



Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center of Biologics Evaluation and Research

Pharmacology/Toxicology Review
Final BLA Memorandum
Division of Hematology
Office of Blood Research & Review

To: File BLA 125506/0/0 (cross-reference: IND 14235)

Reviewer: M. Keith Wyatt, PhD, Pharmacologist, CBER/OBRR/DH

Through: Anne M. Pilaro, PhD, Supervisory Toxicologist, CBER/OBRR/DH
Basil Golding, MD, Division Director, CBER/OBRR/DH

Applicant: Bio Products Laboratories, Inc. (BPL)

Product: Plasma-derived Coagulation Factor X (Human, REPLAFAC[®]TEN)

Purpose: Final discipline review of results from nonclinical studies to support licensure of Factor X for the treatment of hereditary Factor X deficiency

Date received: July 15, 2013

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EXECUTIVE SUMMARY

This memorandum is the final primary review of the nonclinical pharmacology and toxicology results submitted in the original Biological License Application (BLA) for Bio Product Laboratories' (BPL) Coagulation Factor X (REPLAFAC[®]TEN) for the treatment of bleeding episodes, and prevention of bleeding during surgery in patients with hereditary Factor X deficiency. From the Pharmacology/Toxicology (PT) discipline's perspective, this original BLA STN 125506/0/0 is recommended for approval.

Recommendation:

The Pharmacology/Toxicology discipline recommends approval of Coagulation Factor X (REPLAFAC[®]TEN), BLA 125506/0/0. The submitted nonclinical studies and resulting data are adequate to establish the desired pharmacologic and pro-coagulant activity of Factor X. Moreover, the results support the safe use of the Applicant's Factor X for the treatment of bleeding episodes, and the prevention of bleeding during surgery in patients with hereditary Factor X deficiency.

Additional Nonclinical Recommendations and Letter-ready Comments to the Applicant:

There are no recommendations for additional post-marketing nonclinical studies, no complete response comments, and no advice or information requests to be relayed to the Applicant with the Approval Letter.

Labeling recommendation:

Recommendations for revisions to the language in the nonclinical sections of the product's labeling (i.e., Sections 8.1, 13, and 13.1) have been incorporated into the draft version of the labeling, and are pending review by the Applicant. A brief summary of the Applicant's original proposed language, the FDA suggested revisions and justification for the changes is provided as follows:

Applicant's proposed labeling for Section 8**8. USE IN SPECIFIC POPULATIONS****8.1 Pregnancy**

Pregnancy Category C. Animal reproduction studies have not been conducted with Replafacten. It is not known whether Replafacten can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. Replafacten should be administered to pregnant women only if clinically needed.

FDA recommended changes to the Applicant's proposed labeling:

8.1 Pregnancy

Pregnancy Category C. Animal reproduction studies have not been conducted with REPLAFAC[®]TEN. It is also not known whether REPLAFAC[®]TEN can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. REPLAFAC[®]TEN should be given to a pregnant woman only if clearly needed.

Justification: FDA revised the wording to be consistent with the specific language for Pregnancy Category C, as provided in 21 CFR 201.57.

Applicant's proposed labeling for Section 13

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Human plasma coagulation factor X (as contained in REPLAFAC[®]TEN) is a normal constituent of the human plasma and acts in the same way as the endogenous factor X.

Single dose toxicity studies in rats established a no-observed-effect-level of >2400 IU/kg body weight, a greater than 40 fold safety margin. Repeat dose toxicity studies in rats, with repeated administration every 2 days, established a no-observed-effect level at 30 IU/kg body weight. The no-observed-adverse-effect- level was above the highest dose in the study (360 IU/kg body weight), a greater than 6 fold safety margin.

Thrombogenicity testing in rabbits showed that the thrombogenicity of REPLAFAC[®]TEN at doses of 100-400 IU/kg body weight was not significantly different to that of the physiological saline negative control.

Local tolerance studies established that REPLAFAC[®]TEN had good tolerability when administered by the intravenous route at a dose of 600 IU/kg body weight (10 fold safety margin). A slightly less but still fully acceptable tolerability was found following paravenous injections at 211-370 IU/kg body weight (3.5-6.2 fold safety margin). Visible local erythema, oedema and tissue inflammation were present when administered by intra-arterial, or inadvertently by peri-arterial, injections at a dose of 600 IU/kg body weight (10 fold safety margin). These were tolerable with no evidence of ongoing damage. Visible local signs had resolved by day 8.

FDA recommended changes to the Applicant's proposed labeling:

13. NONCLINICAL TOXICOLOGY

[delete the Applicant's language]

13.1. Carcinogenesis, Mutagenesis, Impairment of Fertility

Nonclinical studies evaluating the carcinogenic and mutagenic potential of REPLAFAC[®]TEN have not been conducted. REPLAFAC[®]TEN has not been evaluated in animal fertility studies, and it is not known whether it can affect fertility or sperm development in patients with hereditary Factor X deficiency.

Justification: The Applicant's proposed labeling language at the beginning of Section 13 was deleted based on recommendations in FDA Guidance for Industry entitled *Labeling for Human Prescription Drug and Biological Products-Implementing the PLR Content and Format Requirements*. The FDA guidance recommends only including language in the labeling that describes unique toxicities identified during animal testing, which were not observed or reported during the clinical trial. The statements in 13.1 regarding the lack of nonclinical carcinogenicity and mutagenicity testing have been revised for simplicity and conciseness, and to be consistent with the language used for labeling of other biotherapeutic protein products.

Summary of Key Finding and Synopsis of Results:

There are no outstanding or substantive nonclinical issues preventing approval of BLA 125506/0/0 for plasma-derived Coagulation Factor X (Human, REPLAFAC[®]TEN) and its intended indications. Additionally, there are no Pharmacology/Toxicology post-marketing commitments or requirements that have been identified. There are also no comments or outstanding information requests related to the nonclinical program to be conveyed to the Applicant at the time the product is approved.

General Review Conclusions:

Plasma-derived Coagulation Factor X (Human, REPLAFAC[®]TEN) was determined to be safe for its intended use for the treatment of bleeding episodes, and prevention of bleeding during surgery in patients with hereditary deficiency of coagulation Factor X. The determination that REPLAFAC[®]TEN is safe for its indicated uses was based on results from nonclinical studies conducted in compliance with the Good Laboratory Practice (GLP; 21 CFR part 58) regulations and from non-GLP compliant studies, and from its use during a clinical trial within the United States conducted under IND.

Pharmacological/Toxicological Findings:

The Applicant conducted nonclinical studies which initially supported clinical trials and support the licensing approval of REPLAFAC[®]TEN. The results from these nonclinical studies clearly demonstrated the clotting activity, and predicted the reasonably safe use of Coagulation Factor X (REPLAFAC[®]TEN) for its intended indications in patients with hereditary Factor X deficiency.

