



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



**Pharmacology / Toxicology Primary Discipline Review**

**To:** File (Original BLA STN 125466/0)

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**Through:** Anne M. Pilaro, PhD, Supervisory Toxicologist, DH/OBRR  
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**Subject:** STN 125466/0 – Novo Nordisk’s Original Biological License Application (BLA) NovoEight®, Antihemophilic Factor (Recombinant) Plasma/albumin Free beta (β)–domain deleted (BDD)

**Indication:** On-demand treatment of bleeding episodes, perioperative management, and routine prophylaxis in Hemophilia A patients

**Date:** May 31, 2013

This memorandum is the final primary, pharmacology/toxicology review of the nonclinical program submitted to the original biological license application (BLA) for Novo Nordisk’s NovoEight®, Antihemophilic Factor (Recombinant) Plasma/albumin Free beta (β)–domain deleted (BDD), indicated for prophylaxis or prevention, major surgical prophylaxis and life threatening on-demand treatment in hemophilia A patients. From the toxicology/pharmacology reviewer’s perspective, this original biological application STN 125466/0 is recommended for approval.

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**I. Recommendations**

Based on review of the submitted toxicology and pharmacology data, this original biological application STN 125466/0 is recommended for approval. There were no nonclinical deficiencies

identified in this submission based on review of the pharmacological and toxicological data presented in STN 125466/0 Novo Nordisk Inc's Original Biological License Application (BLA) for NovoEight®, Antihemophilic Factor (Recombinant) Plasma/albumin Free beta (β)-domain deleted (BDD). There are no outstanding issues from the nonclinical standpoint to prevent this BLA from approval, and there are no requests for any further nonclinical evaluation at this time.

## **II. Summary Basis for Regulatory Action (SBRA) for Nonclinical NovoEight Data**

### **Official Summary Basis for Regulatory Action (SBRA)**

#### **4. Non-clinical Pharmacology/Toxicology**

##### *General Review Conclusions*

NovoEight (Antihemophilic factor [recombinant]) was determined to be safe for its intended use for on-demand treatment of bleeding episodes, perioperative management, and routine prophylaxis in Hemophilia A patients based on nonclinical data from Good Laboratory Practices (GLP)-compliant and non-GLP studies, and its clinical use both within and outside of the United States. The nonclinical program consisted of a series of studies to demonstrate the safety and effectiveness of NovoEight in animals including hemophilic mice and dogs, and wild-type FVIII expressing mice, rats, rabbits, and cynomolgus monkeys. In animal studies, the adverse events noted were predominantly exaggerated pharmacological effects of FVIII including thromboembolic events, local reactions at treatment site, and re-bleeding, at doses 50 to 100-fold greater (i.e. 1250 IU-5000 IU/kg NovoEight) than the proposed maximum clinical dose of 150 IU/kg in a repeat dose setting. These adverse events were predictive for human use of product, as confirmed by the adverse events reported in clinical trials. The results from the nonclinical program suggest that treatment of patients with hemophilia A with NovoEight (Antihemophilic factor [recombinant]) will be reasonably safe for use in its proposed indications.

##### *Pharmacological/Toxicological Findings*

The Applicant (Novo Nordisk Inc.) has completed an extensive nonclinical program to demonstrate the safety and effectiveness of Antihemophilic Factor (recombinant). Based on data from GLP-compliant and non-GLP nonclinical studies, NovoEight (Antihemophilic factor [recombinant]) was determined to be safe for its intended use for on-demand treatment and prophylaxis of bleeding episodes and peri-operative management of patients with hemophilia A.

The completed nonclinical program consisted of a series of studies to demonstrate the safety and effectiveness of Antihemophilic Factor (recombinant) in animals. Completed nonclinical studies included safety pharmacology (rats and dogs), effectiveness including pharmacology (monkeys), proof-of-principle (hemophilic mice and dogs), dose range-finding studies (mice), acute toxicity (monkeys, with toxicokinetics), repeat-dose toxicity (rats with toxicokinetics and monkeys with toxicokinetics), immunogenicity (rats), and pharmacokinetics (mice and dogs). Activity of NovoEight® for the proposed indication was tested in nonclinical studies in FVIII-deficient mice and dogs.

Overall, the nonclinical safety profile of N8 had no unexpected findings and did not identify any novel toxicities of concern. Antihemophilic factor (recombinant) was tested acutely in animals at doses up to 5000 IU/kg (50 times the intended median perioperative clinical dose of 100 IU/kg) for one day followed by 24 hour recovery observation period, before animals were euthanized without any adverse events reported. Repeat dose toxicity studies were complete up to 5000 IU/kg daily dosing for 14 days (166 times the intended median prophylactic clinical dose of 30

IU/kg) and product was tolerated, and similar results were confirmed by repeat (prophylactic) clinical use in clinical trials. In animal studies, adverse events were noted as an exaggerated pharmacological effect from product use including thromboembolic events, local reactions at treatment site, and re-bleeding at doses 50-100X (1250 IU-5000 IU NovoEight) greater than the proposed maximum clinical dose (150 IU/kg) in a repeat dose setting. These adverse events were predictive for human use of product as confirmed by adverse events reported in clinical trials. Toxicokinetic profiles demonstrated a linear dose-dependent increase in the levels of Factors VIII followed by a time-dependent decrease in product levels until antibody formation occurs resulting in decreased FVIII activity. The non-clinical safety profile raised no concerns. There is a potential safety concern regarding immunogenicity responses that may occur in patients following repeated product administration due to the presence of recombinant human proteins in NovoEight, and formation of antibodies in animal following repeat use. Therefore, clinical trials to evaluate the safety of repeated use and the immunogenicity of NovoEight will be requested by the clinical reviewer as post-marketing commitments. The safety profile determined for NovoEight™ is sufficient to support approval of the Biological License Application (BLA) in the proposed indication. Previous experience with similar products indicates NovoEight dosing has the potential to elicit thromboembolic events, local irritation, hypersensitivity or neutralizing antibody formation following administration. These potential safety issues are appropriately described in the NovoEight package insert.

The Applicant has not completed animal studies for carcinogenicity, mutagenicity, fertility, reproductive toxicity or teratogenicity, since these studies are not required based on both the indication and the current ICH S6 (R1) guidance, and there were no special toxicity concerns identified for this product regarding impurities or unexpected toxic effects. A toxicological risk assessment analysis was completed on the leachables and extractables associated with NovoEight manufacturing and container closure systems. The results of this risk analysis indicated that the levels of potential leachable/extractable impurities were within the range of the Applicant's specifications, and at acceptable levels based on extensive clinical and nonclinical experience. Based on the results of both the risk assessment and the nonclinical studies conducted with the extractables/leachables from the components used in its manufacturing and storage, NovoEight is approvable for licensure.

