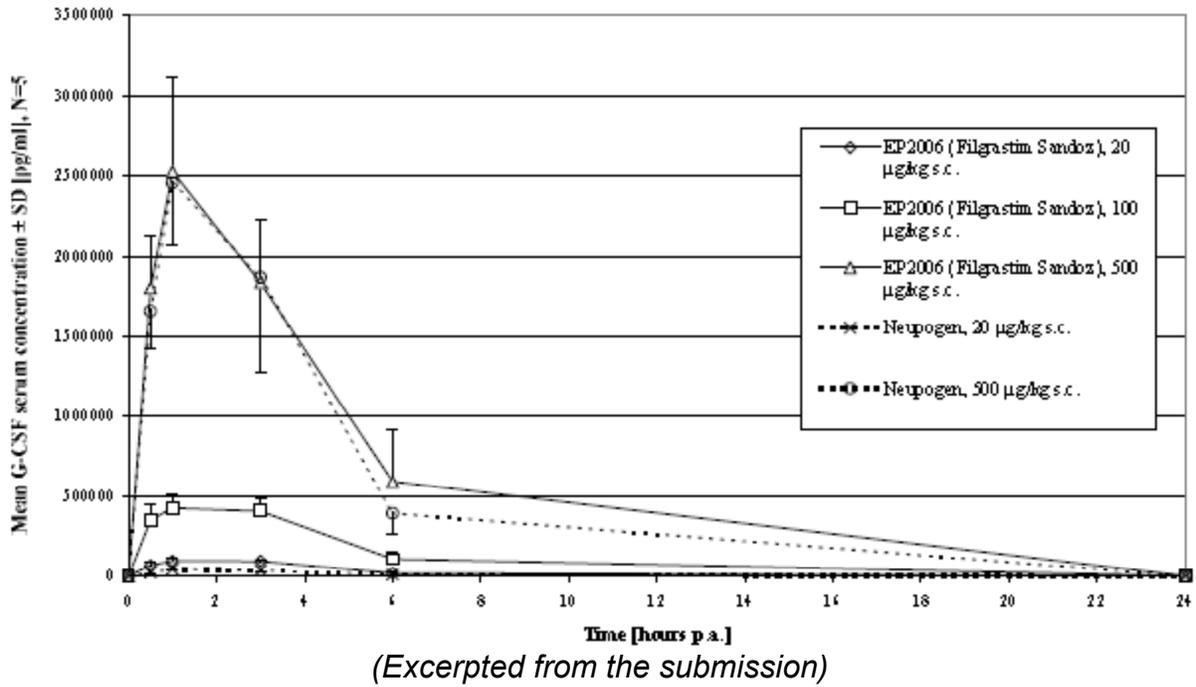
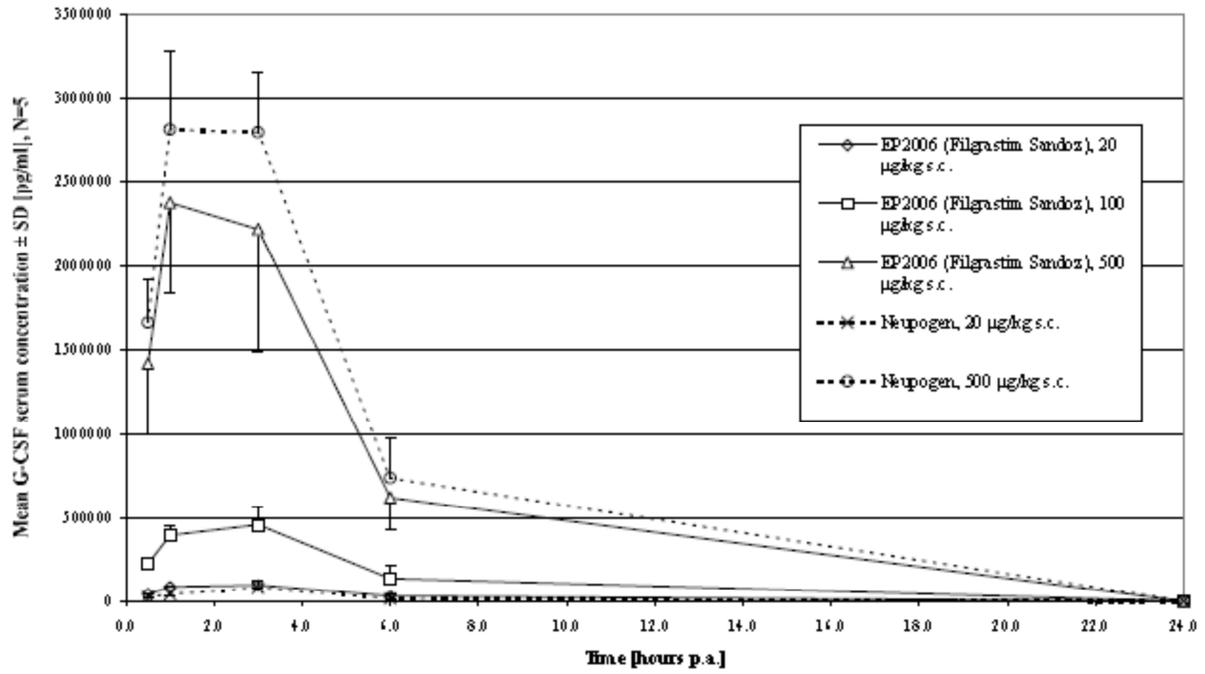


Figure 8 Mean G-CSF concentration in rat serum after repeated dosing of EP2006 or EU-approved Neupogen – Day 13



(Excerpted from the submission)

Table 8 Toxicokinetics in rats exposed to EP2006 or EU-approved Neupogen for 14 days

Study day	Group	Test article	Dose [µg/kg]	t _{1/2} [h]	C _{max} [pg/ml]	t _{max} [h]	AUC _(0-t) [pg*hr/ml]	Vd [ml]	CL(sys) [ml/h]
0	1	EP2006	20	1.28	94366	1.0	573514	64.38	34.87
13	1	EP2006	20	1.81	96545	3.0	690959	75.55	28.95
0	2	EP2006	100	1.57	424793	1.0	2847524	79.45	35.12
13	2	EP2006	100	1.71	458898	3.0	3183869	77.46	31.41
0	3	EP2006	500	1.48	2526998	1.0	14837891	72.15	33.70
13	3	EP2006	500	1.70	2378025	1.0	15787895	77.50	31.67
0	4	Neupogen®	20	1.90	43920	1.0	280973	194.97	71.18
13	4	Neupogen®	20	1.30	80426	3.0	438404	85.56	45.62
0	5	Neupogen®	500	1.54	2457831	1.0	12681229	87.74	39.43
13	5	Neupogen®	500	1.64	2817079	1.0	19129222	62.01	26.14

(Excerpted from the submission)

Dosing Solution Analysis

EP2006 was prepared in buffer to concentrations of 10, 50, and 250 µg/mL. The concentrations of freshly prepared dosing solutions were verified as was the stability of the dosing solutions following storage at -20°C for 5 months.

Study title: 28-day subcutaneous toxicity study of filgrastim Sandoz (EP2006) and filgrastim (EU-approved Neupogen) in the rat followed by a 6-week recovery period

Study no.: EP06-006
Study report location: 4 2 1 1
Conducting laboratory and location:  (b) (4)

Date of study initiation: August 30, 2007
GLP compliance: Statement included and signed
QA statement: Statement included and signed
Drug, lot #, and % purity: EP2006, batch 000675211G, 480 µg/0.5 mL
EU-approved Neupogen, batch 1001143, 480 µg/0.5 mL

Key Study Findings

- Low incidences of slight to marked swollen joints and/or paralysis of the hind legs were observed in males treated with 500 µg/kg of EP2006 or EU-approved Neupogen
- Dose- and time-related increases in white blood cells, notably neutrophils, were observed in males and females treated with ≥ 20 µg/kg EP2006 or EU-approved Neupogen on Days 3, 14 and 28
- Dose-related increases (approximately 2-fold or greater) in alkaline phosphatase were observed in males and females at doses ≥ 20 µg/kg
- Increases in spleen weights reached the level of statistical significance at doses ≥ 100 µg/kg
- Exaggerated pharmacodynamic effects of EP2006 and EU-approved Neupogen included myeloid hyperplasia with neutrophilic granulocytes in the bone marrow, liver and spleen at ≥ 20 µg/kg, increased hematopoietic cells in the spleen at ≥ 20 µg/kg, and myelofibrosis in the bone marrow at 500 µg/kg
- Exposure was similar in animals dosed with EP2006 or EU-approved Neupogen at matched dose levels

Methods

Doses: EP2006: 20, 100, 500 µg/kg
EU-approved Neupogen: 20 and 500 µg/kg

Frequency of dosing: Once daily for 28 days

Route of administration: SC

Dose volume: 2 mL/kg (0, 100 and 500 µg/kg)
1.3 mL/kg (20 µg/kg)

Formulation/Vehicle: L-glutamate buffer (EP2006),
acetate buffer (EU-approved Neupogen)

Species/Strain: Wistar rat

Number/Sex/Group: Treatment: 10/Sex/Group
Recovery: 5/Sex/Group

Age at first dosing: Approximately 5 weeks
(Males: 35 days / Females: 33 days)

Weight: Males: 120.9 to 164.5 g
Females: 126.0 to 149.1 g

Satellite groups: TK: 9/Sex/Group

Unique study design: EP2006 versus EU-approved Neupogen

Deviation from study protocol: None that affected study interpretation

Study Design

Group	Dose [$\mu\text{g}/\text{kg}$ b.w./day, s.c.]	Number and sex of animals	Rat number		
		MS + RP + SA	MS	RP	SA
1	0 (control)	10 + 5 + 9 m 10 + 5 + 9 f	1 - 10 16 - 25	11 - 15 26 - 30	171 - 179 180 - 188
2	20 Filgrastim Sandoz (low dose)	10 + 5 + 9 m 10 + 5 + 9 f	31 - 40 46 - 55	41 - 45 56 - 60	189 - 197 198 - 206
3	100 Filgrastim Sandoz (intermediate dose)	10 + 9 m 10 + 9 f	61 - 70 71 - 80	none	207 - 215 216 - 224
4	500 Filgrastim Sandoz (high dose)	10 + 5 + 9 m 10 + 5 + 9 f	81 - 90 96 - 105	91 - 95 106 - 110	225 - 233 234 - 242
5	20 Neupogen [®] (low reference item dose)	10 + 5 + 9 m 10 + 5 + 9 f	111 - 120 126 - 135	121 - 125 136 - 140	243 - 251 252 - 260
6	500 Neupogen [®] (high reference item dose)	10 + 5 + 9 m 10 + 5 + 9 f	141 - 150 156 - 165	151 - 155 166 - 170	261 - 269 270 - 278

MS: main study
 RP: recovery period
 SA: satellite animals
 m: male
 f: female

(Excerpted from the submission)

Observations and Results

Mortality

Viability and mortality were recorded twice daily Monday-Friday throughout the treatment and recovery periods. Two female TK animals died as a result of stress associated with blood withdrawal, one on Day 5 (at 100 $\mu\text{g}/\text{kg}$ EP2006) and one on Day 28 (at 20 $\mu\text{g}/\text{kg}$ EP2006).

