SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Protaphane. For information on changes after approval please refer to module 8.

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterised by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Acute, life-threatening consequences of diabetes are hypoglycaemia, and hyperglycaemia with ketoacidosis or non-ketotic hyperosmolar syndrome. Long-term complications of diabetes include retinopathy with potential loss of vision, nephropathy leading to renal failure, and peripheral neuropathy causing foot ulcers, gastrointestinal, genitourinary, and sexual dysfunction. The disease is also accompanied by an increased incidence of atherosclerotic cardiovascular, peripheral vascular and cerebrovascular disease.

Type 1 diabetes, which usually is of childhood or adolescence onset, accounts for 5 to 10% of diagnosed diabetes; it is characterised by loss of insulin production due to destruction of pancreatic ß cells as a result of an autoimmune response or idiopathic causes. Patients with type 1 diabetes depend on exogenous insulin for survival.

Type 2 diabetes, which usually is of adult onset, is by far the more common form of diabetes. In the Western World, it constitutes approximately 90% of all cases of diabetes. Type 2 diabetes is characterised by impaired insulin secretion, insulin resistance, increased hepatic glucose output and lipid disorders. Patients with type 2 diabetes generally do not require insulin treatment for survival, although a substantial number (20-30%) of patients need insulin to achieve acceptable metabolic control.

This application seeks marketing authorisation for insulin human for the treatment of patients with diabetes mellitus. The active substance of Protaphane is insulin human manufactured by recombinant DNA technology in Saccharomyces cerevisiae. Protaphane is isophane insulin formulated in a suspension where protamine and insulin are brought together in isophane proportions at a neutral pH forming an amorphous precipitate. Protaphane is long-acting insulin.

Protaphane is intended for marketing in the dose strengths 40 IU/ml and 100 IU/ml in the following pharmaceutical forms:

Protaphane 40 IU/ml, 10 ml vial
Protaphane 100 IU/ml, 10 ml vial
Protaphane Penfill 100 IU/ml, 3 ml
Protaphane InnoLet 100 IU/m, 3 ml
Protaphane NovoLet 100 IU/ml, 3 ml
Protaphane FlexPen 100 IU/ml, 3 ml

The Penfill presentation is a cartridge which is designed to be inserted in a durable device. The cartridges should only be used with devices and needles that are compatible with the Penfill products.

The InnoLet presentation is a multi-dose prefilled pen and delivers a maximum of 50 units per dose in increments of 1 unit. The device is equipped with an *end-of-content* mechanism that ensures that the adjusted dose does not exceed the remaining content of the 3ml cartridge after multiple use. InnoLet is targeted for use by elderly patients with impaired dexterity and/or vision

The NovoLet presentation is a multi-dose disposable prefilled pen, delivering 2-78 units per dose in increments of 2 units.

The FlexPen presentation is also a multi-dose disposable prefilled pen, delivering 1-60 units per dose in increments of 1 unit. For patients, the FlexPen device represents an improvement over the NovoLet device in terms of automatic zeroing and delivery of dose in single unit increments.

2. Part II: Chemical, pharmaceutical and biological aspects

Composition

Protaphane is a neutral suspension of isophane insulin crystals formulated for long action. The formulation contains the following agents for functions as follows: protamine sulphate (protracting agent – forms isophane crystals with the insulin in the formulation), zinc (crystal forming), glycerol (isotonic), disodium phosphate dehydrate (buffer), phenol (preservative) and metacresol (preservative).

Protaphane is presented in 10 ml vials, 3 ml cartridges (Penfill) and in multi-dose prefilled pens (Innolet, NovoLet and FlexPen). Two strengths exist in vial presentations: 100 IU/ml and 40 IU/ml. Only one strength exists in the other presentations: 100 IU/ml. All 100 IU/ml presentations have identical compositions.

The 10 ml vial is a glass container with a laminated isoprene/bromobutyl rubber stopper (disc) and snap-off cap. The glass container is produced from type I Ph.Eur. colourless glass.

The Penfill cartridges consist of a 3 ml type I Ph.Eur., colourless glass cartridge sealed with a laminated isoprene/bromobutyl rubber stopper (disc) and a bromobutyl rubber plunger.

Innolet, NovoLet and FlexPen are multi-dose prefilled pens made of a plastic injector device fitted with 3 ml Penfill cartridges.

Active substance

The active substance of Protaphane, insulin human (rDNA) complies with Ph.Eur. monograph 1999:838 with additional tests as follows:

Identification by Amino acid composition

Nitrogen content

Total viable count (CFU/g)

DNA content

Methods of analysis for the additional tests developed by the applicant are fully described with relevant validations.

Development Genetics

Human insulin is produced using a genetically modified strain of *Saccharomyces cerevisiae*. The strain carries a plasmid which codes for the expression of a single chain insulin precursor attached to a pre-pro leader region of the yeast mating factor (MFα1) gene. The plasmid is constructed based on the yeast 2μ plasmid.

The yeast transformant used to produce the insulin precursor is a transformant of *Saccharomyces cerevisiae* carrying the expression plasmid described above. The applicant has presented the complete DNA sequence of the plasmid. The sequencing presented is assembled from published sequences and in-house sequence determinations as relevant. The gene has also been fully characterised from isolated plasmids from long-term production scale fermentation and cell bank (Original Mother Culture (OMC)).

Constructional stability has been investigated in production strain, prolonged and very long term fermentation and cell bank (OMC).

Cell bank system

The cell bank system consists of Original Mother Culture (OMC), New Mother Culture (NMC), MCB and WCB. Satisfactory details of the preparation of the different types of cell banks have been provided and a clear description given of the numbering and origin of the various cell banks and their sublots.

Production of active substance

The encoded product of secretion during fermentation is a single chain insulin precursor consisting of the first 29 amino acid residues of the insulin B chain linked with three amino acids to the insulin A chain. This single chain precursor is converted enzymatically to an insulin methyl ester, which is subsequently hydrolysed to yield human insulin, consisting of two chains (A and B) linked together with disulphide bridges. The purification process employs several chromatography and precipitation steps for isolation of the precursor, the intermediates, and the active substance respectively. This process is well established and it should be noted that human insulin rDNA has been manufactured by the applicant over a period of many years during which time a number of improvements have been made.

Validation data have been provided for the fermentation, recovery and purification processes. In each case, critical parameters in these processes have been identified and investigated.

Satisfactory analytical data are provided for 10 recently produced batches of human insulin demonstrating a high degree of consistency in the manufacturing process.

Stability of active substance

The applicant has provided results of testing of 20 batches from the ongoing stability programme. Testing parameters include dry substance, insulin polymer, insulin dimer, A21 desamido insulin, other related substances and assay. The data confirm that active substance is stable for 60 months when stored at at the recommended storage temperature.