The nonclinical program consisted of pharmacodynamic and toxicologic studies conducted in rabbits and in rats. The pro-coagulant activity of Factor X was assessed in vitro by spiking Factor X into Factor X-depleted human plasma and measuring the changes in prothrombin (PT) and activated partial thromboplastin times (aPTT). The potential thrombogenicity of Factor X was also assessed in rabbits using the (b) (4) assay as a venous stasis model. Results from these nonclinical studies demonstrated that Factor X clotted blood and did so without thrombogenic effects at the expected clinical dose.

The pharmacokinetic profile of Factor X was assessed at several dose levels on days 1 and 29 of a repeat-dose toxicology study in rats. The results from the PK study demonstrate that the elimination half-life ($t_{1/2}$) for Factor X was similar at both the beginning and at the end of study. This result suggests that Factor X did not accumulate following repeated administration at doses 5-fold greater than the intended clinical dose of 70 IU/kg. The results also indicate that rats were exposed to supraphysiologic doses of Factor X for the duration of the study while only exhibiting slight and clinically irrelevant toxicity. Finally, the maximum plasma concentration (C_{max}) of Factor X in rats was used to calculate a margin of safety (MoS) of 10-fold over the clinical dose, based on its comparison with the C_{max} value for Factor X measured during the Phase 3 clinical trial. The MoS of 10 further predicts the safe use of Factor X for its intended indications.

The potential acute toxicity of Factor X was also assessed in rats. One male rat administered a single dose of 2400 IU/kg Factor X exhibited poor coordination and a hunch posture, but this overt effect resolved 3 hours after dosing. Rats administered a single dose of 600 IU/kg Factor X, which is approximately 8.5-fold greater than the clinical dose, did not display any evidence of acute toxicity. No acute toxicities were observed in rats administered 60 IU/kg Factor X, which is equivalent to the expected clinical dose.

Repeat-dose toxicity studies, including assessments of immunogenicity and immunotoxicity, were also conducted in rats. The pathologic evaluation did not identify any significant findings at the highest Factor X dose, of 360 IU/kg which was 7-fold greater than the expected clinical dose. Administration of human Factor X did not appear to stimulate production of neutralizing antibodies against human Factor X, or to endogenous Factor X in rats. These results are consistent with the absence of inhibitor antibody formation reported during the Phase 3 clinical trial. Also, results from an immunotoxicity study demonstrated that primary immune responses (as measured by

increases in serum IgM levels directed against sheep red blood cells) were not impaired by the co-administration of Factor X at a dose of 360 IU/kg.

Results from a separate local tolerability study conducted in rabbits demonstrated that a single intravenous injection of 600 IU/kg Factor X, which is 8.5-fold greater than the expected clinical dose, was well-tolerated. The results from a neo-antigenicity study demonstrated that dry heat used in the viral inactivation of plasma-derived Factor X (REPLAFACTEN[®]) did not appear to cause the formation of new epitopes or antigenic sites on Factor X.

Conclusion:

The results from the nonclinical studies submitted by the Applicant establish the expected biological activity and predict the safety of plasma-derived Factor X (REPLAFACTEN[®]) for the treatment of bleeding episodes, and prevention of bleeding during surgery in patients with hereditary deficiency of coagulation Factor X.

PHARMACOLOGY/TOXICOLOGY REVIEW

Introduction:

Bio Products Laboratory (BPL, the Applicant) has developed plasma-derived Factor X (referred to as Factor X, REPLAFAC[®]TEN) for the treatment of the rare, autosomal recessive, bleeding disorder hereditary Factor X deficiency. The disease is estimated to occur in 1:1,000,000 individuals and exhibits a carrier frequency of 1:500. Based on the incidence of the disease, Factor X was granted orphan drug status in 2010. The Applicant contends that because Fresh-frozen Plasma (FFP) or Prothrombin Complex Concentrates (PCC), which are also used to treat Factor X deficiency, contain variable amounts of Factor X, the introduction of Factor X (REPLAFAC[®]TEN) will reduce this variability and increase the reproducibility of dosing during bleeding episodes. The safety and efficacy of Factor X was investigated in clinical trials conducted under IND.

To support both the IND and BLA, the Applicant performed and submitted results from a nonclinical thrombogenicity study in rabbits and acute- and repeat-dose toxicology studies in rats. The repeat-dose toxicology study included a simultaneous evaluation of Factor X pharmacokinetics, immunotoxicity, comparative immunogenicity, and neo-antigenicity. Results from a local tolerance study in rabbits were also submitted. A final review of these nonclinical studies and resulting data to support approval of BLA STN 125506/0/0 for plasma-derived Coagulation Factor X (REPLAFAC[®]TEN) is provided in the memorandum that follows.

List of Nonclinical Studies Reviewed:

Pharmacodynamics/Pharmacology Studies

Study #RCC S07740. FACTOR X: In Vivo thrombogenicity Test in Rabbit (Modified (b) (4) Test. Study conducted by (b) (4) . February 29, 2008; GLP-compliant

Toxicology (including Pharmacokinetics) Studies

Study #B74250. Acute Intravenous Extended Toxicity Study in Rats. Conducted by (b) (4) . December 18, 2008; GLP compliant

Study #C16823. 28-Day Toxicity (Intravenous Injection) Study in the (b) (4) Rat. Conducted by (b) (4) . June 22, 2009; GLP compliant

Study #C07092. Local intravenous, paravenous and intraarterial tolerability study in rabbits. Conducted by (b) (4) . June 8, 2009; GLP compliant

Study #FXR 244. Assay of rat plasma samples from (b) (4) study C16823: 28-day toxicity (intravenous injection) study in the (b) (4) Rat for antibodies to the human factor X-version 2. Conducted by BPL, UK. June 4, 2010; non-GLP compliant

Neo-antigenicity studies

Study #FXR 361. Investigating the Immunogenicity of BPL Factor X final product and competitor products. Conducted by BPL, UK. November 19, 2012; Non-GLP compliant

Submitted Miscellaneous Study Reports:

Study #4.2.3.7.3. Excipients and Impurities. Bibliographic information regarding the toxicology of excipients and impurities in FACTOR X in the context of human exposure at the intended FACTOR X dose. Prepared by BPL, UK; Non-GLP compliant

List of studies not reviewed for this submission:

Reviewer comment: The following study reports were submitted to Module 4 of the BLA, but contain CMC-related data that will be reviewed by the CMC discipline.