Clinical Signs

Animals were observed twice daily throughout the treatment and recovery periods. Females were unaffected. A summary of the findings in males is presented in Table 9.

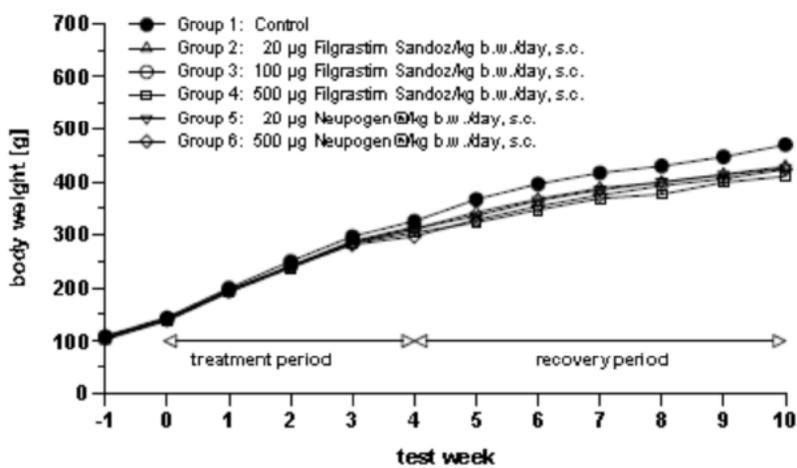
Table 9 Summary of treatment period clinical signs in males – EP06-006

Clinical signs	Number of animals affected					
	Males					
Sex						
Drug	VH	EP2006			EU-approved Neupogen	
Dose ($\mu\text{g}/\text{kg}$)		0	20	100	500	20
No. of animals examined	15	15	10	15	15	15
Observation						
Paralysis	–	–	–	–	–	3
Swollen hindlegs	–	–	–	1	–	3

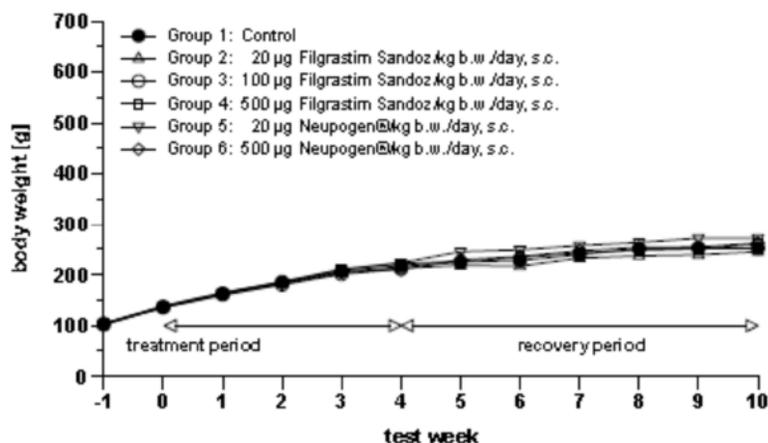
–: finding not present

Body Weights

Body weights were recorded prestudy, on the first day of treatment and weekly during the treatment and recovery periods (Figure 9 and Figure 10).

Figure 9 Body weights EP06-006 study - males

(Excerpted from the submission)

Figure 10 Body weights EP06-006 study - females

(Excerpted from the submission)

Feed Consumption

Food consumption was recorded on a weekly basis and reported in terms of daily intake for the treatments and recovery periods. Unremarkable

Ophthalmoscopy and Auditory Examination

Examinations were performed predose, at the week 4 (all main study animals) and the end of the recovery period (recovery animals). Unremarkable

Neurological Screening (Observational and Functional Tests)

Assessments were conducted once before the start of treatment and in week 4 (approximately 1 to 2 hours after dosing) in all main study animals from the control and 500 µg/kg (EP2006 and EU-approved Neupogen) groups. Unremarkable

Hematology

Blood was collected from 10 main study animals/sex/group at termination of the treatment period (Day 29) and from 5 recovery animals/sex/group at the end of the recovery period (Day 71) for standard hematological parameters. Dose-related increases in reticulocytes and decreases in platelet counts were observed. Blood was also collected from the retrobulbar venous plexus of 9 satellite animals/sex/group on Days 1, 3, 14, 28 and from 5 recovery animals/sex/group on Day 71 for differential white blood cell counts. Dose- and time-related increases in white blood cells, notably neutrophils, were observed in males and females treated with either EP2006 or EU-approved Neupogen (Table 10).

Table 10 Hematology findings - EP06-006 study

Sex	Percent change compared to control									
	Males					Females				
Drug	EP2006			EU-approved Neupogen		EP2006			EU-approved Neupogen	
Dose (µg/kg)	20	100	500	20	500	20	100	500	20	500
No. of animals	10/9/5					10/9/5				

Table 10 Hematology findings - EP06-006 study

Sex	Percent change compared to control									
	Males					Females				
Drug	EP2006			EU-approved Neupogen		EP2006			EU-approved Neupogen	
Dose (µg/kg)	20	100	500	20	500	20	100	500	20	500
No. of animals	10/9/5			10/9/5		10/9/5			10/9/5	
RET1 (% RBC)										
Day 29	↑16	↑24	↑33	↑11	↓7	↑39	↑23	↑39	↑29	↑22
PCT (10 ⁹ /L)										
Day 29	↑1	↓5	↓17	↓5	↓17	↓12	↓17	↓19	↓15	↓22
WBC (10 ⁹ /L)										
Day 3	↑49	↑51	↑120	↑52	↑142	↑23	↑74	↑101	↑38	↑128
Day 14	↑71	↑101	↑223	↑32	↑199	↑14	↑85	↑171	↑26	↑140
Day 28	↑114	↑170	↑423	↑82	↑493	↑61	↑113	↑241	↑47	↑287
Day 71	NR	NA	NR	NR	NR	NR	NA	NR	NR	NR
NEU (10 ⁹ /L)										
Day 3	↑201	↑262	↑459	↑203	↑537	↑142	↑298	↑444	↑174	↑485
Day 14	↑417	↑702	↑1362	↑248	↑1204	↑181	↑535	↑1113	↑229	↑802
Day 28	↑844	↑1302	↑3081	↑567	↑3541	↑535	↑857	↑1780	↑417	↑2139
Day 71	↑20	NA	↑16	↑33	↑7	↑20	NA	↓3	↓18	↓6
LYM (10 ⁹ /L)										
Day 3	↑18	↑7	↑48	↑20	↑58	↓1	↑29	↑32	↑11	↑53
Day 14	↑7	↓9	↑18	↓7	↑19	↓15	↑9	↑10	↓8	↑24
Day 28	↑5	↑3	↑27	↑11	↑40	↓5	↑9	↑30	↓2	↑32
Day 71	↑4	NA	↓8	↑10	↑7	↓14	NA	↑5	↑59	↑20
MONO (10 ⁹ /L)										
Day 3	↑73	↑72	↑170	↑70	↑200	↑34	↑88	↑134	↑48	↑179
Day 14	↑85	↑67	↑96	↑24	↑92	↓8	↑30	↑64	↓12	↑63
Day 28	↑83	↑79	↑166	↑55	↑208	↑57	↑64	↑135	↑4	↑142
Day 71	0	NA	↓7	↑15	↓14	↓4	NA	↑11	↑32	↓38
EOS (10 ⁹ /L)										
Day 3	↑35	↑44	↑123	↑47	↑87	↑10	↑77	↑49	↑12	↑128
Day 14	↑53	↑40	↑88	↑23	↑62	↓14	↑38	↑59	↑2	↑53
Day 28	↑88	↑71	↑143	↑41	↑155	↑51	↑81	↑84	↓12	↑135
Day 71	↓13	NA	↑6	↑27	↑14	↓4	NA	↑11	↑32	↓38
BASO (10 ⁹ /L)										
Day 3	↑47	↑53	↑174	↑63	↑221	↑5	↑73	↑114	↑23	↑159
Day 14	↑143	↑147	↑430	↑47	↑373	↑6	↑65	↑168	↑3	↑177
Day 28	↑140	↑219	↑766	↑115	↑968	↑56	↑129	↑354	↑27	↑332
Day 71	0	NA	↑27	↑27	↑13	0	NA	↑25	↑88	↑13
LUC (10 ⁹ /L)										
Day 3	↑65	↑139	↑262	↑81	↑330	↑36	↑86	↑184	↑58	↑334
Day 14	↑325	↑653	↑1346	↑224	↑1167	↑66	↑131	↑450	↑53	↑441
Day 28	↑190	↑285	↑1221	↑131	↑1322	↑108	↑196	↑354	↑31	↑377
Day 71	↓23	NA	↓25	↑17	↑10	↓9	NA	↑12	↑58	↑13

↑: increased; ↓: decreased; shaded values represent statistical significance, p<0.01; NR: not reported; NA: no animals were assigned to the recovery group at the 100 µg/kg dose level; RET1: reticulocytes; PCT: platelets; WBC: white blood cells; NEU: neutrophils; LYM: lymphocytes; MONO: monocytes; EOS: eosinophils; BASO: basophils; LUC: large unstained cells

Clinical Chemistry

Blood was also collected from the retrobulbar venous plexus of 10 main study animals/sex/group at termination of the treatment period (Day 29) and from 5 recovery animals/sex/group at the end of the recovery period (Day 71) (Table 11).

Table 11 Clinical chemistry findings - EP06-006 study

Sex	Percent change compared to control									
	Males					Females				
Drug	EP2006			EU-approved Neupogen		EP2006			EU-approved Neupogen	
Dose (µg/kg)	20	100	500	20	500	20	100	500	20	500
No. of animals	10/9/5			10/9/5		10/9/5			10/9/5	
AP (U/L)										
Day 29	↑107	↑343	↑685	↑78	↑471	↑127	↑439	↑788	↑152	↑903

↑: increased; shaded values represent statistical significance, p<0.01; AP; alkaline phosphatase

Urinalysis

Urine samples were collected over a period of 16 hours from main study animals at termination of the treatment period (Day 29) and from recovery animals at the end of the recovery period. Unremarkable

Gross Pathology

Protocol specified tissues were examined macroscopically during the scheduled sacrificed of the main study animals on Day 29 and the recovery animals on Day 71 (Table 12 and Table 13).