Other ingredients

All ingredients conform to Ph. Eur. apart from metacresol (no monograph in Ph.Eur.), which conforms to USP.

Product development and finished product

Development Pharmaceutics

The protracting effect of protamine on insulin was originally discovered in 1936. The concept was improved in 1946 through the development of isophane insulin, whereby zinc ions together with phenol and/or cresol were used to form an insulin-protamine complex at neutral pH. In the 1970s, monocomponent porcine and bovine insulin based Protaphane formulations were developed and in the 1980s, the equivalent monocomponent human insulin containing Protaphane product. In 1987, in association with development of the NovoPen dispensing system, the product stability was improved by fine tuning the formulation. The manufacturing process was fine-tuned thereafter to optimise insulin crystal size. Finally, an adjustment in preservative overage (to compensate for potential loss during manufacture) for two presentations was introduced in 1993 to make the formulation for all 100 IU/ml protamine insulin products manufactured by the applicant identical.

Emphasis has been placed on correct insulin crystal size in the product. This is achieved through a combination of optimised zinc and protamine sulphate concentrations in the formulation and through a carefully defined and controlled manufacturing process.

The preservative content has been optimised with respect to overall chemical stability of the product, preservative efficacy and local irritation at the site of injection. Criteria B of the Ph.Eur. antimicrobial preservative efficacy test are met.

Neutral pH is optimal for medical use.

Compatibility of the container components and product is shown to be satisfactory via stability studies.

Sterilisation by filtration is essential given the heat sensitivity of the active ingredient.

Manufacturing process

Protaphane is prepared by mixing solutions of ingredients in a sterile tank. The solutions are sterile filtered into a filling tank resulting in a suspension of protamine insulin human. The precipitate is then allowed to crystallise into oblong tetragonal crystals. Filling occurs in a grade A zone and vials and

cartridges are inspected individually by manual or automated inspection. Pen injector products are assembled thereafter.

Due to the nature of this application i.e. products marketed under MRP since 1988 and based on the extensive experience the applicant has with their products, no new validation studies have been initiated for this application. An overview of the processes used together with a description of the critical production parameters is provided. Summary results have also been provided for various Protaphane products manufactured at various approved sites and in different batch sizes. Available data show a consistent, well-controlled manufacturing process.

Protaphane complies with the requirements of the following Ph. Eur. monographs:

01/2002:0854 Insulin Preparations, Injectable

1997:0833 Insulin Injection, Isophane

In addition to monograph tests the products are tested by in-house methods for confirmation of isophanicity, identity and contents of preservatives and for dose accuracy (prefilled injector products only).

Full methodologies have been provided for all in-house methods. A complete justification of the tests employed has been provided.

Batch analysis data have been provided for 3 recently produced batches of each presentation, except for Protaphane FlexPen where only 2 batches were available at the time of submission. All batches comply with their respective specifications.

Stability of the Product

Stability reports are provided covering the different strengths, presentations and production sites for Protaphane.

Results have been generated by validated, stability indicating methods and indicate satisfactory stability. These results support the shelf life stated in the SPC.

Viral safety and TSE risk assessment

A number of animal derived raw materials are used in the production of human insulin, rDNA. These are peptone, beef extract and pepticase which are used in the preparation and storage of cell banks, and L-threonine and trypsin used in the purification process to convert human insulin precursor to human insulin methyl ester. In addition, protamine sulphate derived from salmon is used as excipient in the finished product.

Pepticase falls outside the scope of the TSE Guideline as it is derived from casein from milk from healthy cows only and no other ruminant materials are used in its preparation.

For peptone (CEP-2000-175) and beef extract (CEP-2000-181) Certificates of Suitability of the EDQM have been submitted.

L-threonine is sourced from avian feathers and porcine gelatine and trypsin from porcine pancreas.

The risk of transmission of TSE from Protaphane to human beings has been appropriately addressed in accordance with CPMP/CVMP Note for Guidance for minimising the risk of transmitting animal spongiform encephalopathy via medicinal products (EMEA/410/01).

Viral safety issues have been addressed and compliance with relevant guidelines are considered to be met.

Discussion on chemical, pharmaceutical and biological aspects

Satisfactory evidence is provided that product manufacture is well controlled, that consistency of production is achieved and that a stable product results. The requirements of the relevant directives and guidelines are met. The pharmaceutical portions of the SPC, package insert and product label are supported by the information provided in the dossier. Several minor quality issues will be addressed by the applicant on an ongoing (post-approval) basis.

3. Part III: Toxico-pharmacological aspects

The preclinical evaluation of the present product is based on the documentation for the active ingredient; insulin human (rDNA). The programme includes recent studies performed with the insulin analogue insulin detemir. In several of these studies insulin human (rDNA) was used as a reference substance.

Pharmacodynamics

- **Primary pharmacology programme.**

The programme includes studies performed in the eighties demonstrating the similarity between recombinant insulin human and semi-synthetic insulin human, later studies supplementing above studies and recent studies where insulin human (rDNA) was used as a reference substance for insulin analogues.

- *In vitro* studies

Insulin is a hormone composed of two polypeptides (two protein chains named A and B chains having respectively 30 and 21 amino-acids). Two disulfide bonds link these two chains. The structure of the insulin is similar of those of several other hormones or growth-factors (including insulin-like growth factors IGF-1 and IGF-2). IGF-1 and IGF-2 have some affinity for the insulin receptor, however, both growth factors have their own receptors. The insulin and IGFs receptors both belong to the tyrosine kinase family receptors. The activation of the receptors is obtained when the endogenous ligand occupies the receptor. Once activated the signal transduction produced by these receptors, which mediates the physiological action of the hormone, starts with an autophosphorylation of the receptor. The *in vitro* studies explored the affinity of insulin analogues for other receptors belonging to the tyrosine kinase family.

The receptor binding activity of insulin human (rDNA) was studied in connection with the pre-clinical development of the insulin aspart (see Table 1 below).

Table 1: Determination of the receptor affinity of insulin human (rDNA).

Affinity for Insulin Receptor	Affinity for IGF1-Receptor
=100%	0.03%

- *In vivo* studies

The effect on blood glucose in diabetic rats after subcutaneous administration was studied in diabetic rats which received by a single subcutaneous injection either insulin human(rDNA), semi-synthetic insulin or vehicle. The effect on blood glucose was measured by blood sampling. Insulin human (rDNA) and semi-synthetic insulin showed dose and time dependant antidiabetic effect.

The pharmacological effect of insulin human (rDNA) 40 U/ml was studied in a cross-over assay in rabbits. A standard crossover study (British Pharm., 1980) of the hypoglycaemic effect after SC administration in Rabbits (n=36) was done. There was no difference between equivalent preparations made from human insulin or semi-synthetic insulin.

- Safety pharmacology programme.

