CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208751Orig1s000

NON-CLINICAL REVIEW(S)

MEMORANDUM TO THE FILE

Date: July 11, 2017
From: Arulasanam Thilagar, PhD; Pharmacology/Toxicology Reviewer
Through: C. Lee Elmore, PhD; Supervisory Toxicologist

Subject: Nonclinical information supporting the safety of IV administration of nicotinamide

To: NDA 208751 for (b) (4)® (Insulin aspart injection), Novo Nordisk

Introduction: The (b) (4)® (hereafter referred to as insulin aspart injection) formulation contains 20.(b) (4) mg/mL nicotinamide as an absorption modifier. The Applicant proposes in draft product labeling that insulin aspart injection may be diluted for subsequent intravenous (IV) infusion. Systemic exposures relevant to IV administration with insulin aspart injection containing nicotinamide are covered by the Applicant's current nonclinical database, and while Pharm/Tox previously recommended approval for insulin aspart injection (see review in DARRTS, signed on 29 August 2016), we have been called upon to address residual clinical concern regarding the safety of IV infusion of nicotinamide, given that animal studies to support approval of insulin aspart injection were conducted predominantly by the SC route.

Here, we provide a nonclinical review of the available safety information supporting insulin aspart injection administration by the IV route, followed by Pharm/Tox's conclusions and recommendations.

Mechanism of action for absorption enhancement properties of nicotinamide: As discussed in the primary NDA review authored by Dr. Tsai-Turton, the effect of nicotinamide on insulin is related to enhancement of insulin monomer formation, rather than an effect secondary to increased local blood flow (e.g., vasodilation) proximal to the SC injection site. According to Dr. Tsai-Turton's review, "The mechanism by which the increased early absorption is achieved for insulin aspart injection [with nicotinamide] was investigated in vitro and in vivo. The in vitro study using an HDMEC monolayer showed that after s.c. administration, nicotinamide increases the monomer fraction and permeation rate of insulin aspart across the endothelium. In vivo study (Study No JKIP130102) in pigs of the effect of nicotinamide on ^{133}Xe washout (a marker of local blood flow) revealed no statistically significant effect of nicotinamide alone. These studies suggest that the nicotinamide present in insulin aspart injection augments the proportion of insulin aspart monomers readily available for absorption following s.c. administration thereby facilitating a more rapid absorption across the endothelium compared to NovoRapid®/NovoLog® whereas a direct effect of nicotinamide on local blood flow is considered unlikely to play a major role (Study No JKIP130102)." The demonstrated mechanism of action of nicotinamide's role in insulin aspart injection, and

in the absence of evidence of any direct effect on the vasculature, is consistent with a determination of low risk of long-term safety of IV nicotinamide.

Nicotinamide as co-API in approved injectable products: Nicotinamide is contained in several FDA-approved parenteral multi-vitamin drugs as a co-API. These drugs may be infused chronically by the IV route. The Applicant provided information on IV nicotinamide infusion in these approved drugs compared to insulin aspart injection (see table below). In insulin aspart injection, the concentration of nicotinamide in the infusion solutions is within the range used in the approved drugs. Moreover, for insulin aspart injection there is a lower exposure rate of nicotinamide (≤4.4 mg/h) compared to that of approved drugs (up to 960 mg/h), since the infusion flow rate for insulin aspart injection is generally much slower. These data are considered to support both the systemic and local safety of longer-term IV infusion of nicotinamide in insulin aspart injection.

Overview of nicotinamide infusion details in approved drugs for IV infusion in comparison to insulin aspart injection

	Nicotinamide Concentration (µg/mL)	Duration of infusion (h/day)	Flow rate (mL/h)	Exposure rate nicotinamide (mg/h)	Daily dose nicotinamide (mg)	Further details
Insulin Aspart Injection 0.5-1U/mL[a]	105-210	≤24	≤42[b]	≤4.4[c]	≤24[b]	Up to 9 days (2)
Infuvite® (3,4,5)	40-80	2-24	≤500	≤20	40	The dose can Be increased 1.5 to 3- fold for a few days/ weeks
Pabrinex® (6)	≤6,000	0.5[d]	160-260	≤960[e]	160-480	Up to 7 days

[a] Insulin aspart injection prepared for IV infusion (diluted 100 to 200-fold according to the proposed label).

[b] Daily insulin doses were 27-115 U/day and bolus doses up to 21 U/h (in a review of 12 protocols in the insulin therapy in critical care units (1). Median treatment duration of 4 days (interquartile range, 2 to 9 days) (2).

[c] Calculation of maximum exposure rate (concentration of nicotinamide x flow rate): 105 µg/mL* 42 mL/h = 4.4 mg/h or 210 µg/mL* 21 mL/h = 4.4 mg/h

[d] Some cases administered 30 minutes every 8 hours or 30 minutes twice daily for up to 7 days (6).

[e] Calculation of maximum exposure rate: 6000 µg/mL* 160 mL/h = 960 mg/h based on 3 pairs of vials (each pair of vials containing 160 mg nicotinamide in 10 mL) diluted with 50 mL infusion solution

Nicotinamide's use as an excipient in approved injectable products: Nicotinamide is listed in the FDA's database "Inactive Ingredient Data Base for Approved Drug Products" for parenteral injection at concentrations higher than those in insulin aspart injection. While neither drug is administered chronically, as proposed under NDA 208751, they add to the body of evidence supporting the safety of nicotinamide administration by the IV route.

Data from the FDA's Inactive Ingredient database (IID)

Excipient	Max content in approved products	Route of administration
Nicotinamide (niacinamide)	2.5%	Intravenous, intramuscular intra-articular, intrasynovial, intralesional*

*Nicotinamide is contained in two parenteral use products, including [redacted] (b) (4) (intramuscular, intravenous, intra-articular, intralesional and intrasynovial routes) and [redacted] (b) (4) (intramuscular route)

Local toxicity studies conducted with insulin aspart injection: The Applicant did not provide any dedicated chronic nonclinical studies to support the safety of long-term IV administration of nicotinamide, but did provide some acute/local nonclinical safety studies. The Applicant provided information on the single-dose local tolerance using intra-articular, IM and IV administration of nicotinamide in rabbits. The local tissue reactions observed when dosing with insulin aspart injection were of similar nature and severity for all three administration routes when comparing to the saline control. Refer to Dr. Tsai-Turton's primary NDA review (29 August 2016) for additional details.

Conclusion and safety recommendation: While the Applicant did not provide dedicated nonclinical studies to evaluate the long-term safety of IV nicotinamide administration in the NDA, the safety of IV usage is supported by 1) the Sponsor's existing database (for the purposes of supporting systemic exposures by IV administration), 2) the mechanism of action of nicotinamide (i.e., increased insulin monomer formation rather than a direct effect on the vasculature, e.g., vasodilation), 3) the prior use of nicotinamide in FDA-approved IV administered drug products (both as API or as an excipient) at higher amounts and for chronic dosing, and 4) local toxicity studies demonstrating the absence of significant local toxicity by the IV (or other) route(s). In conclusion, the available data support the safety of nicotinamide for chronic IV use as an excipient contained in insulin aspart injection and such use may be labeled for this product.

List of Literature References (cited references in the Tables are in Italics)

1. Draft Guidance for Industry and Review Staff: Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route, FDA, March 2008.

2. Powell, M. E., Hill, S. A., Saunders, M. I., Hoskin, P. J., and Chaplin, D. J. Human tumor blood flow is enhanced by nicotinamide and carbogen breathing. Cancer research, 57(23), 5261-5264, 1997.

3. FDA Guidance for Industry, Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, 2005

4. OECD. 3-Pyridinecarboxamide (nicotinamide) CAS No. 98-92-0. SIDS Initial Assessment Report for SIAM 15. 2003.

5. European Chemical Agency (ECHA). Nicotinamide. http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances. Accessed July 2014.

6. Mitterer KE. Rabbit Embryotoxicity Study of LZ612 (Unpublished Report No. 14464/01). Summary document. LPT Laboratory of Pharmacology and Toxicology KG, Hamburg. 2003.

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/s/

ARULASANAM K THILAGAR
07/12/2017

CALVIN L ELMORE
07/12/2017
I concur

DEPARTMENT OF HEALTH & HUMAN SERVICES Food and Drug Administration

Memorandum

PHARMACOLOGY/TOXICOLOGY
MEMO TO FILE

Date: September 20, 2016
From: Miyun Tsai-Turton, PhD, Pharmacology/Toxicology Reviewer
Through: C. Lee Elmore, PhD; Supervisory Toxicologist

Subject: Supplementary Pharmacology/Toxicology Review for NDA 208751 addressing the (b) (4)

To: NDA 208751 for (b) (4)**® (Insulin aspart injection), Novo Nordisk**

Refer to: Pharmacology/Toxicology Review & Evaluation of NDA 208751 (Finalized in DARRTS on August 29, 2016)

Background

(b) (4) (insulin aspart injection) is intended for the treatment of patients with diabetes mellitus. The pharmacology of insulin aspart injection is similar to that of NovoLog®. Insulin aspart injection contains insulin aspart in a new formulation (with two additional excipients: nicotinamide and L-arginine) designed to alter the absorption profile.

On August 31, 2016, Pharmacology/Toxicology was contacted by CMC concerning (b) (4) .

On September 01, 2106, the following Information Request was sent out to the sponsor:

(b) (4)

On September 14, 2016, the sponsor responded to our Information Request. This memo is to evaluate if the sponsor adequately addressed the (b) (4)

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/s/

MIYUN M TSAI-TURTON
09/20/2016

CALVIN L ELMORE
09/20/2016
I concur.

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 208751
Supporting document/s:	SDN0000
Applicant's letter date:	December 8, 2015
CDER stamp date:	December 8, 2015
Product:	(b) (4) (Insulin aspart injection)
Indication:	To improve glycemic control in adults with diabetes
Applicant:	Novo Nordisk
Review Division:	DMEP
Reviewer:	Miyun Tsai-Turton, PhD, MS
Supervisor/Team Leader:	Lee Elmore, PhD
Division Director:	Jean-Marc Guettier, MD
Project Manager:	Callie Cappel-Lynch

PAUDA goal date: October 8, 2016

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 208751 are owned by Novo Nordisk or are data for which Novo Nordisk has obtained a written right of reference. Any information or data necessary for approval of NDA 208751 that Novo Nordisk does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 208751.

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1 Executive Summary

1.1 Introduction

Novo Nordisk (the Applicant) is seeking approval of (b) (4) (hereafter referred to as insulin aspart injection) as a mealtime insulin with altered early glucose lowering compared to NovoLog® (a U.S.-marketed insulin aspart product hereafter referred to as Novolog® or as NovoRapid® for the European marketed version) for the treatment of patients with diabetes mellitus. Insulin aspart injection contains insulin aspart in a new formulation designed to alter the absorption profile. Compared to NovoLog®, insulin aspart injection contains two additional excipients: nicotinamide (vitamin B3, or niacinamide) to enhance absorption and L-arginine hydrochloride (an amino acid) as a stabilizing agent. Nicotinamide appears to result in a slightly earlier initial absorption profile of subcutaneously administered insulin aspart. The Applicant intends for insulin aspart injection to be used in in combination with basal (i.e., long-acting) insulins, (b) (4)

Insulin aspart injection is packaged as a pre-filled pen containing 100 units/mL insulin aspart at a concentration of 600 nmol/mL. Insulin aspart injection is provided in either a 3 mL autoinjector cartridge assembled into a pre-filled pen-injector or 10mL vials for injection. The prefilled pen-injector has a range of 1 to 80 units adjustable in increments of 1 unit.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical development of insulin aspart injection relies upon the nonclinical program conducted to support NDA 020986 (NovoLog®), as well as literature review of two additional excipients (nicotinamide and L-arginine), pharmacokinetic studies, and local tolerance studies. Since a full nonclinical program was previously conducted for NovoLog®, no additional toxicity studies (aside from those pharmacokinetic and local toxicology studies discussed here) were conducted for insulin aspart injection. See product labeling for (b) (4) or Novolog® or the Integrated Summary of this review for a brief description of reproduction, genetic toxicity and carcinogenicity studies conducted for Novolog®.

Various formulations of insulin aspart injection containing nicotinamide and L-arginine were tested in pharmacokinetic studies. The formulations FIA (Q) was chosen as the commercial insulin aspart injection formulation, whereas FIA (A/B/C/D), and other formulations, were evaluated during the development of insulin aspart injection and are generally representative of the FIA (Q) formulation. Pharmacokinetic studies with 4 formulations (FIAs A/B/C/D) showed an earlier absorption and earlier glucose lowering profile in pigs compared to NovoLog®.

Local tolerance studies for insulin aspart injection were performed in the rat, rabbit, and minipigs models. In minipigs, local tolerance was studied after single dose subcutaneous administration of insulin aspart containing various concentrations of L-arginine and nicotinamide. Insulin aspart injection was administered as daily injections of FIA (A, B, C and D) for up to four weeks followed by a two-week recovery period. The tissue reactions observed in these local tolerance studies with minipigs and rats were similar when comparing FIA (A, B, C and D) and NovoLog®. In rabbits, local tolerance was assessed after a single dose intravenous, intramuscular, and intra-arterial administration (assessing for inadvertent dosing in the muscle or blood vessel) of insulin aspart injection. The local tissue reactions observed for all three routes of administration of insulin aspart injection were of comparable to controls (0.9% sodium chloride for injection). While intravenous injection sites showed changes of a slightly higher grade (slight versus minimal perivascular/vascular necrosis) with insulin aspart injection compared to with 0.9% sodium chloride, the results do not suggest any significant safety concern for rare accidental intravenous exposures.

Review of the available literature supports the safe use of nicotinamide and L-arginine as excipients at the levels contained in the insulin aspart injection formulation for chronic subcutaneous administration in humans. No significant safety concerns were identified.

Drug substance-related impurities were comparable between the impurity profiles for insulin aspart injection and NovoLog®. One excipient-related impurity and two leachables were evaluated, and were determined not to pose a safety risk to humans per ICH M7, ICH Q3B and ICH Q3C guidances.

Findings in pharmacokinetic and local tox studies conducted with insulin aspart injection for this application were predominantly related to the expected pharmacological or exaggerated pharmacological actions of insulin aspart, which included clinical signs of hypoglycemia and effects secondary to hypoglycemia. In summary, the extensive nonclinical and clinical experience with the insulin aspart component of insulin aspart injection (identical to the active component of Novolog®), along with the available literature supporting the nicotinamide and L-arginine excipients, the local tolerance studies in rats, rabbits and minipigs and the safety evaluation of impurities, the nonclinical data supports the safety of chronic administration of insulin aspart injection in patients with diabetes.

1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/Toxicology perspective, this application for insulin aspart injection is approvable.

1.3.2 Additional Nonclinical Recommendations

No additional recommendation.

1.3.3 Labeling

The labeling for insulin aspart injection is based on the approved NovoLog product labeling. (b) (4) . The current Novolog® label and the label recommended by FDA DPMH reviewer are available in the Appendix of this document.

The Sponsor's Proposed Labeling

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

(b) (4)

There are no (b) (4) available with FIASP to (b) (4) drug-associated risk (b) (4)

(b) (4)

(b) (4)

Clinical Considerations

Disease-associated maternal and/or embryo/fetal risk

Poorly controlled diabetes in pregnancy increases the maternal risk for diabetic ketoacidosis, pre-eclampsia, and delivery complications (b) (4) Poorly controlled diabetes increases the fetal risk for (b) (4) still birth, macrosomia related morbidity (b) (4)

(b) (4)

Data

Human Data

(b) (4)

Animal Data

(b) (4)

Fertility, embryo-fetal and pre-and postnatal development studies have been performed with (b) (4) insulin aspart (b) (4) and regular human insulin in (b) (4) rats and rabbits. In a fertility and embryo-fetal development study, insulin aspart was administered (b) (4) before mating, during mating, and throughout pregnancy. Further, in a pre- and postnatal development study insulin aspart was given throughout pregnancy and during lactation to rats. In an embryo-fetal development study insulin aspart was given to female rabbits during organogenesis. The effects of insulin aspart did not differ from those observed with subcutaneous regular human insulin. Insulin aspart, like human insulin, caused pre- and post-implantation losses and visceral/skeletal abnormalities in rats at a dose of 200 units/kg/day (approximately 32 times the human subcutaneous dose of 1.0 unit/kg/day, based on human exposure equivalents) and in rabbits at a dose of 10 units/kg/day (approximately three times the human subcutaneous dose of 1.0 unit/kg/day, based on human exposure equivalents). No significant effects were observed in rats at a dose of 50 units/kg/day and in rabbits at a dose of 3 units/kg/day. These doses are approximately 8 times the human subcutaneous dose of 1.0 unit/kg/day for rats and equal to the human subcutaneous dose of 1.0 unit/kg/day for rabbits, based on human exposure equivalents. The effects are considered secondary to maternal hypoglycemia.

8.2 Lactation

Risk Summary

(b) (4) The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for insulin, any potential adverse effects on the breastfed child from insulin aspart or from the underlying maternal condition.

(b) (4)

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

(b) (4)

In 52-week studies, Sprague-Dawley rats were dosed subcutaneously with (b) (4) at 10, 50, and 200 units/kg/day (approximately 2, 8, and 32 times the human subcutaneous dose of 1.0 units/kg/day, based on units/body surface area, respectively). At a dose of 200 units/kg/day, (b) (4) increased the incidence of mammary gland tumors in females when compared to untreated controls. The incidence of mammary tumors for (b) (4) was not significantly different than for regular human insulin. The relevance of these findings to humans is not known. (b) (4) was not genotoxic in the following tests: Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, *in vivo* micronucleus test in mice, and in *ex vivo* UDS test in rat liver hepatocytes. In fertility studies in male and female rats, at subcutaneous doses up to 200 units/kg/day (approximately 32 times the human subcutaneous dose, based on units/body surface area), no direct adverse effects on male and female fertility, or general reproductive performance of animals was observed.

(b) (4)

The Reviewer's Recommended Labeling

Per DPMH recommendation, DMEP Pharm/Tox proposed labeling under Section 8 is described below. (b) (4)

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

There are no (b) (4) available with FIASP to (b) (4) drug-associated risk (b) (4)

(b) (4)

(b) (4)

Clinical Considerations

Disease-associated maternal and/or embryo/fetal risk

Poorly controlled diabetes in pregnancy increases the maternal risk for diabetic ketoacidosis, pre-eclampsia, and delivery complications (b) (4)

[redacted] Poorly controlled diabetes increases the fetal risk for [redacted] still birth, macrosomia related morbidity [redacted].