Study #FXR 163. Investigation of the effect of human factor X on the prothrombin time of rat plasma. Conducted by BPL. October 28, 2008. Non-GLP compliant

Study #FXR 254. Testing of FACTOR X in global Haemostasis Tests. Conducted by BPL. December 03, 2010. Non-GLP compliant

Study #FXR 307. (b) (4) as a method for measuring potential thrombogenicity in FACTOR X. Conducted by BPL. April 19, 2012. Non-GLP compliant

Study #FXR 308. Factor Xa (b) (4) assay fitness for purpose testing for the analysis of Factor Xa levels in Factor X (b) (4) final product. Conducted by BPL. April 24, 2012. Non-GLP compliant

Study #FXR 320. Investigating the NAPTT profile of BPL FACTOR X. Conducted by BPL. May 18, 2012. Non-GLP

Study #RXR 329. Characterization of protein impurities in FACTOR X final product batches. Conducted by BPL. June 7, 2012. Non-GLP compliant

Study #FXR 334. Thrombin Generation Assay as a method to measure the thrombogenic potential of FACTOR X. Conducted by BPL. July 26, 2012. Non-GLP complaint

Study #FXR 349. Characterisation of FACTOR X final product batches for the presence of contaminating proteins. Conducted by BPL. October 31, 2012. Non-GLP compliant

Investigation of the effect of human factor X on the prothrombin time of rat a plasma, BLP, Study # FXR163, October 28, 2008, non-GLP compliant

Reviewer comment: CMC will also review the following studies and include summaries of the reviews in the final CMC review memorandum for BLA 125506/0/0. These studies were also previously reviewed by the Pharmacology/Toxicology discipline. The PT review summaries can be accessed by cross-referencing the PT review memorandum for the original IND.

Study #FXR 309. Review of NAPTT and FCT data for FACTOR X Pilot-Scale and Manufacturing Batches. Conducted by BPL, United Kingdom (UK). April 24, 2012. Non-GLP compliant

Study #FXR 310. Review of Proteolytic Screen data for FACTOR X and Prothrombin Complex Concentrates. Conducted by BPL, UK. May 3, 2012. Non-GLP compliant

Study #FXR 351. Prothrombin Time and activated partial thromboplastin time assay of FACTOR X spiked into factor X deficient plasma. Conducted by BPL, UK. October 23, 2012. Non-GLP compliant

Study #FXR 355. Thrombin Generation Assays of FACTOR X. Conducted by BPL, UK. November 15, 2012. Non-GLP compliant

PHARMACOLOGY:

Study #RCC S07740. FACTOR X: In Vivo Thrombogenicity Test in Rabbit (Modified (b) (4) Test. Study conducted by (b) (4) . February 29, 2008; GLP-compliant (one phase of the study was not GLP-compliant)
Purpose: To determine the potential thrombogenicity of Factor X.
Methods: An initial thrombogenicity screening and dose-finding study was conducted in rabbits.

Once the initial screening study was completed and the results were evaluated, the main study to assess thrombogenic potential was performed in three male and three female rabbits/group, dosed once intravenously with 100, 200, or 400 IU/kg Factor X (Batch # FXD048). Two positive control groups comprised of three males and three females/group were dosed once intravenously with 50 IU/kg thrombin (BPL, Batch # FTCN6755), or 200 IU/kg 9D/INT (a Prothrombin Combination Complex [PCC] product manufactured by BPL, Batch #006). The negative control group was comprised of three male and three female rabbits that were dosed once intravenously with 0.9 % saline solution.

After injection, the jugular vein from each rabbit was ligated to induce stasis. A vein section 1 cm in length was then excised, evaluated microscopically, and scored on a scale of 1 to 5, with 4 representing the most severe thrombus/occlusion formation and mortality given a score of 5. The thrombogenicity scores for each rabbit in each dose group were then totaled. The percent thrombogenicity for each treatment group was calculated by dividing the sum of the observed scores by the maximum possible score per group. The percentage score calculated for treatment and control groups was compared and evaluated statistically for any significant differences.

Results: Overall, the results demonstrated that a single dose of 400 IU/kg Factor X to male and female rabbits was slightly thrombogenic, and yielded a percentage score of 50% (i.e., the observed thrombogenicity score of 6/maximum possible thrombogenicity score of 12 = 50%). This percentage score was slightly larger, but not significantly different than thrombogenic percentage scores of 33% and 17% reported in male and female rabbits, respectively, dosed with the negative saline control. Both percentage scores were significantly lower than the score of 100% reported in male and female rabbits dosed with 9D/INT, the PCC reference standard. Additional percentage scores from each individual rabbit are presented in Table 1 (excerpted from the submission) that follows:

Table 1. In Vivo Thrombogenicity Test in the Rabbit (Modified (b) (4) Test)

Treatment	Males				%	Females				%	TOTAL	%	Chi-Square (Significance respect to...)		
	1	2	3	Sum		1	2	3	Sum				Negative CONTROL	POSITIVE CONTROL	9D/INT
NEGATIVE CONTROL (PHYSIOLOGICAL SALINE)	2	0	2	4	33	0	0	2	2	17	6	25	-	N.S.	p < 0.01
POSITIVE CONTROL (HUMAN THROMBIN) (50 IU/kg)	1	2	2	5	42	2	2	2	6	50	11	46	N.S.	-	p < 0.01
9D/INT (200 UI/kg)	4	4	4	12	100	4	4	4	12	100	24	100	p < 0.01	p < 0.01	-
FACTOR X (100 UI/kg)	1	2	1	4	33	1	2	2	5	42	9	38	N.S.	N.S.	p < 0.01
FACTOR X (200 UI/kg)	1	0	3	4	33	2	0	2	4	33	8	33	N.S.	N.S.	p < 0.05
FACTOR X (400 UI/kg)	3	1	2	6	50	2	2	2	6	50	12	50	N.S.	N.S.	p < 0.01

Reviewer comments:

- 1) The Applicant included an additional reference article in the experimental design, referred to as 9D/INT (PCC), that yielded percentage scores of 100% thereby demonstrating that the assay was valid and confirming the lower thrombogenicity scores generated with doses of 100 and 200 IU/kg Factor X. The lower scores at doses that mimic the expected clinical dose suggests low thrombogenic risk to patients who will be administered Factor X for the proposed indications.
- 2) The Applicant states that Factor X (REPLAFAC[®]TEN) will be given to humans at a maximum dose of 60 IU/kg (i.e., this dose is the expected pre-surgical bolus amount required to raise Factor X levels to approximately 70-90 IU/dL). Based on the low thrombogenicity scores reported at a dose of 200 IU/kg Factor X, a margin of safety (MoS) of 3.3 is calculated. Although this MoS is somewhat low, the margin still suggests minimal thrombogenic risk to patients following administration of Factor X.
- 3) The low incidence of thrombi, which formed in both male and female rabbits at a dose of 100 IU/kg Factor X, suggest plasma-derived Factor X is a high purity product that contains low levels of activated Factor X.

PHARMACOKINETICS AND TOXICOLOGY

Study #B74250. Acute Intravenous Extended Toxicity Study in Rats. Conducted by (b) (4) December 18, 2008; GLP-compliant

Purpose: To evaluate the potential acute toxicity of Factor X.