**Recommendation:** The Pharmacology/Toxicology Reviewer, La'Nissa A. Brown PhD, recommends that the Biological License Application (BLA) 125466/0 for NovoEight™ be approved, based on the results from both the toxicological risk assessment, and the nonclinical studies conducted by the Applicant.

### **III. Nonclinical Sections for Labeling/Package Insert (PI) for NovoEight**

The labeling was revised to reflect current labeling guidelines and the relevant information for prescribing, based on nonclinical data and clinical experience using NovoEight®.

#### **Labeling Revisions to Applicant's Label**

##### **Applicant's Language (Section edited):**

##### **8.1 Pregnancy**

Pregnancy Category C: Animal reproduction studies have not been conducted with NovoEight. Based on the rare occurrence of hemophilia A in women, experience regarding the use of

NovoEight during pregnancy is not available. Prescribe NovoEight to pregnant women only if clinically needed.

**FDA Revision:** Section 8.1 was modified to reflect labeling guidelines as per 21 CFR 201.57.

### **8.1 Pregnancy**

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

**Justification:** The Applicant did not use the proper language from the CFR to describe the Pregnancy Category C designation for NovoEight®.

## **13. NONCLINICAL TOXICOLOGY**

### **Applicant's Language (Removed the entire Section 13, below):**

Preclinical data reveal no special hazard for humans based on conventional studies of safety pharmacology and repeated dose toxicity.

**FDA Revision:** Entire Section 13 Section removed.

**Justification:** Removed entire Section 13 due to redundancy. The adverse findings in animals are not essential for clinical prescribing information; NovoEight® was evaluated in clinical trials and a similar adverse event profile was reported. Clinical adverse events are described in the appropriate sections of the label.

### **Applicant's Language (Section edited):**

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

No studies have been conducted with NovoEight to assess its mutagenic or carcinogenic potential. NovoEight has been shown to be comparable to other recombinant Factor VIII products with respect to its biochemical and physicochemical properties, as well as its non-clinical in vivo pharmacology and toxicology. No known risk related to carcinogenicity, mutagenicity or impairment of fertility has been reported after the use of Factor VIII in humans.

**FDA Revision: Section 13.1**

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

Long-term studies in animals to evaluate the carcinogenic potential of NovoEight, or studies to determine the effects of NovoEight on genotoxicity or fertility have not been performed. An assessment of the carcinogenic potential of NovoEight was completed, and suggests minimal carcinogenic risk from product use.

**Justification:** Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility section was edited to convey important information that was omitted by the Applicant (i.e. an assessment of carcinogenic risk was performed) and needed to be added to the label. Additionally, language that may be perceived as promotional (e.g. "NovoEight has been shown to be comparable to other recombinant Factor VIII products...") has been removed from this section.

*\*The final labeling language may be edited further, as negotiations are ongoing with the Applicant. If additional changes are made to the nonclinical sections of the label after communication with the Applicant, an addendum will be submitted to this file to document the changes and their justification.\**

#### IV. Background

Novo Nordisk Inc. has developed a recombinant, beta ( $\beta$ )–domain deleted Factor VIII product (rFVIII-BDD, codename N8) that is derived from the active form of FVIII, expressed in Chinese Hamster Ovary (CHO) cells cultured in serum-free media. The Applicant's proposed indications are for on-demand treatment of bleeding episodes, perioperative management, and routine prophylaxis in patients with hemophilia A. Novo Nordisk's recombinant Factor VIII B-domain deleted product is being marketed as a competitor for ReFacto®, and is referred to in this review by its codenames NN7008 or N8, or by its non-proprietary names Antihemophilic Factor (recombinant), and rFVIII-BDD. The mechanism of action for NovoEight® is temporary replacement of the missing or diminished activity of clotting factor VIII that is needed for effective hemostasis, based on the intrinsic pathway of the coagulation feedback cascade in patients with hemophilia A. Deletion of the B-domain of FVIII theoretically provides several advantages over full length FVIII including less immunogenicity, activates thrombin well while retaining its ability to bind von Willebrand factor, provides higher replication efficiency in the producer cell culture, and it is a smaller protein in size and appears as single protein band on gel electrophoresis. The Applicant has adequately addressed the nonclinical safety concerns identified for rFVIII-BDD (specifically, potential hemolysis, thromboembolic events, etc.) within clinical trials. The label reflects relevant precautions.

#### V. Proposed Use and Doses

NovoEight® is an Antihemophilic Factor (Recombinant) indicated for:

- Control and prevention of bleeding episodes in adults, adolescents and children (ages 0 – 12) with hemophilia A
- Perioperative management of patients with hemophilia A
- Routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adults, adolescents and children (ages 0 – 12)

Recombinant FVIII-BDD (NovoEight®) will be administered intravenously in a three-fold manner to hemophilia A patients as prescribed by the treating physician as follows: 1) control and prevention (routine prophylaxis – 20-40 IU NovoEight®/kg BW every 2-3 days [at least twice per week]); 2) Perioperative management (major surgical prophylaxis – a pre-dose of 100 IU/kg body weight [BW]), and if necessary, repeat-dose of 100 IU rFVIII-BDD/kg BW up to 4 times a day [up to 10-14 days] until healing is achieved); or 3) as on-demand treatment for life-threatening bleeding events (prescribed by the treating physician based on the type of bleeding episode).

NovoEight® will be supplied in single-dose vials containing 6 strengths; specifically, 250, 500, 1000, 1500, 2000, or 3000 International Units (IU) per vial. The diluent for reconstitution (0.9% Sodium Chloride) is supplied in a pre-filled diluent syringe.

#### VI. General Comments

- Animal studies for carcinogenicity, mutagenicity, and fertility have not been conducted. These studies are not considered necessary for approval as per the ICH S6(R1) guidance,

because NovoEight® is a recombinant, human protein and is not expected to directly interact with or damage DNA.

- There were no reproductive toxicity or teratogenicity studies using NovoEight® conducted in animals. These studies are not considered necessary for approval, because hemophilia A affects only male patients, and NovoEight® will not likely be used in pregnant women.
- There were no special toxicity concerns identified for this product regarding impurities or unexpected toxic effects.
- There is clinical experience using this product, including in 213 patients in the clinical trials conducted to date. Clinical data was used in lieu of requesting additional nonclinical studies to support and corroborate the safety profile of NovoEight® for BLA licensure.