[redacted]

Data
Human Data

[redacted]

Animal Data

[redacted]

Fertility, embryo-fetal and pre-and postnatal development studies have been performed with [redacted] insulin aspart [redacted] and regular human insulin in [redacted] rats and rabbits. In a fertility and embryo-fetal development study, insulin aspart was administered [redacted] before mating, during mating, and throughout pregnancy. Further, in a pre- and postnatal development study insulin aspart was given throughout pregnancy and during lactation to rats. In an embryo-fetal development study insulin aspart was given to female rabbits during organogenesis. The effects of insulin aspart did not differ from those observed with subcutaneous regular human insulin. Insulin aspart, like human insulin, caused pre- and post-implantation losses and visceral/skeletal abnormalities in rats at a dose of 200 units/kg/day (approximately 32 times the human subcutaneous dose of 1.0 unit/kg/day, based on human exposure equivalents) and in rabbits at a dose of 10 units/kg/day (approximately three times the human subcutaneous dose of 1.0 unit/kg/day, based on human exposure equivalents). No significant effects were observed in rats at a dose of 50 units/kg/day and in rabbits at a dose of 3 units/kg/day. These doses are approximately 8 times the human subcutaneous dose of 1.0 unit/kg/day for rats and equal to the human subcutaneous dose of 1.0 unit/kg/day for rabbits, based on human exposure equivalents. The effects are considered secondary to maternal hypoglycemia.

8.2 Lactation

Risk Summary

[redacted] The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for insulin, any potential adverse effects on the breastfed child from insulin aspart or from the underlying maternal condition.

[redacted]

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

[redacted]

(b) (4)

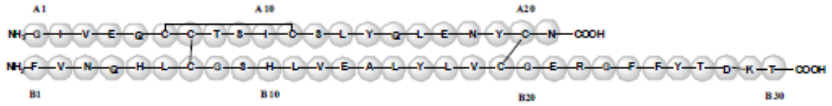
In 52-week studies, Sprague-Dawley rats were dosed subcutaneously with (b) (4) at 10, 50, and 200 units/kg/day (approximately 2, 8, and 32 times the human subcutaneous dose of 1.0 units/kg/day, based on units/body surface area, respectively). At a dose of 200 units/kg/day, (b) (4) increased the incidence of mammary gland tumors in females when compared to untreated controls. The incidence of mammary tumors for (b) (4) was not significantly different than for regular human insulin. The relevance of these findings to humans is not known. (b) (4) was not genotoxic in the following tests: Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, *in vivo* micronucleus test in mice, and in *ex vivo* UDS test in rat liver hepatocytes.

In fertility studies in male and female rats, at subcutaneous doses up to 200 units/kg/day (approximately 32 times the human subcutaneous dose, based on units/body surface area), no direct adverse effects on male and female fertility, or general reproductive performance of animals was observed.

2 Drug Information

2.1 Drug

- CAS Registry Number: 116094-23-6
- Generic Name: insulin aspart
- Code Name: insulin aspart injection, FIA (Q)
- Chemical Name: Asp^{B28} insulin human
- Molecular Formula/Molecular Weight: $C_{256}H_{381}N65O_{79}S_6$, 5825.8 Daltons
- Structure or Biochemical Description: analog of human insulin where the amino acid proline is replaced with aspartic acid in position B28



- Pharmacologic Class: insulin analog

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 106878 (insulin aspart injection), NDA 20986 (NovoLog®), NDA 21172 (NovoLog® Mix 70/30)

2.3 Drug Formulation

Insulin aspart injection 100 U/mL is a clear, colorless solution. The solution is filled in a 3 mL cartridge or in a 10 mL vial. The 3 mL cartridge is assembled into a pre-filled disposable pen injector.

Table 1 Drug Product Composition.

Composition of faster-acting insulin aspart

Name of components	Quantity per ml	Function	Reference to standards
Active substance			
Insulin aspart	600 nmol (100 U)	Drug substance	Novo Nordisk A/S
Excipients			
Phenol	1.50 mg[a]	(b) (4)	Ph. Eur./USP/JP
Metacresol	1.72 mg[a]		Ph. Eur./USP
Glycerol	3.3 mg		Ph. Eur./USP/JP
Zinc (as zinc acetate)	19.6 µg		Ph. Eur./USP/JPE
Disodium phosphate dihydrate	0.53 mg		Ph. Eur./USP
Arginine (as L-arginine HCl)	3.48 mg	Stabilising agent	Ph. Eur./USP/JP
Nicotinamide	20 (b) (4) mg	Absorption modifier	Ph. Eur./USP/JP
Hydrochloric acid	q.s.[b]	pH adjusting agent	Ph. Eur./USP/JP
Sodium hydroxide	q.s.[b]	pH adjusting agent	Ph. Eur./USP/JP
Water for injections	(b) (4)		Ph. Eur./USP/JP
(b) (4)			

[b] To reach pH 7.1

2.4 Comments on Novel Excipients

There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

The impurity profile of insulin aspart injection is comparable to NovoLog®. There are no new drug product-related impurities above threshold limit for qualification, according to Guidance for Industry Q3B(R2) Impurities in New Drug Products. Four degradation products or product types were identified - (b) (4), **insulin aspart related impurities and High Molecular Weight Proteins (HMWP)**. The proposed shelf life specification limits for these degradation products in insulin aspart injection are identical to the specification limits approved for NovoLog®. The in-use acceptance criteria of these degradation products in insulin aspart injection are qualified in nonclinical toxicology studies conducted for NovoLog®.

Impurity levels of insulin aspart degradation products in non-clinical studies and in-use (including shelf life) acceptance criteria

	(b) (4)	Insulin aspart related impurities	HMWP
In-use acceptance criteria (including shelf life) for faster aspart	(b) (4)		
Level of insulin aspart degradation product tested in the pivotal 12-month repeat-dose study in rat[a]			
Dose of insulin aspart degradation product at NOAEL in rats[a] (nmol/kg/day)			
Human intake[b] (nmol/kg/day)[b]			
Dose factor[a,b]			

[a] pivotal 12-month rat study (NovoRapid®/NovoLog® Expert Report on the Toxico – Pharmacological Documentation, 1998, Appendix B, study T-12): NOAEL= (b) (4) nmol/kg/day (b) (4) U/kg/day) in male animals. NOAEL= (b) (4) nmol/kg/day (b) (4) U/kg/day) in female animals. Dose levels are based on the lowest NOAEL of (b) (4) nmol/kg/day.

[b] Human intake is based on a highly insulin resistant patient receiving 200 U/day and a bodyweight of 70 kg (= 17 nmol/kg/day).

One excipient-related impurity was identified - (b) (4) which due to its structural similarity to (b) (4) a structural alert for potential genotoxic effects in *in silico* DEREK/Leadscope analysis was

identified. However, the daily intake of (b) (4) even at a clinical dose of 200 U/day for a highly insulin resistant person will be <(b) (4) µg/person/day, which is below the threshold of toxicological concern (TTC) for long-term administration (>10 year), according to Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk (ICHM7). Therefore, this impurity level is considered acceptable.

Two organic leachables were identified after long-term storage (12-29 months) followed by simulated in-use conditions (1 month at 30°C) - (b) (4) and a reaction product (b) (4) The potential clinical exposure of (b) (4) at 200 U insulin aspart injection is 12,500 fold below the Permissible Daily Exposure (PDE), established in Impurities: (b) (4) The exposure level of (b) (4) at 200 U insulin aspart injection is (b) (4) fold below the TTC (1.5 µg/person/day) per ICHM7. Therefore, both leachables are considered not to be of risk.

Clinical exposure to leachables from 10 mL vials (12 month storage at 5°C followed by 1 months at 30°C – study ongoing)

Compound	CAS no.	Maximal concentration of leachables (µg/mL)[a]	Clinical exposure (µg/day)[b]	PDE (mg/person/day)[c]	Fold to PDE	Fold to TTC (TTC= 1.5 µg/person/day)
(b) (4)						

Clinical exposure to leachables from 3 mL cartridges (29 months storage at 5°C followed by 1 months at 30°C)

Compound	CAS no.	Maximal concentration of leachables[a] (µg/mL)	Cinical exposure (µg/person/day)[b]	PDE[c](mg/person/day)	Fold to PDE	Fold to TTC (TTC= 1.5 µg/person/day)
(b) (4)						

[a]Long-term leachable analysis Module 3.2.P.2.4.

[b]The clinical exposure are based on a highly insulin resistant patient receiving 200 U/day (~ 2 ml/person/day)

[c]The PDE is calculated based on toxicological data from scientific literature applying same principle as in ICH Q3C i.e. PDE= NOAEL × 50 kg bw./ (F1 × F2 × F3 × F4 × F5). The PDE is based on a NOAEL of (b) (4) kg/day derived from a reproductive and developmental toxicity study in rats. This NOAEL was the lowest observed NOAEL including assessment of carcinogenicity [61, 62]. F1 = 5 (extrapolation from rat to human being), F2 = 10 (variation between individuals), F3 = 1 (safety factor for a reproduction and developmental toxicity study covering the complete period of organogenesis), F4 = 10 (safety factor for reproduction toxicity encountered), F5 = 1 (NOAEL has been used).

NA=not applicable

2.6 Proposed Clinical Population and Dosing Regimen

Insulin aspart injection will be marketed as a 100 U/mL formulation for dosing at mealtime or post-meal. Individual total daily insulin requirements are usually between 0.5-1.0 U/kg/day.

2.7 Regulatory Background

- Feb 2010 – IND 106878 submission
- March 2011 – EOP2 mtg
- Nov 2013 – Type C written response: PSP plan (1 nonclinical question – no juvenile animal study was required.)
- June 2014 – Type C written response: NDA submission (1 nonclinical question – division agreed that the nonclinical data would be appropriate in scope to support the NDA submission.)
- Feb 2015 – Proposed PSP was submitted (no planned nonclinical study; no iPSP agreement as of May 2015)
- June 2015 – Type B pre-NDA meeting (no nonclinical questions)
- Dec 2015 – NDA 208751 submission

3 Studies Submitted

3.1 Studies Reviewed

Pharmacodynamic/pharmacokinetic and local tolerance studies are reviewed.

3.2 Studies Not Reviewed

n/a

3.3 Previous Reviews Referenced

n/a

4 Pharmacology

4.1 Primary Pharmacology

The pharmacology of insulin aspart is well-established and has been previously reviewed extensively. The pharmacology of insulin aspart (NovoLog®), has been demonstrated to be similar to that of human insulin, with the primary difference being the more earlier absorption profile for Novolog® compared to insulin. Insulin aspart injection has a slightly earlier absorption compared to that observed with NovoLog®. After absorption, insulin aspart has the same pharmacodynamic activity at the insulin receptor as that from Novolog®. A pharmacokinetic/pharmacodynamic study in pigs (Study No HaRe131104) showed that the blood glucose over time profile of insulin aspart injection had an earlier absorption profile of insulin aspart and with earlier onset of the glucose-lowering effect compared to NovoLog®.

4.2 Secondary Pharmacology

n/a

4.3 Safety Pharmacology

n/a

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Study No HaRe131104: Pharmacokinetics/Pharmacodynamics (PK/PD) in LYD pigs

Insulin aspart injection containing nicotinamide and L-arginine showed slightly earlier plasma glucose lowering when compared to the NovoRapid® (non-US approved version of Novolog®). Insulin aspart injection demonstrates earlier absorption of insulin aspart as evaluated with the measure $AUC_{0\text{-}15min}/AUC_{0\text{-}60min}$ ratio when compared to the NovoRapid® formulation. There were no differences in PK/PD profiles between these formulations, either with or without (b) (4)

Study title: NNC0121-0000-0014 - Pharmacokinetic/pharmacodynamic profiles after subcutaneous dosing of insulin aspart in different formulations to LYD pigs

Study objective: This study was designed to compare the pharmacokinetic (PK) and pharmacodynamic (PD) parameters of insulin aspart in the NovoRapid® formulation versus the formulations to support development of insulin aspart injection.

Study description: The PD and PK profiles after single subcutaneous dose of insulin aspart as NovoRapid® or insulin aspart injection (NNC 0121-0000-0014) were studied in LYD (the crossbred Landrace, Yorkshire & Duroc) pigs. Four insulin aspart injection formulations were compared to NovoRapid®. These insulin aspart injection formulations contained (b) (4) mM nicotinamide, (b) (4) mM L-arginine and (b) (4) There were 4 different concentrations of (b) (4) at (b) (4)). Each insulin aspart formulation was dosed subcutaneously on two occasions to 8 LYD pigs in a non-blinded crossover manner. Blood was sampled over a 300 min period post-dosing and insulin aspart was quantified by luminescent oxygen channeling immunoassay (LOCI). There were 10 days of dosing with washout periods of 2-3 days between doses.

Formulations:

- A: NovoRapid®
- B: NNC 0121-0138-0014-4X
- C: NNC 0121-0142-0014-1X
- D: NNC 0121-0347-0014-1X
- E: NNC 0121-0348-0014-1X

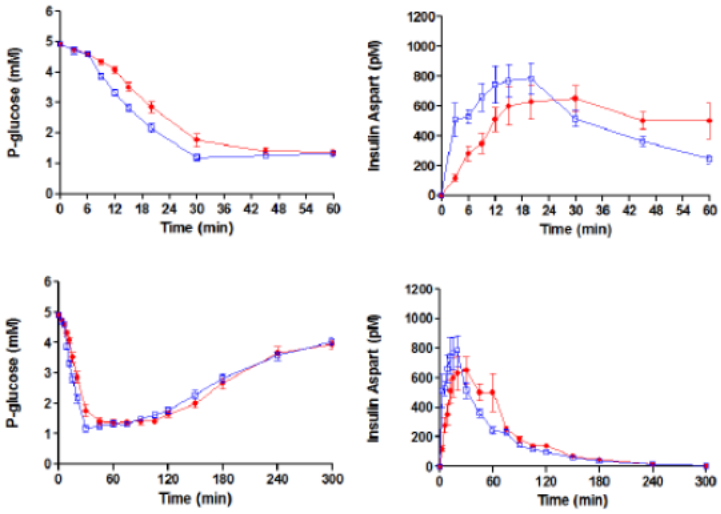
	Insulin Aspart (mM)	Zn (mM)	Phenol (mM)	m-cresol (mM)	Glycerol (% w/w)	(b) (4)	Arg/Hcl (mM)	Nicotinamide (mM)	(b) (4)	NaCl (mM)	pH
A	0.6								(b) (4	(b) (4)	7.4
B	0.6										7.1
C	0.6										7.1
D	0.6										7.1
E	0.6										7.1

Study findings:

- Based on as the ratio (area under the curve (AUC) for 0-15 min post-injection versus AUC 0-60 min post-injection (i.e., $AUC_{0\text{-}15min}/AUC_{0\text{-}60min}$), insulin aspart formulations containing nicotinamide and L-arginine showed an earlier absorption of insulin aspart when compared to NovoRapid® ($AUC_{0\text{-}15min}/AUC_{0\text{-}60min}$ for insulin aspart injection was 27% vs. 15% for NovoRapid®; p< 0.001).
- (b) (4) Therefore, (b) (4) was excluded in the final to be marketed formulation.
- An earlier decrease in plasma glucose (P-glucose) was observed, which was consistent with the earlier absorption of insulin aspart.
- Total exposure (AUC_{inf}) of insulin aspart was similar between insulin aspart injection and NovoRapid®.
- T_{max} was 30 min for NovoRapid®. T_{max} was between 12 and 15 min for insulin aspart injection FIA (B/C/D/E) formulations.

Figure 1 Study No. HaRe131104 – PK/PD profile: NovoRapid® vs. insulin aspart injection formulation B

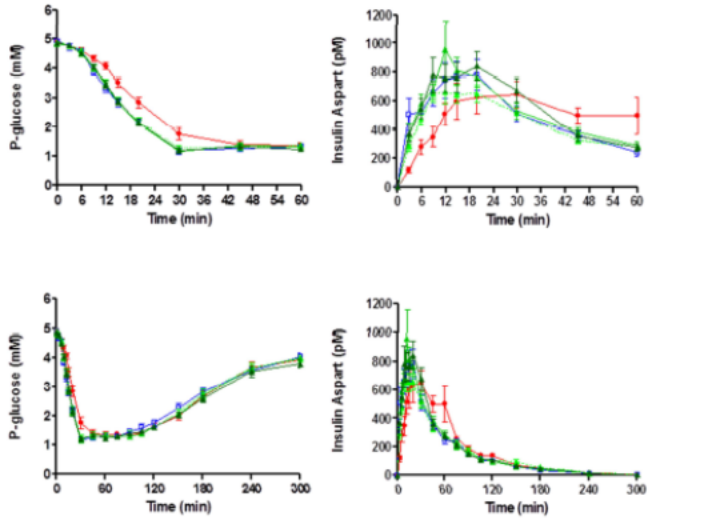
PK/PD profiles (mean +/- SEM; n=2x8) of insulin aspart in the NovoRapid® formulation and a formulation with (b) (4) nM nicotinamide and 20 mM L-arginine after sc administration of 1 nmol/kg to LYD pigs



The upper two figures show profiles for the first hour after dosing and the lower two figures show the full 5 hrs profiles. ● A: NovoRapid® and □ B: 0121-0138-0014-4X

Figure 2 Study No. HaRe131104 – PK/PD profile: NovoRapid® vs. insulin aspart injection formulations

PK/PD profiles (mean +/- SEM; n=2x8) of insulin aspart in the NovoRapid® formulation and in four formulations with (b) (4) nicotinamide and 20 mM L-arginine and different (b) (4) concentrations after sc administration of 1 nmol/kg to LYD pigs



The upper two figures show profiles for the first hour after dosing and the lower two figures show the full 5 hrs profiles. ● A: NovoRapid®; □ B: 0121-0138-0014-4X, ▲ C: 0121-0142-0014-1X, ▲ D: 0121-0347-0014-1X, ▲ E: 0121-0348-0014-1X

Table 2 Study No HaRe131104 – Mean PK Parameters.