Table 12 Summary of macroscopic findings in males – EP06-006

Macroscopic findings		Number of animals affected					
Sex		Males					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)		0	20	100	500	20	500
No. of animals examined		10/5	10/5			10/5	
Organ	Finding						
Spleen	Day 29	0	0	5	8	1	6
	Day 71	0	0	NA	1	0	0

NA: no animals were assigned to the recovery group at the 100 µg/kg dose level; n = 10 main study animals on Day 29 and 5 recovery animals on Day 71

Table 13 Summary of macroscopic findings in females – EP06-006

Macroscopic findings		Number of animals affected					
Sex		Females					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)			20	100	500	20	500
No. of animals examined		10/5	10/5			10/5	
Organ	Finding						
Spleen							
	Day 29	0	0	2	7	0	4
	Day 71	0	0	NA	0	0	0

NA: no animals were assigned to the recovery group at the 100 µg/kg dose level; n = 10 main study animals on Day 29 and 5 recovery animals on Day 71

Organ Weights

Table 14 Organ Weights - EP06-006 study

Sex	Percent change compared to control									
	Males					Females				
	EP2006			EU-approved Neupogen		EP2006			EU-approved Neupogen	
Drug	20	100	500	20	500	20	100	500	20	500
Dose (µg/kg)	10/5			10/5		10/5			10/5	
No. of animals	10/5			10/5		10/5			10/5	
Spleen										
Day 29										
Absolute	↑37	↑97	↑184	↑31	↑165	↑19	↑65	↑146	↑23	↑120
Relative	↑39	↑98	↑193	↑30	↑174	↑20	↑72	↑141	↑27	↑135
Day 71										
Absolute	↑9	NE	↑22	↑3	↑10	↓11	NE	↑4	↑16	↑15
Relative	↑17	NA	↑36	↑12	↑21	↑2	NA	↑1	↑7	↑13

Shaded values represent statistical significance, $p < 0.01$; NA: no animals were assigned to the recovery group at the 100 µg/kg dose level; NE: not evaluated

Histopathology

Adequate Battery

Yes

Peer Review

No

Histological Findings

Table 15 Summary of microscopic findings in males – EP06-006

Microscopic findings		Number of animals affected					
Sex		Males					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)			0	20	100	500	20
No. of animals examined		10	10	10	10	10	10
Organ	Finding						
Bone marrow Sternum	Myeloid hyperplasia, neutr. gra.	0	9	NE	10	8	8
Bone marrow I (right femur)	Myeloid hyperplasia, neutr. gra.	1	10	NE	10	10	10
	Myelofibrosis	1	0	NE	7	0	6
Bone marrow II (left femur)	Myeloid hyperplasia, neutr. gra.	1	10	NE	10	10	10
	Myelofibrosis	1	1	NE	8	1	7
Epididymides	Lumen: immature spermatozoa	0	0	NE	0	0	1
	Interstitial lymphocytic infiltrate.	0	0	NE	2	1	2
Injection site	Mixed cell infiltration	0	2	NE	3	1	0
	Granulation tissue	0	0	NE	4	5	0
	Lympho-histiocytic infiltrations	0	0	NE	1	0	2
	Fibrosis	0	0	NE	1	1	0
Kidney I	Hydronephrosis	0	0	NE	0	0	2
Kidney II	Hydronephrosis	2	1	NE	1	2	2
Liver	Myeloid hyperplasia, neutr. gra.	0	8	NE	9	5	9
Lymph node (mesenteric)	Lymphoid hyperplasia	6	8	NE	8	9	7
Lymph node (submandibular)	Lymphoid hyperplasia	7	10	NE	4	10	8
Spleen	Myeloid hyperplasia, neutr. gra.	0	5	NE	10	5	9
	Hematopoietic cells	2	6	NE	10	5	7
Testis I	Atrophy of the germinal epithelium	0	0	NE	1	1	1
	Giant cells	0	0	NE	0	0	1
Testis II	Atrophy of the germinal epithelium	0	0	NE	1	1	1
	Giant cells	0	0	NE	0	0	1

Neutr. gra: neutrophilic granulocytes; NE: not examined

Table 16 Summary of microscopic findings in females – EP06-006

Microscopic findings		Number of animals affected					
Sex		Females					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)			0	20	100	500	20
No. of animals examined		10	10	10	10	10	10
Organ	Finding						
Bone marrow Sternum	Myeloid hyperplasia, neutr. gra.	0	9	NE	10	10	10
Bone marrow I (right femur)	Myeloid hyperplasia, neutr. gra.	0	10	NE	10	10	10
	Myelofibrosis	0	0	NE	0	0	1

Table 16 Summary of microscopic findings in females – EP06-006

Microscopic findings		Number of animals affected					
Sex		Females					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)			20	100	500	20	500
No. of animals examined		10	10	10	10	10	10
Organ	Finding						
Bone marrow II (left femur)	Myeloid hyperplasia, neutr. gra.	0	6	NE	9	10	10
Forestomach	Mixed cell infiltrates subepithelium	0	0	NE	0	1	2
Injection site	Granulation tissue	7	4	NE	6	3	9
Kidney I	Mineralization	10	10	NE	10	10	10
	Hydronephrosis	2	2	NE	1	2	2
Kidney II	Hydronephrosis	0	0	NE	1	1	0
Liver	Myeloid hyperplasia, neutr. gra.	0	0	NE	10	2	10
Liver (scarlet red)	Involution	0	2	NE	10	10	10
Lymph node (mesenteric)	Lymphoid hyperplasia	10	10	NE	8	8	10
Lymph node (submandibular)	Lymphoid hyperplasia	9	9	NE	7	9	10
Spleen	Myeloid hyperplasia, neutr. gra.	0	0	NE	10	3	8
	Hematopoietic cells	0	1	NE	5	3	8

Neutr. gra: neutrophilic granulocytes; NE: not examined

Special Evaluation

Bone Marrow

Bone marrow samples from the terminal (main study) and recovery sacrifices were evaluated for myeloid to erythroid (M:E) ratios. Dose-related increases in M:E ratios were observed for animals dosed with EP2006 or EU-approved Neupogen. The increases reached the level of statistical significance in main study males treated with 20 µg/kg EP2006 and those given 500 µg/kg EU-approved Neupogen and in main study females treated with 500 µg/kg EP2006 or EU-approved Neupogen.

Immunogenicity

Serum Immunoglobulins

Blood samples were taken via the retrobulbar venous plexus from 3 main study animals/sex/group at study termination on Day 29 and from 3 recovery rats/sex/group at the end of the recovery period on Day 71 for the determination of plasma IgA, IgE, IgG, and IgM concentrations. Unremarkable

Anti-rhG-CSF Antibodies

Blood samples were taken via the retrobulbar venous plexus from 8-9/satellite animals/sex/group before the first dose and on Days 7, 14 and 28, and from 5 recovery animals/sex/group before the sacrifice on Day 71 for the determination of anti-rhG-CSF antibodies. The incidence of anti-rhG-CSF antibodies was greater in EU-approved

Neupogen-treated animals compared with EP2006-treated animals at 20 and 500 µg/kg, respectively (Table 17).

Table 17 Incidence summary of anti-rhG-CSF antibody positive animals

Group	Treatment	Number of rats with specific anti-rhG-CSF antibodies	
		Males	Females
1	Placebo	0/8	0/8
2	EP2006 (20 µg/kg)	1/14	4/14
3	EP2006 (100 µg/kg)	3/8*	3/9
4	EP2006 (500 µg/kg)	4/14	1/14
5	EU-approved Neupogen (20 µg/kg)	7/14	6/14
6	EU-approved Neupogen (500 µg/kg)	8/14	9/14

* One animal died before test day 7

Toxicokinetics

Serum rhG-CSF levels were assessed in samples taken from rats treated subcutaneously with 20, 100, or 500 µg/kg of EP2006 or with 20 or 500 µg/kg of EU-approved Neupogen on Days 3, 14 and 28 of the treatment period. A summary of the toxicokinetic values are presented in Table 18.

Peak serum EP2006 and EU-approved Neupogen levels occurred at approximately 1 to 3 hours postdose.

Increases in exposure (C_{max} and $AUC_{0-24\text{ hr}}$) were roughly proportional to increases in dose, and exposure was similar in animals given the same doses levels of EP2006 or EU-approved Neupogen on Days 3, 14 and 28.

Table 18 Toxicokinetics in rats exposed to EP2006 or EU-approved Neupogen for 28 days

Dosage	Non-compartment analysis						
	Sex	C _{max} ^{#1} [ng/mL]	t _{max} ^{#1} [h]	t _{1/2} [h]	K _{el} [1/h]	AUC _{0-24h} [ng*h/mL]	AUC _{0-∞h} [ng*h/mL]
Test day 3							
20 µg Filgrastim Sandoz	m	37.0	1.0	2.7	0.3	244.2	244.7
	f	49.0	1.0	2.2	0.3	49.0	49.0
100 µg Filgrastim Sandoz	m	262.2	1.0	1.8	0.4	1563.9	1564.1
	f	241.6	0.5	1.9	0.4	1402.5	1402.7
500 µg Filgrastim Sandoz	m	1735.0	1.0	1.8	0.4	7535.8	7536.6
	f	2018.0	1.0	1.8	0.4	6820.5	6821.2
20 µg Neupogen®	m	44.0	1.0	2.1	0.3	263.7	263.8
	f	60.8	3.0	1.0	0.7	325.0	325.0
500 µg Neupogen®	m	1507.9	1.0	1.8	0.4	8138.0	8138.8
	f	1877.2	1.0	1.8	0.4	8647.3	8648.1
Test day 14							
20 µg Filgrastim Sandoz	m	38.0	3.0	1.8	0.4	267.6	267.6
	f	41.9	3.0	1.3	0.5	241.6	241.6
100 µg Filgrastim Sandoz	m	238.2	3.0	1.7	0.4	1857.3	1857.4
	f	235.5	3.0	1.7	0.4	1718.7	1718.8
500 µg Filgrastim Sandoz	m	1535.1	3.0	1.7	0.4	11241.8	11242.4
	f	1656.5	1.0	1.7	0.4	9172.7	9173.3
20 µg Neupogen®	m	32.4	3.0	2.2	0.3	263.1	263.3
	f	24.6	3.0	2.7	0.3	220.3	220.8
500 µg Neupogen®	m	1259.7	3.0	1.7	0.4	11123.6	11124.1
	f	1290.2	1.0	1.8	0.4	9655.4	9656.0
Test day 28							
20 µg Filgrastim Sandoz	m	34.2	3.0	1.9	0.4	233.0	233.0
	f	39.6	3.0	2.6	0.3	200.9	202.3
100 µg Filgrastim Sandoz	m	221.2	3.0	1.7	0.4	1861.4	1861.9
	f	235.1	3.0	1.5	0.5	1391.0	1391.0
500 µg Filgrastim Sandoz	m	1833.4	3.0	3.1	0.2	13794.7	13798.6
	f	1369.7	3.0	3.3	0.2	7504.9	7508.4
20 µg Neupogen®	m	37.1	3.0	2.2	0.3	211.9	212.4
	f	37.1	3.0	1.4	0.5	221.6	221.6
500 µg Neupogen®	m	1931.0	1.0	3.3	0.2	12630.6	12653.5
	f	1523.8	1.0	3.3	0.2	9605.9	9610.9

m: Male

f: Female

#1: Values obtained from serum analysis, all other values calculated by toxicokinetic analysis