## **VII. List of Non-Clinical Studies in BLA STN 125466/0**

### **Primary Pharmacodynamics**

1. **Study Report TEI m070101** - In vivo dose –response with FVII (Refacto®) and single doses of N8 and Advate in FVIII knock-out mice
2. **Study Report Telm070501** - N8 (rFVIII) In vivo dose –response of N8 and Advate in the tail bleeding Model in Hemophilia A mice
3. **Study Report SAQ070601** - The effect of N8 and Advate in a joint bleeding model hemophilia A mice
4. **Study Report AGRU240608** – N8 (rFVIII) Kinetic analysis of FVIII binding to different anti-FVIII monoclonal antibodies by -----(b)(4)-----
5. **Study Report EgPe0805** – (b)(4) analysis of N8 binding to von Willebrand factor and comparison with Advate and ReFacto
6. **Study Report MKja080602** – N8 (rFVIII) The effect of N8 in -----(b)(4)-----  
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7. **Study Report Mkja080601** – N8 (rFVIII) ----(b)(4)---- of N8 and commercially available FVIII compounds

### **Pharmacokinetics**

8. **Study Report MiE070601** – N8 (rFVIII) Pharmacokinetic and pharmacodynamic study N8 and Advate in two Hemophilia A dogs
9. **Study Report EgPe070901** – N8 (rFVIII) N8 interaction with von Willebrand factor assessed using -----(b)(4)-----
10. **Study Report DKpf070802** - Pharmacokinetics of N8, Advate and ReFacto administered i.v. to FVIII KO mice
11. **Study Report 208356** - NNC 0155-0000-0004: A Quantitative Whole Body autoradiography Study on (b)(4) and vWF KO Mice after single dose of N8

### **Toxicology**

12. **Study Report 208012** – NN1008: 14-Day Intravenous Administration Toxicity and Safety Pharmacology Study in the Male --- (b)(4) --- Money Followed by a 6 Day Recovery Period
13. **Study Report 211030** – 14 Day Intravenous (bolus) Administration Toxicity Study in Rat

14. **Study Report 207402** – NN7008: Single Intravenous Dose Escalation and Toxicokinetic Study in the Male ---(b)(4)--- Monkey

#### Other/Special Toxicity Studies

15. **Study Report 211272** – NNC 0155-0000-0004: Local Tolerance Study in Rabbits 4 Days after Perivenous, Intravenous and Intra-arterial Injection
16. **Study Report 210401** – NNC 0155-0000-0004: 2 and 4 week Immunogenicity Study in --- (b)(4) --- Rats
17. **Study Report Mkja070801** - N8 (rFVIII): Species cross-reactivity of N8 the --- (b)(4) --- monkey coagulation

### **VIII. Summary of Non-clinical Studies in STN 125466/0**

In summary:

PEL (pharmacologically effective level) = 5 IU/kg

tSF (tentative safety factor) = approximately 42-fold over the NOAEL for the prophylaxis regimen proposed, using the median clinical dose of 30 IU/kg NovoEight

NOAEL = 1250 IU/kg for repeat dose regimen and 5000 IU/kg for acute dosing

#### **Common Abbreviations**

TAT = thrombin-antithrombin	DDM = D-dimer	FIB = fibrinogen
PT = prothrombin time	HR = heart rate	volm. = volume
aPTT = activated partial thrombin time	KO = knock-out	gr. = group
ECG = electrocardiography	tSF = tentative safety factor	
BP = blood pressure		
CVS = cardiovascular system parameters (cardiotoxic signs, BP, ECG)		
i.v., IV = intravenous	NOAEL = no observed adverse effect level	
wt. = weight	TK = toxicokinetics	
TEG = thromboelastography	macro. = macroscopic sign(s)	
s.s. = statistically significant	PEL = pharmacologically effective level	
WBCT = whole blood clotting time (coagulation), <i>i.e.</i> FIB, aPTT, PT		
M = Male	PK = pharmacokinetics	
NOEL = No observed effect level	TTH = time to hemostasis	
h = hour(s)	min. = minute(s)	
wk = week(s)	F = female	
ADA = anti-drug antibodies		
C <sub>max</sub> = Maximum observed concentration	T <sub>max</sub> = maximum concentration	
AUC <sub>0-24</sub> = Area under the concentration-time curve (calculated using the trapezoidal rule from 0 hours to 24 hours)		
* The <b>vehicle (buffer)</b> for the test article was NaCl [18 mg/mL], CaCl <sub>2</sub> [0.25 mg/mL], histidine [1.5 mg/mL], polysorbate 80 [0.1 mg/mL] and sucrose [3.0 mg/mL]. The pH was approximately 6.9.		

#### Primary Pharmacodynamics

Several studies were omitted from the nonclinical discipline review, because they were in vitro studies and were a component of the assay validations. These studies are identified below, and were reviewed by the CMC reviewer and validated according to FDA standards and guidelines for CMC.

**Study Report Telm070501- N8 (rFVIII) In vivo dose – response of N8 and Advate in the tail bleeding model in hemophilia A mice**

The aim of this study was to evaluate a range of N8 doses for use in dose selection for further nonclinical studies and clinical trials. This study was an *in vivo* analysis to examine the dose response effects (efficacy) and potency of N8 compared to Advate® in FVIII knock-out (KO) mice versus wild type (WT) mice (-(b)(4)-) in tail bleeding model. FVIII knock-out mice (n=8/gr, M&F, total=80 mice) were dosed with 1, 5, 20, or 200 IU/kg BW or vehicle (buffer), N8 (batch 10B), or Advate® and vehicle only (WT mice only, n=8). As expected, the vehicle dosed KO mice had statistically significantly (s.s.) higher bleeding times and blood loss compared to the normal, wild-type (WT) group. FVIII KO animals dosed with 200 IU/kg of either FVIII variants showed normalization of blood loss and bleeding times following treatment, similar to WT mice. Statistically significant blood loss and bleeding times persisted in all FVIII treatment groups compared to WT type mice at doses up to 200 IU/kg. Dose-dependent, partial effects (trend - not s.s.) were seen in the lower dose FVIII variant treated groups, while the group treated at 1 IU/kg N8 showed no pharmacological effect. There did not appear to be any differences in the potency of the FVIII variants under the conditions tested. In addition, similar results in bleeding time and blood loss normalization were shown when comparing N8 to Refacto® and Advate® at 200 IU/kg in an NN internal study (2007, Study No TEIm070101; data [study report] not submitted, but was summarized in submission). There were no notable differences in FVIII activity measured following FVIII variant treatment in either of the studies based on chromogenic assay (35 mins. post-dose).