Mean PK parameters for insulin aspart in five formulations

Formulation	Additive	T_{max}* (min)	C_{max} (pM)	$AUC_{(0\text{-}15/0\text{-}60)}$ (%)	AUC_{inf} (pM*min)
A: NovoRapid		30	968	15	51607
B: 0121-0138-0014-4X	(b) (4) mM nicotinamide 20 mM L-arginine	12	987	27**	46200
C: 0121-0142-0014-1X	(b) (4) mM nicotinamide 20 mM L-arginine (b) (4)	12	834	27**	46461
D: 0121-0347-0014-1X	(b) (4) mM nicotinamide 20 mM L-arginine (b) (4)	15	1173	27**	49067
E: 0121-0348-0014-1X	(b) (4) M nicotinamide 20 mM L-arginine (b) (4)	14	1054	26**	49854

*Median,
**p<0.0001 ANOVA (see Table 3)

Study No JKIP130102: Pharmacokinetics in pigs

Even though there was no clear effect of nicotinamide alone on ^{133}Xe washout evaluated for 30 and 120 min after s.c. administration, there was a trend that insulin aspart formulated with nicotinamide increased the ^{133}Xe washout rate at 30 min after injection in all 3 studies. Since ^{133}Xe washout rate, both early and overall, was not significantly affected by nicotinamide without insulin aspart, a direct effect of nicotinamide on local blood flow at the subcutaneous injection site could not completely account for the increased early absorption in insulin aspart injection.

Study title: NNC0121-0000-0014 - Xenon 133 washout after SC injection with formulations of insulin aspart (NNC0121-0000-0014) and nicotinamide

Study objective: This study was to evaluate the ^{133}Xe (Xenon 133) washout response of the insulin aspart injection formulations containing nicotinamide in order to determine if nicotinamide could influence subcutaneous local blood flows in pigs.

Study description: The method used was based on disappearance (washout) of ^{133}Xe at the s.c. injection site. Washout of ^{133}Xe method has been used previously to study blood flow changes. In this study, the ^{133}Xe signal was not an absolute blood flow measurement; however the signal correlated with blood flow. The rationale for testing this hypothesis was that nicotinamide has been shown to affect blood flow in certain models. NovoRapid® and four insulin aspart injection (NNC0121-0000-0014) formulations with nicotinamide, or prostaglandin E1 (as positive control) were tested in three different studies (Study Nos. JKIP130102, JKIP130201, JKIP130402). There were 8 animals in each study and all insulin formulations and vehicles were dosed 0.6 nmol/kg.

Formulation details for study JKIP130102

NNC and batch no.	Components
0121-9112-0014-27X	(b) (4)
NovoRapid® vehicle	
0121-0116-0014-1X	
Nicotinamide vehicle	1 % (w/vol) glycerol (b) (4) M nicotinamide; pH 7.44

Formulation details for study JKIP130201

NNC and batch no.	Components
0121-9112-0014-27X	(b) (4)
NovoRapid® vehicle	
0121-0116-0014-1X	
Nicotinamide vehicle	1 % (w/vol) glycerol; (b) (4) nicotinamide; pH 7.40

Formulation details for study JKIP130402

NNC and batch no.	Components
0121-9112-0014-33X	(b) (4)
NovoRapid® vehicle	
0121-0116-0014-3X	
Nicotinamide vehicle	1 % (w/vol) glycerol; (b) (4) nicotinamide; pH 7.40
0121-0037-0014-3X	(b) (4)
Prostaglandin E1 vehicle	4 µg/ml prostaglandin E1 (incl 0.04% ethanol), 0.12% ethanol, 7mM phosphate, 2.1% glycerol, pH 7.40

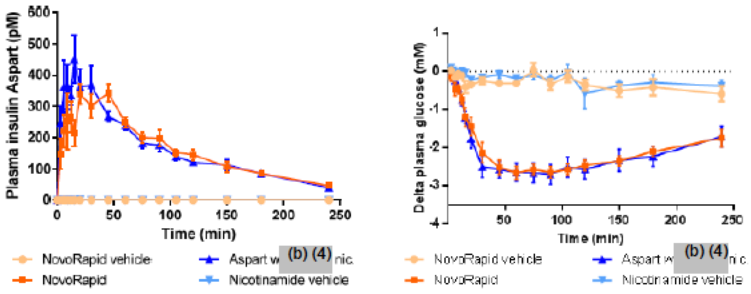
Study findings:

- Insulin aspart formulated with (b) (4) mM nicotinamide or prostaglandin E1 (4 µg/mL) showed an earlier initial absorption than NovoRapid®. Statistical significance for nicotinamide ($p<0.05$) was reached in Study No JKIP130402, but not in Study No JKIP130102 ($p=0.069$) and Study No JKIP130201 ($p=0.089$). While, statistical significance was not reached in two of the three studies, all showed the same trend.
- The effects of (b) (4) mM nicotinamide were demonstrated in Study No. HARE131104), where an earlier absorption was seen with insulin aspart injection formulations.
- Insulin aspart combined with nicotinamide showed an early increased ^{133}Xe rate when compared to NovoRapid®. However, it was unclear if this effect could account for the increased absorption.

Figure 3 JKIP Studies – PK/PD profile: NovoRapid® vs. insulin aspart injection containing nicotinamide

Study No JKIP130102

The mean±SEM (n=8) of the insulin profiles (left) and glucose profiles (right) for NovoRapid®, NovoRapid® vehicle, insulin aspart with (b) (4) mM nicotinamide, and nicotinamide vehicle

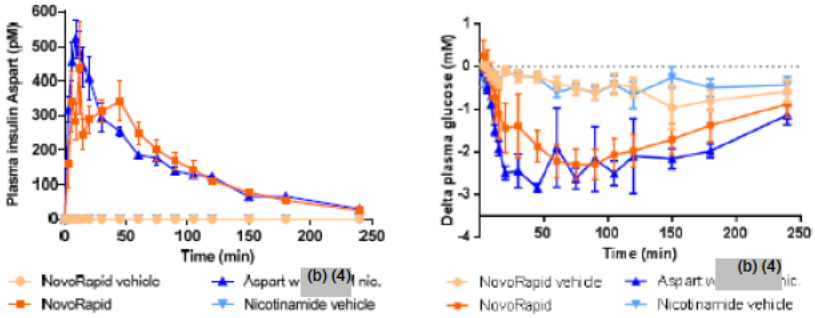


Pharmacokinetic parameters of each group

Formulation		T_{max} (min)	C_{max} (pmol/l)	AUC_{INF} (min*pmol/l)	$AUC_{t=0-15min}/AUC_{t=0-60min}$ (%)
Insulin aspart w (b) (4) nM Nicotinamide	**Mean**		**606***	**43642**	**24 (p=0.069)**
	Median	**18**			
NovoRapid®	**Mean**		**447**	**43481**	**17**
	Median	**25**			

Study No JKIP130201

The mean±SEM (n=6) of the insulin profiles (left) and glucose profiles (right) for NovoRapid®, NovoRapid® vehicle, insulin aspart with (b) (4) M nicotinamide, and nicotinamide vehicle

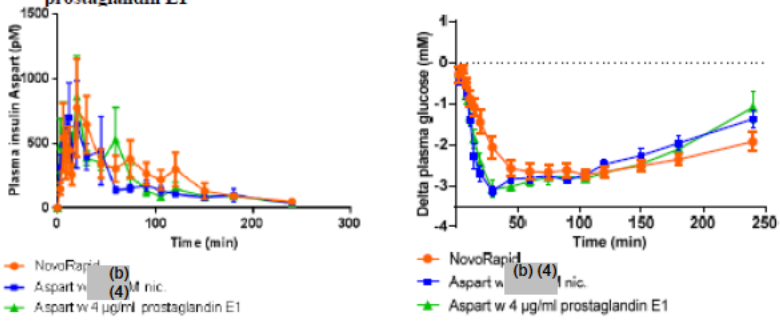


Pharmacokinetic parameters of the groups

Formulation		T_{max} (min)	C_{max} (pmol/l)	AUC_{inf} (min*pmol/l)	$AUC_{t=0-15min}/AUC_{t=0-60min}$ (%)
Insulin aspart w (b) (4) nM Nicotinamide	**Mean**		**598**	**38382**	**32 (P=0.089)**
	Median	**9**			
NovoRapid®	**Mean**		**605**	**37557**	**23**
	Median	**29**			

Study No JKIP130402

The mean±SEM (n=8) of the insulin profiles (left) and glucose profiles (right) for NovoRapid®, insulin aspart w (b) (4) nM nicotinamide, insulin aspart w 4µg/ml prostaglandin E1

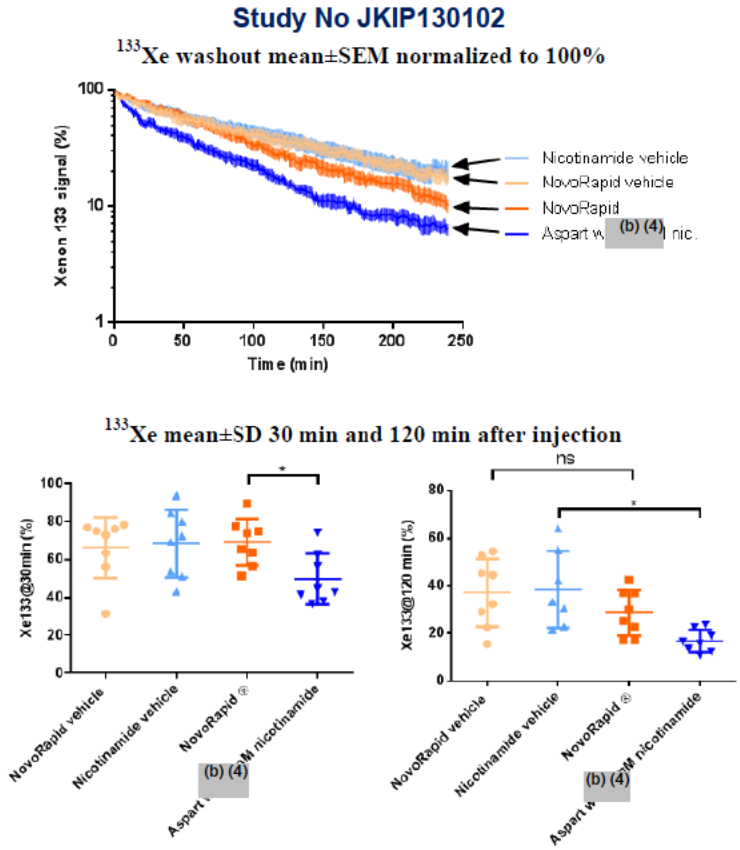


Pharmacokinetic parameters of the groups

Formulation		T_{max} (min)	C_{max} (pmol/l)	AUC_{inf} (min*pmol/l)	$AUC_{t=0-15min}/AUC_{t=0-60min}$ (%)
Insulin aspart w (b) (4) M Nicotinamide	Mean		1155	47401	34* (p<0.05)
	Median	9			
Insulin aspart w prostaglandin E1	Mean		1055.5	53770	23
	Median	25			
NovoRapid®	Mean		954.25	646023	17
	Median	30			

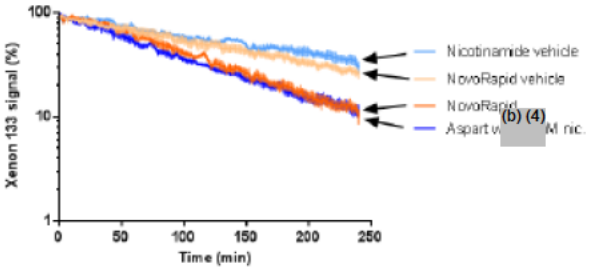
- No statistically significant differences in ^{133}Xe washout rate were observed between the NovoRapid® vehicle and the nicotinamide vehicle.
- The studies showed a trend for insulin aspart formulated with nicotinamide for increased ^{133}Xe washout rate at 30 min after injection in all 3 studies. However, the only statistically significant effect was observed in Study No. JKIP130102.
- The increase in ^{133}Xe washout rate with the insulin aspart injection formulations with nicotinamide was less than the effect of prostaglandin E1 as shown in Study No. JKIP130402.
- Since ^{133}Xe washout rate, both early and overall, was not significantly affected by nicotinamide without insulin aspart, a direct effect of nicotinamide on local blood flow at the subcutaneous injection site could not completely account for the increased early absorption in insulin aspart injection.

Figure 4 JKIP Studies – ^{133}Xe washout 30 min and 120 min after injection: NovoRapid® vs. insulin aspart injection containing nicotinamide

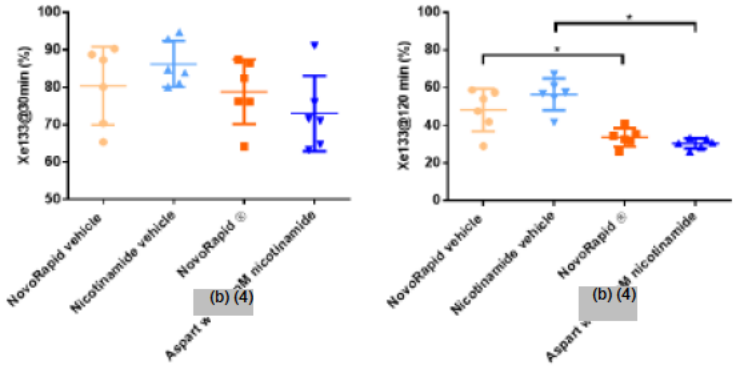


Study No JKIP130201

^{133}Xe washout mean±SEM normalized to 100%

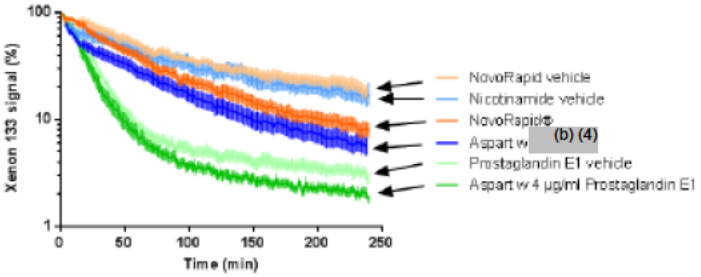


^{133}Xe mean±SD 30 min and 120 min after injection

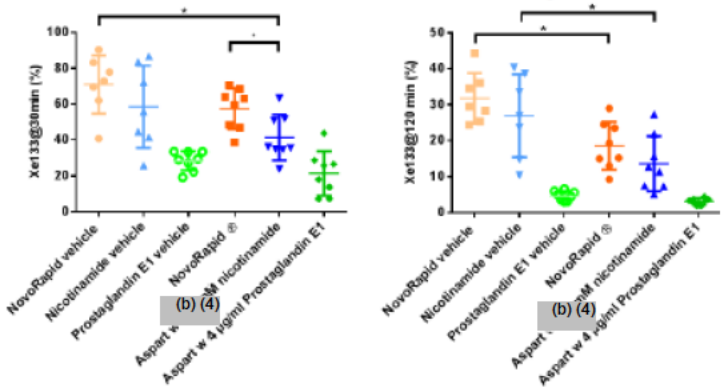


Study No JKIP130402

133Xe washout mean±SEM normalized to 100%



^{133}Xe mean±SD 30 min and 120 min after injection



6 General Toxicology

6.1 Single-Dose Toxicity

No studies have been conducted with insulin aspart injection. Studies were conducted with insulin aspart to support approval of NovoLog®.

6.2 Repeat-Dose Toxicity

No studies have been conducted with insulin aspart injection. Studies were conducted with insulin aspart to support approval of NovoLog®.

7 Genetic Toxicology

No studies have been conducted with insulin aspart injection. Studies were conducted with insulin aspart to support approval of NovoLog®.

8 Carcinogenicity

No studies have been performed with insulin aspart injection. The carcinogenic potential of insulin aspart to support approval of NovoLog® was assessed in two 52-week studies in rats.

9 Reproductive and Developmental Toxicology

No studies have been conducted with insulin aspart injection. Studies were conducted with insulin aspart to support approval of NovoLog®.

10 Special Toxicology Studies

GLP Local Tolerance Studies

Study No 209322 – Study in minipigs

The local tissue reactions observed in the female minipigs dosed with insulin aspart injection (A, B, C and D) were similar to those observed in the animals dosed with NovoRapid®.

Study title: Faster-acting insulin aspart (FIA) local toxicity 2 and 5 days after subcutaneous in minipigs

Study objective: This study was designed to assess the local tolerance of four different formulations of insulin aspart injection compared to NovoRapid® in minipigs.

Study description: This study assessed the local tolerance in minipigs of four different early formulations in the development of insulin aspart injection FIA (A, B, C and D) after a single subcutaneous injection compared to NovoRapid®. Insulin aspart injection formulations (A, B, C and D) contained (b) (4) mg L-arginine/mL and (b) (4) mg nicotinamide/mL. Female minipigs were dosed on Days 1 and 4 of the study (4/group). The animals were sacrificed on Day 6. In addition, one animal from each group was injected with 0.1 mL of 0.9% sodium chloride. The animals were anaesthetized prior to dosing and each injection site was only used once. On days of dosing, the injection sites were observed continuously for five minutes post-injection and re-inspected 2 hours after injection as well as daily throughout the study. At necropsy, all injection sites from all animals were sampled, processed for histological examination and microscopically examined.

Table 3 Study No. 209322 – Local Tolerance (Minipigs) – Study Design.

Formulation code	Batch number	Insulin aspart	Arginine hydrochloride	Nicotinamide	Sodium chloride	Glycerol	(b) (4)
		U/ml	mM	mM	mM	mg/ml	
A	412-N09620	100					(b) (4)
B	412-N09621	100					
C	412-N09622	100					
D	412-N09623	100					
E	XQ50090	100					
F	12CBH16	Sodium chloride 0.9%					

Group	Injection site No 1 Day 1 and No 4 Day 4	Animal Nos		Injection site No 2 Day 1 and No 3 Day 4	Animal Nos		Dose volume per injection site* (µL)	Colour code
1	A	1-4		F	1		100	White
2	B	5-8		F	5		100	Blue
3	C	9-12		F	9		100	Green
4	D	13-16		F	13		100	Red
5	E	17-20		F	17		100	Yellow

*Material as supplied

Study findings:

- No adverse test item-related clinical signs were observed during the in-life period, including reactions at the injection sites.
- Two days after subcutaneous injection, the main findings at all injection sites were minimal to slight hemorrhages and minimal to slight inflammatory changes (neutrophilic granulocytes and macrophages).
- Five days after subcutaneous injection, the main findings at all injection sites were minimal to moderate hemorrhages and minimal to moderate inflammatory changes (neutrophilic granulocytes and macrophages).
- Overall, the histopathological evaluation of the injection sites showed mild to moderate inflammatory changes and mild to moderate hemorrhage. The tissue reactions observed in the animals dosed with insulin aspart injection (A, B, C and D) were similar to those observed in the animals dosed with NovoRapid®.