In the Irwin test, a few mice showed a slight reduction in exploratory and spontaneous activity. In the Animex test, which is more sensitive, mice showed a decrease in motor activity at the highest dose (5 U/kg). Reduced performance in the rotarod test was also observed in mice at the highest dose (5 U/kg) in one study, but no effects were observed at 100 U/kg in a later study. The locomotion activity in rats were slightly reduced at 100 U/kg, which was the only dose tested.

Newer studies support the original ones.

The time from disappearance to reappearance of the righting reflex (sleeping time) induced by pentobarbital in mice was prolonged after treatment with 5 U/kg. The same applies to hexobarbital after treatment with 100 U/kg; the effect was reversed with glucose administration. A dose of 100 U/kg after administration of ethanol significantly increased the mortality and sleeping time. No antagonistic effect on

pentylenetetrazol-induced convulsions in mice was observed at 100 U/kg, and this treatment did not act as a pro-convulsant either. Insulin human (rDNA) did not show any inhibitory effects on acetic acid induced writhing in mice at 100 U/kg (P-27), indicating absence of analgesic potential. The Body temperature in mice was unaffected by 100 U/kg (P-28). Neither insulin human (rDNA) nor semi-synthetic insulin human produced any "curarizing" effect on neuromuscular transmission after treatment of rats up to 5 U/kg IV. No effects attributed to treatment were observed in an in vitro preparation of guinea-pig ileum and *vas deferens*.

No effects on cardiovascular and respiratory system attributed to treatment were observed in cats and in pigs. The gastro-intestinal motility of mice was unaffected. A transient fall in diuresis was observed in rats, however, this effect was reversed after SC administration of glucose. A bromsulphtalein-test showed no indications of pathological effects to liver parenchyma in pigs. Blood platelets of human Rich Platelet Plasma were not affected after in vitro treatment with insulin human (rDNA).

Effects seen in the original and newer safety pharmacology studies can all be related to hypoglycaemia.

Pharmacokinetics

Insulin isophane preparations have been used since 1946. The pharmacokinetic profile of isophane insulin NPH is already well characterised. In classic trials carried out from 1936 addition of protamine and zinc was found to prolong the insulin effect from 2-4 times in the normal rabbit (Scott and Fisher 1936).

Toxicology

The active component of Protaphane is insulin human (rDNA). The single doses toxicity of insulin human (rDNA) was in an early study compared to semisynthetic insulin human and Protaphane, insulin human (rDNA) was later included as a reference substance in a series of repeated dose and reproduction toxicity studies of an insulin analogue. Some other studies confirmed the lack of local toxicity and tried to evaluate the possible immunogenicity of the product.

- **Single dose toxicity studies.**

Mice and Rats were given a single dose of insulin human (rDNA) subcutaneously at dosage up to 4000 U/kg. In higher dosage groups insulin human was compared to semi-synthetic insulin. Apart from few sporadic hypoglycaemic reactions on the day of dosing, no treatment related signs were seen

Repeated doses toxicity.

Insulin human (rDNA)

The subacute toxicity was examined in rats and dogs during a 4 weeks SC study in Wistar Rats and a 13 weeks SC study in Beagle Dogs.

Insulin human (rDNA) was administrated subcutaneously for 1 year to Sprague Dawley Rats. At necropsy, there was an increased incidence of mammary gland cysts and mammary tumours were found at microscopic examination. The incidence of total number of mammary tumours as well as fibroadenomas and adenocarcinomas were, however, not significant from the control group. There were no other treatment-related effects in any organ, including the pituitary.

Beagle dogs were given insulin human (rDNA) 1 U/kg twice daily SC for 12 months. Besides one case of abnormal weight gain, there were no other important effects of the treatments.

Protaphane, insulin human (rDNA), isophane formulation

The subacute and chronic toxicities of Protaphane, insulin human (rDNA) were examined in rats and dogs. In the 3-month study Sprague-Dawley rats were given Protaphane, insulin human (rDNA) subcutaneously. Apart from severe and fatal cases of hypoglycaemia only a few signs were observed. They are all consistent with other studies performed in rats.

In a 6-month study Sprague Dawley rats received insulin isophane. No deaths were seen. No treatment related effects other than well known small changes in some clinical pathology data were found.In the 3-month dog study, animals were given Protaphane, insulin human (rDNA) subcutaneously. All animals survived the dosing period. Besides subcutaneous haemorrhage and inflammatory cell

infiltration at injection sites, no treatment related signs were observed. Neither the 6-month nor the 12-month study showed any drug related toxicity.

- **Genotoxicity.**

The genotoxic potential of insulin human (rDNA) was evaluated through a bacterial reverse mutation test in 4 strains of *Salmonella typhimurium*, a clastogenic activity test in cultured human lymphocytes, a mutagenic activity test on the HGPRT-locus in chinese hamster V79 cells and a micronucleus test in bone marrow erythrocytes. In all the tests insulin human (rDNA) was found non-mutagenic.

Insulin human (rDNA) was included as reference substance in a gene mutation study in mouse lymphoma L5178Y cells (TFT-resistance). Negative findings were obtained with no signs of cytotoxicity.

- **Carcinogenicity.**

MCF-7 human breast cancer cells were incubated with different concentrations of insulin aspart, insulin human (rDNA) and an experimental insulin analogue. Dose response curves from seven studies were the same for insulin aspart and insulin human (rDNA), whereas the experimental insulin analogue had at least 10-times their mitogenic potential.

In an exploratory 12-month test and in the formal 12-month toxicity study in the Sprague-Dawley rat the effects of chronic administration of insulin aspart and insulin human (rDNA) on mammary tissues in the Rat were explored. In these studies some animals developed neoplasms of mammary tissue. All animals in all treatment groups showed hyperplasia of mammary glandular epithelial cells. In both tests most mammary gland tumours were fibroadenomas all had a typical histological appearance. The small number of adenocarcinomas had remained local and had not metastasised. The pituitary glands appeared normal.

A study exploring the effects of repeated subcutaneous injection of insulin aspart and insulin human (rDNA) for 52 weeks in rats has been conducted. This study has been performed in Sprague-Dawley rats. A dose-related increase in palpable subcutaneous masses has been observed at 30 and 75 U/kg twice daily. A statistically significant ($p<0.01$) increased incidence of female animals bearing mammary gland tumours at 75 U/kg/bid were found. The increase was evident in benign/malign combined as well as in malign tumours alone. No evidence of mammary gland hyperplasia or of tumours was seen in the test up to 12 months in the dog.

Particularly under certain experimental conditions insulin may induce mammary tumours in the female Sprague Dawley rat (a sensitive species, strain and sex) probably related to a mitogenic and growth-promoting action of insulin mediated by the insulin receptor.