**Reviewer Comment:** Based on the efficacy and potency results in head-to head comparisons of N8 with Refacto® or Advate®, it appears that N8 would pose no greater safety risk than currently marketed FVIII products.

**Study Report SAQ070601 - The effect of N8 and Advate in a joint bleeding model hemophilia A mice**

The purpose of this study was to simulate the treatment effects of N8 as treatment for joint bleeding in patients with severe hemophilia, through an induced, knee joint bleeding model in hemophilic A mice (hemophilic synovitis model), monitored on Day 0, 1 or 3. FVIII Knock-out mice (n=24/gr.) were dosed with vehicle-buffer, or 200 IU/kg Advate® or N8 (batch 10B), 5 mins. prior to injury (i.e. needle inserted in the knee joint). The results indicate that, based on visual assessment, treatment with both FVIII variants significantly reduced bleeding in mice at approximately the same rate and extent comparatively.. Control (vehicle) animals scored maximum on visual bleeding score (VBS) assessment pre- and post-dosing, as expected. There were no changes to the hematology panel noted following treatment with any test article.

**Reviewer Comments:** This is a biologic activity study intended to illustrate that N8 was as effective as Advate®. Based on the results, N8 is reasonably safe for use in patients with hemophilia A for the treatment and prevention of bleeding, and N8 treatment would pose no apparent greater risk than currently marketed FVIII products in treating joint bleeding.

**Study Report TEI m070101- In vivo dose – response with FVII (Refacto®) and single doses of N8 and Advate in FVIII knock-out mice**

The purpose of this study is to evaluate the *in vivo* effect of FVIII derivatives in hemophilia A mice. FVIII Knock-out mice (n=6/gr.) were dosed with a single dose of 1, 10, 50, 100 or 200 U/kg Refacto®, 200 U/kg N8, or 200 U/kg Advate® or vehicle (buffer) for controls (negative:



WT mice and positive: FVIII knock-out mice). The blood samples from treated mice in the study were tested with the chromogenic activity assay for bleeding time and blood loss. The results indicate that the FVIII variants did not display notable, s.s. differences in normalization of bleeding time or blood loss in FVIII K.O. mice. The study demonstrates that N8 would likely be as effective as currently marketed FVIII products in normalization of coagulation function.

**Study Report EgPe070901 – N8 (rFVIII): N8 interaction with von Willebrand factor assessed using -----(b)(4)-----**

The aim of this study was to confirm that N8 (as compared to. ReFacto®) will retain its ability to bind to von Willebrand factor, its carrier protein (chaperone protein), and to determine the extent of this interaction using -----(b)(4)----- . The association and dissociation rates were dose-dependently determined, based on the amount of FVIII-variants added to sample. The vWF binding properties of N8 (batch (b)(4)) appear to correspond to the published and observed values for ReFacto®, with a  $K_D$  (binding association constant) ranging from 0.2-0.5 nM. This was an *in vitro* activity analysis assay; therefore validation of this study is deferred to the product (CMC) reviewer.

**Study Report AGRU240608 – N8 (rFVIII) Kinetic analysis of FVIII binding to different anti-FVIII monoclonal antibodies by -----(b)(4)-----**

The purpose of this study was to use plasmon surface resonance to measure interaction kinetics (i.e. conformational changes based on binding kinetics) between (b)(4) anti-FVIII monoclonal antibodies and N8 (batch (b)(4)), Advate® and ReFacto®. The  $K_D$  (binding association constants) appeared similar between the (b)(4) FVIII variants tested in this study, indicating that the targeted epitopes of these antibodies were present and accessible. It can be inferred that there are no differences in the tertiary structure of N8, Advate® and ReFacto®, based on conformational epitope recognition. This was an *in vitro* analysis which is at best supportive of data; validation and application of this study are therefore deferred to the CMC reviewer.

**Study Report EgPe0805 – (b)(4) analysis of N8 binding to von Willebrand factor and comparison with Advate and ReFacto**

This *in vitro* analysis was conducted to determine the competitive binding nature of N8 to von Willebrand factor versus Advate® and ReFacto®. This was an *in vitro* analysis assay conducted as part of the product characterization; therefore, the evaluation and validation of this study are deferred to the CMC reviewer.

**Study Report MKja080602 – N8 (rFVIII): The effect of N8 in -----(b)(4)----- assays**

This study detailed using -----(b)(4)----- assays to determine the *in vitro* effects of N8 on plasma. This was an *in vitro* analysis assay; therefore the evaluation and validation of this study are deferred to the CMC reviewer.

**Study Report Mkja080601 – N8 (rFVIII): -----(b)(4)----- of N8 and commercially available FVIII compounds**

This study investigated the reactivity of anti-FVIII monoclonal antibodies ----- (b)(4)----- with N8 (Batch (b)(4)) vs. Refacto®, Advate® and Haemate® (plasma derived). The results indicate that N8 can be characterized using the anti-FVIII monoclonal antibodies, and that the ---(b)(4)--- peptides were similar between N8 and the FVIII variants

tested, using -----(b)(4)----- analysis (----- (b)(4)-----). This was an *in vitro* analysis assay; therefore the validation of this study is deferred to the CMC reviewer.

### Pharmacokinetics

#### **Study Report MiE070601 – N8 (rFVIII): Pharmacokinetic and pharmacodynamic study N8 and Advate in two hemophilia A dogs**

The aim of this study is to evaluate the pharmacokinetic/pharmacodynamic (PK/PD) effects of N8 vs. ADVATE® in hemophilia A dogs in a cross-over study manner. Two hemophilic (FVIII knock-out) dogs were dosed by i.v. bolus injection with 100 IU/kg N8 to monitor clot formation, while observing clinical signs, whole blood clotting time (WBCT), and thromboelastography (TEG) based on chromogenic and clotting assays. The results of the PK profiles for N8 and Advate® in dogs are superimposable, and AUC values are comparable. It can be inferred that N8 would present no greater risk in safety pharmacology than other, currently marketed products based on cumulative experience with hemophilia dogs and replacement therapy testing (please see Table 3, below from a literature review for PK profiles with other FVIII replacement factors in hemophilia dogs).