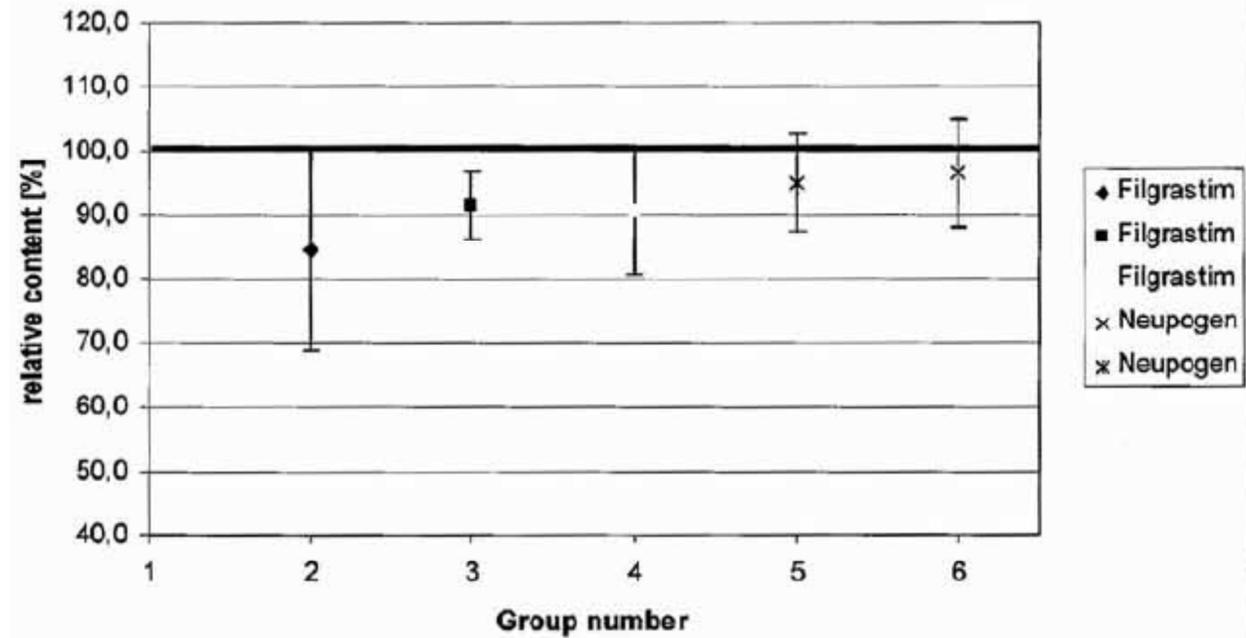
■: Shaded areas indicate differences to the results of all animals

(Excerpted from the submission)

Dosing Solution Analysis

One hundred seventy-four total samples were collected for possible analysis and 10 samples/dose group (only 9 samples from Group 4; 500 µg/kg EP2006) were randomly selected for G-CSF content analysis. The individual recoveries for each group were within the acceptance range; except for one sample from Group 2 (20 µg/kg EP2006) which is considered an outlier (Figure 11).

Figure 11 Comparison of relative content for groups 2 - 6



(Excerpted from the submission)

Study title: A 28-day subcutaneous toxicity study of EP2006 (filgrastim Sandoz) in the rat followed by a 6-week recovery period

Study no.: EP06-001
Study report location: 4.2.3.2
Conducting laboratory and location:  (b) (4)
Date of study initiation: November 13, 2003 (males)
September 30, 2003 (females)
GLP compliance: Statement included and signed
QA statement: Statement included and signed
Drug, lot #, and % purity: EP2006, batch RS21, 0.96 mg/mL
EU-approved Neupogen, batch
NO577AA, 0.48 mg/0.5mL

Key Study Findings

- Low incidences of mild to marked swollen joints and/or hind legs were observed, predominantly in animals treated with 500 µg/kg of EP2006 or EU-approved Neupogen
- Dose- and time-related increases in white blood cells, notably neutrophils, were observed in males and females treated with ≥ 20 µg/kg EP2006 or EU-approved Neupogen on Days 3, 14 and 28
- Dose-related increases (approximately 2-fold or greater) in alkaline phosphatase were observed in males and females at doses ≥ 100 µg/kg
- Increases in spleen weights reached the level of statistical significance at doses ≥ 20 µg/kg
- Microscopic findings of note included hyperplasia of lymphoid tissues, femur bone compacta riddled with small islands of bone marrow and myelofibrosis in the femur bone
- Exposure was similar in animals dosed with EP2006 or EU-approved Neupogen at matched dose levels

Methods

Doses: EP2006: 20, 100, 500 µg/kg/day
 EU-approved Neupogen: 20, 500 µg/kg/day
 Frequency of dosing: Once daily for 28 days
 Route of administration: SC
 Dose volume: 2 mL/kg
 Formulation/Vehicle: Acetate-formulation buffer
 Species/Strain: Wistar rat
 Number/Sex/Group: Treatment: 10/Sex/Group
 Recovery: 5-6/Sex/Group
 Age at study initiation: Approximately 6 weeks
 Weight at first dose: Males: 75-92 g
 Females: 70-88 g
 Satellite groups: None
 Unique study design: EP2006 versus EU-approved Neupogen
 Deviation from study protocol: None that affected study interpretation

Study Design

Groups	Treatment	Dose [µg/kg b.w.]		Number of animals	
0	Placebo	-	Control	15 males	15 females
1	EP2006	20	Low dose	15 males	15 females
2	EP2006	100	Mid dose	10 males	10 females
3	EP2006	500	High dose	15 males (+1)	15 females (+1)
4	Neupogen [®]	20	Low dose	15 males	15 females
5	Neupogen [®]	500	High dose	15 males (+1)	15 females (+1)

(Excerpted from the submission)

Observations and Results**Mortality**

Viability and mortality were recorded twice daily on treatment days and once daily during the recovery period. There were no unscheduled deaths during the treatment period (Days 0-27) or during the recovery period (Days 28-70). One (replacement) male treated with 500 µg/kg EP2006 and one given 500 µg/kg EU-approved Neupogen were removed from the study on Day 16.

Clinical Signs

Observations were recorded twice daily during the treatment period and once daily during recovery (Table 19 and Table 20).

Table 19 Summary of treatment period clinical signs in males – EP06-001

Clinical signs	Number of animals affected					
	Males					
Sex						
Drug	VH	EP2006			EU-approved Neupogen	
		20	100	500	20	500
Dose (µg/kg)	0	20	100	500	20	500
No. of animals examined	15	15	10	15	15	15
Observation						
Apathy	–	–	–	1	–	–
Marked swelling of the tongue	–	–	–	1	–	–
Mild dehydration	–	–	–	2	–	–
Dragging of one hindlimb	–	–	–	1	–	–
Dragging of both hindlimbs	–	–	–	1	–	–
Mild swelling of right hindfoot	–	–	–	2	–	1
Swelling of sole of right hindfoot	–	–	–	1	–	–
Reddened sole of foot	–	–	–	2	–	–
Mild swelling of left ankle joint	–	–	1	–	–	–
Swelling of left hindfoot	–	–	–	1	–	1
Mild swelling of left hindfoot	–	–	–	–	–	1
Mild swelling of both hindfeet	–	–	–	–	–	3
Marked swelling of both hindfeet	–	–	–	1	–	1

–: finding not present

Table 20 Summary of treatment period clinical signs in females – EP06-001

Clinical signs	Number of animals affected					
	Females					
Sex						
Drug	VH	EP2006			EU-approved Neupogen	
		20	100	500	20	500
Dose (µg/kg)	0	20	100	500	20	500
No. of animals examined	15	15	10	15	15	15
Observation						
Limping	–	–	–	–	–	1
Dragging	–	–	–	–	–	1
Dragging left hindfoot	–	–	–	1	–	–
Dragging right hindfoot	–	–	–	1	–	–
Swelling of hindfoot	–	–	–	–	1	–
Swelling of right hindfoot	–	–	–	–	–	2
Mild swelling of right hindfoot	–	–	–	–	–	1
Marked swelling of right hindfoot	–	–	–	–	–	1
Swelling of left hindfoot	–	–	–	1	1	–
Mild swelling of left hindfeet	–	–	–	1	–	–
Marked swelling of left hindfoot	–	–	–	–	–	1
Swelling of both hindfeet	–	–	–	2	1	2
Mild swelling of both hindfeet	–	–	–	–	–	1
Cyanosis of the hindfeet	–	–	–	–	–	1

–: finding not present

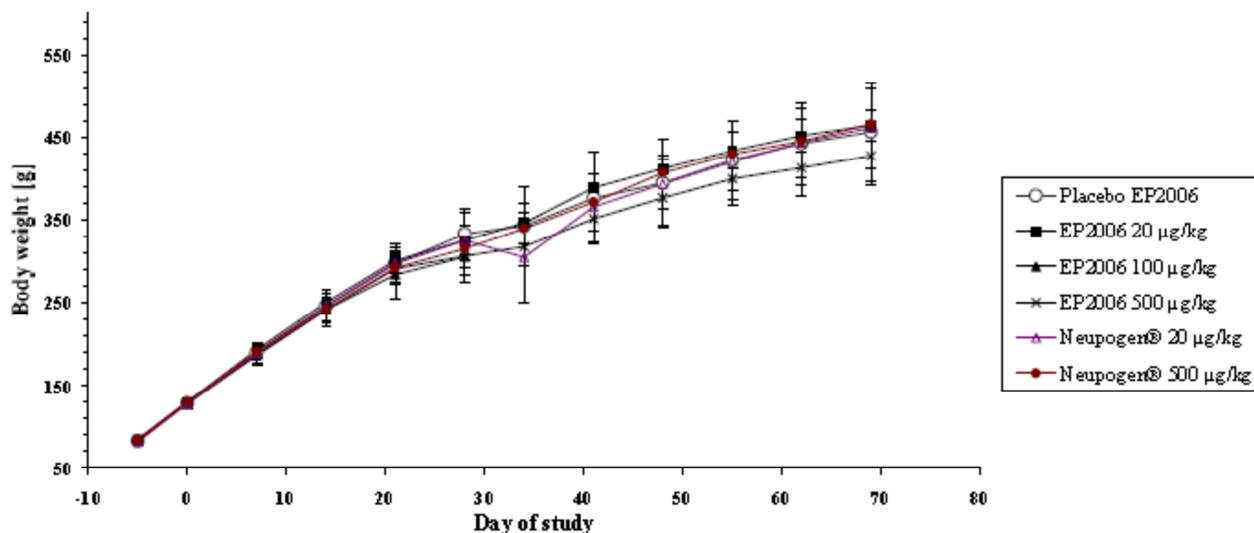
Irwin Test

A modified Irwin test was used to assess behavior and physiological function in rats treated with either 0 (control) or 500 µg/kg of EP2006 or EU-approved Neupogen once after the first treatment (Day 0) and once in the last week of treatment (Day 23). On Day 23 skin turgor was lower in males and females treated with EP2006 when compared to controls. Some females treated with 500 µg/kg of EP2006 also showed a more prominent startle response or mild paleness.