An increase in the number of benign mammary adenomas and fibroadenomas has been shown in Sprague Dawley rats. In one 12 month study, there was a statistically significant increase of female animals bearing benign and malign mammary gland tumours at the highest dose. There was no increase of mammary gland hyperplasia or tumours in the 12 month dog study.

- **Reproduction Toxicity.**

Fertility and Embryo-Foetal Development studies have been conducted in the Sprague Dawley Rat. Fertility was not affected. Males showed slight reduction in the epididymal sperm count. Dams treated with high dose (200 U/kg/day) of insulin human (rDNA) showed pre- and post-implantation loss, and a specific pattern of anatomical abnormalities of the foetuses was seen. The findings are regarded as a consequence of the severe maternal hypoglycaemia.

The pre- and post-natal development of Sprague Dawley rats born from pregnant females exposed to insulin human (rDNA) has been studied. Maternal hypoglycaemia with a few deaths and effects on weight gain and food consumption were observed in the dams.

Newborn pups showed slightly increased weight gain, which had become normalised by weaning. There were a few other variations in F_1 animals but no major effect was found.

Embryo-foetal development of rabbits born from pregnant females exposed to insulin human (rDNA) has also been studied. The high doses of insulin led to increased food consumption and accelerated weight gain, which persisted to the end of the experiment. There was a dose-related reduction in plasma glucose.

In the mid- and low doses it had recovered by 4h after the first dose. Top-dose group (5 U/kg) showed embryonic deaths and related depression of litter size and weight. At 1.5 U/kg and above, foetuses showed skeletal abnormalities. These effects were considered to be due to the induced maternal hypoglycaemia.

In Segments I/II study, fertility was not affected in rats given insulin human (rDNA). Males had a slightly reduced epididymal sperm count. Pre- and post-implantation loss was increased and a proportion of foetuses had characteristic abnormalities attributed to reduction of maternal blood glucose. In an embryo-foetal development study in rabbits, an increase in early embryonic deaths with associated decrease in litter size and litter weight was observed at 10 U/kg/day. A dose-dependent increase in foetuses with skeletal abnormalities was seen.

During gestation, abortion and foetal death and malformations were seen, but only during severe maternal hypoglycaemia and are already known to occur in incorrectly treated diabetic women.

- **Local Tolerance.**

The local toxicity after subcutaneous injection has been studied in pigs. Pigs were given subcutaneous injections of different preparations including biphasic dual-acting insulin human 30, Protaphane, insulin human (rDNA), i.e. pure isophane insulin human NPH, three media preparations and 0.9% saline. It was shown that all insulin products and formulations caused identical changes including light to moderate subcutaneous mixed inflammatory cell infiltration. The response to biphasic dual-acting insulin human 30 and Protaphane, insulin human (rDNA) was similar but slightly more extensive. The saline and all media preparations caused almost no change.

In the same way, pigs received either biphasic, dual-acting insulin human 30 or Protaphane, insulin human (rDNA). The changes noted above were confirmed. No differences between the insulin products were seen.

The local tolerance was studied in rabbits after IM injections of insulin human. It was concluded that insulin human (rDNA) caused damages which were similar to those found after injection of isotonic saline solution.

A test for local irritation in rabbits showed that there were no differences in the damages caused by isotonic saline solution and by insulin human (rDNA) products.

- **Immunotoxicity studies.**

Insulin antibodies, even in moderate and low amounts, may prevent rapid rise in free blood insulin, thereby leading to higher postprandial glucose levels, or cause increased risk of hypoglycaemia when insulin is released from circulating insulin antibody complexes. The purity of the injected insulin has been shown to be of crucial importance on the amount of insulin antibody formed. Thus, 5-times crystallised porcine insulin induces more insulin antibodies than the same preparation containing mono component insulin.

The immunogenicity of insulin human (rDNA) has been studied in Rabbits. Freund's adjuvant and 20 U of respectively insulin human (rDNA), semi-synthetic insulin and 5 times crystallized porcine insulin were injected intramuscularly to groups of rabbits twice a week. Serum insulin binding was estimated until 97 days. No statistically significant differences between the immunogenicity of insulin human and semi-synthetic insulin was found, whereas they both were demonstrated to be significantly less immunogenic that 5-times crystallized porcine insulin. It was concluded, that insulin human fulfils the demand of low potential to induce insulin antibodies in accordance with other mono component insulins.

There was no statistically significant difference between the immunogenicity in rabbits of insulin human and semi-synthetic human insulins. These insulins were found to be significantly less immunogenic than 5 times crystallised pork insulin. The potential for human antibody production against insulin human is thus considered to be low.

- **Ecotoxicity/Environmental Risk Assessment.**

Insulin human (rDNA) is considered readily degradable; hence does not suggest any environmental risk for clinical use. The containers and devices in which it is supplied are appropriate for disposal by the means normally employed for simple medical devices.

Discussion on toxico-pharmacological aspects

The main purpose in the studies for primary and secondary pharmacodynamics was to demonstrate the similarity between the new insulin human (rDNA) and marketed semi-synthetic variant. Effects seen in the safety pharmacology studies can all be related to hypoglycaemia.

As the majority of the insulin human preparation is of same composition as the semi-synthetic insulin preparations, no pharmacokinetic studies were conducted in the original preclinical programme. Linearity concerning AUC/dose was confirmed in different species, meaning that there was no drug accumulation.

No specific safety pharmacology studies on Protaphane, insulin human (rDNA) have been carried out. Effects seen in the original and newer safety pharmacology studies on insulin human (rDNA) can all be related to hypoglycaemia. The toxic effects seen in the single dose and repeated dose toxicity studies were attributed to the hypoglycaemic activity and thus an exaggerated pharmacological effect caused by the high doses of the insulin. Increased weight, depressed activity, convulsions and death were some of these effects.

No specific studies were conducted on toxicity of Protaphane, insulin human (rDNA), as the active component is insulin human. However, some experiments where Protaphane, insulin human (rDNA) was used as a reference substance to the testing of insulin analogue the results do not give reason for new safety concerns.

The noted effects on embryos and foetuses were only seen at severe maternal hypoglycaemia and are already known to occur in incorrectly treated diabetic women.

All conducted genotoxicity studies were negative for mutagenic potential. An increase in the number of benign mammary adenomas and fibroadenomas has been shown in Sprague Dawley rats. It is concluded that the increased incidence of mammary tumours seen in rats is probably caused by mitogenic and growth-promoting action via the insulin receptor, but is probably also related to the fact that Sprague Dawley rats are especially sensitive and were given large doses. There was no increase of mammary gland hyperplasia or tumours in the 12-month dog study.

Finally, a test for local irritation in rabbits showed that there were no differences in the damages caused by isotonic saline solution and by Protaphane, insulin human (rDNA). The potential for human antibody production against recombinant human insulin is thus considered to be low.