Table 4 Study No 209322 – Local Tolerance (Minipigs) – Microscopic Findings.

Microscopic findings – two days after injection (K2)

Two days after injection (K2)						
Dose group	**Test form. A**	**Test form. B**	**Test form. C**	**Test form. D**	**Test form. E** (NovoRapid®)	**Test form. F** (control, NaCL)
Number of injection sites examined	4	4	4	4	4	5
Inflammation, focal/multifocal, primarily macrophages and neutrophilic granulocytes, subcutis						
Total	1	0	0	1	0	1
Minimal (Grade 1)	1	-	-	1	0	1
Focal accumulation of inflammatory cells, neutrophilic granulocytes/ Macrophages, subcutis						
Total	3	3	2	3	4	0
Minimal (Grade 1)	2	3	2	3	4	-
Slight (Grade 2)	1	-	-	-	-	-
Haemorrhage, subcutis, focal						
Total	4	3	4	3	3	1
Minimal (Grade 1)	2	1	3	2	3	1
Slight (Grade 2)	2	2	1	1	-	-
Needle canal						
Total	4	2	2	3	2	2

Microscopic findings – five days after injection (K5)

Five days after injection (K5)						
Dose group	**Test form. A**	**Test form. B**	**Test form. C**	**Test form. D**	**Test form. E** (NovoRapid®)	**Test form. F** (control, NaCL)
Number of injection sites examined	4	4	4	4	4	5
Inflammation, focal/multifocal, primarily macrophages and neutrophilic granulocytes, subcutis						
Total	2	1	3	3	3	1
Minimal (Grade 1)	-	1	3	3	1	-
Slight (Grade 2)	1	-	-	-	1	1
Moderate (Grade 3)	1	-	-	-	1	-
Focal accumulation of inflammatory cells, neutrophilic granulocytes/ Macrophages, subcutis						
Total	2	3	1	1	0	1
Minimal (Grade 1)	2	3	1	1	-	1
Haemorrhage, subcutis, focal						
Total	2	2	4	4	4	2
Minimal (Grade 1)	-	2	1	3	3	2
Slight (Grade 2)	2	-	2	-	1	-
Moderate (Grade 3)	-	-	1	1	-	-
Needle canal						
Total	1	3	3	1	3	1

Study No. 212147 – Local toxicity in the rabbit

The local tissue reactions in all groups were considered to be acceptable and of similar nature and severity between insulin aspart injection and 0.9% sodium chloride controls.

Study title: FIA (Q) and FIA (R) Local Tolerance Study in Rabbits 4 Days after Intramuscular, Intravenous and Intraarterial Injection

Study objective: This study was to assess the local tolerance of two different formulations of insulin aspart injection (Q and R) in rabbits via three administration routes.

Study description: Local tolerance at the injection sites was assessed four days after a single intramuscular, intravenous or intra-arterial injection of insulin aspart injection in rabbits compared to 0.9% sodium chloride. Female New Zealand white rabbits (4/group) were given a single dose of 0.1 mL insulin aspart injection in the right thigh (intramuscular) or ear (intravenous or intraarterial), respectively, and 0.1 mL 0.9% sodium chloride in the left thigh (intramuscular) or ear (intravenous or intra-arterial) of the same animal. The injection sites were observed and scored prior to treatment, during the first 5 minutes after dosing, 30 minutes after dosing and 2 hours after dosing. Thereafter the injection sites were observed daily. Even though FIA (R) was included in this study, it was not selected for further development.

Table 5 Study No 212147 – Local Tolerance (Rabbit) – Study Design.

Names of ingredients	FIA (Q)	FIA (R)	Function	Reference to standards
Drug substance				
Insulin aspart	600 nmol~100 U	600 nmol~100 U	Drug substance	Novo Nordisk A/S
Other ingredients				
Zinc (as Zinc acetate)	19.6 µg	(b) (4)	(b) (4)	USP/JPE/Ph. Eur.
Phenol[1]	1.50 mg			USP/JPE/Ph. Eur
m-Cresol[1]	1.72 mg			USP/Ph. Eur
Disodium hydrogen phosphate dihydrate	0.53 mg			USP/Ph. Eur
Glycerol	3.3 mg			USP/JP/Ph. Eur
Arginine (as L-Arginine hydrochloride)	3.48 mg		Stabilising agent	USP/Ph. Eur
Nicotinamide	20.8 mg		Absorption modifier	USP/Ph. Eur
Sodium hydroxide[2]	q.s	q.s	pH adjusting agent	USP/JP/Ph. Eur
Hydrochloric acid[2]	q.s	q.s	pH adjusting agent	USP/JP/Ph. Eur
Water, WFI	(b) (4)			USP/JP/Ph. Eur

(b) (4)

[2] To reach target pH (7.1) for the FIA products

Group	Route of treatment	Test item	Dose conc. (U/ml)	Dose volume (ml/injection site)	No of animals Female
1	Intramuscular, right thigh	FIA (Q)	100	0.1	1-4
	Intramuscular, left thigh	NaCl 0.9%	0		
2	Intravenous, right ear	FIA (Q)	100	0.1	5-8
	Intravenous, left ear	NaCl 0.9%	0		
3	Intraarterial, right ear	FIA (Q)	100	0.1	9-12
	Intraarterial, left ear	NaCl 0.9%	0		
4	Intramuscular, right thigh	FIA (R)	100	0.1	13-16
	Intramuscular, left thigh	NaCl 0.9%	0		
5	Intravenous, right ear	FIA (R)	100	0.1	17-20
	Intravenous, left ear	NaCl 0.9%	0		
6	Intraarterial, right ear	FIA (R)	100	0.1	21-24
	Intraarterial, left ear	NaCl 0.9%	0		

Study findings:

- A single dose of 0.1 mL (10 U) per animal of insulin aspart injection administered either intramuscularly (in the thigh), intravenously or intra-arterially (in the ear) did not result in any mortality or clinical signs.
- The injections caused mild histopathological changes, characterized by focal necrosis, inflammation and hemorrhage at the injection sites treated with saline (left ear) as well as FIA(Q) or FIA(R) (right ear).
- In the intramuscular and intra-arterial injection sites, changes were comparable in nature and severity between insulin aspart injection and 0.9% sodium chloride.

- In the intravenous injection sites, changes of a slightly higher grade (minimal to slight perivascular/vascular necrosis) were noted following injection with insulin aspart injection compared to intravenous injection with 0.9% sodium chloride.
- These changes seemed to be mild and mostly caused by the injection procedure.

Table 6 Study No 212147 – Local Tolerance (Rabbit) – Microscopic Findings.

Intramuscular injection, Group 1 FIA (Q) / 0.9% NaCl

Dose group	**Group 1 0.9 % NaCl/ FIA (Q)**	
Route of administration	**IM**	
Sex	**F**	
Number of animals examined (Rabbits)	4	
3 levels, pooled examination.	L	R
Inflammation, focal		
Total	3	2
Grade 1 - minimal	3	2
Myofibre necrosis/regeneration, focal		
Total	2	1
Grade 1 - minimal	2	1

L = Left Site - 0.9 % NaCl
R = Right Site – FIA (Q).

Intramuscular injection, Group 4 FIA (R) / 0.9% NaCl

Dose group	**Group 4 0.9 % NaCl/ FIA (R)**	
Route of administration	**IM.**	
Sex	**F**	
Number of animals examined (Rabbits)	4	
3 levels, pooled examination.	L	R
Inflammation, focal		
Total	4	2
Grade 1 - minimal	3	1
Grade 2 - slight	1	1
Haemorrhage, focal		
Total	1	0
Grade 2 - slight	1	-
Myofiber necrosis, focal		
Total	2	2
Grade 1 - minimal	2	2

L = Left Site - 0.9 % NaCl
R = Right Site – FIA (R).

Groups 2 and 3, intravenous/intraarterial injection FIA (Q) / 0.9 % NaCl

Dose group	Group 2 0.9 % NaCl/ FIA (Q)		Group 3 0.9 % NaCl/ FIA (Q)	
Route of administration	**IV**		**IA**	
Sex	**F**		**F**	
Number of animals examined (Rabbits)	4		4	
Introduction of the needle	L	R	L	R
Infiltration of inflammatory cells, focal, mainly lymphoplasmocytic cells and fibroblasts/oedema				
Total	1	3	2	2
Grade 1 - minimal	1	3	-	1
Grade 2 - slight	-	-	2	1
Necrosis, perivascular				
Total	0	2	1	0
Grade 1 - minimal	-	2	1	-
Necrosis, vascular				
Total	0	3	0	0
Grade 1- minimal	-	2	-	-
Grade 2 - slight	-	1	-	-
Haemorrhage, focal, perivascular				
Total	0	0	1	1
Grade 1- minimal	-	-	1	1
Tip of the needle	L	R	L	R
Necrosis, perivascular				
Total	0	0	0	1
Grade 1 - minimal	-	-	-	1
Haemorrhage, focal, perivascular				
Total	0	0	1	1
Grade 1- minimal	-	-	1	-
Grade 2 - slight	-	-	-	1

Dose group	Group 2 0.9 % NaCl/ FIA (Q)		Group 3 0.9 % NaCl/ FIA (Q)	
Route of administration	**IV**		**IA**	
Sex	**F**		**F**	
Number of animals examined (Rabbits)	4		4	
Proximal to the tip of the needle	L	R	L	R
Infiltration of inflammatory cells, focal, mainly lymphoplasmocytic cells and fibroblasts/oedema				
Total	0	0	1	0
Grade 1 - minimal	-	-	1	-
Necrosis, perivascular				
Total	0	0	1	0
Grade 1 - minimal	-	-	1	-
Haemorrhage, focal, perivascular				
Total	0	0	1	0
Grade 2 - slight	-	-	1	-

L = Left Site - 0.9 % NaCl
R = Right Site – FIA (Q)

Groups 5 and 6, intravenous/intraarterial injection FIA (R) / 0.9% NaCl

Dose group	Group 5 0.9 % NaCl/ FIA (R)		Group 6 0.9 % NaCl/ FIA (R)	
Route of administration	**IV**		**IA**	
Sex	**F**		**F**	
Number of animals examined (Rabbits)	4		4	
Introduction of the needle	L	R	L	R
Infiltration of inflammatory cells, focal, mainly lymphoplasmocytic cells and fibroblasts/oedema				
Total	0	2	1	1
Grade 1 - minimal	-	2	1	1
Necrosis, vascular				
Total	0	1	0	0
Grade 1- minimal	-	1	-	-
Haemorrhage, focal, perivascular				
Total	0	0	1	2
Grade 1- minimal	-	-	1	2
Crust, focal				
Total	0	1	0	0
Grade 1- minimal	-	1	-	-
Intimal proliferation, focal				
Total	0	0	1	0
Grade 1- minimal	-	-	1	-
Tip of the needle	L	R	L	R
Infiltration of inflammatory cells, focal, mainly lymphoplasmocytic cells and fibroblasts/oedema				
Total	0	1	0	2
Grade 1 - minimal	-	1	-	1
Grade 2 - slight	-	-	-	1
Necrosis, perivascular				
Total	0	1	0	1
Grade 2 - slight	-	1	-	1
Necrosis, vascular				
Total	0	1	0	0
Grade 1- minimal	-	1	-	-

Dose group	Group 5 0.9 % NaCl/ FIA (R)		Group 6 0.9 % NaCl/ FIA (R)	
Route of administration	**IV**		**IA**	
Sex	**F**		**F**	
Number of animals examined (Rabbits)	4		4	
Haemorrhage, focal, perivascular				
Total	0	0	1	1
Grade 1- minimal	-	-	1	1
Proximal to the tip of the needle	L	R	L	R
Infiltration of inflammatory cells, focal, mainly lymphoplasmocytic cells and fibroblasts/oedema				
Total	1	0	0	1
Grade 1 - minimal	1	-	-	-
Grade 2 - slight	-	-	-	1
Haemorrhage, focal, perivascular				
Total	0	0	0	2
Grade 1 - minimal	-	-	-	2

L = Left Site - 0.9 % NaCl
R = Right Site – FIA (R)

Study No. 212251 – Local toxicity in the rat

Single- and multiple (4 weeks) daily subcutaneous injections of NovoRapid® or insulin aspart injection (Q and R) to rats resulted in minimal-moderate local responses of comparable severity and nature across all groups. However, in animals dosed with 0.9% sodium chloride controls, the reaction was less

pronounced. Following a 2-week recovery period, ongoing recovery occurred in all groups with no differences observed between NovoRapid® and insulin aspart injection formulations (Q and R).

Study title: FIA (Q) and FIA (R) A 4-Week Multiple Dose Local Tolerance Study with a 2-Week Recovery in Sprague Dawley Rats

Study objective: This study was designed to assess the local tolerance of two different formulations of insulin aspart injection (Q and R) in rats via once daily subcutaneous injection for 28 days compared to NovoRapid®.

Study description: This study assessed the local tolerance of insulin aspart injection compared to the marketed formulation of insulin aspart, NovoRapid®/NovoLog®, when administered by subcutaneous injection to rats daily for 28 days at alternate injection sites in the neck (injection site 1) and upper back (injection site 2). The last dose was administered subcutaneously in the left hind leg (injection site 3) on day 28 in order to assess the acute local reaction after a single dose. A 2-week recovery period was included and injections with 0.9% sodium chloride (NaCl) were used as control. In the insulin aspart injection formulation, nicotinamide (vitamin B3) and L-arginine (amino acid) were present compared to NovoRapid®/NovoLog®. The only difference between insulin aspart injection FIA (Q) and FIA (R) was (b) (4) Sprague Dawley rats were dosed daily for 28 days where the dose volume was kept constant at 0.17 mL/animal (100 U/mL), corresponding to a dose of 102 nmol/animal (17 U/animal). FIA (Q) was selected for future clinical phase III studies.

Table 7 Study No 212251 – Local Tolerance (Rat) – Study Design.

Group	Test/control item	Dose level (nmol/animal) In paranthesis IU/animal	Starting dose level (nmol/kg) In paranthesis IU/kg	Dose concentration* (nmol/mL)	Animal Nos (Main study)		Animal Nos (Recovery)	
					Male	Female	Male	Female
1	0.9% NaCl (Injection control)	0	0	0	1-5	6-10	41-43	44-46
2	NovoRapid®	102 (17)	600 (100)	600	11-15	16-20	47-49	50-52
3	FIA (Q)	102 (17)	600 (100)	600	21-25	26-30	53-55	56-58
4	FIA (R)	102 (17)	600 (100)	600	31-35	36-40	59-61	62-64

*Material as supplied

Names of ingredients	FIA (Q)	FIA (R)	NovoRapid®	Function	Reference to standards
Drug substance					
Insulin aspart	600 nmol~100 U	600 nmol~100 U	600 nmol~100 U	Drug substance	Novo Nordisk A/S
Other ingredients					
Zinc total	19.6 µg	(b) (4)	19.6 µg	(b) (4)	USP/JPE/Ph. Eur.
Phenol[1]	1.50 mg		1.50 mg		USP/JPE/Ph. Eur
m-Cresol[1]	1.72 mg		1.72 mg		USP/Ph. Eur
Disodium hydrogen phosphate dihydrate	0.53 mg		1.25 mg		USP/Ph. Eur
Glycerol	3.3 mg		-		USP/JP/Ph. Eur
Sodium Chloride	-		0.58 mg		
Arginine (as L-Arginine hydrochloride)	3.48 mg		-	Stabilising agent	USP/Ph. Eur
Nicotinamide	20.8 mg		-	Absorption modifier	USP/Ph. Eur
Sodium hydroxide[2]	q.s	q.s	q.s	pH adjusting agent	USP/JP/Ph. Eur
Hydrochloric acid[2]	q.s	q.s	q.s	pH adjusting agent	USP/JP/Ph. Eur
Water, WFI	(b) (4)				USP/JP/Ph. Eur

[2] To reach target pH (7.1) for the FIA products and pH (7.4) for NovoRapid.

Study findings:

- Daily subcutaneous treatment for 28 days with 0.9% sodium chloride, NovoRapid®/NovoLog® and insulin aspart injection did not result in clinical signs. Body weight gain and food consumption were similar in all groups.
- Macroscopic findings included subcutaneous red discoloration (slight to marked) in the majority of injection sites in the neck (injection site 1; alternating dosage every second day, Days 1-27) and left hind leg (injection site 3; treated on the last day of the study, Day 28). A slight to moderate red subcutaneous discoloration was also recorded in few injection sites on the upper back (injection site 2; alternating dosage every second day, Days 1-27). The changes were comparable between NovoRapid®/NovoLog® and insulin aspart injection dosed animals, whereas the changes were less pronounced in 0.9% sodium chloride dosed animals.
- Microscopic findings included minimal to moderate local responses characterized by subcutaneous myofiber degeneration/regeneration at the injection sites. The local reactions observed with NovoRapid®/NovoLog® and insulin aspart injection were similar in nature and severity. No findings were observed in the lymph nodes evaluated. In general, lower incidences of all findings, except for subcutaneous inflammatory cell infiltration, were observed in the 0.9% sodium chloride control group compared to the other groups.
- Following the two-week recovery period, on-going recovery was observed in all groups and no differences were observed between NovoRapid®/NovoLog® and insulin aspart injection.

Table 8 Study No 212251 – Local Tolerance (Rat) – Microscopic Findings: Main Study.