Methods: Six male and six female (b) (4) rats/group were dosed with a single intravenous injection of 60, 600 or 2400 IU/kg Factor X (Lot #FXD048), or a saline control. The rats were monitored for up to 14 days, sacrificed and subjected to necropsy. Interim groups, comprised of three male and three female rats at each dose level were sacrificed and subjected to necropsy three days after the single dose, and the remainder of the animals were sacrificed at study termination. Following necropsy, large organs, gross lesions and injection sites were collected and evaluated macroscopically and histopathologically.

During the study, rats were monitored daily for changes in clinical signs and for body weights on days 2, 8, and 15. Hematology, including PT and aPTT, and serum chemistry were evaluated in all rats prior to the study and on day 3 (day 2 post-dose), and day 15 (day 14 post-dose).

Results: One male (animal ID: 13) in the 600 IU/kg Factor X dose group died spontaneously before treatment and one male (animal ID: 11) in the 60 IU/kg group died on day 15 during blood sampling; therefore, both mortalities were not considered to be related to Factor X. One male dosed with 2400 IU/kg Factor X exhibited poor coordination and a hunched position shortly after injection, but these clinical signs resolved 3 hrs after administration. No significant difference in group mean body weights was reported between rats dosed with Factor X or the negative control saline solution.

No significant changes in hematologic parameters were reported in rats in the interim sacrifice groups on day 3. Significant decreases in red blood cell (RBC) volume and hemoglobin concentration were reported in all Factor X-treated groups, but the RBC volumes and hemoglobin concentrations were within acceptable reference ranges. According to the Applicant, the differences in hematologic parameters were not considered toxicologically or clinically relevant.

Reviewer comment: This Reviewer agrees with the Applicant's assessment that the hematologic differences were not toxicologically relevant.

Significant decreases in glucose levels in male rats dosed with 600 IU/kg or 2400 IU/kg Factor X were reported on day 3, but the levels were within the historical ranges. On day 15, significant decreases in mean aspartate aminotransferase (ASAT) levels were reported in male rats in the 2400 IU/kg dose group, but the mean value was within the reference range.

Pathology identified grade 2 and grade 3 hematopoietic spleen and renal tissues in most males and most females in all Factor X treatment groups on day 3. The increased grades were not dependent on increasing Factor X dose levels. Liver tissue exhibited grade 2 or 3 inflammatory foci in most males and females in all treatment groups on day 3.

A male rat dosed with 60 IU/kg Factor X that died during sampling had lung foci and alveolar hemorrhage which, according to the pathology report, was common in rats that age so was deemed unrelated to treatment with Factor X.

Reviewer comment: Based on changes in the clinical signs and results from pathology, a no-observed-effect-level (NOEL) of 2400 IU/kg for Factor X claimed by the Applicant is appropriate. Moreover, a NOEL of 600 IU/kg can still be used to calculate a MoS of 7.5, which suggests Factor X will present minimal risk to patient safety.

Study #C16823. 28-Day Toxicity (Intravenous Injection) Study in the (b) (4) Rat. Conducted by (b) (4) . June 22, 2009; GLP compliant

Purpose: To evaluate the potential sub-chronic toxicity and immunogenicity of Factor X; (2) to determine the PK of Factor X; and (3) to assess the potential immunotoxicity of Factor X by monitoring primary IgM immune responses and effects on T and B cell populations.

Methods: The potential sub-chronic toxicity of Factor X was assessed by dosing 25 male and 25 female (b) (4) rats/group intravenously (slow bolus over 2 minutes) with 30, 120, or 360 IU/kg Factor X (lot #FXSN 7850, specific activity 535 IU/5.1 mg/vial), every other day for 28 days. Nineteen male and 19 female rats in the negative control group were dosed with saline solution. Additional group identification and experimental design information is provided in Table 2 (excerpted from the submission) that follows:

Table 2. Group Identification and Rat Numbers Assigned to Treatment

Allocation and Dose Levels		Group 1 Control*	Group 2	Group 3	Group 4
		0 IU/kg/dose	30 IU/kg/dose	120 IU/kg/dose	360 IU/kg/dose
Males	A	01 - 10	20 - 29	45 - 54	70 - 79
	B	11 - 13	30 - 38	55 - 63	80 - 88
	C	14 - 19	39 - 44	64 - 69	89 - 94
Females	A	95 - 104	114 - 123	139 - 148	164 - 173
	B	105 - 107	124 - 132	149 - 157	174 - 182
	C	108 - 113	133 - 138	158 - 163	183 - 188

- * Control animals were treated with the vehicle, phosphate buffered saline (PBS), only
- A Toxicity testing (termination after 29 treatment days)
- B Animals for toxicokinetics and for terminal immunogenicity/plasma samples (were sacrificed at the end of treatment)
- C Animals for primary immune response analysis and for immunogenicity/plasma samples on day 14 of treatment period (were sacrificed at the end of treatment)

Rats in all groups were monitored for changes in clinical signs twice daily. Changes in body weight, ophthalmology, and food consumption were monitored weekly. Blood samples were collected from all rats in each dose group of allocation Group C on day 14, and from all rats in allocation Group A on day 28 and evaluated for changes in hematology, serum chemistry, and Prothrombin times (PT)/Activated partial thromboplastin time (aPTT). Urinalysis was performed once on a volume of urine collected on day 28 from rats in all groups over an 18 hour period. Urinalysis was not performed on day 14 of the study. Male and female rats from dose groups 1 and 4 in allocation Group C and in allocation Group A were sacrificed on day 14 and day 28, respectively, and subjected to necropsy. Selected organs and tissues were evaluated for changes in pathology and histopathology.

Differences in food consumption, body weight, and organ weight ratios were evaluated for significance by various statistical methods.

Plasma samples from 3 male and 3 female rats/group in allocation Group B were collected before dosing, and then over a 48-hr time-course after dosing on day 1 and day 29. The concentration of Factor X in plasma samples was determined using a validated (b) (4). The concentration results were analyzed using commercially available software to determine standard PK parameters.

Plasma samples were collected from all rats in each dose group from allocation Groups B and C, on day 14 and day 28 and evaluated for the formation of binding and neutralizing anti-Factor X antibodies. This immunogenicity sub-study was performed in-house by the

Applicant and has been reviewed separately in this memorandum (cross-referenced to Study #FXR 244).

The potential of Factor X to induce immunotoxicity was also assessed in rats during the repeat-dose study, by measuring changes in IgM levels and leukocyte populations following the administration of sheep red blood cells as an antigen challenge. Specifically, all male and all female rats in each dose group in allocation Group C were dosed with a 0.5 mL suspension containing 4×10^8 sheep erythrocytes (SRBC)/mL on day 24 of the 28-day repeat-dose toxicity study. On day 29, plasma was collected from all rats/group and evaluated for the formation of IgM antibodies against SRBCs using an (b) (4)

Blood samples collected on day 29 were also evaluated by flow cytometry for changes in the distribution of T cells using the $CD3^+/CD4^+$ and $CD3^+/CD8^+$ cell surface markers. Changes in the B-cell and monocyte populations were assessed by flow cytometry monitoring the $CD45RA$ and $CD11b^+$ markers, respectively. The levels of IgM and the distribution of the T-cell, B-cell and monocyte populations in samples on day 28 were compared with IgM levels and the same populations in blood collected from rats that were not administered SRBCs, in allocation Group A, on day 28.