**Study Report MiE070601 PK profiles for N8 and Advate® in hemophilia A Dogs**

Drug order dosing)	(In of	Animal (chromogenic assay)	Dose (IU/kg)	$t_{1/2}$ hr.	CL mL/kg/hr	Vss mL/kg	MRT (hr)	AUC <sub>t</sub> (h U/mL)
N8, rFVIII-BDD		(b)(4) animal	100	7.2	13.4	123	9	7.2
ADVATE®			100	7.9	9.5	98	10	9.9
ADVATE®		(b)(4) Animal	100	8.2	4.5	47	11	22.0
N8, rFVIII-BDD			100	10.5	4.3	62	14	22.9

Table 3 (below) is courtesy of Brinkhous K. et. al., 2002.

**Table 3 Mean Pharmacokinetic Parameter Estimates in Hemophilia A Dogs**

Product	Dose (IU/kg)	C 0.25 h (IU/mL)	Cl (mL/hr/kg)	Vd <sub>ss</sub> (mL/kg)	MRT (hr)	T <sub>1/2</sub> (hr)	k <sub>e</sub> (hr <sup>-1</sup> )	AUC <sub>0-4</sub> (IU/mL·hr)
ReFacto	125 (n=3)	2.4	3.9	63.5	15.9	11.7	0.06	33.1
Octonativ-M7	125 (n=3)	2.5	5.8	84.4	14.3	10.8	0.06	22.3
ReFacto	500 (n=2)	8.3	5.7	68.2	11.5	8.1	0.09	84.9
Octonativ-M7	500 (n=2)	5.9	9.6	112.8	11.5	8.6	0.08	52.0

C 0.25 = Activity measured 0.25 hr after administration.

Cl = clearance; Vd<sub>ss</sub> = Volume of distribution at steady state; MRT = Mean Residence Time; T<sub>1/2</sub> = Elimination half-life;

k<sub>e</sub> = Elimination rate constant; AUC<sub>0-4</sub> = area under the plasma FVIIIc-time curve from time 0 to infinity.

Note: Concentrations have been corrected for the effect of dilution of the blood by the citrate anticoagulant.

**Reviewer Comment:** The results are somewhat predictive of expected PK activity of N8, based on previous experience with similar FVIII-products in hemophilia A dogs and historical experience with this model and antihemophilic replacement therapy.

**Study Report DKpf070802 - Pharmacokinetics of N8, Advate and ReFacto administered i.v. to FVIII KO mice**

The purpose of this study was to evaluate the pharmacokinetics of FVIII variants in FVIII-KO mice. KO Mice (n=6:7 M:F/gr, T=68 animals) were intravenously dosed with 8, 80, 180, or 280 IU/kg N8 N8 (Batch (b)(4)), or 280 IU/kg ReFacto® or Advate®, and observed for 0-48 hrs post-dosing. N8 pharmacokinetics appeared to be dose-linear between 80-280 IU/kg/dose, but variable at 8 IU (data not shown). Both the chromogenic and FVIII antigen assays were utilized in this study to determine FVIII levels. It appears that the pharmacological effects of N8 are not easily determined or meaningful at lower doses, and that a minimum effective dose between approximately 180 to 280 IU/kg of N8 should be targeted in treatment of patients with hemophilia A, to ensure maximum treatment effect.

**\*All results presented below are based on results from the chromogenic assay**

Product	Dose IU/kg	Cmax (IU/mL)	AUC h*IU/L	T <sub>1/2</sub> (h)	CL (mL/h/kg)	Vss (mL/kg)
N8	80	1.04	7.1	4.9	11	73
N8	180	1.96	20	7.2	9	90
N8	280	2.74	26	7.8	11	117
Advate®	280	4.26	28	7.3	10	108
ReFacto®	280	2.87	29	6.7	10	97

**\*Results in the following table are based on 280 IU/ kg dosing of each FVIII variant**

Product	Assay	Cmax (IU/mL)	AUC h*IU/L	T <sub>1/2</sub> (h)	CL (mL/h/kg)	Vss (mL/kg)	MRT (h)
N8	FVIII chromogenic assay	2.74	26	3	7.8	11	11
Advate®		4.26	28	1	7.3	10	11
ReFacto®		2.87	29	1	6.7	10	9.9
N8	FVIII (b)(4)	2.01	20	8	9.6	14	13
Advate®		2.64	17	17	11	17	15
ReFacto®		1.99	20	10	8.2	14	12

It appears that FVIII variants display similar values for all PK parameters examined in the study.

**Reviewer Comments:** There were variations in the values for the parameters tested based on which assay was employed; however, these differences did not appear to be significant between the FVIII treatment groups when evaluated using the same assay. It does not appear that N8 would cause any greater risk to patients than currently marketed FVIII products, based on the clearance and distribution of the product compared to currently marketed products.

**Study Report 208356 - NNC 0155-0000-0004: A Quantitative Whole Body autoradiography Study on (b)(4) and vWF KO Mice after single dose of N8**

The aim of this study was to examine the tissue distribution of radioactivity by quantitative whole body radiography following acute intravenous administration of [<sup>125</sup>I] N8 to -----(b)(4)-----

---(b)(4)--- mice (wild-type), or to von Willebrand Factor (vWF) knock-out (KO) mouse model. vWF KO mice were dosed to investigate the distribution of N8 in the absence of vWF, which is the chaperone and stabilizer of FVIII in circulation, via formation of a non-covalently bound complex that prevents degradation of inactive FVIII in circulation. Mice (n=9 M/gr./strain) were dosed with 5.0 mL/kg BW containing approximately 59 IU/kg and 30.4  $\mu$ Ci/mL (radiochemical) of [ $^{125}$ I] N8, and were sampled (terminal sacrifice; cold shock) at 15, 30, and 90 minutes post-dose. Animals were treated and tissue cryo-slices were prepared, for  $^{125}$ I-N8 distribution by whole body autoradiography.

For ----(b)(4)---- mice, the average concentrations of N8 as determined by -(b)(4)- were 2.0U/mL, 1.8U/mL and 1.8 U/mL at 15, 30 and 90 min respectively. According to the autoradiography results, the mean concentration of radioactivity in the blood was similar up until 90 minutes post dosing, at which time it dropped to approximately 73%. N8 product was widely distributed in the -----(b)(4)----- WT mice, with peak levels occurring mainly at 90 minutes after dosing. Initial distribution was rapid and in general, blood, liver, kidney, lungs and spleen contained the highest levels of radioactivity at all sampling times.