4. Part IV: Clinical aspects

Diabetes is a group of metabolic disorders characterised by hyperglycaemia due to defects in insulin secretion and/or insulin action. The two most common forms of diabetes mellitus are type 1 and type 2 diabetes. Type 1 diabetes is characterised by an absolute deficiency of insulin due to destruction of the pancreatic β-cells. Although the rate of β-cell destruction is variable, all type 1 diabetic patients will eventually require exogenous insulin for survival. In contrast, type 2 diabetes is characterised by insulin resistance, relative impairment of insulin secretion and increased hepatic glucose output. In general, patients with type 2 diabetes do not require exogenous insulin for survival. Nevertheless, during the course of the disease, a large minority of these patients will be treated with exogenous insulin to correct persistent hyperglycaemia.

The goal of insulin treatment is to mimic the physiologic pattern of insulin secretion, which under normal conditions consist of a basal secretion and meal related short peaks. The most commonly used insulin regimen is the so-called basal-bolus regimen in which basal insulin requirements are provided by one or two injections of long-(intermediate) acting insulin and mealtime requirements are provided by meal related injections of fast-/rapid-acting insulin human/insulin analogues. Instead of separate injections of long- (intermediate) acting and fast-acting insulins, the two insulin preparations may be mixed (by the patient or as ready-made premixed insulin) before injection. It is generally accepted that the basal-bolus regimen offers the best glycaemic control. However, many patients, especially type 2 diabetic patients who produce significant amounts of insulin themselves, may be adequately controlled on twice-daily injections of long-(intermediate) acting insulins or mixtures of fast-acting and long-(intermediate) acting insulins.

Protaphane, insulin human (rDNA) is isophane insulin formulated as a suspension where protamin and insulin are brought together in such proportions (isophane proportions) at a neutral pH that it forms a crystalline protamine insulin suspension. Therefore, Protaphane, insulin human (rDNA) is a long

acting insulin. The approximate action profile following a subcutaneous injection shows that the onset of action is obtained approximately 1 hour and a half after the injection. The maximum effect is obtained between 4 and 12 hours and the duration of the effect can last up to 24 hours.

Clinical pharmacology

Pharmacodynamics in healthy subject

Three pharmacodynamic and/or pharmacokinetic studies form the main documentation for the pharmacodynamics and pharmacokinetics of Protaphane, insulin human (rDNA). All studies included healthy subjects only.

Table 2: Clinical pharmacology studies.

Study	*Population (Number of subjects)*	*Design*	*Dose regimens*
NN304/DCD/ 001/D	Healthy young male subjects (11 subjects)	Open-labelled, randomised, 5-period cross-over, single-centre	Single doses of Protaphane, -insulin human 0.3 U/kg and insulin analogue,
NN304/DCD/ 4/A	Healthy young male subjects (10 subjects)	Double-blind, randomised, placebo-controlled, 5-period cross-over, single-centre	Single doses of Protaphane, insulin human (rDNA) 0.3 and 0.6 U/kg, insulin analogue, and placebo.
NN304/1028	Healthy young male subjects (16 subjects)	Open-labelled, randomised, 4-period cross-over, single-centre	Single doses of Protaphane, insulin human (rDNA) 0.2 U/kg s.c., fast-acting insulin human 0.075 U/kg i.v., and insulin analogue i.v.

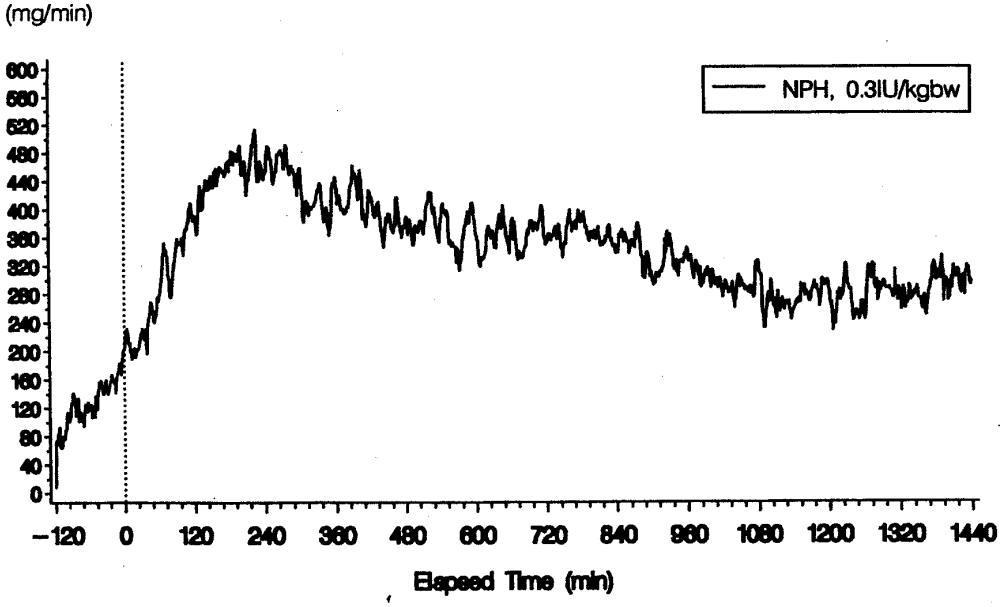
All these studies were conducted as part of the clinical development programme for an insulin analogue, in which Protaphane, insulin human (rDNA) was used as a comparator and not target of the primary analyses.

Individual studies

Study NN304/DCD/001/D is an open-labelled randomised 5-period crossover trial comparing the pharmacokinetic and pharmacodynamic properties of an insulin analogue at ascending dose levels to those of Protaphane, insulin human (rDNA) during euglycaemic clamps in healthy male volunteers.

This study compared the pharmacodynamic and pharmacokinetic properties of an insulin analogue with Protaphane, insulin human (rDNA) using the euglycaemic clamp technique. Eleven healthy subjects were given single doses of Protaphane, insulin human (rDNA) 0.3 U/kg. The insulin was administered subcutaneously in the abdominal wall. Figure 1 depicts the glucose infusion rate after administration of Protaphane, insulin human (rDNA) 0.3 U/kg. As can be seen, the maximum effect of Protaphane, insulin human (rDNA) is observed about 3-6 hours after injection. At the end of the study period, the glucose infusion rate was still well over baseline level.