Body Weights

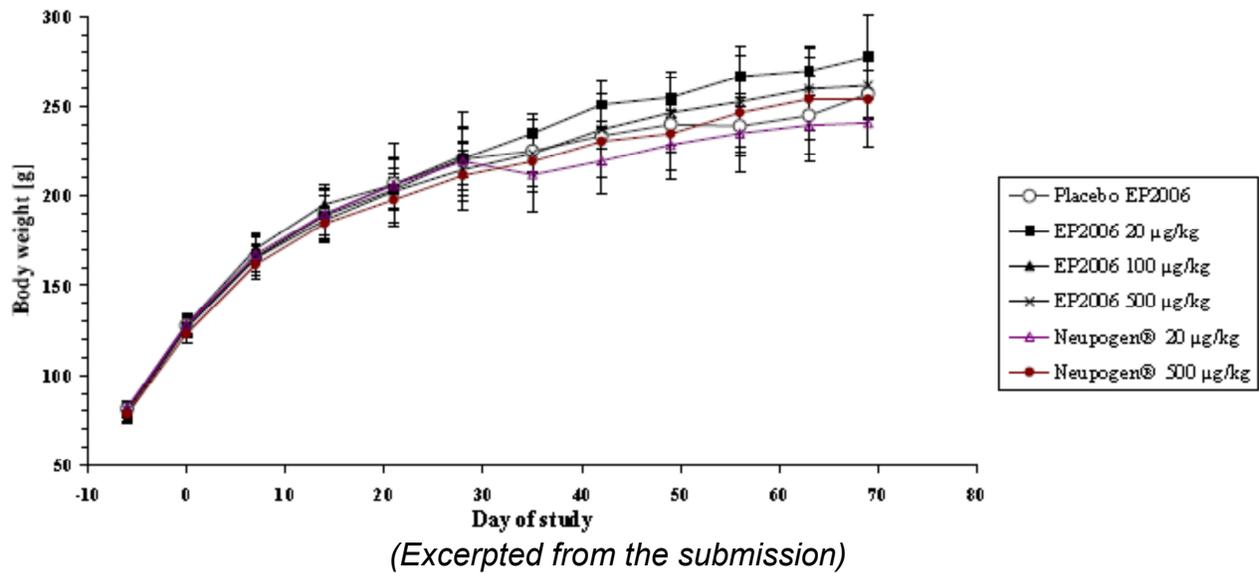
Body weights were recorded once prestudy, daily during the treatment period and twice weekly during the recovery period. The graphs below depict the weekly mean values (Figure 12 and Figure 13).

Figure 12 Body weights EP06-001 study - males



(Excerpted from the submission)

Figure 13 Body weights EP06-001 study - females



Feed Consumption

Food consumption was assessed on a weekly basis (Figure 14 and Figure 15).

Figure 14 Food consumption EP06-001 study - males

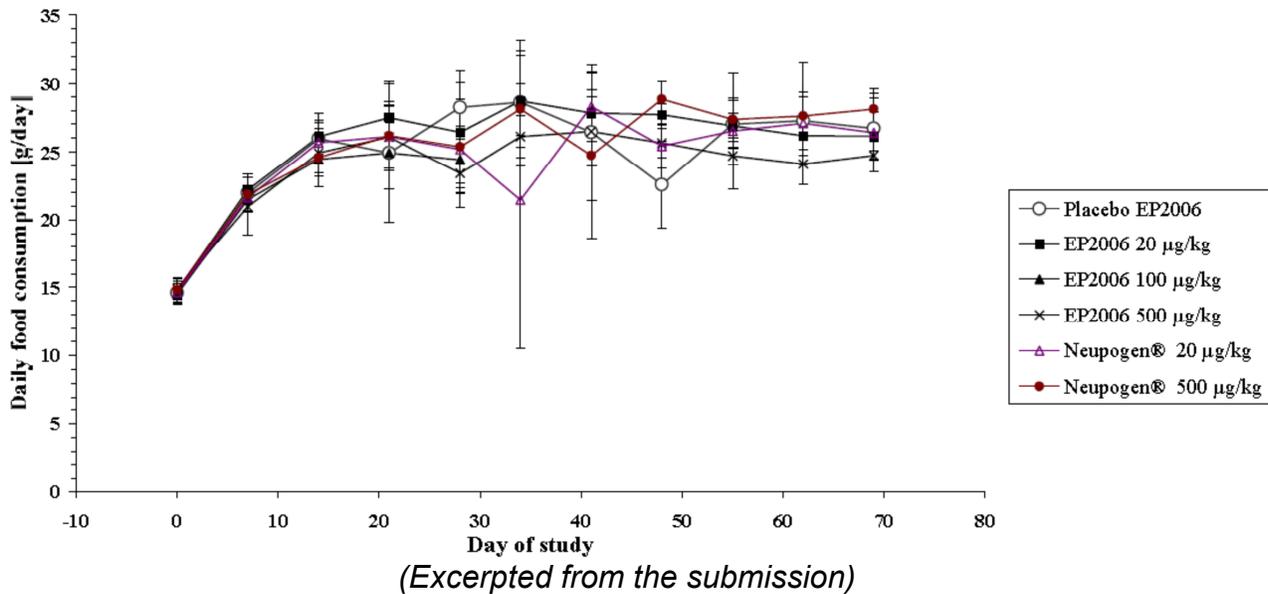
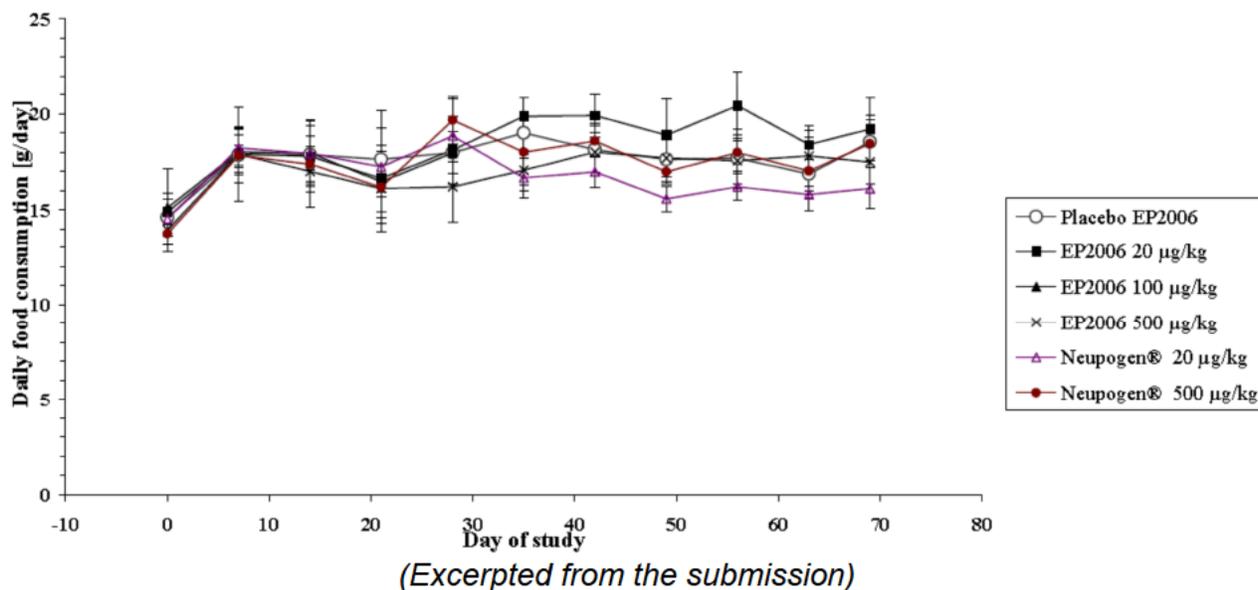


Figure 15 Food consumption EP06-001 study - females

Ophthalmoscopy

Ophthalmic assessments were carried out on Days -4 and 23. There was a single clinical finding on Day 23 of “irregularities ocular fundus” noted in a female given 500 µg/kg of EP2006.

Hematology

Blood was collected for hematology (Table 21) and clinical chemistry (Table 22) from 5 animals/sex/group on Days 3, 14, 28 (end of treatment period) and 70 (end of recovery). In general, reticulocytes were increased and platelets were decreased following treatment with EP2006 or EU-approved Neupogen without clear associations to dose level or the number of days of treatment. Dose- and time-related increases in white blood cells, notably neutrophils, were observed in males and females treated with either EP2006 or EU-approved Neupogen.

Table 21 Hematology findings - EP06-001 study

Sex	Percent change compared to control									
	Males					Females				
	EP2006			EU-approved Neupogen		EP2006			EU-approved Neupogen	
Dose (µg/kg)	20	100	500	20	500	20	100	500	20	500
No. of animals	5	5	5	5	5	5	5	5	5	5
WBC (10 ³ /µL)										
Day 3	↑26	↑19	↑26	↑10	↑2	↑9	↑16	↑32	↓4	↑43
Day 14	↑49	↑98	↑131	↑59	↑144	16	↑47	↑135	↑15	↑139
Day 28	↑56	↑74	↑269	↑61	↑310	↑16	↑94	↑193	↑42	↑139
Day 70	↓4	NC	↓15	↑2	↓24	↑5	NC	↑3	↑14	↑31

Table 21 Hematology findings - EP06-001 study

Sex	Percent change compared to control									
	Males					Females				
	EP2006			EU-approved Neupogen		EP2006			EU-approved Neupogen	
Drug	20	100	500	20	500	20	100	500	20	500
Dose ($\mu\text{g}/\text{kg}$)	20	100	500	20	500	20	100	500	20	500
No. of animals	5	5	5	5	5	5	5	5	5	5
NEU ($10^3/\mu\text{L}$)										
Day 3	↑89	↑137	↑211	↑79	↑153	↑45	↑80	↑150	↑10	↑180
Day 14	↑312	↑618	↑876	↑312	↑1035	↑200	↑431	↑1113	↑219	↑1119
Day 28	↑391	↑648	↑1826	↑326	↑1887	↑214	↑557	↑1321	↑86	↑1093
Day 70	0	NC	↓19	0	↓24	↑58	NC	↓16	↑26	↑11
LYM ($10^3/\mu\text{L}$)										
Day 3	↑15	↓9	↓16	↓2	↓29	↓1	0	↑3	↓12	↑16
Day 14	↑11	↑11	↓4	↑25	↓4	↓13	↓3	↓2	↓13	↓9
Day 28	↓4	↓28	↑6	↑12	↑58	↓10	↑31	↑39	↑31	↑10
Day 70	↓3	NC	↓18	↑2	↓19	↑4	NC	↑10	↑8	↑40
MONO ($10^3/\mu\text{L}$)										
Day 3	↑33	↑78	↑111	↑11	↑44	↑43	↑43	↑86	↑43	↑29
Day 14	↑0	↑90	↑130	↓20	↑50	↑71	↑14	↑143	0	↑86
Day 28	↑60	↑100	↑210	↑90	↑100	↑25	↑125	↑225	↑150	↑150
Day 70	↓18	NC	0	↓18	↓64	↑17	NC	0	↑17	↑17
EOS ($10^3/\mu\text{L}$)										
Day 3	↑100	↑100	↑100	0	0	0	0	↑100	0	↑100
Day 14	↑100	↑200	↑300	↑100	↑200	0	0	↑100	0	↑100
Day 28	0	0	↑150	0	↑250	↑100	↑200	↑300	↑100	↑200
Day 70	0	NC	0	0	↓50	↑11	NC	↑11	↑121	↑16
BASO ($10^3/\mu\text{L}$)										
Day 3	0	↑100	↑150	0	0	0	↑100	↑100	↑100	↑100
Day 14	0	↑100	↑150	0	↑50	↑100	0	↑200	0	↑100
Day 28 [‡]	↑400	↑600	↑1100	↑300	↑500	0.1	0.1	0.3	0.2	0.4
Day 70	0	NC	↑100	0	↓100	0	NC	0	0	0
PLT ($10^3/\mu\text{L}$)										
Day 3	↓4	↓7	↓10	↓18	↓8	↓8	↓7	↓5	↓2	↓5
Day 14	↑24	↑16	↓4	↑24	↑6	↓23	↓11	↓10	↓6	↓8
Day 28	0	↓3	↓24	↓6	↓16	↓10	↓2	↓2	↑16	0
Day 70	↓21	NC	↓3	↓5	↓9	↑3	NC	↑1	↑4	↑9
RE (%)										
Day 3	↑9	↑17	↑15	↑19	↑19	↓1	↓2	↑5	↓14	↓4
Day 14	↑12	↑15	↑20	↑17	↑28	↑67	↑47	↑73	↑60	↑100
Day 28	↑6	↓21	↑9	↑15	↓17	↑23	↑20	↑7	↑10	↓7
Day 70	↓8	NC	↓2	↑21	↓27	↑16	NC	↑8	↓9	↓7