In the vWF KO mice, the average concentrations of N8 as determined by -(b)(4)- were 1.0U/ml, 0.9U/ml, and 0.2 U/ml at 15, 30 and 90 min, respectively. According to autoradiography, mean radioactivity in the blood was decreased to approximately 57% at 30 minutes, and to 32% at 90 minutes, compared to the concentration measured in blood after 15 minutes. In vWF KO mice, product widely distributed throughout body with peak levels in the liver, thyroid glands, kidney and adrenals mainly at 90 minutes after dosing. The levels in the central nervous system were generally low, but were quantifiable throughout the study period.

It appears that there is a time- and dose-dependent distribution of  $^{125}$ I-N8 in mice following dosing. As time progresses, radioactivity levels decrease and are allocated to red blood cell-rich depots or high blood flow areas such as the spleen, liver, and thyroid gland. This study was completed in October 2008 in Malenovv, Denmark at NovoNordisk A/S's GLP test facility.

### Toxicology

#### **Study Report 207402 – NN7008: Single Intravenous Dose Escalation and Toxicokinetic Study in the Male ----(b)(4)---- Monkey**

The aim of this study was to investigate the toxicity and pharmacokinetics/toxicokinetics of N8 in male monkeys following single dosing with N8 (NNCD 0155-0000-0004, Batch (b)(4)). Monkeys (n=2M/gr., T=6 animals) were dosed acutely on two separate days with 50 and 500 IU/kg BW; [Days 1 and 8], 250 and 1250 IU/kg BW [Days 4 and 12], and 2500 and 5000 IU/kg BW [Days 19 and 26]) intravenously, while monitoring clinical signs (BW, mortality, behavior, electrocardiography [ECG], mean arterial pressure [MAP], etc.) and pathology. Prothrombin time (PT) was extended in dose-dependent manner, while aPTT was reduced in treatment animals; both effects were reversible at 24 hrs after dosing. The D-dimer (DDM) and thrombin-antithrombin (TAT) profiles were normal, to moderately higher than baseline. The NOAEL was determined to be 5000 IU, which is approximately 100-fold greater than the intended dose for licensure.

This study included histopathology and hematology panel with the following:

**Examined**

adrenals  
aorta  
brain  
caecum  
colon  
duodenum  
eyes and optic nerves  
femur (with bone marrow and articular surface)  
gall bladder  
gross lesions  
heart  
ileum#  
injection sites – left and right saphenous  
jejunum#  
kidneys  
liver  
lungs (with main stem bronchi, bronchioles, bronchial lymph nodes)  
mesenteric lymph nodes  
mandibular lymph nodes  
muscle  
oesophagus  
ovaries  
pancreas  
pituitary  
prostate  
salivary gland – mandibular, sublingual, parotid  
skin and subcutaneous tissue  
sciatic nerves  
seminal vesicles  
skin  
spinal cord cervical  
spinal cord lumbar  
spinal cord thoracic  
spleen  
sternum with bone marrow  
stomach  
testes and epididymides  
thymus  
thyroids with parathyroids  
tongue  
trachea  
ureters  
urinary bladder

# Peyer's patch was presented from either jejunum or ileum.

**Not examined – retained in fixative**

animal identification  
bone marrow smear (sternum)  
lacrimal glands  
larynx  
rectum (with anus)

**Hematology Panel consisted of:**

haemoglobin concentration  
packed cell volume  
mean cell volume  
mean cell haemoglobin concentration  
red cell distribution width  
platelet crit  
platelet distribution width  
prothrombin time

red blood cell count  
reticulocytes  
mean cell haemoglobin  
haemoglobin distribution width  
platelet count  
mean platelet volume  
total and differential white cell count  
fibrinogen

activated partial thrombin time (aPTT)  
d-dimers (DDM)

Thrombin-antithrombin complex (TAT)

**Clinical Chemistry Panel consisted of:**

Alkaline phosphatase (ALP)  
Aspartate aminotransferase (AST)  
Urea  
Glucose (Gluc)  
Total protein (Total Prot.)  
Sodium (Na)  
Chloride (Cl)  
Inorganic phosphorus (Phos)

Alanine aminotransferase (ALT)  
Total bilirubin (Bili)  
Creatinine (Creat)  
Total cholesterol (Chol)  
Albumin (Alb)  
Potassium (K)  
Calcium (Ca)  
Albumin/Globulin ratio (A/G ratio)

**Urinanalysis** (appearance, volume, electrolytes, specific gravity & microscopic examination)

**Ophthalmoscopy**

**Cardiovascular parameters** (HR, BP, lead II ECG, etc.)

**Necropsy-post mortem** (Macroscopic pathology, histology, organ weight-major organs only)

A reduction, as compared to pre-dose values and historical control data, in the circulating levels of several red cell parameters (hemoglobin, RBC, Packed cell count) was notable at 24 hours following treatment at dose levels of 500, 1250, or 5000 IU/kg. The Applicant claims that these changes were resulting from the repeated blood sampling, were not correlated to microscopic findings and thus are not considered adverse. There was a dose-dependent increase in FVIII activity (includes endogenous FVIII) as expected in toxicokinetics testing. The terminal half-life was 6.4-12 hrs in the higher dosed animals. The AUC, Vss, and Cl could not be measured in all animals consistently. Based on the histopathology evaluation, there did not appear to be any significant toxicity concerns not commonly associated with FVIII administration. This study should be considered a preliminary MTD determination study due to the small number of animals treated and design of the study as related to the dosing regimen. This study was not in compliance with Good Laboratory Practice standards. This study was completed February 2009, at -----(b)(4)-----.

**Reviewer Comments:** There were no control (positive or negative) or comparator (i.e. other FVIII variants) animals groups used in this study to determine relevance of results and findings. The study design of dose escalation that employed repeat use of animals (i.e. different doses in the same animals without sufficient washout period for complete product clearance) was not optimal. There were also no immunogenicity observations made in this study. It does not appear that N8 causes any overt toxicity, but the long-term effects of product use remain unknown and it is not predictive based on the lack of data presented in this study.