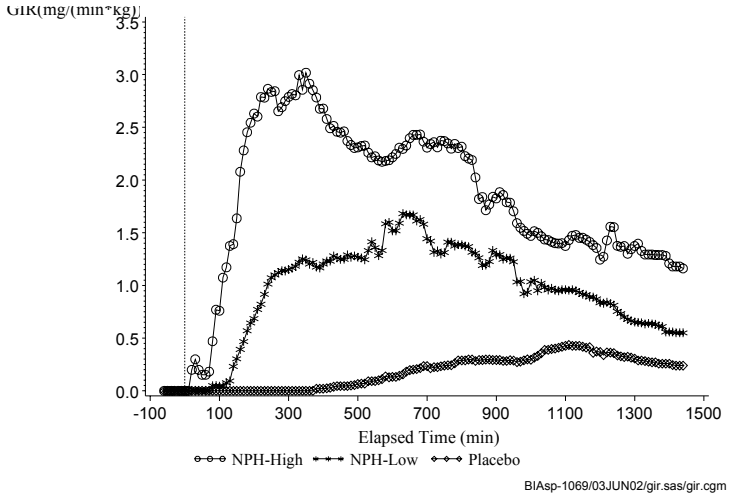
Figure 1: Mean Glucose infusion rate profile after Protaphane, insulin human (rDNA) SC (Study NN304/DCD/001/D).



The study **NN304/DCD/4/A** is a double-blind randomised placebo-controlled 5-period crossover trial aimed at comparing the pharmacokinetic and pharmacodynamic properties of an insulin analogue with Protaphane, insulin human (rDNA) including placebo during euglycaemic glucose clamps in healthy male volunteers.

The objective of this study was to compare the pharmacodynamic and pharmacokinetic properties of the insulin analogue with isophane insulin human NPH using the euglycaemic clamp technique. A placebo treatment was included as well. Ten healthy subjects were given single doses of Protaphane, insulin human (rDNA) 0.3 and 0.6 U/kg and placebo on separate study days. The insulins were given subcutaneously in the abdominal wall. Figure 2 shows the glucose infusion rate after administration of Protaphane, insulin human (rDNA) 0.3 and 0.6 U/kg. This table also summarises the pharmacodynamic results obtained after the administration of the two doses of Protaphane, insulin human (rDNA) . The effect of Protaphane, insulin human (rDNA) peaks about 8-10 h. This was observed a little earlier with the highest dose. The mean area under the glucose infusion rate curve (determined between 0 and 24 hours of administration) for the highest dose of Protaphane, insulin human (rDNA) is nearly double that seen for the lowest dose suggesting of a dose-response relationship. The Glucose infusion rate values for the two doses of Protaphane, insulin human (rDNA) were still well greater than the placebo level and the baseline level at the end of the study period.

Figure 2: Glucose infusion rates after injection of Protaphane, insulin human (rDNA) 0.3 and 0.6 U/kg, and placebo. (Study NN304/DCD/4/A).



Pharmacokinetics

Pharmacokinetic data concerning Protaphane, insulin human (rDNA) were obtained from the same pharmacodynamic studies performed in healthy volunteers (studies NN304/DCD/001/D, NN304/DCD/4/A and NN304-1028 see Table 3).

- **Absorption and bioavailability**

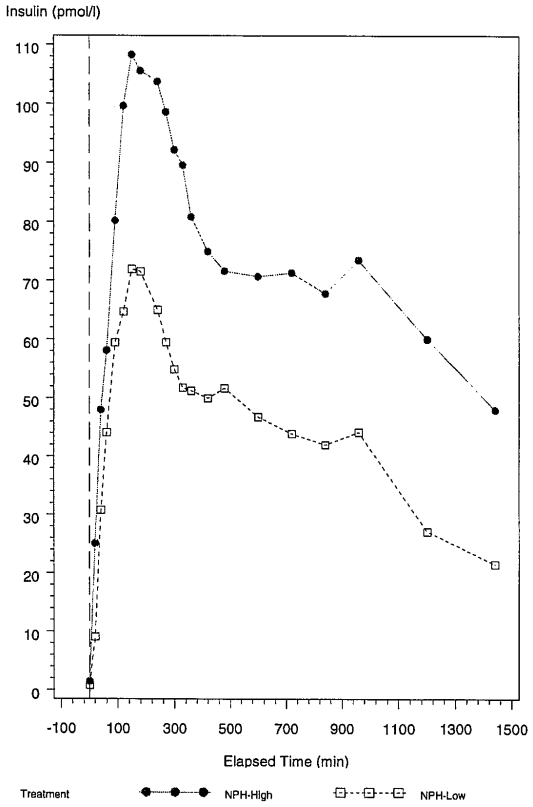
Study NN304/DCD/4/A showed that as expected, for two parameters the mean area under the time-concentration curve (measured between 0 and 24 hours, denoted by AUC_{0-24}) and the highest insulin concentration value observed in plasma (denoted by C_{max}) the values for exogenous insulin were significantly higher for the highest dose of Protaphane, insulin human (rDNA) as compared to the lowest dose administered to the subjects, approximately 1.7-fold and 1.5-fold, respectively. Mean time to peak concentration was about 5 hours for both doses. In addition, the exogenous insulin levels had not returned to baseline levels at the end of the study period (see Figure 3).

Table 3: Summary of Protaphane, insulin human (rDNA) results from Study NN304/DCD/4/A.

	NPH-Low	NPH-High
AUC_{0-24} ((pmol/l)*min)		
N	10	10
Mean	60229	101975
SD	20057	31531
Min	22150	62936
Max	90529	163862
C_{max} (pmol/l)		
N	10	10
Mean	80.7	120.3
SD	33.1	45.0
Min	30.0	76.8
Max	147.7	219.7
MRT_{0-24} (min)		
N	10	10
Mean	617.3	686.8
SD	128.3	113.3
Min	418.5	515.7
Max	775.2	921.8
t_{max} (min)		
N	10	10
Mean	321.0	288.0
SD	314.3	322.9
Min	90.0	150.0
Max	960.0	1200.0

Trial ID.: NN304/DCD/004/A
Spadille Biostatistics ApS (tab07.sas/7.asc) 07JAN98
Cross-reference: EOT Table 7.

Figure 3: Exogenous insulin profiles after Protaphane, insulin human (rDNA) (Study NN304/DCD/4/A).



- **Bioavailability**

The objective of the study NN304-1028 was to measure the relative bioavailability of Protaphane, insulin human (rDNA) administered subcutaneously. In a randomised crossover design sixteen healthy males were given single doses of Protaphane, insulin human (rDNA) 0.2 U/kg SC, fast-acting insulin human (rDNA) 0.075 U/kg as a 30-min IV infusion. Blood samples for plasma glucose, total insulinand C-peptide were drawn for 12 h after IV dosing and 24 hours after SC dosing.

The estimated bioavailability of Protaphane, insulin human (rDNA) was 49% (95% confidence interval = [38 ; 62%] based on an ANOVA analysis. Other pharmacokinetic results included mean t_{max}, which was about 7 hours for Protaphane, insulin human (rDNA) subcutaneously.