Results:

Toxicology: No deaths were reported during this study. No significant changes in food consumption, body weight, and ophthalmology were reported at any Factor X dose level tested. Analysis of serum chemistry showed significant increases in the mean sodium, chloride and phosphorous levels in males dosed with 120 and 360 IU/kg Factor X on day 28. Serum chemistry in female rats showed significant increases in sodium content at dose amounts of 30 IU/kg Factor X and above, and in phosphorus and calcium content at a dose of 360 IU/kg Factor X on day 28.

Urinalysis did not reveal any significant differences in males and females at any dose on day 28. No significant changes were reported in the organ weight or relative organ weight in males and females at any dose on day 28. A significant decrease in mean ovary weight was reported at a dose of 360 IU/kg Factor X, but the decrease did not occur in a Factor X dose-dependent manner so was not considered clinically relevant by the Applicant.

In addition to the changes in serum chemistry previously mentioned, several high-dose males (animal IDs: 70, 73 and 79), and one high-dose female (animal ID: 170) had elevated aspartate aminotransferase (AST) levels. However, pathologic examination of the liver only identified limited, unremarkable findings in males and females in the high-dose group so, according to the Applicant, this significant change in serum chemistry was not attributable to hepatic or renal damage.

Elevated bilirubin levels were observed in females at a dose of 360 IU/kg Factor X on day 28. Increases in bilirubin were associated with slight increases in alanine aminotransferase (ALAT) activity but no definitive histopathology findings; therefore, the Applicant concluded that liver function was not adversely affected by Factor X at the highest dose of 360 IU/kg.

Results from the hematologic evaluation identified significant increases in the mean relative basophil levels in males administered 360 IU/kg Factor X by day 28. The mean white blood cell level was significantly increased in females dosed with 360 IU Factor X on day 28. The mean relative neutrophil level was significantly decreased in females at the highest Factor X dose on day 28. However, mean absolute basophil, lymphocyte, and monocyte levels were all significantly increased in females at the highest Factor X dose of 360 IU/kg on day 28. The Applicant stated that in the absence of any histomorphological changes in spleen or bone marrow, the significant changes in the hematological parameters were not considered adverse.

Pathology

Microscopic analyses of lung tissues identified three instances of grade 1 and one finding of grade 2 alveolitis in male rats dosed with the highest Factor X dose of 360 IU/kg. The spleens from one male and one female rat in the high dose group exhibited grade 3 hematopoiesis at day 14, but these effects were not deemed excessive by the Applicant compared with the level of hematopoiesis in the spleens of rats dosed with the saline control. The spleens of two additional male and female rats in the high dose group also exhibited grade 2 hematopoiesis at day 28, but the Applicant did not consider these findings representative of any pathology related directly to Factor X.

Two males from the high dose group (animal IDs: 70 and 72) and one high-dose female (animal ID: 166) exhibited grade 1 proliferation in the bile duct. The female rat #166 also had a bilirubin level that was significantly elevated. According to the Applicant, because liver did not possess any other microscopic findings and red blood cell counts remained within normal ranges, the changes in bilirubin and proliferation in the bile duct were considered incidental, and not indicate of any adverse reactions in the liver.

Reviewer comments:

1. The hepatic toxicity and slightly elevated alanine aminotransferase (ALAT) and AST levels that were detected at the highest-dose of 360 IU/kg Factor X is a safety concern. However, these nonclinical effects only occurred at doses that were approximately 7-fold larger than a pre-surgical dose of 49.2 IU/kg administered without adverse effect during the Phase 3 clinical trial entitled "Ten03". Although the potential for hepatotoxicity in patients is a concern, the absence of any remarkable liver toxicity in rats at the lower Factor X doses of 30 and 120 IU/kg tend to suggest Factor X will present minimal risk to patient safety.

2. Although ALAT and AST levels were slightly elevated in rats dosed with 360 IU/kg, the safety of Factor X is qualified based on previous clinical experience demonstrating that patients in the Phase 3 clinical trial did not exhibit elevated ALAT levels or any evidence of hepatotoxicity.
3. Pathologic evaluation of male rats in all Factor X dose groups identified lung, thymus and pancreatic tissues which were dark red or had developed cellular foci, but these effects are considered random by this Reviewer, and not dependent on Factor X dose levels. The incidence of affected tissues was lower in female rats in all Factor X dose groups; moreover, none of the pathologic findings were considered remarkable by the Applicant or this Reviewer further qualifying the safety of Factor X (REPLAFAC[®]TEN).

The PK study demonstrated that the mean $t_{1/2}$ of Factor X was 6.4, 5.2, and 5.6 hrs in male rats dosed with 30, 120 and 360 IU/kg, respectively, on day 1. On day 29, the mean $t_{1/2}$ increased to 11, 7.5, and 6.2 hrs at the same Factor X doses, but the Applicant indicated these $t_{1/2}$ values were not reliable. Additional PK results are provided in Table 3 (excerpted from the submission) that follows:

Table 3. Results from PK studies conducted with Factor X on Day 1 and 29 of the Rat Repeat-dose Toxicity Study

Group	Dose [IU/kg/adm]	Ratio*	AUC _{0,t} [ng·h/mL]	Ratio*	AUC _{0,t} /Dose	Ratio*	C _{max} [ng/mL]	Ratio*	C _{max} /Dose	Ratio*	t _{1/2} [h]	Ratio*	
Males													
Day 1	2	30	-	14598	-	487	-	3398	-	113	-	6.4**	-
	3	120	4.0	42492	2.9	354	0.7	11808	3.5	98	0.9	5.2**	-
	4	360	3.0	144497	3.4	401	1.1	36383	3.1	101	1.0	5.6	-
Group 4/Group 2			12		9.9		0.8		11		0.9		-
Day 29	2	30	-	37937	-	1265	-	5862	-	195	-	11**	-
	3	120	4.0	77324	2.0	644	0.5	12898	2.2	107	0.6	7.5**	-
	4	360	3.0	215587	2.8	599	0.9	44499	3.5	124	1.2	6.2	-
Group 4/Group 2			12		5.7		0.5		7.6		0.6		-
Females													
Day 1	2	30	-	11349	-	378	-	3053	-	102	-	5.4**	-
	3	120	4.0	44836	4.0	374	1.0	14800	4.8	123	1.2	4.9**	-
	4	360	3.0	160570	3.6	446	1.2	51484	3.5	143	1.2	4.7**	-
Group 4/Group 2			12		14		1.2		17		1.4		-
Day 29	2	30	-	49116	-	1637	-	5344	-	178	-	13	-
	3	120	4.0	113821	2.3	949	0.6	21911	4.1	183	1.0	6.6	0.5
	4	360	3.0	189623	1.7	527	0.6	36193	1.7	101	0.6	4.9	0.7
Group 4/Group 2			12		3.9		0.3		6.8		0.6		0.4

*: Ratio group 3/2 and group 4/3

** : Less reliable value

Reviewer comments:

- 1) Mean AUC values were not decreased by day 28 of the study, suggesting that the formation of binding and neutralizing anti-Factor X antibodies was minimal and that rats were continuously exposed to Factor X at the corresponding dose levels for the duration of the study.