**Study Report 211030 – N8: 14 Day Intravenous (bolus) Administration Toxicity Study in Rat**

The aim of this study was to investigate the repeat dose toxicity and toxicokinetics of N8 (batch --- (b)(4) ---) following 14-day repeat administration with a 6-, treatment-free recovery period in rats. ----- (b)(4) ----- rats (n=5 M & F/dose in main gr., 5 M & F/dose in recovery gr.) were dosed daily (0 [vehicle buffer], 50, 250 and 1250 IU/kg BW N8 [Batch No. --- (b)(4) ---]) with 14-day recovery treatment-free period while monitoring clinical signs (BW, ECG, BP, food consumption, etc.), behavior (CNS, neurological, etc.), toxicokinetics (PK/TK/ immunogenicity)

and overt toxicity (gross pathology). The toxicokinetics were examined on Days 16 and 23. All animals were sacrificed on Day 16 or Day 23 as designated. There were no overt toxicities noted in this study. AUC could not be confirmed due to decreased endogenous FVIII activity following repeat dosing with N8. The terminal half-life was approximately 4.55 hrs based on median-dosed gr's.; however, AUC and other toxicokinetic parameters could not be confirmed for this study due to the development of antibodies interfering with endogenous FVIII activity from Day 14 until end of the study.

There were local tolerance reactions (i.e. slight inflammation, irritation noted, mild to moderate at treatment site) as expected in both the control and treatment groups. There was one incidence of thrombosis in a single animal from the high dose group (male animal). Spleen weights in male animals only decreased in the 250 and 1250 IU/kg groups in a dose-dependent, s.s. manner, although there were no microscopic or macroscopic correlates identified to corroborate the change.

There was development of anti-N8 antibodies reported in 110/114 animals correlating to increased aPTT times with reduced endogenous FVIII levels, beginning around Day 10-14 and persisting into end of recovery period, particularly in high dosed group animals (1250 IU/kg group). There were no significant changes in clinical signs (ECG, MAP, respiration, etc.) observed following treatment with N8 at any dose. Side effects observed at high doses were expected, as related to treatment with this product. Necropsy and histopathology were performed on animals in the manner as mentioned above (acute toxicity testing). Based on the line listed data for individual animals, the findings were acceptable as related to toxicity assessment, and within the range of the control animals. These minor changes also appeared to be reversible (time- and dose-dependent) as noted by the discontinuation of product use during recovery period. It appears that N8 was well tolerated in all animals tested at all dose levels. The NOEL for this study is 1250 IU/kg which is ~ 42X the proposed clinical median dose for prophylaxis treatment regimen (~30 IU/kg). The NOEL did not account for the formation of antibodies (i.e. the immunogenic response expected from foreign protein in animal model), therefore the NOEL defined for this study is the non-immunogenic dose level.

This study was completed February 2011 at -----(b)(4)-----  
-----, following the Organization for Economic Cooperation and Development (OECD) Principles on Good Laboratory Practice (GLP) Regulations.

**Study Report 208012 – NN1008: 14-Day Intravenous Administration Toxicity and Safety Pharmacology Study in the Male -----(b)(4)----- Monkey Followed by a 6-Day Recovery Period**

The aim of this study was to investigate the toxicity and toxicokinetics of N8 (batch ---(b)(4)---) following 14-day repeat administration with a 6-day, treatment-free recovery period in ---(b)(4)--- monkeys. Male monkeys (n=5 M/dose in main gr.; 3 M/dose in recovery gr. T=29 animals) were dosed daily (0 [buffer], 50, 1000 and 5000 IU/kg BW) with 6-day recovery treatment-free period while monitoring clinical signs (BW, ECG, BP, food consumption, etc.), behavior (CNS, neurological, etc.), toxicokinetics (PK/TK/ immunogenicity) and overt toxicity (gross pathology). The toxicokinetics were examined consistently in only the 1000 and 5000 IU/kg groups followed up to 24 hr; AUC had mostly dose-linear appearance. The terminal half-life was 5-12 hrs based on the high-dosed groups.

One animal, animal (b)(4) (which replaced animal (b)(4) on day 14 [at day 15] for humane reasons: impaired mobility in rt. foreleg) was removed prematurely on Day 19 and euthanized. This

monkey developed an acquired hemophilia due to cross-reacting, neutralizing antibodies exhibited by bruising and swelling in the inguinal region and scrotum, and impaired mobility in rt. hind leg. These incidences may be of concern, although doses are significantly higher for these occurrences (5000 IU/kg/dose, or 10 to 50-fold higher than the proposed highest clinical dosing). All other animals were sacrificed on Day 15 (n=4/gr.) and on Day 21 (n=3/gr.-treatment only) and did not appear to have any overt thrombogenic findings. There were local tolerance issues (slight inflammation, irritation/rash-noted, bruising [sores] and mild to moderate swelling) as expected from the repeat dosing, handling and treatment regimen. This study was GLP compliant (except for product formulation and formulation analysis, which was completed offsite).

There was development of anti-N8 antibodies in 22/24 animals, with neutralizing, cross-reacting antibodies developing in 18/24 [22] animals correlating to increased aPTT times, beginning around day 10-14 and persisting into the end of the recovery period. The antibody formation was at reasonable level based on dosing levels compared to the control levels. FIB, DDM, and TAT levels were normal in treated animals (2-3 outliers in each N8 dosed animals leading to slight alterations, but no notable or s.s. differences occurred), while PT increased in treated animals (notable increase in 5000 IU gr. at end of recovery period). There were incidences of hemorrhage that were dose-related in all treatment animals (e.g., joints, lung and slight to moderate in severity) as expected from development of cross-reacting antibodies. Mean red blood cell levels and packed red blood cell volm. were altered in 1000 IU/kg gr. but were not s.s (similar to changes observed in acute toxicity testing). A reduction, when compared to control, in the circulating levels of several red cell parameters occurred (mean hemoglobin, red blood cells [RBCs], packed cell volm.) and an increase in some hematology parameters (hemoglobin distribution width, red cell distribution width, PLT dist. width, PT and elevated mean RET, absolute RET) were noted in high dose treated animals, but not s.s. in differences. These animals were treated well above expected clinical maximum dose levels. There were no significant changes in clinical signs (electrocardiogram [ECG], mean arterial pressure [MAP], respiration, etc.) observed following treatment with N8 at any dose. Side effects observed at high doses were expected as related to treatment with this product. Necropsy and histopathology were performed on animals in the manner as mentioned above for the acute toxicity study. Based on the line listings, the findings were acceptable as related to toxicity assessment, and the results were within range of control animals. These changes also appear reversible (time- and dose-dependent) as noted by the discontinuation of product use during recovery period. In addition, human PK data was collected in UK clinical trial (Study NN7008-3522). This study was completed February 2008 at -----(b)(4)----- following the Organization for Economic Cooperation and Development (OECD) Principles on Good Laboratory Practice (GLP) Regulations.