FIA(Q)

Treatment related findings, FIA (Q), Injection sites 1 and 2

Dose Group	Group 1		Group 2		Group 3	
Main study	Control 0.9 % NaCl		Group 2 Novorapid® (600nmol/kg/day)		Group 3 600 nmol FIA(Q) /kg/day	
Sex	M	F	M	F	M	F
Number of animals examined	5	5	5	5	5	5
*Injection Site 1, Neck (alternating dosing every second day), pooled examination**						
Necrosis, focal, sc						
Total	2	1	2	2	5	3
Grade 1 – minimal	2	-	1	1	2	2
Grade 2 – slight	-	1	1	1	2	1
Grade 3 – moderate	-	-	-	-	1	-
Inflammatory cells, focal, sc						
Total	4	5	4	5	5	5
Grade 1 – minimal	2	2	-	2	-	-
Grade 2 – slight	2	3	2	3	3	5
Grade 3 – moderate	-	-	2	-	2	-
Haemorrhage, focal, sc						
Total	0	2	2	2	5	1
Grade 1 – minimal	-	1	-	-	2	-
Grade 2 – slight	-	-	2	2	3	1
Grade 3 – moderate	-	1	-	-	-	-
Fibrosis, subcutis, focal/multifocal						
Total	0	1	4	4	3	5

Dose Group	Group 1		Group 2		Group 3	
Main study	Control 0.9 % NaCl		Group 2 Novorapid® (600nmol/kg/day)		Group 3 600 nmol FIA(Q) /kg/day	
Sex	M	F	M	F	M	F
Number of animals examined	5	5	5	5	5	5
Grade 1 – minimal	-	1	1	3	-	5
Grade 2 – slight	-	-	3	1	3	-
Degeneration/regeneration myofibers, focal/multifocal						
Total	1	1	1	3	3	4
Grade 1 – minimal	1	1	1	1	2	3
Grade 2 – slight	-	-	-	2	1	1
Injection site 2, Site Upper Back (alternating dosing every second day), pooled examination *						
Ulcerations/erosions, focal						
Total	0	0	0	1	0	0
Grade 1 – minimal	-	-	-	1	-	-
Necrosis, focal, sc						
Total	0	0	2	2	1	1
Grade 1 – minimal	-	-	2	1	1	1
Grade 2 – slight	-	-	-	1	-	-
Inflammatory cells, focal, sc						
Total	4	2	5	5	3	5
Grade 1 – minimal	4	2	4	4	2	3
Grade 2 – slight	-	-	1	1	1	2
Haemorrhage, focal, sc						
Total	0	0	0	0	1	2
Grade 1 – minimal	-	-	-	-	1	2
Fibrosis, subcutis, focal/multifocal						
Total	0	0	0	2	1	2
Grade 1 – minimal	-	-	-	2	1	2
Degeneration/regeneration myofibers, focal/multifocal						
Total	2	2	2	2	4	3
Grade 1 – minimal	2	2	2	2	2	3
Grade 2 – slight	-	-	-	-	2	-

Treatment related findings FIA (Q), Injection site 3

Dose Group	Group 1		Group 2		Group 3	
Main study	Control 0.9 % NaCl		Group 2 Novorapid® (600nmol/kg/day)		Group 3 600 nmol FIA(Q) /kg/day	
Sex	M	F	M	F	M	F
Number of animals examined	5	5	5	5	5	5
Injection Site 3, left hind leg (single dose, last day of the study, Day 28) pooled examination *						
Ulcerations, focal						
Total	0	0	0	1	0	0
Grade 1 – minimal	-	-	-	1	-	-
Necrosis, focal, sc						
Total	0	2	5	4	2	2
Grade 1 – minimal	-	2	5	3	1	2
Grade 2 – slight	-	-	-	1	1	-
Inflammatory cells, focal, sc						
Total	5	5	5	5	5	4
Grade 1 – minimal	5	4	2	1	2	4
Grade 2 – slight	-	1	3	4	3	-
Haemorrhage, focal, sc						
Total	0	1	4	4	5	3
Grade 1 – minimal	-	1	3	1	3	2
Grade 2 – slight	-	-	1	3	1	1
Grade 3 – moderate	-	-	-	-	1	-
Degeneration/regeneration myofibers, focal/multifocal						
Total	0	0	3	1	0	0
Grade 1 – minimal	-	-	3	1	-	-

FIA(R)

Treatment related findings FIA (R), Injection sites 1 and 2

Dose Group	Group 1		Group 2		Group 4	
Main study	Control 0.9 % NaCl		Group 2 Novorapid® (600nmol/kg/day)		Group 4 600 nmol FIA(R) /kg/day	
Sex	M	F	M	F	M	F
Number of animals examined	5	5	5	5	5	5
*Injection Site 1, Neck (alternating dosing every second day), pooled examination**						
Necrosis, focal, sc						
Total	2	1	2	2	5	3
Grade 1 – minimal	2	-	1	1	2	1
Grade 2 – slight	-	1	1	1	3	2
Inflammatory cells, focal, sc						
Total	4	5	4	5	5	5
Grade 1 – minimal	2	2	-	2	-	2
Grade 2 – slight	2	3	2	3	4	3
Grade 3 – moderate	-	-	2	-	1	-
Haemorrhage, focal, sc						
Total	0	2	2	2	1	3
Grade 1 – minimal	-	1	-	-	-	1
Grade 2 – slight	-	-	2	2	1	-
Grade 3 – moderate	-	1	-	-	-	2
Fibrosis, subcutis, focal/multifocal						
Total	0	1	4	4	5	5
Grade 1 – minimal	-	1	1	3	3	2
Grade 2 – slight	-	-	3	1	2	3
Degeneration/regeneration myofibers, focal/multifocal						
Total	1	1	1	3	2	4
Grade 1 – minimal	1	1	1	1	2	4
Grade 2 – slight	-	-	-	2	-	-
*Injection Site 2, Upper Back (alternating dosing every second day), pooled examination (3 sections/injection site)**						
Ulcerations/erosions, focal						
Total	0	0	0	1	1	0
Grade 1 – minimal	-	-	-	1	1	-
Necrosis, focal, sc						
Total	0	0	2	2	1	1
Grade 1 – minimal	-	-	2	1	1	1
Grade 2 – slight	-	-	-	1	-	-

Dose Group	Group 1		Group 2		Group 4	
Main study	Control 0.9 % NaCl		Group 2 Novorapid® (600nmol/kg/day)		Group 4 600 nmol FIA(R) /kg/day	
Sex	M	F	M	F	M	F
Number of animals examined	5	5	5	5	5	5
Inflammatory cells, focal, sc						
Total	4	2	5	5	5	3
Grade 1 – minimal	4	2	4	4	5	2
Grade 2 – slight	-	-	1	1	-	1
Haemorrhage, focal, sc						
Total	0	0	0	0	0	1
Grade 1 – minimal	-	-	-	-	-	-
Grade 3 – moderate	-	-	-	-	-	1
Fibrosis, subcutis, focal/multifocal						
Total	0	0	0	2	3	1
Grade 1 – minimal	-	-	-	2	3	1
Degeneration/regeneration myofibers, focal/multifocal						
Total	2	2	2	2	1	4
Grade 1 – minimal	2	2	2	2	1	4

Treatment related findings FIA (R), Injection site 3

Dose Group	Group 1		Group 2		Group 4	
Main study	Control 0.9 % NaCl		Group 2 Novorapid® (600nmol/kg/day)		Group 4 600 nmol FIA(R) /kg/day	
Sex	M	F	M	F	M	F
Number of animals examined	5	5	5	5	5	5
Injection Site 3, left hind leg (single dose, last day of the study, Day 28) pooled examination *						
Ulcerations, focal						
Total	0	0	0	1	0	0
Grade 1 – minimal	-	-	-	1	-	-
Necrosis, focal, sc						
Total	0	2	5	4	2	2
Grade 1 – minimal	-	2	5	3	1	2
Grade 2 – slight	-	-	-	1	1	-
Inflammatory cells, focal, sc						
Total	5	5	5	5	5	5
Grade 1 – minimal	5	4	2	1	4	3
Grade 2 – slight	-	1	3	4	1	2
Haemorrhage, focal, sc						
Total	0	1	4	4	2	4
Grade 1 – minimal	-	1	3	1	2	3
Grade 2 – slight	-	-	1	3	-	1
Grade 3 – moderate	-	-	-	-	-	-
Degeneration/regeneration myofibers, focal/multifocal						
Total	0	0	3	1	0	2
Grade 1 – minimal	-	-	3	1	-	2

Table 9 Study No 212251 – Local Tolerance (Rat) – Microscopic Findings: Recovery.

Treatment related findings FIA (Q), Injection sites 1,2 and 3- Recovery

Dose Group	Group 1		Group 2		Group 3	
Main study	Control 0.9 % NaCl		Group 2 Novorapid® (600nmol/kg/day)		Group 3 600 nmol FIA(Q) /kg/day	
Sex	M	F	M	F	M	F
Number of animals examined	3	3	3	3	3	3
*Injection Site 1 Neck (alternating dosing every second day), pooled examination**						
Inflammatory cells, focal, sc						
Total	2	2	2	2	3	3
Grade 1 – minimal	1	2	2	2	3	3
Grade 2 – slight	1	-	-	-	-	-
Fibrosis, subcutis, focal/multifocal						
Total	0	0	1	1	2	1
Grade 1 – minimal	-	-	1	1	2	1
Injection Site 2 Upper Back (alternating dosing every second day), pooled examination *						
Inflammatory cells, focal, sc						
Total	1	0	1	0	1	1
Grade 1 – minimal	1	-	1	-	1	1
Degeneration/regeneration myofibers, focal/multifocal						
Total	1	0	0	0	0	0
Grade 1 – minimal	1	-	-	-	-	-
Injection Site 3, left hind leg (single dose, last day of the study, Day 28) pooled examination *						
Inflammatory cells, focal, sc						
Total	1	0	0	1	0	0
Grade 1 – minimal	1	-	-	1	-	-
Degeneration/regeneration myofibers, focal/multifocal						
Total	0	1	0	0	0	0
Grade 1 – minimal	-	1	-	-	-	-

Treatment related findings FIA (R), Injection sites 1,2 and 3- Recovery

Dose Group	Group 1		Group 2		Group 4	
Main study	Control 0.9 % NaCl		Group 2 Novorapid® (600nmol/kg/day)		Group 4 600 nmol FIA(R) /kg/day	
Sex	M	F	M	F	M	F
Number of animals examined	3	3	3	3	3	3
Injection Site 1 Neck (alternating dosing every second day), pooled examination *						
Inflammatory cells, focal, sc						
Total	2	2	2	2	3	3
Grade 1 – minimal	1	2	2	2	1	3
Grade 2 – slight	1	-	-	-	2	-
Fibrosis, subcutis, focal/multifocal						
Total	0	0	1	1	1	0
Grade 1 – minimal	-	-	1	1	1	-
Injection Site 2 Upper Back (alternating dosing every second day), pooled examination *						
Inflammatory cells, focal, sc						
Total	1	0	1	0	2	2
Grade 1 – minimal	1	-	1	-	1	2
Grade 2 – slight	-	-	-	-	1	-
Fibrosis, subcutis, focal/multifocal						
Total	0	0	0	0	1	0
Grade 1 – minimal	-	-	-	-	1	-
Injection Site 3, left hind leg (single dose, last day of the study, Day 28) pooled examination *						
Inflammatory cells, focal, sc						
Total	1	0	0	1	1	0
Grade 1 – minimal	1	-	-	1	1	-
Degeneration/regeneration myofibers, focal/multifocal						
Total	0	1	0	0	0	0
Grade 1 – minimal	-	1	-	-	-	-

Impurities

Study No 8293371-8300937 - (b) (4)

Study title: DEREK in silico evaluation of (b) (4)
Study objective/description: (b) (4) was identified as excipient-related impurity in (b) (4) It was subjected to a computational toxicology assessment using two complementary (Q) SAR prediction methodologies, Derek Nexus and Leadscope. (b) (4) DEREK Nexus / Leadscope toxicological analysis was performed on (b) (4) and the results have been extrapolated to (b) (4).

Study findings:

- The combined DEREK Nexus and Leadscope *in silico* analysis of (b) (4) caused an alert for potential carcinogenicity based on its structural similarity to (b) (4)

(b) (4)

- (b) (4) is a genotoxicant, a rodent carcinogen and classified as an IARC (International Agency for Research on Cancer) Group 2A carcinogen. However, there is no evidence that

(b) (4) can cause cancer in humans. According to ICH M7, the Threshold of Toxicological Concern (TTC) for an individual impurity is dependent on duration of exposure and a TTC <1.5 µg/person/day is considered to be associated with a negligible risk for long-term administration in humans (> 10 years). Since the levels of (b) (4) in insulin aspart injection is below a TTC of 1.5 µg/person/day, it is considered of no toxicological concern.

Acceptable daily intakes for an individual impurity based on length of treatment.

Duration of treatment	≤ 1 month	> 1 – 12 months	> 1 – 10 years	> 10 years to lifetime
Daily intake (µg/day)	120	20	10	1.5

- DEREK Nexus and Leadscope *in silico* analysis also concluded (b) (4) to be 'plausible for skin sensitization' based on structural similarity with compounds, which have been positive in a local lymph node assay and in the guinea pig maximization test. However, based on published literature on concentrations and skin sensitization reactions and the subcutaneous route of administration, (b) (4) is considered unlikely to cause a sensitization reaction in human subjects.
- Also, a subcutaneous multiple dose local tolerance 28 study (Study No 212251) in rats has been conducted using insulin aspart injection formulation. There were no signs of a skin sensitization occurring during in-life observations. The microscopy of the lymph nodes also did not reveal any proliferation or other signs of immunological activation. Skin sensitization by (b) (4) in insulin aspart injection is therefore not considered to be a cause for concern.

Others

Study No 8303170-a - Literature Review of Nicotinamide

In a highly insulin-resistant patient receiving 200 U/day, the highest dose of nicotinamide to be administered with insulin aspart injection is approximately 42 mg/person/day (comparable to dietary intake; the mean and maximum daily intake of nicotinamide in food = 34-57 mg/person/day). Nonclinical literature suggests that nicotinamide does not cause general toxicities and is not teratogenic, genotoxic or carcinogenic.

Animal data

PD profile: Nicotinamide is part of the vitamin B3 complex. It is required as a nutrient to prevent the niacin deficiency disorder pellagra. It can also act as a constituent of the enzyme cofactors NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate). This oxidation-reduction reaction is essential to cellular energy production and utilization, steroid biosynthesis, DNA replication/repair, cellular differentiation, and the general metabolism of carbohydrates, amino acids, and fatty acids.

PK profile: The PK of nicotinamide is dependent on dose, species and route of treatment, and metabolic pathway which differ in different animal species including rat, rabbit, guinea-pig, pig, cat and dog. In rabbits (IP at 1000 mg/kg, orally up to 2000 mg/kg), the blood concentration of nicotinamide increased over several hours and plateaued. In cats/dogs (IV at 500 mg/kg), the blood concentration of nicotinamide was high initially and decreased linearly over several hours. The parent compound is rapidly absorbed, circulated in plasma in an unbound form, and distributed throughout the extracellular fluid after oral, SC, and IP administration in animals, as well as humans (15 to 40 L for a 70 kg person). Nicotinamide is mainly metabolized in the liver to N-methyl nicotinamide or nicotinamide N-oxide. There are no unique metabolites reported for humans.

Toxicological profile:

Single does toxicity: Nicotinamide has very low acute toxicity in rats and mice when dosed via oral, IP, IV, or SC with LD_{50}s ranged from approx. 1.6 to 7 g/kg).

Lethal single dose (LD_{50}) values for nicotinamide

Species	Route	Dose
Mouse	Intraperitoneal	2050 mg/kg
Mouse	Oral	3100 mg/kg
Mouse	Oral	2500 mg/kg
Mouse	Intravenous	1800 mg/kg
Mouse	Subcutaneous	2000 mg/kg
Rat	Oral	3530 mg/kg (males) 3620 mg/kg (females)
Rat	Oral	7100 mg/kg (males) 5500 mg/kg (females)
Rat	Oral	>2500 mg/kg
Rat	Subcutaneous	1680 mg/kg
Rabbit	Dermal	>2000 mg/kg

Repeated dose toxicity: In rats, when nicotinamide was dosed orally in the diet (35, 70, and 140 mg/kg/day for 8-12 weeks) or via gavage (0, 215, and 1000 mg/kg/day for 4 weeks), changes in growth rate and relative liver weight (changes not dose-related) were seen. In the dietary study, nicotinamide caused enhanced growth rate at 70 mg/kg/day, but inhibited growth rate at 140 mg/kg/day. It also caused a relative liver weight decrease at 70 mg/kg/day. In the oral gavage study, decreased body weight gain and food consumption, increased liver weight, accompanied by mild centrilobular hypertrophy and extramedullary hematopoiesis of the spleen were observed. The NOAEL obtained from this oral gavage study was 215 mg/kg/day based on the minor effects on the liver and spleen. In comparison to highly insulin resistant patients receiving 200 U of insulin aspart injection, these patients are expected to receive a maximum amount of nicotinamide of approx. 41.6 mg/person/day (~ 0.69 mg/kg/day based on a 60 kg person). One intraperitoneal study (60, 200, or 600 mg/kg/day for 5 weeks), male rats had reduced food consumption and body weight gain in a dose-dependent manner. Liver hypertrophy was noted in all groups associated with fatty liver. Kidney hypertrophy was also noted at 500 mg/kg/day.

Overview of repeat dose toxicity studies with nicotinamide

Study type	Species	Route of administration	Dose levels
35 days dose range finding study	Mice	Oral, through drinking water	Up to 3330 mg/kg/day for males, and 2640 mg/kg/day for females
4-week toxicity study	Rat	Oral gavage	215 and 1000 mg/kg/day
12-week toxicity study	Rat	Dietary (oral)	35, 70 and 140 mg/kg/day
7-day infusion with pump	Rat	Subcutaneously	50 mg/kg/day
5-week study	Rat	Intraperitoneal	60, 200 and 600 mg/kg/day

Reproductive toxicity: In vitro studies showed that nicotinamide (5 μM) had no effects on hamster embryo development. However, Nicotinamide (5 μM in MEM medium or 8.2 μM in Ham's F10 medium) inhibited mouse embryo development (decreased cleavage rates and/or reduced morphological development) and viability. There was no teratogenic effect to chick embryos at doses of 2-19 mg/egg. In pregnant BL6 mice, reductions in the amount of amniotic fluid and fetus/maternal liver weight were seen when given nicotinamide at 61 mg/kg via SC administration from GDs 6 to 14. In pregnant JCL:ICR mice given a single IP injection of 0.18 μCi ^{14}C-nicotinamide on GD 9, levels of radioactive nicotinamide were higher in the placenta (16x) and fetus (5x) than the maternal blood. In pregnant rats given nicotinic acid at 0, 4, 200, 100 mg/kg/day from GDs 6 to 15 via oral administration, there were no teratogenic effects at doses up to 1000 mg/kg/day. Based on slightly decreased body weight gain of the dams and significantly

decreased placental weight at 1000 mg/kg/day, the NOAEL for development toxicity in the study was 200 mg/kg/day. In pregnant rabbits given doses up to 450 mg/kg/day, there were no teratogenic effects and the NOAEL for the dams and fetuses was 50 mg/kg/day. In rats (1000 mg/kg/day) and rabbit (450 mg/kg/day), post-implantation loss and increased number of resorptions (early and total resorptions) were noted. Nicotinic acid is converted to nicotinamide in vivo. Even though nicotinamide and nicotinic acid are identical in their vitamin functions, nicotinamide does not have the same biological effects as nicotinic acid (e.g., flushing, lipid-lowering effect etc.).