↑: increased; ↓: decreased; shaded values represent statistical significance, $p < 0.05$; NC: not collected; WBC: white blood cells; NEU: neutrophils; LYM: lymphocytes; MONO: monocytes; EOS: eosinophils; BASO: basophils; PLT: platelets; RE: reticulocyte; ‡: female Day 28 values are absolute values as the control value was 0.

Clinical Chemistry

Table 22 Clinical chemistry findings - EP06-001 study

Sex	Percent change compared to control									
	Males					Females				
	EP2006			EU-approved Neupogen		EP2006			EU-approved Neupogen	
Drug										
Dose (µg/kg)	20	100	500	20	500	20	100	500	20	500
No. of animals	5	5	5	5	5	5	5	5	5	5
AP (U/L)										
Day 3	↑7	↓42	↓32	↓54	↓34	↑9	↑4	↑15	↓3	↑26
Day 14	↑25	↑61	↑106	↑12	↑127	↑21	↑63	↑97	↑26	↑116
Day 28	↑6	↑25	↑144	0	↑151	↑68	↑113	↑208	↑62	↑276
Day 70	↑3	NC	↑5	↓8	↑19	0	NC	↑2	↓5	↑4

↑: increased; ↓: decreased; shaded values represent statistical significance, p<0.01; NC: not collected

Urinalysis

Samples were collected from animals on Day 24 of the treatment period and Day 70 of the recovery period. The urinalysis results were unremarkable.

Gross Pathology

Protocol specified tissues were examined macroscopically during the scheduled sacrificed of the main study animals on Day 28 and the recovery animals on Day 70 (Table 23 and Table 24).

Table 23 Summary of macroscopic findings in males – EP06-001 study

Macroscopic findings		Number of animals affected					
Sex		Males					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)			0	20	100	500	20
No. of animals examined (main study/recovery)		10	10	10	10	10	10
Organ	Finding						
Dermal tissue	Mild hematoma(s) / subcutaneous hematoma	2	1	4	–	1	3
	Mild subcutaneous hemorrhage(s)	–	–	–	2	1	1
Liver	Markedly marbled	–/–	–/1	–/NC	–/1	–/–	–/1
		–	1	NC	1	–	1
Spleen	Mild white surficial patched	–	4	2	4	3	4
	Dark	–/–	–/1	–/NC	–/1	–/1	–/1
		–	1	NC	1	1	1
	Slightly-markedly enlarged	–	2	3	6	4	9
	Swelling	–	–	1	1	–	–
Axillary LN	Unilaterally slightly enlarged	–	–	–	–	–	1
Mesenteric LN	Slightly enlarged	1	–	–	1	1	1
Mandibular LN	Unilaterally slightly enlarged	–/–	1/1	–/NC	–/–	1/–	1/–
		–	1	NC	–	–	–
Hindlimbs	Swelling of joint ankles(s)	–	–	–	2	–	1
	Mild swelling of hindfoot (-feet)	–	–	–	–	–	4

Table 23 Summary of macroscopic findings in males – EP06-001 study

Macroscopic findings		Number of animals affected					
Sex		Males					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)			0	20	100	500	20
No. of animals examined (main study/recovery)		10	10	10	10	10	10
Organ	Finding						
Thymus	Red spots	-/	-/	-/	-/	-/	-/
		-	-	NC	-	-	1

-: finding not present; single values are findings on Day 28 and multiple values represent findings on Day 28/findings on Day 70; NC: not collected

Table 24 Summary of macroscopic findings in females – EP06-001 study

Macroscopic findings		Number of animals affected					
Sex		Females					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)			0	20	100	500	20
No. of animals examined (main study/recovery)		10/5	10/5	10/5	10/6	10/5	10/6
Organ	Finding						
Dermal tissue	Subcutaneous hemorrhage(s)	-	-	1	-	-	-
	Slight hematoma / mild hematoma	-	-	-	-	1	1
	White hemorrhages	-	-	-	-	-	1
Liver	White spots one lobe	1	-	-	-	-	-
	Slightly marbled	-/1	-/2	-/NC	-/2	-/1	-/1
	Yellow spots	-/	-/	-/NC	-/1	-/1	-/
	Dark red	-/	-/	-/NC	-/	-/1	-/
	Spotted	-/	-/	-/NC	-/	-/1	-/
Spleen	Slightly enlarged	-	2	1	4	3	5
	Slightly reduced in size	-/	-/	-/NC	-/	-/1	-/
	Small white cysts	-/	-/	-/NC	-/2	-/	-/
	Spleen-colored nodule juxtaposed to spleen	-/	-/1	-/NC	-/	-/	-/
	White surficial patches	-	-	2	1	2	4
Adrenals	Unilaterally reduced in size	-	-	-	1	-	-
	Slightly enlarged	-/	-/1	-/NC	-/2	-/1	-/1
Mesenteric LN	Slightly enlarged	-/1	-/	-/	1/	1/	2/
	Reddening	-	-	-	-	1	-
Mandibular LN	Unilaterally prominent / slightly enlarged	-/2	1/1	-/NC	-/1	3/1	2/1

Table 24 Summary of macroscopic findings in females – EP06-001 study

Macroscopic findings		Number of animals affected					
Sex		Females					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)			0	20	100	500	20
No. of animals examined (main study/recovery)		10/5	10/5	10/5	10/6	10/5	10/6
Organ	Finding						
	Unilaterally reddened	–/1	–/–	–/NC	–/–	–/–	–/–
Thymus	Red spots	1	–	–	–	–	–
	Slightly enlarged	–	2	2	1	–	–
	Unilaterally reddened	–	1	–	–	–	–
Salivary glands	Enlarged	–	–	–	–	–	2
Hindlimbs	Swelling hindfoot (-feet)	–	–	–	2	1	2

–: finding not present; single values are findings on Day 28 and multiple values represent findings on Day 28/findings on Day 70; NC: not collected

Table 25 Organ Weights - EP06-001 study

Sex	Percent change compared to control									
	Males					Females				
	EP2006			EU-approved Neupogen		EP2006			EU-approved Neupogen	
Dose (µg/kg)	20	100	500	20	500	20	100	500	20	500
No. of animals	5	5	5	5	5	5	5	5	5	5
Liver										
Main Study										
Absolute	↓10	↓20*	↓13**	↓14**	↓15*	↓13*	↓7	↓12*	↓6	↓17**
Relative	↓9	↓17*	↓12*	↓12*	↓12*	↓15**	↓8	↓12*	↓11*	↓19**
Recovery										
Absolute	↓2	NC	↓3	↑13	↑2	↑7	NC	↑2	↑2	↓1
Recovery	↓3	NC	↓2	↑13	↑1	↑3	NC	↑4	↑4	↓4
Spleen										
Main Study										
Absolute	↑51*	↑61**	↑130**	↑42**	↑145**	↑46	↑29**	↑62**	↑38**	↑82**
Relative	↑40	↑67	↑131	↑44	↑151	↑14	↑31**	↑63**	↑31**	↑77**
Recovery										
Absolute	↑7	NC	↑7	↑7	↑6	↑39	NC	↑17	↓2	↑6
Recovery	↑6	NC	↑8	↑8	↑4	↑33	NC	↑18	0	↑3

*/**: statistically significant, p<0.05/p<0.01; NC: not collected

Histopathology

Adequate Battery

Yes

Peer Review

No

Histological Findings

Table 26 Summary of microscopic findings in males – EP06-001

Microscopic findings		Number of animals affected					
Sex		Males					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)			0	20	100	500	20
No. of animals examined (main study/recovery)		10	10	9	10	10	10
Organ	Finding						
Kidney	Several small cysts	1	–	–	–	1	–
	Mild congestion	–	4	–	3	9	6
	Focal dilated tubuli	–/–	–/–	–/NC	–/1	–/–	–/–
	Dilation of pelvis with atrophy of the medulla	–	–	2	–	1	–
	Chronic pyelitis	–	–	1	–	–	–
	Unilaterally some small increased connective tissue fields	–	–	–	–	1	–
	Unilaterally chronic pyelonephritis with increased connective tissue or scar and chronic inflammation cell infiltrates	–/–	–/–	–/–	–/–	1/–	–/1
Adrenals	Mild congestion	–	–	–	1	–	–
Spleen	Mild hyperplasia of lymphatic nodules	–	2	–	–	–	–
	Mild congestion	–/–	3/1	6/NC	7/1	3/–	10/–
	Mild hyperplasia of the red pulpa	–	–	6	6	–	3
	At the surface small band of cell infiltrates	–	–	–	1	–	–
Small intestine	Hyperplasia lymph follicle	8/3	7/5	–/NC	10/3	7/4	8/4
	Mild dilation	–	–	2	–	2	1
Colon	Hyperplasia lymph follicle	–/–	–/1	–/NC	2/–	1/2	–/2
	Dilation	–	–	–	–	3	3
Liver	(Single/several) (small) focal cell infiltrations	1/–	1/–	1/NC	5/1	2/1	8/2
	Mild congestion	–/–	2/1	2/NC	9/1	4/1	9/–
Lung	Single small/moderate focal cell infiltrations	1	–	2	1	3	4
	Single focal cell infiltrations	–	1	–	3	1	–
	Several small focal cell infiltrations	–/–	–/1	–/NC	–/1	–/1	–/–
	Diffuse cell infiltrations	–	–	–	1	1	4
	Mild hemorrhages	–	–	–	–	–	2
	Mild congestion	–	–	–	1	–	–
	Focal congestion, small hemorrhages	–/–	–/–	–/NC	–/–	–/1	–/–
Lymph node mesenteric/mandibular	Lymphatic hyperplasia	1	1	–	4	3	1
	Hyperplasia lymph follicle	–/–	–/–	–/NC	–/–	–/–	–/1
	Sinus histiocytosis	–/2	6/3	–/NC	8/3	4/–	1/1
Skin	Small/moderate cell infiltrations in hypodermis	4	2	4	5	8	7