- **Distribution**

No formal distribution studies were performed with Protaphane, insulin human (rDNA). Insulin is not bound to plasma proteins unless circulating antibodies directed against insulin are present.

- **Elimination**

Protaphane, insulin human (rDNA) cannot be administered intravenously, thus clearance after intravenous administration could not be studied. As the half-life of intravenously injected insulin human (rDNA) is relatively short, the terminal half-life of insulin human (rDNA) following subcutaneous injection is a measure of the terminal absorption rather than the elimination of insulin from plasma per se. The terminal elimination half-life of insulin human (rDNA) following SC injection of Protaphane, insulin human (rDNA) has not been calculated in the listed studies.

Metabolism

Metabolism of Protaphane, insulin human (rDNA) was not formally investigated. Protaphane, insulin human (rDNA) is absorbed as standard insulin human (rDNA). From previously published data it is known that insulin is catabolised by various proteases. The degradation products are not active.

Excretion

Excretion of Protaphane, insulin human (rDNA) was not formally investigated. As insulin is eliminated by metabolism, excretion of unchanged drug is minimal or non-existent.

- **Pharmacokinetics in special populations**

Patients with impaired renal or hepatic function

The applicant has not submitted any data on the pharmacokinetics in patients with impaired renal/hepatic function.

Pregnancy and lactation

No studies have been performed. Diabetes is associated with an increased risk of complications during pregnancy and congenital malformations in the baby. Optimising metabolic control before and during pregnancy can reduce this risk. For most of the patients with type 2 diabetes and all patients with type 1 diabetes, insulin is the only way of optimising metabolic control. Insulin can be administered during pregnancy and lactation.

- **Interaction studies.**

No formal interaction studies have been performed.

- **Conclusion on pharmacokinetic studies.**

Protaphane, insulin human (rDNA) is a long-acting human insulin. It is a suspension of crystalline protamine insulin. Onset of action is within 1½ h, and the peak effect is reached after about 3-12 hours. This corresponds quite well with the kinetic results. The duration of action seems to be around 24 h or even more. The absolute bioavailability was estimated at 49%. There are no pharmacodynamic/kinetic data specifically on Protaphane, insulin human (rDNA) with regard to the effect of age, gender, ethnic origin, hepatic and renal impairment. As with all subcutaneously injected insulins, the terminal elimination half-life is determined by absorption rather than elimination. The terminal elimination half-life of Protaphane, insulin human (rDNA) has not been documented. Insulin human (rDNA) is eliminated through degradation in various organs and tissues. There are no active metabolites. The numerous products, which interact with insulin on the dynamic level by affecting glucose metabolism, are identified. There are no known pharmacokinetic interactions.

Clinical efficacy

The treatment of diabetes mellitus with insulin has been established for many decades. It is a life saving treatment for patients with type 1 diabetes and is required by many patients with type 2 diabetes.

A number of different insulin regimens have been proposed for treatment of diabetes. It is generally accepted that the so-called basal-bolus insulin regime (one or two injections of NPH insulin covering basal insulin requirements in combination with three injections of fast-/rapid-acting insulin to cover meal-related insulin requirements) generally yields the best glycaemic control in diabetes. However, a number of patients, especially patients with type 2 diabetes can be adequately regulated by twice daily injections of Protaphane, insulin human (rDNA) with or without concomitant injection of fast-/rapid-acting insulin.

The application is based on recently performed studies where Protaphane, insulin human (rDNA) has been used in clinical trials performed to compare the efficacy and safety of Protaphane, insulin human (rDNA) with other insulin analogues,. Some older studies have been performed to characterise the pharmacokinetic profile of insulin human and to demonstrate efficacy and safety of genetically engineered insulin human (rDNA) as compared to semi-synthetic produced human insulin with particularly clinical and laboratory assessments of direct toxicity, and immunisation or allergy caused by possible contaminants. Finally since an important post-marketing experience with human insulins

has been gathered considering that these products have been marketed since 1988 some additional safety data have been included and submitted in Periodic Safety Update Reports.

Main studies (phase III = therapeutic confirmatory trials).

Table 4: Phase III trials

Study	Population (Number of subjects)	Design	Objective
BiAsp - 1069	Type 2 diabetic patients (403 patients).	Multinational randomised controlled double blind parallel groups trial.	Efficacy and safety comparison of twice daily biphasic, dual-acting insulin aspart 30 or Protaphane, insulin human (rDNA) in subjects with type 2 diabetes
INS/UK/002/UK	Type 2 diabetic patients (73 patients).	Open, randomised, cross-over study.	Compare twice daily Protaphane, insulin human (rDNA) and once daily long-acting insulin in a formulation of zinc crystals in non-insulin dependent diabetic subjects.
NN304-1038	Type 1 diabetic patients (59 patients).	Multicentre open randomised cross-over trial.	Compare the blood glucose lowering effect of an insulin analogue with Protaphane, insulin human (rDNA) in type 1 diabetes subjects.
NN304/DCD/006/D	Type 1 diabetic patients (22 patients).	Multicentre uncontrolled open trial.	Titration study of an insulin analogue in a basal bolus regime in type 1 diabetic patients.

- **Study Bi-Asp-1069**

The **study Bi-Asp-1069** has been conducted with patients with type 2 diabetes. This study was aimed at comparing the efficacy and safety of biphasic dual-acting insulin aspart 30 administered twice daily and Protaphane, insulin human (rDNA) in patients with type 2 diabetes. It is a multinational randomised double-blind controlled parallel group trial.

1. Description of the study

The primary objective of the trial was to compare the glycaemic control of biphasic dual-acting insulin aspart 30 with that of Protaphane, insulin human (rDNA) in patients with type 2 diabetes. Secondarily the study compared the safety profile of both products (biphasic dual-acting insulin aspart 30 with that of Protaphane, insulin human (rDNA)).

The trial was a multinational randomised controlled double-blind parallel groups study. The patients enrolled in the study consisted of male and female patients with type 2 diabetes mellitus (as defined by WHO criteria) aged 18 years old or more. Additional inclusion criteria included a body mass index (denoted by BMI) $\leq$ 35.0 kg/m^2, and glycosylated haemoglobin (HbA_{1c}) $\leq$ 11.0 %. Both insulin naïve patients and patients treated with Protaphane, insulin human (rDNA) (monotherapy or combination with oral hypoglycaemic agents) were included.

The patients were randomised to receive twice daily injections (subcutaneously) of either biphasic dual-acing insulin aspart 30 or Protaphane, insulin human (rDNA). Both Protaphane, insulin human (rDNA)and biphasic dual acting insulin aspart 30 were administered immediately before breakfast and dinner (usually Protaphane, insulin human (rDNA) in this type of insulin regimen is administered 30 minutes before the meals). Insulin experienced patients received their usual pre-trial dose of Protaphane, insulin human (rDNA) or a corresponding dose of biphasic dual-acting insulin aspart 30. Insulin naïve patients were initially treated with a starting dose of 8 to 16 IU per day. For both types of

patients insulin doses were adjusted according to blood glucose measurements in accordance with standard diabetes treatment guidelines.