- 2) The PK results suggest rats were exposed to supraphysiologic levels of Factor X during this repeat dose toxicity study. Although some splenic findings were observed during the study, the effects only occurred at exposures much larger than those expected clinically (approximately 7-fold greater than the expected human dose, on an IU/kg basis), which suggests that Factor X will be reasonably safe in patients with hereditary factor X deficiency.
- 3) A comparison of the MoS for Factor X calculated using the mean C_{max} from rats and patients is presented in Table 4 (generated by this Reviewer) that follows:

Table 4. MoS for Factor X Based on the C_{max} Determined in Male Rats and Patients

	C_{max} , rat day 1 30 IU/kg	C_{max} , rat day 1 120 IU/kg	C_{max} , rat day 1 360 IU/kg	C_{max} , rat day 30 30 IU/kg	C_{max} , rat day 30 120 IU/kg	C_{max} , rat day 30 360 IU/kg
C_{max} , human dose 25 IU/kg Initial dose	$\frac{3398}{5747} = 1^a$	$\frac{11808}{5747a} = 2^a$	$\frac{36838}{5747} = 6.4^a$			
C_{max} , human dose 25 IU/kg Repeat dose				$\frac{3053}{5100} = 1^a$	$\frac{14800}{5100} = 2.9^a$	$\frac{51484}{5100} = 10^a$

^a Swelling and extramedullary hematopoiesis in the spleen were observed at all Factor X doses, but these effects were not considered clinically relevant by this Reviewer

- 4) The MoS of 10 calculated using the C_{max} derived during the nonclinical and clinical studies reduces concern about the safety of Factor X, and predicts that Factor X will present limited risk to the hereditary Factor X deficient patient population.
- 5) The frequency of dosing during the repeat-dose study was greater than the dosing frequency that will be used to treat hereditary Factor X deficiency in patients. The minimal toxicity observed in rats subjected to more frequent administration of doses larger than the expected clinical dose further suggests Factor X will be reasonably safe for the intended indications.
- 6) Published reports indicate the biological half-life of Factor X contained in PCC or FFP varies among individuals and with the frequency of dosing, but the $t_{1/2}$ for Factor X in these comparator products is normally between 20-40 hrs in patients. A similar $t_{1/2}$ for Factor X was reported after the clinical trial conducted under IND; however, the $t_{1/2}$ of Factor X determined non-clinically in rats was much lower and ranged from just 4.7 and 13 hrs.

Immunotoxicity:

The primary IgM immune response to SRBCs was not significantly reduced in male and female rats dosed with 30, 120, or 360 IU/kg Factor X compared with IgM responses in rats treated with the saline control. Additional IgM results from female rats (results from male rats were similar and not shown) are provided in Table 5 (excerpted from the submission) that follows:

Table 5. Mean Anti-SRBC IgM Titer in Female Rats

Test Group	mg/Kg/day	Mean Relative Titer (%)*	Standard Deviation	S**	P
1	0	479.0	142.8		0.319
2	30	359.4	169.5	-	
3	120	495.7	265.8	-	
4	360	586.1	217.4	-	

*: relative Titer = ((titer of the test sample x 100) / titer of the corresponding standard)

**.: statistical analysis: „-,“ = not significant; „+“ = significant.

S: statistical significance

P: p-value

According to the Applicant, no significant changes in the T-cell, B-cell, or monocyte populations were identified in rats dosed with 30, 120, or 360 IU/kg Factor X compared with T-cell, B-cell, or monocyte populations in control treated rats. The population of leukocytes with the CD45RA marker was increased 32% in high-dose males, but the increase was not statistically significant and was consistent with historical standards, according to the Applicant. The Applicant added that in the absence of any histomorphologic changes in immune organs, such as spleen, the change in leucocyte CD45RA population was not considered adverse. The mean leukocyte population results in male and female rats were similar; therefore only the results from female rats are provided in Table 6 (excerpted from the submission) that follows:

Table 6. Leukocyte Counts in Female Rats Dosed with 30, 120 and 360 IU/kg FX

Dose*	CD3 ⁺ -Cells				CD3 ⁺ /CD4 ⁺ -Cells				CD3 ⁺ /CD8 ⁺ -Cells			
	Mean	SD	S**	P	Mean	SD	S**	P	Mean	SD	S**	P
1	63.18	4.42		0.093	45.68	4.04		0.159	17.96	1.71		0.095
2	59.63	6.15	-		43.78	5.41	-		16.59	1.56	-	
3	65.11	6.58	-		48.96	6.55	-		17.43	1.61	-	
4	54.43	10.68	-		40.43	8.41	-		14.64	3.60	-	

Dose*	CD45RA ⁺ -Cells				CD11b ⁺ -Cells			
	Mean	SD	S**	P	Mean	SD	S**	P
1	23.97	3.29		0.307	22.04	4.26		0.811
2	27.30	4.95	-		23.03	4.90	-	
3	23.75	6.03	-		21.86	4.21	-	
4	32.27	10.31	-		23.87	2.27	-	

*: dose groups; „1 = control group, 2-4 = low, mid and high dose group

** : statistical analysis: „-,“ = not significant; „+“ = significant

SD: standard deviation

S: statistical significance

P: p-value

Study #C07092. Local intravenous, paravenous and intra-arterial tolerability study in rabbit. Conducted by (b) (4) . June 8, 2009. GLP compliant.

Purpose: To assess the potential of Factor X to induce local intolerance reactions.

Methods: The right ear of one male and two female rabbits/group were injected intravenously (Group 1), paravenously (Group 2) or intra-arterially (Group 3) with 600 IU/kg Factor X. The left ear on the same rabbits was injected with a control saline solution using the same routes of administration previously applied to the right ear. All doses were administered as a 5 mL infusion over a 30 min duration. Rabbits in Group 2 received lower Factor X doses of between 1.76 - 3 mL because of technical problems. The injection sites were monitored for local toxicity for 8 days.

Clinical signs were evaluated immediately after completion of infusion and then again at 3 hrs, 6 hrs, and one day after dosing. Thereafter, clinical signs were monitored twice daily on days 2, 3, and 4 and then daily on days 5 through 8. Following the study, all rabbits were sacrificed. The injection sites were subjected to macroscopic and histopathologic evaluation.