Overview of reproductive toxicity studies with nicotinamide

Study type	Species	Route of administration	Dose levels
Fertility*	Rat	Oral gavage	215 and 1000 mg/kg/day
Embryofoetal development	Mice	Subcutaneous	61 mg/kg/day GD[1] 6-14
Embryofoetal development	Rat	Oral gavage	1000 mg/kg/day GD[1] 6-17
Embryofoetal development	Rabbit	Oral gavage	0, 50, 150 and 450 mg/kg/day GD[1] 6-20

[1]GD = gestation day *Not a dedicated fertility study.

Genotoxicity: Nicotinamide was negative in an Ames assay (50 mg/plate), in vitro chromosome aberration (up to 50 mg/plate), and in vivo micronucleus studies (up to 1470 mg/kg). Nicotinamide induced sister chromatid exchanges in CHO cells at 1.0 to 10 mM, possibly by poly(ADP-ribose) polymerase inhibition. However, with the negative genotoxicity in the standard assays, the biological significance of this finding was unclear.

Overview of genotoxicity studies with nicotinamide

Study type	Species/strain	Route of administration	Dose levels
AMES	*Salmonella typhimurium*	*In-vitro*	33 µg to 10000 µg/plate
AMES	*Saccaromyces cerevisiae* D4, and *Salmonella typhimurium*	*In-vitro*	Up to 5%
AMES	*Salmonella typhimurium*	*In-vitro*	3.3 mg/plate
AMES	*Salmonella typhimurium*	*In-vitro*	50 mg/plate
Chromosome aberration	Chinese hamster fibroblast cell line	*In-vitro*	2 mg/mL
Chromosome aberration	Chinese hamster ovary cells	*In-vitro*	3 mg/mL (25 mM)
Chromosome aberration	Chinese hamster ovary cells	*In-vitro*	1.0 to 10 mM
Chromosome aberration	Human lymphocytes	*In-vitro*	9.8 to 5000 µg/mL
Two independent Micronucleus tests	Mice	Intraperitoneal	681, 1000 and 1470 mg/kg

Carcinogenicity: In Swiss albino mice, no carcinogenic effects were noted after giving 1% nicotinamide in drinking water in a lifetime study. However, nicotinamide has been shown to modulate the induction of tumors by certain carcinogens. In rats, nicotinamide (IP 350-500 mg/kg) and diethylnitrosamine increased pancreatic islet tumors when compared to diethylnitrosamine alone. On the other hand, nicotinamide (IP 2x 350 mg/kg 3 hours apart) decreased renal oncogenic activity of streptozotocin. The relevance of these findings to clinical use as an excipient is questionable.

Carcinogenicity study in mice with nicotinamide

Study type	Species/strain	Route of administration	Dose levels
Carcinogenicity (lifetime dosing)	Swiss albino mice	Drinking water	1% nicotinamide in the drinking water (3330 or 2640 mg/kg for males and females, respectively)

Local tolerance: Nicotinamide was slightly irritating to the skin of New Zealand white rabbit at 2.5% dissolved in saline. A skin sensitization test was negative at 5% during induction and 20% during challenge in a guinea pig Buehler test.

Human data

Nicotinamide (niacinamide or vitamin B3) is present in the insulin aspart injection formulation at a concentration of (b) (4) mM (corresponding to 20 (b) (4) mg/mL or (b) (4) % w/v). This equates to a maximum total dose of nicotinamide of approximately 41.6 mg/person/day (~0.5 mg/kg/day) in a highly insulin-resistant patient receiving 200 U/day (100 U/mL).

In humans, nicotinamide is easily absorbed parenterally and from all parts of the GI tract following oral administration, as well as by SC and intramuscular injection. Oral bioavailability of nicotinamide has been indirectly approximated to 85% from available literature, which means an acceptable intake of 560-900 mg/person/day, nicotinamide exposure is a factor of 11-18 fold higher than the maximum exposure of nicotinamide (42 mg/person/day ~ <0.5 mg/kg/day) in a highly insulin resistant patient receiving 200 U of insulin aspart injection, assuming 100% bioavailability of nicotinamide after sc dosing. Peak concentrations are achieved within 1 hr of oral ingestion and widely distributes throughout the body. It can pass the blood brain barrier and can be taken up by cells of the brain via a high-affinity transport system. PK data also suggests that metabolic clearance pathways are saturated at oral doses >1000 mg/person/day. Excretion is primarily via the urine. Nicotinamide has been used as an oral radiosensitizer in the treatment of cancer to enhance radiation damage, acne treatment (at levels of 4% applied topically), and orally in the treatment of T1DM (doses at 3-6 g tolerated with no side effects). Minor abnormalities in liver enzymes could occur infrequently at very high doses (8000-1000 mg/person/day), with side effects including nausea and vomiting.

Nicotinamide is listed in the U.S. FDA's database "Inactive Ingredient Search for Approved Drug Products" in products for injections for use at 2.5% as intravenous/intramuscular injection and at 1.25% as a topical gel. Nicotinamide is Generally Recognized as Safe (GRAS) for use as a food additive (which includes its use in infant formula), nutrient or dietary supplement. In addition, nicotinamide is approved by the FDA for use as a food additive to enrich corn meal, rice, macaroni and noodle products, whereas it is approved by the EPY for use in pesticide products applied to growing crops (as a synergist with a maximum limitation of 0.5% of formulation). Lastly, nicotinamide is also contained in the European Pharmacopoeia (Ph. Eur.), U.S. Pharmacopoeia (USP) and Japanese Pharmacopoeia (JP).

Nicotinamide is well-established for oral human use with a recommended upper limit ranging from 560-1500 mg/person/day. At 1500 mg/day, it is estimated to be approximately 30-fold higher than the exposure following treatment with 200 U of insulin aspart injection. In addition, there is a wealth of oral safety data relating to nicotinamide use in humans, and repeated oral doses up to 3000 mg/person/day appeared to be well tolerated. Diabetic patients (age 5-35 years) have been dosed (orally) for 1 year at 1500 mg/person/day with no apparent adverse effects. Likewise, children (age 3-12 years) have been dosed orally for 2.1 years on average at 1260 mg/person/day with no observable adverse effects.

Table 10 Study No 8303170a – Literature Review: Safety Margin for Nicotinamide.

Overview of the most relevant nonclinical safety studies, published human guidance levels and clinical trials for nicotinamide

Study (reference)	Dose	Dose ratio(s) [1]
4-week oral gavage study in rats (cited in 21, 55])	NOAEL = 215 mg/kg/day = 35 mg/kg/day based on HED	70 [2]
Rabbit (oral gavage) embryofoetal development study [47], (cited in 55])	NOEL = 50 mg/kg/day = 16 mg/kg/day based on HED	32 [2]
Genotoxicity. *In-vitro* and *in-vivo* tests (cited in [21, 22, 38, 39])	All tests negative	Not applicable
Carcinogenicity (life time) study in mice (drinking water) [41]	No carcinogenic effect at 3330 mg/kg/day (males) and 2640 mg/kg/day (females) = 270 mg/kg/day (males) and 215 mg/kg/day (females) based on HED	540 (males) 430 (females)
Human guidance levels (oral) [29, 42, 45, 48, 49, 50]	560-1500 mg/day	11-30 [3]
Human clinical studies (oral) [24, 32, 44]	Oral doses up to 3000 mg/person/day appear to be well tolerated	61 [3]

HED = Human equivalent dose

[1] Dose ratio are based on a highly insulin resistant patient receiving 200 U/day resulting in 42 mg nicotinamide/day (~0.5 mg/kg/day) and assuming 100% bioavailability of nicotinamide after s.c. dosing

[2] Values are based on HED

[3] Values are adjusted for oral bioavailability of nicotinamide in humans (85%)

Study No 8303170-b - Literature Review of L-Arginine

In a highly insulin-resistant patient receiving 200 U/day, the highest dose of L-arginine to be administered with insulin aspart injection is considerably lower (~ 7 mg/person/day; ~ 1 mg/kg/day) than an Observed Safe Level of arginine of 20,000 mg/day for oral administration in normal healthy adults. Based on available nonclinical literature, L-arginine is not expected to induce adverse effects in toxicity studies and has no adverse effect on animal reproduction. L-arginine is not genotoxic, and is not considered to possess carcinogenic potential as it is a naturally occurring amino acid.

Animal data

PD profile: L-arginine is the precursor of nitric acid through conversion by endothelial nitric oxide synthase and has a number of effects on the cardiovascular system (e.g., blood vessel dilation in rats and rabbits). In addition, L-arginine has other activities on blood clotting (inhibition of platelet aggregation in rats/rabbits), endocrine function (stimulation of insulin secretion), and weight loss (reduction of adipose tissue and serum glucose concentrations). All of the effects of L-arginine described above are not expected at the levels proposed for use in insulin aspart injection formulations.

Safety Pharm: No published data is available on the CNS, CV, or respiratory system with L-arginine. However, in a renal safety pharmacology assessment of the thrombolytic agent, Tenecteplase, which contains arginine as an excipient at a level of 550 mg for 52.5 mg of active drug, there were slightly increased urine volume, retention of sodium, decreased chloride excretion, and increased potassium excretion. These findings were considered secondary to the angioedemic response due to the presence of arginine (observed at 30 mg/kg active – corresponding to 314 mg/kg L-arginine). Such possible angioedemic effects in dogs were observed at levels greatly in excess of those proposed for use in insulin aspart injection.

PK profile: Like other amino acids, supplemental arginine can be readily absorbed from the GI tract and is actively transported across the intestine. In rats, ^{14}C-arginine via IP injection can be distributed in the skin, liver, large intestines, and stomach. The bulk of radiolabelled arginine was eliminated in the urine. Under physiological conditions, excretion via the kidneys does not play a role because of tubular reabsorption of amino acids in the kidney. However, at high IV doses, substantial urinary excretion of L-arginine does occur when the reabsorption pathway is saturated.

Toxicological profile:

Repeated dose toxicity: In 28-day SC rat study (n=10/group) two doses approx. 5 hrs apart), L-arginine caused slight to moderate hemorrhage at the injection site for 7/10 animals. Such observations were common following SC administration, due to the dosing procedure. In 13-week oral rat studies, L-arginine caused no obvious toxicity up to approx. 4000 mg/kg/day. There were findings, including reduced water intake in females, increased hemoglobin in males, decreased orthochromatic erythroblast ratio and increased mast cell ratio in males, decreased urea nitrogen in males, and slightly elevated urinary glucose in males. However, these findings were not associated with pathological changes. The NOAEL was 3331-4181 mg/kg/day. In a 52 week oral dietary study in the rat with LAE (LAE is metabolized to n-lauryl-L-arginate and subsequently to L-arginine), higher doses (800-995 mg/kg/day) caused lesions in the non-glandular epithelium of the stomach. However, the molecular weight of LAE is approximately 50% more than L-arginine HCl (421.0 g/mol vs. 210.7 g/mol). In single/repeat dose up to 2 weeks IV dog study (in part of assessing Tenecteplase and Alteplase, thrombolytic agents), high levels (314 mg/kg) of L-arginine via IV administration could cause some angioedemic effect. In addition, L-arginine given to other species such as non-diabetic BioBreeding rats, lean Zucker, obese ZDF rats, sheep, pigs, also did not result in any adverse effects. All in all, the relevance these described findings above for SC administration at the very low levels is not clear.

Overview of selected repeat dose toxicity studies with L-arginine and LAE

Study type	Species	Route of administration	Dose levels (mg/kg/day)	NOAEL (mg/kg/day)
4-week study*	Rat	Oral	2120 - 4182	3850 (males) 4182 (females)
13-week toxicity	Rat	Diet (oral)	752 – 3565	3331 (males) 3565 (females)
13-week toxicity study	Rat	Diet (oral)	843 - 3879	3318 (males) 3879 (females)
13-week toxicity study*	Rat	Diet (oral)	343 - 3500	343 (males) 398 (females)
52-week study*	Rat	Diet (oral)	106 - 995	271 (males) 347 (females)

*dosed with LAE

Reproductive toxicity: I) Reproduction studies: In a repeat dose SC rat study, L-arginine was administered at 724 mg/kg/day prior to mating (63 days for males and 14 days for females), during mating, throughout gestation and lactation for females, which had no significant effects on reproduction performance or pre- or post-natal development. In study in pigs, L-arginine (as a dietary supplement, approx. 325 mg/kg/day) was given to pregnant females from gestation day (GD) 30 to 110. There was a significant increase in the number of live births (by 22%) and live birth weights (by 24%) compared to controls. II) Embryo-fetal studies: In an IP rat embryo-fetal study (Naidu CR, 1973), L-arginine doses of 0 (n=9) or 15 mg/kg/day (n=18) were given to rats on GDs 1 to 6 and sacrificed on GDs 12 and 15. The study showed that 68/158 fetuses had anomalies (i.e., malformations of hind limb development – loss of distal part of leg or loss/malformation of feet) and there were 5 resorption sites. At 15 mg/kg/day, it was approx. 15 fold higher than max exposure of 1 mg/kg/day (estimated maximal L-arginine dose in a highly resistant patient receiving 200 U/day of insulin aspart injection. However, with small number of animals and dose route used (IP) in this study, the relevance of these findings was unclear. This study was not noted in the table below. In a dietary rat study, L-arginine was given as a dietary supplement (approx. 1040 mg/kg/day) to rats either throughout pregnancy or during GDs 1 to 7. There was a significant increase in litter size, with a greater number of surviving embryos. No adverse effects on reproductive performance were observed.

Overview of selected reproduction toxicity studies with LAE

Study type	Species	Route of administration	Dose levels	NOAEL (mg/kg/day)
Fertility (segment 1)**	Mice and Rabbit	Intravenous	6000 mg/kg	No data
Embryofoetal development (Segment 2)*	Rat	Gavage	138, 415 and 1382 mg/kg	1382
Embryofoetal development (Segment 2)	Rat	Dietary/oral	1040 mg/kg	1040
Embryofoetal development (Segment 2)	Rabbit	Intravenous	300 mM (81.66 mg Arginine HPO_4/mL vehicle)	No findings in study
Embryofoetal development (Segment 2)*	Rabbit	Gavage	69, 207 and 691mg/kg	691
One generation (segment 3)	Rat	Subcutaneous	724 mg/kg	724
Two generation (segment 3)*	Rat	Dietary/oral	Up to 946 mg/kg	443

*dosed with LAE. ** Possibly not a dedicated fertility study.

Genotoxicity: Two in vitro tests, an Ames test (up to 26 mg/pate L-arginine glutamate) and a chromosomal aberration assay in CHO cells (up to 4 mg/mL L-arginine-glutamate), showed no evidence of genotoxicity with or without addition of S9. Excessive levels of arginine (10-100 μg/mL added to original content of 146 μg/mL) caused a small (<10%) increase in sister chromatid exchanges in human lymphocytes. Similar finding could be seen with a range of other amino acids with increases of 0.2-2 fold over control levels. The concentrations described above were approximately 10-fold greater than the maximum amount of L-arginine exposure of (b) (4) μg/mL with insulin aspart injection depending on formulations. In addition, adding 5 mM L-arginine to cultured human fibroblasts and keratinocytes (irradiated with UVA) increased UVA-mediated cytotoxicity and DNA damages, as measured in a Comet assay. This could be attributed to the nitric oxide generation.

Overview of selected Genotoxicity studies with L-arginine and LAE

Study type	Species/strain	Test system	Dose levels
AMES	*Salmonella typhimurium*	*In vitro*	Up to 26000 µg/plate
AMES	*Salmonella typhimurium* and *Escherichia coli*	*In vitro*	Up to 5000 µg/plate
AMES	*Salmonella typhimurium* and *Escherichia coli*	*In vitro*	Up to 5000 µg/plate
AMES*	Salmonella typhimurium	*In vitro*	Up to 150 µg/plate
Mouse lymphoma*	L5178Y cells	*In vitro*	1 to 50 µg/mL
Chromosome aberration	Chinese hamster cells	*In vitro*	Up to 4000 µg/mL
Chromosome aberration	Human lymphocytes	*In vitro*	Up to 1740 µg/mL
Chromosome aberration	Human lymphocytes	*In vitro*	Up to 1740 µg/mL
Bone marrow** micronucleus	Mouse erythrocytes	*In vivo*	2000 mg/kg

*dosed with ethyl lauroyl arginate hydrochloride
** dosed with n-lauroyl-L- arginate (LAS)

Carcinogenicity: No published data was found. However, arginine has been shown to either inhibit tumor growth in animal cancer models or stimulate tumor protein synthesis and growth in cultures cells or immune-deficient animals.

Local tolerance: Retinal damages such as retinal necrosis and atrophy was seen in rabbit eye after intravitreal injection of thrombolytic agent, Tenecteplase. Injection site effects such as slight to severe inflammatory reactions and slight hemorrhage were observed after SC injection of L-arginine in rats and dogs. However, actual dose levels were not available.

Human data
L-arginine is a naturally occurring amino acid. L-arginine (as L-arginine HCl) is present in the insulin aspart injection formulation at 20 mM (corresponding to 3.48 mg/mL as L-arginine HCl or 0.35% w/v). This would equate to a maximum total dose of L-arginine HCl of 6.96 mg/day (~7 mg/day) or ~0.1 mg/kg/day in a highly insulin resistant patient receiving 200 U/day (100 U/mL).