Table 26 Summary of microscopic findings in males – EP06-001

Microscopic findings		Number of animals affected					
Sex		Males					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)			0	20	100	500	20
No. of animals examined (main study/recovery)		10	10	9	10	10	10
Organ	Finding						
	Mild hemorrhage(s)	2	1	–	5	6	–
	Marked hemorrhage	–	–	–	–	1	–
Urinary bladder	Dilation	1/ 2	3/ –	–/ NC	3/ –	2/ –	2/ –
	Subacute inflammation	1	–	–	–	–	–
Testes	One site: partial atrophy	1	–	–	–	–	–
	One site: single degenerative seminiferous tubules	–	–	–	1	–	–
	Unilaterally reduced spermatogenesis	–	–	–	1	1	–
Epididymis	Unilaterally focal chronic inflammation	–	–	–	–	1	–
Seminal vesicles	Small focal hemorrhages	–	–	–	–	1	–
Prostate	Mild chronic inflammation	–	–	–	1	–	–
Bone femur	Compacta riddled with small islands of bone marrow	–	7	–	8	8	8
	Mild myelofibrosis near metaphysis	–	2	–	2	–	1
Bone marrow sternum	Mild/moderate granulocytic hypercellularity	–	10	–	10	10	10
Hindlimbs	Moderate cell infiltrations in the soft tissue starting from the bone structure	–	3	–	3	1	–
	Severe cell infiltrations in the surrounded soft tissue starting from bone structure	–	–	–	–	–	2

–: finding not present; single values are findings on Day 28 and multiple values represent findings on Day 28/findings on Day 70; NC: not collected

Table 27 Summary of microscopic findings in females – EP06-001

Microscopic findings		Number of animals affected					
Sex		Females					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)			0	20	100	500	20
No. of animals examined (main study/recovery)		10	10	9	10	10	10
Organ	Finding						
Kidney	Mild/moderate hyperemia	2	1	–	–	–	–
	Mild/moderate congestion	–/ 2	–/ –	–/ NC	2/ –	7/ 3	5/ 1
Adrenals	Mild congestion	–	–	–	1	–	–
Liver	Enclosed liver tissue with an extreme induration, old hemorrhages with hemosiderin deposits	–	1	–	–	–	–

Table 27 Summary of microscopic findings in females – EP06-001

Microscopic findings		Number of animals affected					
Sex		Females					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)			0	20	100	500	20
No. of animals examined (main study/recovery)		10	10	9	10	10	10
Organ	Finding						
	Necrotic structureless areas, giant cells	–	1	–	–	–	–
	Small/several focal cell infiltrates	–/3	–/2	–/NC	8/4	2/2	7/6
	Single small focal cell infiltrations	–	–	–	–	–	2
	Mild congestion	–/3	1/2	–/NC	–/4	–/3	–/6
Spleen	Mild/moderate hyperemia	5	1	–	–	–	–
	Mild/moderate congestion	–/–	–/1	1/NC	3/2	5/–	–/1
	Fibrotic tissue on the surface	–	–	1	–	–	–
	Increased granulocytic hypercellularity, cell infiltrations on the surface and fat tissue	–	–	2	–	–	–
	Granulocytic hypercellularity	–	–	–	–	–	1
	Mild increased lymphatic tissue	–	–	–	3	4	1
	Increased lymphatic tissue, surficial small deposits of lymphatic tissue	–	–	–	1	–	–
Lung	Moderate cell infiltrations	–/–	–/1	–/NC	–/–	–/–	–/–
	Mild/moderate focal cell infiltrations	4/1	1/1	1/NC	1/2	4/1	3/2
	Moderate diffuse cell infiltrations	–/–	–/1	1/–	5/–	2/–	1/–
	Moderate focal cell infiltrations partly with foam cells	–	2	–	–	–	–
	Moderate hemorrhages	–/–	–/1	–/NC	–/–	–/–	–/–
	Marked focal cell infiltrations	–	–	–	–	1	–
	Marked large cell infiltrations	–/–	–/1	–/–	–/–	–/–	–/–
	Mild focal hemorrhages	–	–	–	1	–	–
	Mild congestion	–	–	–	1	–	–
Small intestine	Hyperplasia lymph follicle	7/4	7/1	–/NC	5/5	3/3	6/4
	Dilation	–/–	–/1	–/NC	–/2	–/1	–/–
	Moderate dilation	–	–	1	5	2	1
Colon	Hyperplasia lymph follicle	–/1	–/1	–/NC	–/3	–/–	–/1
	Moderate hyperplasia lymph follicle	–	1	–	–	–	1
Skin	Mild cell infiltrations	6	6	–	6	3	–
	Moderate cell infiltrations in the hypodermis	–	–	–	–	–	2
	Mild hemorrhages	5	3	–	2	2	–
	Moderate hemorrhages in the subcutaneous space	–	–	1	–	–	–

Table 27 Summary of microscopic findings in females – EP06-001

Microscopic findings		Number of animals affected					
Sex		Females					
Drug		VH	EP2006			EU-approved Neupogen	
Dose (µg/kg)			0	20	100	500	20
No. of animals examined (main study/recovery)		10	10	9	10	10	10
Organ	Finding						
Urinary bladder	Mild interstitial cystitis	1	–	–	–	–	–
Lachrymal gland	Mild hemorrhage	–/–	–/1	–/NC	–/–	–/–	–/–
Lymph node	Mild lymphatic hyperplasia	–	3	–	9	7	5
mesenteric/mandibular	Sinus histiocytosis	–/1	–/1	–/NC	–/–	–/1	–/1
Bone femur	Compacta upper part riddled with small islands of bone marrow	–/–	–/1	–/NC	4/1	–/1	5/4
	In metaphysis upper part mild to moderate Myelofibrosis	–/–	–/–	–/–	3/–	1/–	2/1
Bone marrow sternum	Mild/moderate granulocytic hypercellularity	–	10	–	10	10	10
Thymus	Mild increased lymphatic tissue	–	–	2	5	3	–
Hindlimbs	Destruction of the bone trabecles and compacta	–	–	–	1	1	–
	Moderate Myelofibrosis	–	–	–	1	1	1
	Increased lymphatic tissue	–	–	–	–	1	–
Uterus	Moderate congestion	–	–	1	–	–	–

–: finding not present; single values are findings on Day 28 and multiple values represent findings on Day 28/findings on Day 70; NC: not collected

Special Evaluation

Immunogenicity

Serum Immunoglobulins

Blood samples were taken via the retroorbital plexus from 5 rats/sex/group on Day 28 (all groups) and on Day 70 (except for group 2) for the determination of serum IgA, IgE, IgG and IgM concentrations. No differences were noted between the placebo group and the G-CSF treated animals. The levels of IgA, IgE and IgM detected in Day 70 samples were similar to the levels detected in Day 28 samples. IgG levels were elevated in Day 70 samples compared with the levels detected in Day 28 samples.

Serum anti-rhG-CSF antibodies

Blood samples were taken via the retroorbital plexus from 5 rats/sex/group on Day 7 and 28 (all groups) and on Day 70 (except for group 2) for the determination of serum anti-rhG-CSF antibodies (Figure 16). No conclusions can be drawn from the data as anti-rhG-CSF antibodies were detected in treatment and control rats, irrespective of

drug, dose or gender. It was noted in the study report that nonspecific binding of serum antibodies to the test articles may have occurred.

Figure 16 Mean serum anti-rhG-CSF antibody levels following at 7 and 28 days of EP2006 or EU-approved Neupogen and after a 6-week recovery (Day 70)

Group	Treatment	Dose µg/kg	Anti-rhG-CSF antibody level (nU/mL)					
			Day 7		Day 28		Day 70	
			M	F	M	F	M	F
0	Placebo	–	149	Blq	176	97	991	797
1	EP2006	20	64	Blq	760	155	774	654
2	EP2006	100	Blq	98	119	133	ND	ND
3	EP2006	500	76	Blq	154	83	714	1232
4	EU- approved Neupogen	20	88	Blq	197	137	721	1081
5	EU- approved Neupogen	500	84	Blq	138	69	862	803

M: male; F: female; Blq: below limit of quantification; ND: not determined

Toxicokinetics

Serum rhG-CSF levels were assessed in samples taken from rats treated subcutaneously with 20, 100, or 500 µg/kg of EP2006 or with 20 or 500 µg/kg of EU-approved Neupogen on Day 28 of the treatment period and on Day 70 of the recovery period. The concentration-dose plots for Day 28 are shown in Figure 17 (EP2006) and Figure 18 (EU-approved Neupogen).

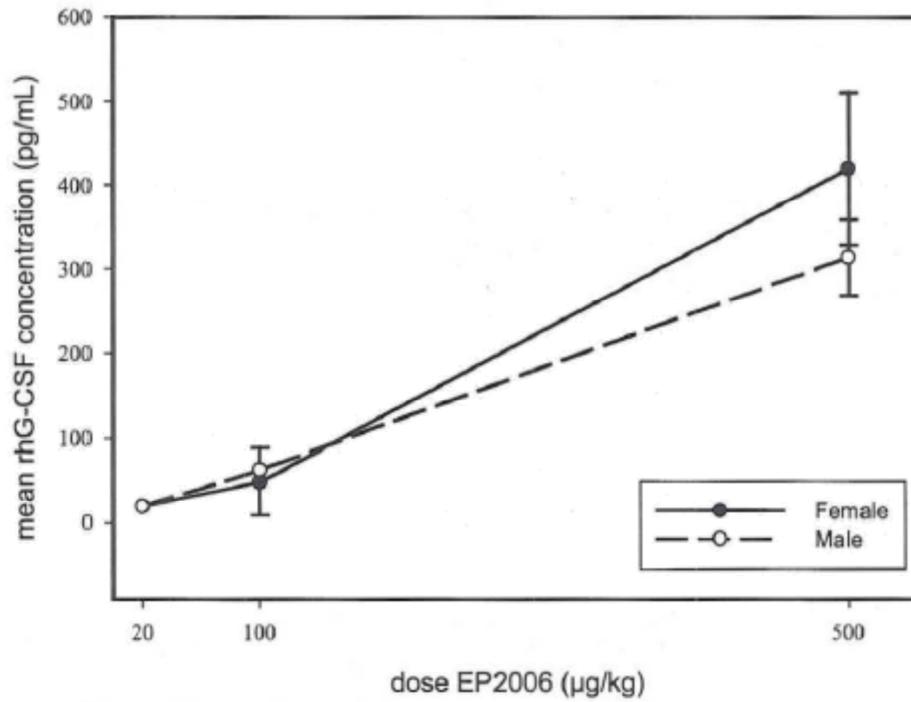
Day 28

- At the 20 µg/kg dose level:
 - Serum rhG-CSF levels were below the limit of quantification for males and females treated with either EP2006 or EU-approved Neupogen.
- At the 100 µg/kg dose level (EP2006 only):
 - Serum rhG-CSF levels increased linearly from 100 to 500 µg/kg.
- At the 500 µg/kg dose level:
 - Males given EP2006 exhibited lower mean serum rhG-CSF levels than males given EU-approved Neupogen.
 - Females exhibited similar mean serum rhG-CSF levels following exposure to either EP2006 or EU-approved Neupogen.