Results: The results indicate that Factor X dosed intravenously at 600 IU/kg in a 5-mL volume was well-tolerated. Administration of Factor X by the paravenous and intra-arterially routes was not well-tolerated. The paravenous and intra-arterial injections sites exhibited localized erythema and edema, but these effects resolved by approximately 8 days after administration. Slight phlebitis developed in one Group 1 female after intravenous delivery, but the effect was not treatment related according to the Applicant. Pathologic evaluation identified phlebitis/periphlebitis, inflammation, and slight acanthosis in the right ear of two group 1 rabbits, but similar findings were identified in the left ear of control treated rabbits so the effect was not considered to be related to

Factor X. The results from the pathologic evaluation also showed that Factor X delivered by the paravenous route was tolerated, but only up to a Factor X dose of between 211-370 IU/kg.

Reviewer comment: This Reviewer agrees with the Applicant's claim that Factor X doses up to 600 IU/kg were well tolerated when delivered intravenously. Overall, the results at 600 IU/kg Factor X, or approximately 10-fold greater than the expected clinical dose, suggest minimal risk of local reactivity in hereditary Factor X deficiency patients treated intravenously with Factor X.

Study #FXR 244. Assay of rat plasma samples from (b) (4) study C16823: 28-day toxicity (intravenous injection) study in the (b) (4) Rat for antibodies to the human factor X. Conducted by BPL. June 4, 2010. Non GLP

Purpose: To measure the titer of binding and neutralizing rat anti-human anti-Factor X antibodies that developed in rats on day 14, and day 28 of the 28-day repeat-dose toxicology study.

Methods: Blood samples were collected from ten male and ten female rats/dose group/allocation group on day 14 (Allocation C) and day 28 (Allocation B) of the 28-day repeat-dose toxicology study (cross-reference Study #16823 in this memorandum for the review of the 28-day study). On the final day of the study (day 28), blood samples were collected at 8, 24, and 48 hrs after the final Factor X dose. All blood samples collected at 8, 24, and 48 hrs were evaluated for the formation of inhibitor anti-Factor X antibodies.

Initially, rat plasma was screened for the presence of human anti-Factor X antibodies using an (b) (4)



The Applicant was unable to quantitate the titer of anti-Factor X antibody in rat plasma because a human anti-Factor X antibody needed to generate a standard curve for quantitation was not commercially available. In lieu of an anti-Factor X antibody and standard curve, the Applicant used a commercially available kit to further evaluate positive samples for neutralizing antibodies. Based on the results from this kit, the neutralizing activity (in Bethesda Units, BU) in plasma samples was calculated.

Results: Results from the initial screening determined that most rats administered Factor X at all dose levels (including the control) developed non-neutralizing, binding antibodies against Factor X. Although the positive results reported in rats in the negative control group invalidate the assay, the mean incidence and results from the study are provided in Table 7 (excerpted from the submission) that follows:

Table 7. Incidence and Results from the Bethesda Assay Conducted on Rat Plasma Samples Collected on Day 14 of the 28-day Repeat-dose Toxicity Study

Group	Sex	By Sex				Combined			
		Average BU/ml		Incidence (>0.5BU/ml)		Average BU/ml		Incidence (>0.5BU/ml)	
		Method 1	Method 2	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2
1 (control)	Female	0.395	0.513	1/6	4/6	0.470	0.551	5/12	8/12
	Male	0.545	0.588	4/6	4/6				
2 (30 IU/Kg)	Female	0.373	0.485	1/6	3/6	0.548	0.541	6/12	8/12
	Male	0.723	0.597	5/6	5/6				
3 (120 IU/Kg)	Female	0.497	0.463	1/6	1/6	0.618	0.538	5/12	7/12
	Male	0.738	0.613	4/6	6/6				
4 (360 IU/kg)	Female	0.475	0.520	1/6	3/6	0.314	0.475	3/12	5/12
	Male	0.153	0.430	2/6	2/6				

Reviewer comments:

1. Based on results from plasma collected from rats in the control and treatment groups, the Applicant claims that rats administered 30, 120 and 360 IU/kg Factor X every other day for 28 days did not develop neutralizing human anti-Factor X antibodies. This Reviewer disagrees because positive results in the negative control group invalidate the assay.
2. Although the results from the inhibitor antibody assays are difficult to because plasma from control treated rats produced BU > 0.5, these results may not impact safety because several reports in the literature suggest hereditary Factor X deficient patients do not normally develop inhibitor antibodies during or after replacement therapy (Brown and Kouides (2008) *Haematology*). Brown and Kouides state that hereditary Factor X deficient patients, in this consanguineous cohort, do not possess mutations that result in premature stop codons. Consequently, most members express at least some full-length Factor X, although with limited catalytic function, resulting greater immune tolerance to Factor X replacement therapy. Therefore, these patients are not expected to develop inhibitor anti-Factor X antibodies following treatment with plasma-derived Factor X.

3. Although the inhibitor assay was considered invalid, the safety of Factor X is established by clinical experience demonstrating that patients in the Phase 3 clinical trial did not develop inhibitor anti-Factor X antibodies.

Study #FXR 361. Investigating the Immunogenicity of BPL Factor X Final Products and Competitor Products. Conducted by BPL. November 19, 2012. Non-GLP

(b) (4)



(b) (4)

(b) (4)



MISCELLANEOUS

Study #4.2.3.7.3. Excipients and Impurities

Purpose: To provide bibliographic information that qualifies the safety of excipients and impurities in Factor X.

Method: The Applicant listed (b) (4), citric acid, (b) (4), sodium chloride, and sucrose as excipients in Factor X, or in the diluent used to reconstitute Factor X. The Applicant then provided results from toxicologic studies in the literature and an assessment of exposure following single, or repeated dosing to qualify the safety of these excipients in Factor X

The Applicant listed (b) (4) as impurities of Factor X. The Applicant then provided a risk assessment based on the amount of each impurity in single and repeated doses of Factor X. The Applicant also provided results from carcinogenicity, mutagenicity, and developmental/reproductive studies conducted with the impurities to qualify their safety.

Reviewer comment: The toxicologic assessment is adequate to qualify the safety of excipients and impurities in Factor X and successfully predicts minimal risk to hereditary Factor X deficiency patients who will be administered Factor X.

INTEGRATED SUMMARY AND SAFETY EVALUATION

Synopsis and Introduction

The following section summarizes the findings documented in the final primary review of the nonclinical pharmacology and toxicology data submitted in the Applicant's original BLA for REPLAFAC[®]TEN, Coagulation Factor X (plasma-derived, human Factor X; code name Factor X). Bio Products Laboratory has developed a plasma-derived Factor X for the treatment of bleeding episodes, and prevention of bleeding during surgery in patients with the rare, autosomal recessive, bleeding disorder referred to as hereditary Factor X deficiency. The disease is estimated to occur in 1:1,000,000 male or female individuals, and exhibits a carrier frequency of 1:500. Based on the extremely low incidence of the disease, Factor X was granted orphan drug status in 2010. The Applicant contends that because Fresh-frozen plasma (FFP) or Prothrombin complex concentrates (PCC), which are also used to treat hereditary Factor X deficiency, contain variable amounts of Factor X, the introduction of Factor X purified from plasma will reduce this variability and increase the reproducibility of dosing during bleeding episodes and surgery. From the Pharmacology/Toxicology discipline's perspective, this original BLA STN 125506/0/0 for Factor X (REPLAFAC[®]TEN) is recommended for approval.