L-arginine (as Arginine) is listed in the US Food and Drug Administration (FDA)'s database "Inactive Ingredient Search for Approved Drug Products" in products for injections (intramuscular, intravenous), with a maximum potency/concentration of up to 88% and in oral tablet form up to 25 mg/tablet. L-amino acids are generally regarded as safe as direct food additives for human consumption by the US FDA. L-arginine has GRAS notice for specific food use up to 0.6% and is approved by the US FDA as a food additive up to 6.6% by weight of total protein. L-arginine is contained in Ph. Eur., USP and JP.

In humans, L-arginine has been well tolerated when given via oral, intravenous, and intra-arterial routes in does ≤ 3000 mg. Available published human data ranging from 7 days to 3 years of exposure has determined an Observed Safe Level of L-arginine of 20,000 mg/day for oral administration in healthy adults. Reported clinical adverse effects include nausea, vomiting, flushing, headache, and local venous irritation. However, these effects were associated with rapid intravenous infusion at large doses (at least 1000 fold greater than recommended doses). Humans are exposed to L-arginine through the diet with calculated levels of 4000-6000 mg/day and L-arginine is used as a diagnostic aid or in drug formulations at up to approximately 500 mg/kg for intravenous use. Oral bioavailability of L-arginine in humans has been measured in the range of 21-68%. The plasma levels of arginine in humans have been reported to range from 13-16 µg/mL (75-89 µmol/L) as the average plasma arginine concentration of adult humans

receiving enteral nutrition was measured as 37 µg/mL (210 µmol/L). The arginine plasma levels of 13-16 µg/mL would be approximately 5 fold above what would result from L-arginine exposure from insulin aspart injection (2.8 µg/mL) under an assumption that the entire daily dose of insulin aspart injection would be administered as a single dose and is 100% systemically available and distributed into the plasma In addition, it has been reported that there is an increased risk of death in myocardial infraction patients who received L-arginine supplementation (9 g/day). Such supplementation could lead to an increase in homocysteine production, resulting in worsening of endothelial function and atherosclerosis.

Table 11 Study No 8303170b – Literature Review: Safety Margin for L-arginine.

Overview of the most relevant nonclinical safety studies, endogenous human plasma levels and clinical trials for L-arginine

Study (reference)	Dose/concentrations	Dose ratio(s)[1]
13-week (oral) toxicity studies in rats [4, 20]	NOAEL = 3318-3879 mg/kg/day = 535-626 mg/kg/day based on HED	5350-6260 [2]
Rat (oral) embryofetal development study [13]	NOAEL = 1040 mg/kg/day = 168 mg/kg/day based on HED	1680 [2]
Genotoxicity. *In vitro* tests [14, 20]	All tests negative	Not applicable
Carcinogenicity	No studies available. A carcinogenic potential is not expected for L-arginine being an endogenous amino acid	
Endogenous plasma levels of arginine in humans [3] Calculated L-arginine plasma exposure from faster aspart	13-16 µg/mL 2.8 µg/mL	L-arginine exposure from faster aspart 5 fold lower than endogenous plasma levels of L-arginine (13µg/mL/2.8 µg/mL)
Human exposure via the diet [6]	4000-6000 mg/day	120-180 [3]
Observed Safe Level [5]	20,000 mg/day	600 [3]

HED = Human equivalent dose

[1] Dose ratio are based on a highly insulin resistant patient receiving 200 U/day resulting in 7 mg L-arginine/day (~0.1 mg/kg/day) and assuming 100% bioavailability of L-arginine after s.c. dosing

[2] Values are based on HED

[3] Values are adjusted for oral bioavailability of L-arginine in humans (measured in the range of 21%-68%; 21% has been used for calculation)

11 Integrated Summary and Safety Evaluation

Background

NDA 208751 is for a new insulin aspart injection formulation to treat diabetic patients. The insulin aspart injection formulation is developed for chronic subcutaneous treatment. Insulin aspart injection proposed as a 100 U/mL formulation for dosing at meals or post-meal. Individual total daily insulin requirements are usually between 0.5-1.0 U/kg/day. Compared to NovoLog®, insulin aspart injection contains 2 additional excipients: nicotinamide (also known as niacinamide or vitamin B3) and L-arginine hydrochloride. The addition of nicotinamide in the insulin aspart injection formulation results in an earlier initial absorption of insulin aspart following subcutaneous injection, leading to a greater early glucose-lowering effect compared to NovoLog®. The addition of L-arginine hydrochloride supports the stabilization of the insulin aspart injection formulation.

The active ingredient insulin aspart in insulin aspart injection has been marketed as NovoLog® for more than a decade. At the molecular level, insulin aspart in insulin aspart injection and in NovoLog® are identical and therefore have the same biological activity at the insulin receptor. A full nonclinical safety program was conducted for insulin aspart prior to the approval of NovoLog®. The findings for NovoLog® were predominantly related to exaggerated pharmacological effects of insulin (clinical signs of hypoglycemia due to changes in blood glucose levels, decreased food consumption and lowered body weight).

During the development program of insulin aspart injection, several formulations with varying concentrations of the excipients nicotinamide and L-arginine (FIA (A/B/C/D/E/Q/R)), were investigated for selection of the most optimal clinical formulation. A letter in parentheses following the abbreviation FIA is used to denote different formulations of insulin aspart injection tested during development. The chosen formulation for registration is FIA (Q), i.e. FIA (Q) = insulin aspart injection or insulin aspart injection. In

addition, there was a change (b) (4) . The minor changes in (b) (4) are not considered to be associated with any clinical risk. The other excipients used in insulin aspart injection are identical to those used in NovoLog® and are applied at the same or lower concentrations, as shown in the table below (note: "Faster Aspart" represents the final clinical formulation.

Overview of FIA (A-D), faster aspart and NovoRapid®/NovoLog® formulations tested nonclinically in local tolerance studies

	FIA (A-D)	Faster Aspart	NovoRapid®/ NovoLog®	Function of Ingredient
Insulin aspart	100 U/mL (600 nmol/mL)	100 U/mL (600 nmol/mL)	100 U/mL (600 nmol/mL)	Drug substance
Zinc	(b) (4)	19.6 µg/mL[2)]	19.6 µg/mL[1)]	(b) (4)
Phenol		1.50 mg/mL	1.50 mg/mL	
Metacresol		1.72 mg/mL	1.72 mg/mL	
Glycerol		3.3 mg/mL	16 mg/mL	
Sodium chloride		-	0.58 mg/mL	
Disodium phosphate Dihydrate		0.53 mg/mL	1.25 mg/mL	
Arginine (as L-arginine HCl)		3.48 mg/mL	-	Stabilising agent
Nicotinamide		20. (b) (4) mg/mL	-	Absorption modifier
(b) (4)		-	-	(b) (4)
pH		7.1	7.4	n.a.
(b) (4)				

Since a full nonclinical program was done for NovoLog®, no additional nonclinical toxicity studies were conducted as insulin aspart injection is a new formulation of the marketed product NovoLog®. The toxicological safety assessment of insulin aspart injection was therefore based on the previous registration documentation for insulin aspart, a literature safety review of the additional excipients (nicotinamide and L-arginine), an impurity assessment and local tolerance studies.

Pharmacology

No separate primary pharmacodynamic studies have been conducted with insulin aspart injection, but plasma glucose was measured as part of a PK/PD study in pigs and compared to NovoRapid®/NovoLog® (**Study No HaRe131104**). In this PK/PD study in pigs with insulin aspart injection, plasma glucose profiles were measured. The blood glucose profiles mirrored the increased early absorption of insulin aspart with an earlier onset of glucose-lowering effect compared to NovoRapid®/NovoLog®. No further pharmacology studies have been conducted since insulin aspart injection is a new formulation of insulin aspart. The nonclinical primary pharmacodynamic studies conducted for insulin aspart as part of development of NovoLog® demonstrated, in both *in vivo* and *in vitro* studies, that insulin aspart behaved in a manner that closely resembled human insulin and that the dissociation of binding to the insulin receptor of insulin aspart is equivalent to human insulin.

Pharmacokinetics

In **Study No HaRe131104**, Pharmacokinetic and pharmacodynamic profiles after subcutaneous dosing of insulin aspart were studied in pigs using insulin aspart injection and compared to NovoRapid®/NovoLog®. Early absorption of insulin aspart, evaluated as the ratio between the area under the curve (AUC) between 0-15 min and between 0-60 min ($AUC_{0\text{-}15min}/AUC_{0\text{-}60min}$), was statistically significantly increased for insulin aspart injection compared to NovoRapid®/NovoLog®. In accordance with the increased early absorption of insulin aspart, a more rapid decrease in plasma glucose was observed after insulin aspart injection administration.

The mechanism by which the increased early absorption is achieved for insulin aspart injection was investigated in *vitro* and *in vivo*. The *in vitro* study using an HDMEC monolayer showed that after s.c. administration, nicotinamide increases the monomer fraction and permeation rate of insulin aspart across

the endothelium. *In vivo* study (**Study No JKIP130102**) in pigs of the effect of nicotinamide on ^{133}Xe washout (a marker of local blood flow) revealed no statistically significant effect of nicotinamide alone.

These studies suggest that the nicotinamide present in insulin aspart injection augments the proportion of insulin aspart monomers readily available for absorption following s.c. administration thereby facilitating a more rapid absorption across the endothelium compared to NovoRapid®/NovoLog® whereas a direct effect of nicotinamide on local blood flow is considered unlikely to play a major role (**Study No JKIP130102**).

Toxicology
The nonclinical safety assessment of the insulin aspart injection formulation is based on the previous registration documentation for insulin aspart, a literature safety review of the additional excipients (nicotinamide and L-arginine), an impurity assessment and local tolerance studies in rats, rabbits and minipigs with insulin aspart injection (i.e., FIA (Q)) and earlier formulations evaluated in the development of insulin aspart injection (e.g.,FIA (A-D). For approval of NovoLog®, a full nonclinical safety program was conducted for insulin aspart. The findings were predominantly related to the pharmacological action of insulin or exaggerated pharmacology of insulin. No findings of toxicological concern were observed.

No studies with regards to genotoxicity, carcinogenicity, and reproductive and developmental toxicity have been conducted with insulin aspart injection. There were studies conducted and addressed with NovoLog®. There are no outstanding issues with insulin aspart injection. Following information was extracted from NovoLog® label. Genotoxicity: NovoLog® was not genotoxic in the following tests: Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, in vivo micronucleus test in mice, and in ex vivo UDS test in rat liver hepatocytes. Carcinogenicity: Standard 2-year carcinogenicity studies in animals were not conducted to evaluate the carcinogenic potential of NovoLog. However, in 52-week studies, Sprague-Dawley rats were dosed subcutaneously with NovoLog® at 10, 50, and 200 U/kg/day (approximately 2, 8, and 32 times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area, respectively). At a dose of 200 U/kg/day, NovoLog® increased the incidence of mammary gland tumors in females when compared to untreated controls. The incidence of mammary tumors for NovoLog® was not significantly different than for regular human insulin. The relevance of these findings to humans was not known. Reproductive/developmental toxicity: in fertility studies in male and female rats, at subcutaneous doses up to 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on U/body surface area), no direct adverse effects on male and female fertility, or general reproductive performance of animals was observed. Subcutaneous reproduction and teratology studies have been performed with NovoLog® and regular human insulin in rats and rabbits. In these studies, NovoLog® was given to female rats before mating, during mating, and throughout pregnancy, and to rabbits during organogenesis. The effects of NovoLog® did not differ from those observed with subcutaneous regular human insulin. NovoLog®, like human insulin, caused pre- and post-implantation losses and visceral/skeletal abnormalities in rats at a dose of 200 U/kg/day (approximately 32 times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area) and in rabbits at a dose of 10 U/kg/day (approximately three times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area). The effects are probably secondary to maternal hypoglycemia at high doses. No significant effects were observed in rats at a dose of 50 U/kg/day and in rabbits at a dose of 3 U/kg/day. These doses are approximately 8 times the human subcutaneous dose of 1.0 U/kg/day for rats and equal to the human subcutaneous dose of 1.0 U/kg/day for rabbits, based on U/body surface area.

Excipients
Nicotinamide (niacinamide) and L-arginine, the two excipients added compared to NovoLog®, are both listed in the FDA's database "Inactive Ingredient Search for Approved Drug Products" in products for injections and are contained in Ph. Eur., USP (niacinamide) and JP. The addition of nicotinamide appeared to result in a slightly earlier absorption of insulin aspart following subcutaneous injection. Arginine (as L-Arginine hydrochloride) was added for stabilization. The review of the available nonclinical literature and the well-established clinical experience support the safe use of nicotinamide and L-arginine hydrochloride at the levels used in insulin aspart injection.

Niacinamide (Study Report No 8303170a)

Nicotinamide demonstrated very low potential for acute toxicity in rats and mice when dosed orally, intraperitoneally, intravenously or subcutaneously (LD_{50} values range from approximately 1600 to 7000 mg/kg). In repeat-dose toxicity studies, minor changes such as liver hypertrophy (mostly males) and reduced extramedullary hematopoiesis (females) were observed at 1000 mg/kg/day. The NOAEL was reported to be 215 mg/kg/day. Although limited, the available animal repeat dose toxicity data do not raise any significant safety concerns. No published non-rodent toxicity studies were identified.

Even though data on reproductive toxicology are limited, there are no data suggesting any teratogenic risk of nicotinamide. Nicotinamide had no effect on reproductive organs after four weeks of oral dosing in rats and was not teratogenic in chick embryos, mice, rats, and rabbits. Post-implantation loss and increased number of resorptions (early and total resorptions) was noted at high doses in rats (1000 mg/kg) and rabbits (450 mg/kg). In a GLP-compliant rabbit embryofetal development study at oral does up to 450 mg/kg/day there was no evidence of a teratogenic potential and the NOEL for the dams and fetuses was 50 mg/kg/day. A reduction in the amount of amniotic fluid/fetus and maternal liver weight was seen when nicotinamide was given to pregnant mice. Levels of radioactive nicotinamide were higher in the placenta and fetus than the maternal blood when administered to pregnant mice. No data are available for fertility, but the available repeat-dose toxicity studies in rodents were negative for effects on the gonads. No data are available on pre- and postnatal studies.

Nicotinamide is not genotoxic. Nicotinamide was negative in the Ames, *in-vitro* chromosome aberration and *in-vivo* micronucleus studies. It induced sister chromatid exchanges (SCEs) *in-vitro*, possibly by poly (ADP-ribose) polymerase inhibition. However, with the negative genotoxicity in the standard assays, the biological significance of this finding is unclear.

In Toth 1983 paper, the study was conducted to see if nicotinamide could induce tumors by administrating at high dose levels for life to Swiss mice. The study results showed that no carcinogenic effects were noted in mice from 6 weeks of age following administration of 1% nicotinamide in drinking water (calculated to be 3330 or 2640 mg/kg/day for males and females, respectively). Nicotinamide was non-irritating to slightly irritating to intact or abraded rabbit skin and negative for skin sensitivity.

Lastly, there is a well-established safety database relating to the oral use of nicotinamide in humans where oral doses up to 3000 mg/person/day were well-tolerated. Nicotinamide is well-established for human use with oral guidance levels ranging from 560-1500 mg/person/day and there is a significant amount of clinical data supporting its safe use. The highest dose of nicotinamide in a highly insulin resistant patient receiving 200 U/day of insulin aspart injection is 42 mg/person/day (~<0.5 mg/kg/day).

Dose ratios for nicotinamide based on NOEL/NOAEL in the most relevant non-clinical safety studies, published human guidance levels and tolerated dose in controlled clinical studies

Study (reference)	Dose	Dose ratio(s)*
4-week oral gavage study in rats (cited in 1, 65)	NOAEL = 215 mg/kg/day = 35 mg/kg/day for humans based on HED	70
Rabbit (oral gavage) embryofoetal development study (46, cited in 65)	NOEL = 50 mg/kg/day = 16 mg/kg/day for humans based on HED	32
Genotoxicity. *In-vitro* and *in-vivo* tests (cited in 1, 2, 31, 32)	All tests negative	Not applicable
Carcinogenicity (life time) study in mice (drinking water) (36)	No carcinogenic effect at 3330 mg/kg/day (males) and 2640 mg/kg/day (females) = 270 mg/kg/day (males) and 215 mg/kg/day (females) for humans based on HED	540 (males) 430 (females)
Human (oral) guidance levels (16, 40, 44, 48, 49, 50)	560-1500 mg/day	11-30**
Human (oral) clinical studies (5, 19, 42)	Oral doses up to 3000 mg/person/day appear to be well tolerated	61**

HED = Human equivalent dose

* Dose ratio are based on a highly insulin resistant patient receiving 200 U/day resulting in 42 mg nicotinamide/day and assuming 100% bioavailability of nicotinamide after s.c dosing

** Values are adjusted for oral bioavailability of nicotinamide in humans (85%)

L-arginine (Study Report No 8303170b)

L-arginine showed low acute toxicity in rats when dosed intraperitoneally and orally (LD_{50} values range from approximately 3793 to 16,000 mg/kg). The repeat-dose toxicity studies do not raise a safety concern where the two 13-week oral studies in the rat with L-arginine showed no apparent toxicity at doses up to almost 4000 mg/kg/day. Since long term repeat-dose toxicity studies are absent, general safety after long-term exposure is based primarily on clinical experience.

According to available literature data on fertility, embryofetal development and multi-generation studies, L-arginine and ethyl lauroyl arginate hydrochloride (LAE) appear to produce no adverse effects on animal reproduction when dosed at high doses (up to 1382 mg/kg/day).

As an endogenous amino acid, L-arginine is not considered genotoxic, which is supported by the *in vitro* and limited *in vivo* genotoxicity assessments reported in available literature.

No carcinogenicity studies were identified in the literature. However, carcinogenic potential is not expected for L-arginine as an endogenous amino acid present in human plasma at levels of 13-16 µg/mL and given that insulin aspart injection will result in only a slight increase in plasma arginine concentration (increase of 2.8 µg/mL) in the worst-case scenario (e.g., a highly insulin resistant patient receiving 200 U/day). Based on local tolerance studies, L-arginine is considered to be non-irritant and non-sensitizing.

Lastly, L-arginine has generally been well tolerated in humans when administered via oral, intravenous and intra-arterial routes in doses (singly or intermittently) at up to 30,000 mg. Available published human data ranging from 7 days to 3 years of exposure has determined an Observed Safe Level of L-arginine of 20,000 mg/day for oral administration in healthy adults. Oral bioavailability in humans has been measured in the range of 21%-68%. Humans are exposed to L-arginine through the diet with calculated levels of 4000-6000 mg/day and L-arginine is used as a diagnostic aid or in drug formulations at up to approximately 500 mg/kg for intravenous use.