Day 70

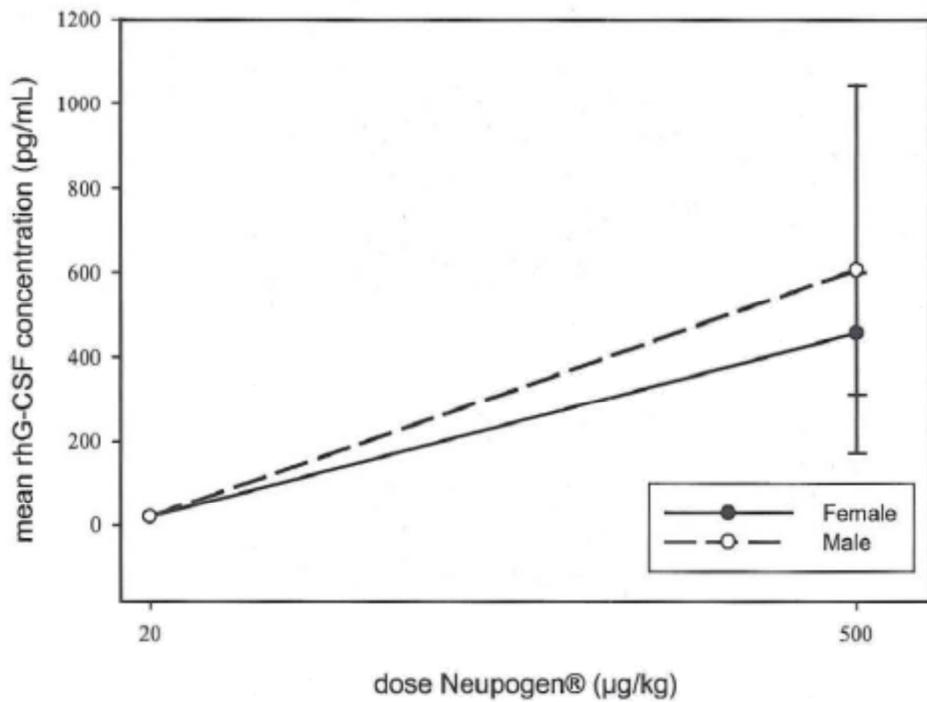
- Serum rhG-CSF levels were below the limit of quantification in all study groups.

Figure 17 Serum rhG-CSF concentrations following 28 days of EP2006



(Excerpted from the submission)

Figure 18 Serum rhG-CSF concentrations following 28 days of EU-approved Neupogen



(Excerpted from the submission)

Dosing Solution Analysis

EP2006 was prepared in buffer to concentrations of 10, 50, and 250 µg/mL. The concentrations of freshly prepared dosing solutions were verified (Table 28) as was the stability of the dosing solutions following storage at -20°C for 5 months (Table 29).

Concentration:

Table 28 Concentration of EP2006 in dosing solutions

	Results for 10 µg/ml [µg/ml]	Results for 50 µg/ml [µg/ml]	Results for 250 µg/ml [µg/ml]
First Dilution	10,83	42,9	222
Second Dilution	8,92	45,4	222
Third Dilution	9,37	43,7	223
Min	8,92	42,9	222
Max	10,83	45,4	223
Average	9,70	44,0	222

(Excerpted from the submission)

Stability:

Table 29 Percent of EP2006 remaining in dosing solutions (retained at -20°C for 5 months) compared with freshly prepared solutions

	Results for 10 µg/ml [% content fresh]	Results for 50 µg/ml [% content fresh]	Results for 250 µg/ml [% content fresh]
Freshly prepared dosing solutions			
Average	100,0	100,0	100,0
Min	92,0	97,5	100,0
Max	111,6	103,2	100,5
Dosing solutions used for toxicity study			
Average	110,3	107,7	104,5
Min	93,0	101,6	87,4
Max	125,8	114,1	118,5

(Excerpted from the submission)

7 Genetic Toxicology

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

Not applicable.

7.2 *In Vitro* Assays in Mammalian Cells

Not applicable.

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

Not applicable.

7.4 Other Genetic Toxicity Studies

Not applicable.

8 Carcinogenicity

Not applicable.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Not applicable.

9.2 Embryonic Fetal Development

Not applicable.

9.3 Prenatal and Postnatal Development

Not applicable.

10 Special Toxicology Studies

Local Tolerance

Sandoz submitted a local tolerance test of two formulations of EP2006 (batches 0304011S and 0304016S) in addition to EU-approved Neupogen (batch NO0693AB) versus saline in New Zealand White rabbits (Study EP06-003). Animals were assigned to groups as outlined in the table below. Various injection sites and routes were used to test the local tolerance of undiluted doses of 48 million International Units/0.5 mL, including the trunk (SC), ear (IV, PV, and IA) and the thigh (IM). The right sides of the animals were used for dosing with EP2006 or EU-approved Neupogen and the left sides was reserved for saline administration. Clinical observations were recorded daily over an observation period of 48 hours (groups 1, 3, 5, 7, 9 and 11) and 96 hours (groups 2, 4, 6, 8, 10 and 12) before sacrifice. Erythema and edema formation were evaluated on a scale with grades 0 to 4 (Table 30), the incidence and severity of pain reactions and hematomas were assessed, and macroscopic and microscopic observations were recorded.

Study Design

Group	No. of animals	Route of administration	Right side	Left side	Observation period
1	3	i.v., s.c.	Neupogen [®]	saline	48 h
2	3	i.v., s.c.	Neupogen [®]	saline	96 h
3	3	p.v., i.a., i.m.	Neupogen [®]	saline	48 h
4	3	p.v., i.a., i.m.	Neupogen [®] e	saline	96 h
5	3	i.v., s.c.	EP2006 (0304011S)	saline	48 h
6	3	i.v., s.c.	EP2006 (0304011S)	saline	96 h
7	3	p.v., i.a., i.m.	EP2006 (0304011S)	saline	48 h
8	3	p.v., i.a., i.m.	EP2006 (0304011S)	saline	96 h
9	3	i.v., s.c.	EP2006 (0304016S)	saline	48 h
10	3	i.v., s.c.	EP2006 (0304016S)	saline	96 h
11	3	p.v., i.a., i.m.	EP2006 (0304016S)	saline	48 h
12	3	p.v., i.a., i.m.	EP2006 (0304016S)	saline	96 h

(Excerpted from the submission)

Results

Table 30 Mean grade of erythema and edema formation in rabbits administered EP2006 of EU-approved Neupogen

Compound /Route	Test Material (Animal's Right Side)								Saline (Animal's Left Side)							
	Erythema				Edema formation				Erythema				Edema formation			
	Hours after dose				Hours after dose				Hours after dose				Hours after dose			
EP2006 (batch 0304011S)	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
IV	1.00	0.50	0	0	0.17	0	0	0	0.67	0.42	0	0	0	0.08	0	0
SC	0	0	0	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0
PV	1.92	1.42	0.67	0.33	0.58	0.42	0.33	0	1.42	1.00	1.00	0	0.17	0.08	0.17	0
IA	1.58	0.83	0.33	0	0.33	0.33	0	0	1.75	1.42	0.67	0.33	0.42	0.67	0	0.33
IM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EP2006 (batch 0304016S)	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
IV	0.42	0.17	0	0	0.08	0	0	0	0.42	0.17	0	0	0	0.17	0	0
SC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PV	1.33	0.75	0.17	0	0	0.33	0.33	0	0.92	0.67	0.17	0	0	0.33	0	0
IA	0	0.33	0.17	0	0	0	0	0	1.08	0.75	0	0	0	0.17	0	0
IM	0	0	0	0	0	0	0	0	0	0	0	0	0.17	0	0	0
EU-approved Neupogen (batch NO0693AB)	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
IV	0.83	0.75	0	0	0.17	0.17	0	0	1.17	0.92	0.50	0.33	0.33	0.33	0	0
SC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PV	1.67	1.58	0.67	0.17	0	0.17	0	0	1.33	1	1	0.5	0	0	0	0
IA	1.5	1.67	1	0.83	0	0.17	0	0	1	1.08	0.67	0.67	0	0.25	0	0
IM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Grade of erythema: 0 (no erythema), 1 (very slight erythema: barely perceptible), 2 (well defined erythema), 3 (moderate to severe erythema), 4 (severe erythema (beef redness) to eschar formation);
 grade of edema: 0 (no edema), 1 (very slight edema), 2 (slight edema: edges of the area well defined by raising), 3 (moderate edema: edges raised approximately 1 mm), 4 (severe edema: edges raised by more than 1 mm and beyond the area of exposure)

Table 31 Incidence of microscopic findings in local tolerance study

Route		Sacrificed after 48 hours					Sacrificed after 96 hours				
		IV	SC	PV	IA	IM	IV	SC	PV	IA	IM
No. of animals		3	3	3	3	3	3	3	3	3	3
Findings											
Saline (control used with EP2006 batch 0304016S)											
Ear	Very slight / focal hemorrhages	1	-	1	1	-	-	-	-	3	-
	Slight hemorrhages	-	-	1	1	-	-	-	2	-	-
	Small thrombus	1	-	-	-	-	-	-	-	-	-
	Very slight serous inflammation around vessel	-	-	-	2	-	-	-	-	2	-
	Starting fibrosis	-	-	-	-	-	-	-	-	1	-
Skin	Slight subcutaneous hemorrhages	-	-	-	-	-	-	1	-	-	-
	Moderate subcutaneous hemorrhages	-	-	-	-	-	-	-	-	-	2
//											
EU-approved Neupogen											
Ear	Very slight hemorrhages	2	-	-	1	-	-	-	-	1	-
	Slight hemorrhages	-	-	-	1	-	-	-	-	-	-
	Very slight serous inflammation	-	-	-	2	-	-	-	-	1	-
Saline (control used with EU-approved Neupogen)											
Skin	Very slight hemorrhages	-	-	-	3	-	-	-	-	-	-
	Very slight serous inflammation	-	-	-	2	-	-	-	-	1	-
	Slight serous inflammation	-	-	-	-	-	-	-	-	1	-

-: finding not present

11 Integrated Summary and Safety Evaluation

See Executive Summary

12 Appendix/Attachments

None

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

/s/

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01/30/2015

HAW-JYH CHIU
01/30/2015