Summary of Key Findings and Synopsis of Results

General Review Conclusions

Plasma-derived Coagulation Factor X (Human, REPLAFAC[®]TEN) was determined to be safe for its intended use for the treatment of bleeding episodes, and prevention of bleeding during surgery in patients with hereditary deficiency of coagulation Factor X. The determination that REPLAFAC[®]TEN is safe for its indicated uses was based on results from nonclinical studies conducted in compliance with the Good Laboratory Practice (GLP; 21 CFR part 58) regulations, and from non-GLP compliant studies, and from its use during a clinical trial within the United States conducted under IND.

Pharmacological/Toxicological Findings

The Applicant conducted nonclinical studies which initially supported clinical trials and now support the licensing approval of REPLAFAC[®]TEN. Individual review summaries of these nonclinical studies and the resulting data are presented in this Final Pharmacology/Toxicology discipline review BLA memorandum. The results from these nonclinical studies clearly demonstrated the clotting activity, and predicted the reasonably safe use of Factor X (REPLAFAC[®]TEN) for its intended indications in patients with hereditary factor X deficiency.

The nonclinical program consisted of pharmacodynamic and toxicologic studies conducted in rabbits and in rats. The pro-coagulant activity of Factor X was assessed in vitro by spiking Factor X into Factor X-depleted human plasma and measuring the

changes in prothrombin (PT) and activated partial thromboplastin times (aPTT). The potential thrombogenicity of Factor X was also assessed in rabbits using the (b) (4) assay as a venous stasis model. Results from these nonclinical studies demonstrated that Factor X was capable of mediating hemostasis and did so without thrombogenic effects.

The pharmacokinetics profile of Factor X was assessed at several dose levels on days 1 and 29 of a repeat-dose toxicology study in rats. The results from the study indicate that AUC increased approximately 20% during the study. This result indicates that rats were exposed to supraphysiologic doses of Factor X for the duration of the study while only exhibiting slight and clinically irrelevant toxicity. Moreover, the C_{max} of Factor X in rats was used to calculate a margin of safety (MoS) of 10, based on its comparison with the C_{max} of Factor X determined clinically during the Phase 3 clinical trial conducted under IND. The MoS of 10 further predicts the safe use of Factor X for its intended indications. Additional MoS values are presented in Table 9 (prepared by the Reviewer) that follows:

Table 9. MoS for Factor X based C_{max} Determined in Male Rats and Patients

	C_{max} , day 1 30 IU/kg	C_{max} , ay 1 120 IU/kg	C_{max} , day 1 360 IU/kg	C_{max} , day 30 30 IU/kg	C_{max} , day 30 120 IU/kg	C_{max} , day 30 360 IU/kg
C_{max} Human 25 IU/kg Time 0	$\frac{3398}{5747} = 1$	$\frac{11808}{5747} = 2$	$\frac{36838}{5747} = 6.4$			
C_{max} human 25 IU/kg Repeat dose				$\frac{3053}{5100} = 1$	$\frac{14800}{5100} = 2.9$	$\frac{51484}{5100} = 10$

Swelling and extramedullary hematopoiesis in the spleen were observed at all Factor X doses, but these effects were not considered clinically relevant by this Reviewer

The potential acute toxicity of Factor X was also assessed in rats. One male rat intravenously administered a single dose of 2400 IU/kg Factor X exhibited poor coordination and a hunch posture, but this overt effect resolved 3 hours after dosing. Rats administered a single dose of 600 IU/kg Factor X, which is approximately 8.5-fold greater than the expected clinical dose, did not display any evidence of acute toxicity. No acute toxicities were observed in rats administered 60 IU/kg Factor X which is the expected clinical dose.

A 28-day repeat-dose toxicity study, which included assessments of immunogenicity and immunotoxicity, was also conducted in rats.. Splenic enlargement, with microscopic evidence of extramedullary hematopoiesis was reported in all treatment groups, including the lowest Factor X dose of 30 IU/kg. Administration of human Factor X did not appear to stimulate production of neutralizing antibodies against either the human Factor X itself, or against endogenous Factor X. Results from the immunotoxicity study conducted in rats demonstrated that primary immune responses, as measured by increases in IgM titers directed against a challenge with sheep red blood cells, were not impaired following

the administration of all Factor X doses, including the highest dose of 360 mg/kg given every other day for 28 days. Additional results from the pharmacodynamic and toxicology studies are summarized in Table 10 (prepared by the Reviewer) that follows:

Table 10. Summary of Nonclinical Pharmacodynamics and Toxicology Studies Conducted with Plasma-derived Factor X

Study	Study #	Dose IU/kg	Species	Results	NOAEL (IU/kg)
Thrombogenicity	S07740	100-400	rabbit	Thrombogenic at 400 IU/kg	200
Acute-dose toxicity	B74250	60-2400	rat	Acute toxicity at 2400 IU/kg	600 ^a
28-day repeat-dose toxicity	C16823	30-360	rat	Splenic inflammation /bleeding	360 ^a
Local Intolerance	C07092	600	rabbit	Tolerated	600
Immunogenicity	FXR 244		rat	Negative	360
Immunotoxicity	C16823	30-360	rat	Negative	360
Neo-antigenicity	FXR 361	Dry heat or Pasteurization	in vitro	Negative	

^a Swelling and extramedullary hematopoiesis in the spleen were observed at all Factor X doses, but these effects were not considered clinically relevant by this Reviewer

Results from a separate local tolerability study in rabbit demonstrated that single intravenous administration of 600 IU/kg Factor X, which is 8.5-fold larger than the expected clinical dose, was well tolerated. The results from a neo-antigenicity study demonstrated that dry heat used in viral inactivation of the plasma-derived product did not result in the formation of new epitopes or antigenic sites on Factor X so the dry heat inactivation process is considered safe.

Conclusions

The results from the nonclinical studies submitted by the Applicant are adequate to establish the expected biological activity and predict the safety of Factor X (REPLAFAC[®]TEN) for the treatment of bleeding episodes, and prevention of bleeding during surgery in patients with hereditary deficiency of coagulation Factor X. From the Pharmacology/Toxicology discipline's perspective, no safety or efficacy issues were identified during the final BLA review; therefore, the discipline recommends approval of BLA 125506/0/0 for plasma-derived Coagulation Factor X (REPLAFAC[®]TEN).