Lastly, the estimated maximum L-arginine dose in a highly insulin resistant patient receiving 200 U/day of insulin aspart injection will be approximately 6.96 mg/person/day (~0.1 mg/kg/day). Based on available nonclinical literature, L-arginine does not induce adverse effects in toxicity studies and has no adverse effect on animal reproduction. L-arginine is also not genotoxic, and is not considered to possess a

carcinogenic potential since it is a natural occurring amino acid ingested daily in large amounts. Assuming that an entire daily dose of insulin aspart injection of 200 U is administered as a single dose and L-arginine is 100% systemically available; the resulting L-arginine exposure (2.8 µg/mL) would still be 5 times lower than 13-16 µg/mL endogenous plasma levels of L-arginine.

Dose ratios for L-arginine based on NOAEL in the most relevant non-clinical safety studies, endogenous human plasma levels and clinical trials

Study (reference)	Dose	Dose ratio(s)*
13-week (oral) toxicity studies in rats (6, 69)	NOAEL = 3331-4181 mg/kg/day = 537-674 mg/kg/day for humans based on HED	5370-6740
Rat (oral) embryofoetal development study (49)	NOAEL = 1040 mg/kg/day = 168 mg/kg/day for humans based on HED	1680
Genotoxicity. *In vitro* tests (50, 69)	All tests negative	Not applicable
Carcinogenicity	No studies available. A carcinogenic potential is not expected for L-arginine being an endogenous amino acid	
Endogenous plasma levels of arginine in humans (5) Calculated L-arginine plasma exposure from faster aspart	13-16 µg/mL 2.8 µg/mL	L-arginine exposure from faster aspart 5 fold lower than endogenous plasma levels of L-arginine (13µg/mL/2.8µg/mL)

Study (reference)	Dose	Dose ratio(s)*
Exposure via the diet (8)	4000-6000 mg/day	120-180**
Observed Safe Level (7)	20,000 mg/day	600**

HED = Human equivalent dose
* Dose ratio are based on a highly insulin resistant patient receiving 200 U/day resulting in 7 mg L-arginine/day and assuming 100% bioavailability of L-arginine after s.c dosing
** Values are adjusted for oral bioavailability of L-arginine in humans (measured in the range of 21%-68%; 21% has been used for the calculation)

Local Tolerance

In **Study No 209322**, single-dose local tolerance following subcutaneous administration was assessed in minipigs using various concentrations of L-arginine and nicotinamide. In **Study No 212251**, single-dose and multiple-dose local tolerance was also assessed in rats with insulin aspart injection given as daily subcutaneous injections for four weeks followed by a two-week recovery period. The studies showed injection site reactions similar to the marketed formulation of insulin aspart, NovoRapid®/NovoLog®. In addition, in **Study No 212147**, single-dose local tolerance was assessed following intravenous, intramuscular and intra-arterial administration of insulin aspart injection in rabbits. The injection site reactions for insulin aspart injection and 0.9% sodium chloride controls were comparable in severity for all three administration routes.

Impurities

The impurity profile of insulin aspart injection is comparable to that of NovoLog® with no new drug product related impurities above threshold limit for qualification according to guidelines (*ICH Q3B(R2)*). The proposed insulin aspart injection drug product shelf-life specification limits for insulin aspart related degradation products were identical to the specification limits established for NovoLog®. The in-use acceptance criteria (including shelf life) were likewise qualified by the nonclinical toxicology studies with NovoLog®.

Insulin aspart degradation products

There is one new product-related impurity compared to NovoLog® reported during stability studies of the insulin aspart injection drug product. The impurity identified as [redacted] (b) (4) is a reaction product between [redacted] (b) (4). The content of [redacted] (b) (4) at end of shelf life and end of in-use is estimated to be < [redacted] (b) (4) %, which is below the threshold of qualification stated in ICH Q3B(R2) and therefore no further qualification is considered to be required.

Besides what is identified above, four groups of insulin aspart degradation products are included in the drug product specifications: [redacted] (b) (4) and insulin aspart related impurities and high molecular weight proteins (HMWP). The proposed insulin aspart injection drug

product shelf-life specification limits for insulin aspart degradation products are identical to the specification limits approved for NovoLog®. The in-use acceptance criteria (including shelf life) are likewise qualified by the nonclinical toxicology studies with NovoLog®

Impurity levels of insulin aspart degradation products in non-clinical studies and in-use (including shelf life) acceptance criteria

	(b) (4)	Insulin aspart related impurities	HMWP
In-use acceptance criteria (including shelf life) for faster aspart			(b) (4)
Level of insulin aspart degradation product tested in the pivotal 12-month repeat-dose study in rat[a]			
Dose of insulin aspart degradation product at NOAEL in rats[a] (nmol/kg/day)			
Human intake[b] (nmol/kg/day)[b]			
Dose factor[a,b]			

[a] pivotal 12-month rat study (NovoRapid®/NovoLog® Expert Report on the Toxico – Pharmacological Documentation, 1998, Appendix B, study T-12): NOAEL= (b) (4) nmol/kg/day ((b) (4) U/kg/day) in male animals. NOAEL= (b) (4) nmol/kg/day (b) (4) U/kg/day) in female animals. Dose levels are based on the lowest NOAEL of (b) (4) nmol/kg/day.

[b] Human intake is based on a highly insulin resistant patient receiving 200 U/day and a bodyweight of 70 kg (= 17 nmol/kg/day).

Excipient-related impurities

One excipient-related impurity, (b) (4), originating from (b) (4) was identified. It caused a structural alert for potential carcinogenicity in the DEREK/Leadscope *in silico* analysis based on its structural similarity to (b) (4) The maximum exposure to (b) (4) would be < (b) (4) µg/day in a highly insulin-resistant patient receiving 200 U/day, which is below a TTC of 1.5 µg/person/day (ICH M7). This excipient related impurity is therefore considered of no toxicological concern.

Leachables

Two leachables, (b) (4) and (b) (4), from the container closure systems were identified after long term storage and in-use conditions. The clinical exposure of (b) (4) in a highly insulin-resistant patient receiving 200 U/day is 12,500 fold below the calculated PDE ((b) (4) mg/person/day per ICH Q3C) and the observed level of (b) (4) is (b) (4) fold below the TTC (1.5 µg/person/day). The two leachables are therefore not considered to pose a safety concern.

Conclusions

Based on the experience with the active ingredient insulin aspart within diabetes management as NovoLog®, the literature safety review on the added excipients (nicotinamide and L-arginine), the local tolerance studies in rats, rabbits and minipigs and the safety evaluation of impurities, the nonclinical data support the safe use of insulin aspart injection for the chronic treatment of diabetes mellitus.

12 Appendix/Attachments

NovoLog Label

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category B. All pregnancies have a background risk of birth defects, loss, or other adverse outcome regardless of drug exposure. This background risk is increased in pregnancies complicated by hyperglycemia and may be decreased with good metabolic control. It is essential for patients with diabetes or history of gestational diabetes to maintain good metabolic control before conception and throughout pregnancy. Insulin requirements may decrease during the first trimester, generally increase during the second and third trimesters, and rapidly decline after delivery. Careful monitoring of glucose control is essential in these patients. Therefore, female patients should be advised to tell their physician if they intend to become, or if they become pregnant while taking NovoLog.

An open-label, randomized study compared the safety and efficacy of NovoLog (n=157) versus regular human insulin (n=165) in 322 pregnant women with type 1 diabetes. Two-thirds of the enrolled patients were already pregnant when they entered the study. Because only one-third of the patients enrolled before conception, the study was not large enough to evaluate the risk of congenital malformations. Both groups achieved a mean HbA_{1c} of ~ 6% during pregnancy, and there was no significant difference in the incidence of maternal hypoglycemia.

Subcutaneous reproduction and teratology studies have been performed with NovoLog and regular human insulin in rats and rabbits. In these studies, NovoLog was given to female rats before mating, during mating, and throughout pregnancy, and to rabbits during organogenesis. The effects of NovoLog did not differ from those observed with subcutaneous regular human

insulin. NovoLog, like human insulin, caused pre- and post-implantation losses and visceral/skeletal abnormalities in rats at a dose of 200 U/kg/day (approximately 32 times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area) and in rabbits at a dose of 10 U/kg/day (approximately three times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area). The effects are probably secondary to maternal hypoglycemia at high doses. No significant effects were observed in rats at a dose of 50 U/kg/day and in rabbits at a dose of 3 U/kg/day. These doses are approximately 8 times the human subcutaneous dose of 1.0 U/kg/day for rats and equal to the human subcutaneous dose of 1.0 U/kg/day for rabbits, based on U/body surface area.

8.3 Nursing Mothers

It is unknown whether insulin aspart is excreted in human milk. Use of NovoLog is compatible with breastfeeding, but women with diabetes who are lactating may require adjustments of their insulin doses.

8.4 Pediatric Use

NovoLog is approved for use in children for subcutaneous daily injections and for subcutaneous continuous infusion by external insulin pump. NovoLog has not been studied in pediatric patients younger than 2 years of age. NovoLog has not been studied in pediatric patients with type 2 diabetes. Please see *Section 14* ***CLINICAL STUDIES*** for summaries of clinical studies.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Standard 2-year carcinogenicity studies in animals have not been performed to evaluate the carcinogenic potential of NovoLog. In 52-week studies, Sprague-Dawley rats were dosed subcutaneously with NovoLog at 10, 50, and 200 U/kg/day (approximately 2, 8, and 32 times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area, respectively). At a dose of 200 U/kg/day, NovoLog increased the incidence of mammary gland tumors in females

when compared to untreated controls. The incidence of mammary tumors for NovoLog was not significantly different than for regular human insulin. The relevance of these findings to humans is not known. NovoLog was not genotoxic in the following tests: Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, in vivo micronucleus test in mice, and in *ex vivo* UDS test in rat liver hepatocytes. In fertility studies in male and female rats, at subcutaneous doses up to 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on U/body surface area), no direct adverse effects on male and female fertility, or general reproductive performance of animals was observed.

13.2 Animal Toxicology and/or Pharmacology

In standard biological assays in mice and rabbits, one unit of NovoLog has the same glucose-lowering effect as one unit of regular human insulin. In humans, the effect of NovoLog is more rapid in onset and of shorter duration, compared to regular human insulin, due to its faster absorption after subcutaneous injection (see *Section 12* CLINICAL PHARMACOLOGY Figure 2 and Figure 4).

DPMH Proposed Label on Section 8.1

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

There are no studies available with FIASP (b) (4) in pregnant women. (b) (4) available information from published randomized controlled trials (b) (4) with insulin aspart (b) (4) [see Data]. There are risks to the mother and fetus associated with poorly controlled diabetes in pregnancy [*see Clinical Considerations*].

In animal reproduction studies (b) (4) administration of subcutaneous insulin aspart to (b) (4) pregnant rats and rabbits during the period of organogenesis did not cause adverse developmental effects at exposures 8 times and equal to the human subcutaneous dose of 1.0 unit/kg/day, respectively. Pre- and post-implantation losses and visceral/skeletal abnormalities were seen at higher exposures; (b) (4) are considered secondary to maternal hypoglycemia [*see Data*].

The estimated background risk of major birth defects is 6-10% in women with pre-gestational diabetes with a HbA1c >7 and has been reported to be as high as 20-25% in women with a HbA1c >10. The estimated background risk of miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Clinical Considerations

Disease-associated maternal and/or embryo/fetal risk

Poorly controlled diabetes increases the fetal risk for (b) (4) still birth, macrosomia related morbidity (b) (4)

Data

Human Data

[redacted] published randomized controlled trials of 441 pregnant women with diabetes mellitus treated with insulin aspart starting during the late 2nd trimester of pregnancy did not identify [redacted] association of insulin aspart with major birth defects or adverse maternal or fetal outcomes. However, these studies cannot definitely establish the absence of any risk because of methodological limitations, including a variable duration of treatment and [redacted] small size of the majority of the trials.

[redacted]

Animal Data

[redacted]

Fertility, embryo-fetal and pre-and postnatal development studies have been performed with [redacted] insulin aspart [redacted] and regular human insulin in [redacted] rats and rabbits. In a fertility and embryo-fetal development study, insulin aspart was administered [redacted] before mating, during mating, and throughout pregnancy. Further, in a pre- and postnatal development study insulin aspart was given throughout pregnancy and during lactation to rats. In an embryo-fetal development study insulin aspart was given to female rabbits during organogenesis. The effects of insulin aspart did not differ from those observed with subcutaneous regular human insulin. Insulin aspart, like human insulin, caused pre- and post-implantation losses and visceral/skeletal abnormalities in rats at a dose of 200 units/kg/day (approximately 32 times the human subcutaneous dose of 1.0 unit/kg/day, based on human exposure equivalents) and in rabbits at a dose of 10 units/kg/day (approximately three times the human subcutaneous dose of 1.0 unit/kg/day, based on human exposure equivalents). No significant effects were observed in rats at a dose of 50 units/kg/day and in rabbits at a dose of 3 units/kg/day. These doses are approximately 8 times the human subcutaneous dose of 1.0 unit/kg/day for rats and equal to the human subcutaneous dose of 1.0 unit/kg/day for rabbits, based on human exposure equivalents. The effects are considered secondary to maternal hypoglycemia.

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/s/

MIYUN M TSAI-TURTON
08/28/2016

CALVIN L ELMORE
08/29/2016
I concur

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: NDA 208751 **Applicant: Novo Nordisk** **Stamp Date: December 8, 2015**

Drug Name: Insulin aspart injection (Fiasp®) **NDA/BLA Type: 505(b)(1)**

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

Novo Nordisk submitted NDA 208751 for a faster acting insulin aspart formulation (Fiasp®, referred to in this memo as Faster aspart) as a meal-time insulin with a greater early glucose-lowering effect compared to NovoRapid ®/NovoLog ®. Faster aspart is insulin aspart in a new formulation, which contains two additional excipients compared to prior formulations: nicotinamide (vitamin B3) and L-arginine hydrochloride (an amino acid). Nicotinamide is absorption enhancer (a faster initial absorption of insulin aspart following subcutaneous injection) and L-arginine hydrochloride is a stabilizer. Both excipients are listed in the Inactive Ingredient Search for Approved Drug Products in products for injections.

Overview of the faster aspart and NovoRapid®/NovoLog® formulations

Ingredients	Faster aspart	NovoRapid®/NovoLog®	Function of ingredient
Insulin aspart	100 U/mL (600 nmol/mL)	100 U/mL (600 nmol/mL)	Drug substance
Zinc	19.6 µg/mL[1)]	19.6 µg/mL[2)]	(b) (4)
Phenol	1.50 mg/mL	1.50 mg/mL	
Metacresol	1.72 mg/mL	1.72 mg/mL	
Glycerol	3.3 mg/mL	16 mg/mL	
Sodium chloride	-	0.58 mg/mL	
Disodium phosphate dihydrate	0.53 mg/mL	1.25 mg/mL	
Arginine (as L-arginine HCl)	3.48 mg/mL	-	Stabilising agent
Nicotinamide	20 (b) (4) mg/mL	-	Absorption modifier
pH	7.1	7.4	Not applicable
(b) (4)			

Faster aspart 100 units/ml will be provided in a pre-filled disposable PDS290 Faster Aspart pen injector (3 ml), in Penfill® cartridges (3 ml) for use in Novo Nordisk insulin delivery systems (outside of US), and in vials (10 ml). The prefilled pen injector has a dose range of 1-80 units in increments of 1 unit.

Regulatory History as IND 106878

- Feb 2010: IND submission
- March 2011: EOP2 mtg
- Nov 2013 – Type C written response: PSP plan (1 nonclinical question – no juvenile animal study would be needed.)
- June 2014 – Type C written response: NDA submission (1 nonclinical question – we agreed that their nonclinical data would be appropriate for NDA submission.)
- Feb 2015 – Proposed PSP plan submitted (no planned nonclinical study; no iPSP agreement as May 2015)
- June 2015 - Type B pre-NDA meeting (no nonclinical question)
- Aug 2015 – iPSP initial agreement
- Dec 2015 –NDA submission (NDA 208751)

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required and requested IND studies in accord with 505 (b)(1) and (b)(2) including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		SC injection
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		Sections 8 & 13

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		Comparable to NovoRapid®/NovoLog® with no new drug product-related impurities above threshold limit for qualification (ICH Q3B) Excipient related impurity ((b) (4) - < (b) (4) µg/person/day which below the TTC for long term use (>10 yr) (ICH M7) Leachables ((b) (4)) from the container closure systems ▪ (b) (4) at 200 U faster aspart is 12500 X below the established PDE (ICH Q3C) ▪ (b) (4) at 200 U faster aspart is (b) (4) X below the TTC (1.5 µg/person/day) (ICH M7) ▪ Both have no toxicological concern.
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?	X		PK/PD in LYD pigs (faster aspart vs. NovoRapid®/NovoLog®) Local tolerance studies in rat, rabbit, and minipig via various routes of administration Literature review of excipients safety assessment – nicotinamide, L-arginine HCl, zinc acetate

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

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

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

LABELING

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

(b) (4)

There are no (b) (4) available with FIASP to (b) (4) drug-associated risk (b) (4)

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

(b) (4)

In 52-week studies, Sprague-Dawley rats were dosed subcutaneously with (b) (4) at 10, 50, and 200 units/kg/day (approximately 2, 8, and 32 times the human subcutaneous dose of 1.0 units/kg/day, based on units/body surface area, respectively). At a dose of 200 units/kg/day, (b) (4) increased the incidence of mammary gland tumors in females when compared to untreated controls. The incidence of mammary tumors for (b) (4) was not significantly different than for regular human insulin. The relevance of these findings to humans is not known. (b) (4) was not genotoxic in the following tests: Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, *in vivo* micronucleus test in mice, and in *ex vivo* UDS test in rat liver hepatocytes. In fertility studies in male and female rats, at subcutaneous doses up to 200 units/kg/day (approximately 32 times the human

subcutaneous dose, based on units/body surface area), no direct adverse effects on male and female fertility, or general reproductive performance of animals was observed.

(b) (4)

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/s/

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01/27/2016

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