**Reviewer Comments:** It appears that the Applicant did not examine a feasible recovery time period from treatment, with only a short treatment free period of 6 days (up to 14 days treatment free is usually employed to determine immunogenicity and toxicity reversibility). There was a re-bleeding event in a high-dose animal that led to early sacrifice of that one animal (Animal (b)(4)). There is concern for hemolysis based on the reduction in RBCs in both the acute and repeat-dose toxicity studies. In addition, the study report was amended with justification provided by Applicant for hemolysis. There was no head-to-head comparison with N8 product and currently marketed FVIII products (ex. ReFacto® or Advate®). The use of N8 should not cause any greater risk in patients than currently market products, with note to the clinical discipline to monitor concerns including joint re-bleeding and hemolysis.



**Other/Special Toxicity Studies****Study Report 211272 – NNC 0155-0000-0004: Local Tolerance Study in Rabbits 4 Days after Perivenous, Intravenous and Intra-arterial Injection**

The aim of this study was to determine if NovoEight will be well tolerated in local injection sites in rabbits followed by 4 days observation. -----(b)(4)----- rabbits (n = 4 M/gr.) were dosed with 500 IU N8/mL in right ear, and vehicle (0.15 mL/kg) in left ear by various routes of administration: intra-arterial (i.a.), perivenous (p.v.), or intravenous (i.v.) which is the intended clinical route of administration. Injection sites were observed daily specifically for hemorrhage, bruising, erythema, and swelling. Each parameter was scored using the following severity scale: 0 - not present, 1 - minimal, 2 - slight, 3 - moderate, 4 - marked or 5 - massive/extensive. Animals tested for p.v., i.v. and i.a. demonstrate similar results between test articles and vehicle over course of the study. Of note, clinical signs of swelling, irritation, and hemorrhage were noted for use of both vehicle (control) and NovoEight product at site of administration (local site) for all routes of administration, with mild histopathological findings (average scores approximately 1-2), and comparable incidence and severity between groups with same routes of administration. These findings were considered as a part of clinical monitoring and advised in label. These results were consistent with similar products and with the N8 results in repeat dose toxicity studies in other animal species. This study was completed October 2011 at -----(b)(4)----- following the Organization for Economic Cooperation and Development (OECD) Principles on Good Laboratory Practice (GLP) Regulations.

**Study Report 210401 – NNC 0155-0000-0004: 2 and 4 week Immunogenicity Study in --- (b)(4) --- Rats**

The aim of this study was to evaluate immunogenicity following N8 use in -----(b)(4)----- Rats. Animals (n=3 M & F/group) were dosed by i.v. bolus with 1250 IU/kg daily for 14 or 28 days as designated by group, and serum samples were investigated after 2 and 4 weeks of dosing for antibody development. Blood samples were taken on Days 1, 8, 14, 15, 16 at pre-dose and 30 minutes post dose (Group 1 only) and Days 1, 8, 15, 22, 28, and 30, at pre-dose and 30 minutes post dose (Group 2 only). Animals were observed daily for clinical observations, body weight (weekly), food and water consumption, local reactions (injection site) and overt toxicity. Antibody development was analyzed by -----(b)(4)----- using radioactive <sup>125</sup>I-labelled rN8. All animals exhibited antibody development (both anti-N8, and cross-reacting to endogenous FVIII). There was an increase in antibody titer levels specifically for anti-N8 on Day 30, as compared to Day 16. There were no signs of adverse events based on clinical observations noted in this study. Based on these study results, further repeat-dose toxicity nonclinical studies were limited to two weeks duration to minimize antibody formation, and ensure the best study results. This study was a non-GLP compliant study.

**Study Report Mkja070801 - N8 (rFVIII): Species cross-reactivity of N8 the cynomolgus monkey coagulation**

The purpose of this study was to predict the antigenicity of N8 in cynomolgus monkey plasma and human plasma, using the -----(b)(4)----- . This *in vitro* assay intended to support the safety of N8 administration. The (b)(4) results demonstrated a dose-dependent change in thrombin generation in human and monkey plasma, although the curve varied between species (i.e. the data were not superimposable). Additionally, these results confirm cross-reactivity

between monkey plasma and N8 as can be expected in coagulation system testing in monkeys with human protein.

### **Leachables and Extractables Analysis**

A risk assessment analysis was completed on the leachables and extractables associated with the manufacturing and storage of NovoEight. The following manufacturing and storage items were assessed in a nine month study: sodium chloride prefilled syringes and chlorobutyl rubber sealed vials (-----)(b)(4)-----). The manufacturing process and materials are comparable to those used in other licensed products such as NovoSeven®.

A toxicological risk assessment analysis was also completed on the leachables and extractables associated with NovoEight manufacturing and container closure systems. The results of analysis indicated that the levels of the leachables/extractables materials observed were within the range of specifications and at acceptable levels of potential leachable/extractable impurities were within range of the Applicant's specifications, based on extensive clinical experience. Based on the results of both the toxicological risk assessment and the nonclinical studies conducted with the extractables/leachables from the components used in its manufacturing and storage, NovoEight is approvable for licensure.

### **Integrated Safety Pharmacology**

Hemophilic animal models (FVIII knock-out mice and dogs) dosed with 4 IU-250 IU/kg NovoEight demonstrate that N8 corrects hemostasis in a dose-dependent manner. The safety of NovoEight has been demonstrated in monkeys, dogs, mice, rats and rabbits up to 5000 IU/kg daily repeat dosing, for up to 14 days. The pharmacological effective level (PEL) is 5 IU/kg, and the NOAEL is 5000 IU/kg for the acute dose setting. The MTD was also 5000 IU/kg in ----(b)(4)---- monkeys treated daily for 14 days, which was highest dose and frequency tested for repeat dose use, although immunogenicity was noted. Adverse events associated with NovoEight were thromboembolic events and re-bleeding, specifically in monkeys dosed with 1250 IU/kg or 5000 IU/kg. Local reactions at the treatment site were noted in all animals in a dose-dependent manner. Anti-drug antibodies (ADA) developed in animals repeatedly dosed with NovoEight, and were an expected immunologic response from foreign protein exposure. All adverse events were expected, exaggerated pharmacological effects following high doses of FVIII. Nonclinical findings support the safety profile of NovoEight for the proposed indication, based on the safety and effectiveness of the product.