2. Primary endpoints

The primary efficacy endpoint was the glycosylated haemoglobin (HbA_{1c}) measured after 4, 8, 12 and 16 weeks of treatment. Secondary parameters were derived from 8-point blood glucose profiles obtained after 1, 2, 4, 8, 12 and 16 weeks of treatment.

The assessment of the safety profile of the products was based on the collection of all adverse events (including all hypoglycaemic episodes) and the measurement of different biological parameters. Episodes of hypoglycaemia were classified as follows: minor (symptoms of hypoglycaemia with or without confirmation by measurement of blood glucose levels), major (symptoms of hypoglycaemia that either required third party assistance or additionally required treatment intervention with intravenous glucose or glucagon.

3. Statistical analysis

Sample size calculations were based on a requirement of a power of 80% to detect a difference of 0.3 percentage points in HbA_{1c} levels. The efficacy analysis was based on the intention-to-treat (ITT) population. Supplementary analysis was performed with the per-protocol (PP) population. A repeated measures ANOVA was used to analyse the primary efficacy variable. Hypoglycaemic episodes were analysed using a log-linear Poisson regression model.

4 Study population

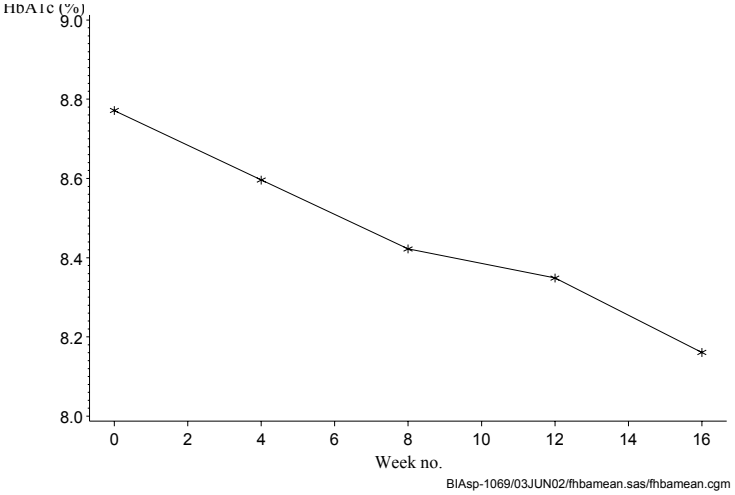
Four hundred and five patients were randomised in the study and 403 actually received the study medication. Three hundred and ninety two patients completed the trial. Withdrawals were equally distributed among the two treatment groups.

The two treatment groups of patients were comparable especially with regard to demographic parameters and baseline disease characteristics. The average disease duration of the patients enrolled in the study was 10 years. Forty percent were insulin naïve. Baseline HbA_{1c} levels were approximately 8.8%. Of the insulin naïve patients the vast majority were previously on oral hypoglycaemic agents. Only very few patients were truly treatment naïve. In both treatment groups the total daily insulin dose increase during the study, more so in the biphasic, dual-acting insulin aspart 30 than in the Protaphane, insulin human (rDNA) group.

5 *Efficacy results*

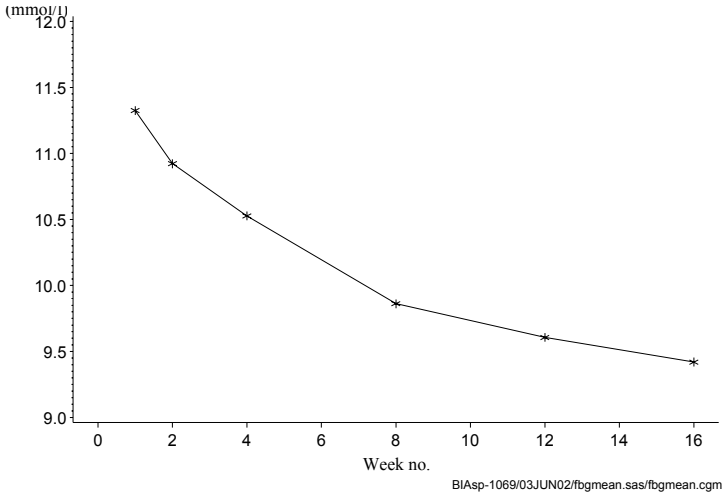
At 16 weeks HbA_{1c} levels have decreased to approximately 8.2% in both treatment groups. There was no significant difference between treatments. The results were similar for both the intent to treat and the per protocol analyses (the intent to treat population is described in ICH E9 as including the patients who were followed-up, assessed and the data analysed irrespective of their compliance to the planned course of treatment. The per protocol population defined in ICH E9 as including the patients who complied sufficiently with the protocol to ensure that the data obtained with these patients reflected the effects of treatment). In both treatment groups, HbA_{1c} exhibited a continuous fall during the 16 weeks of treatment without reaching a stable plateau. An analysis of interaction in the ANOVA of the effect of pretreatment has been performed. The patients previously treated with a combination of NPH-insulin and oral hypoglycaemic agents responded less favourably than patients previously on insulin-NPH monotherapy or on oral hypoglycaemic agents monotherapy. There were no differences between treatments. Stable HbA_{1c} levels were not reached during the 16 weeks of treatment suggesting that the study duration was probably too short to fully evaluate the potential benefits of the treatment with Protaphane, insulin human (rDNA).

Figure 4: HbA1c mean profiles over the 16 weeks of the study measured in the intent to treat population.



Average blood glucose levels were approximately 11 mmol/l at study entry. There was a continuous decrease in average blood glucose levels during the 16 weeks of treatment. At 16 weeks of treatment average blood glucose levels were approximately 9.5 mmol/l.

Figure 5: Development of average blood glucose levels over 16 weeks.



The present study demonstrates that in a subset of patients, Protaphane, insulin human (rDNA) administered twice daily can improve glycaemic control.

Study INS/UK/002/UK

The **study INS/UK/002/UK** has been performed in non-insulin dependent diabetic patients and was aimed at comparing the safety profile of Protaphane, insulin human (rDNA) with human zinc insulin (very long acting insulin).

1. Description of the study

The study INS/UK/002/UK is an open multi-centre randomised and cross-over study. The primary objective was aimed at comparing the safety profile with respect to the number of hypoglycaemic episodes experienced by the patients of Protaphane, insulin human (rDNA) administered twice daily with human zinc insulin (very long-acting insulin) administered once daily. The secondary objectives were to compare the efficacy of both regimens in term of metabolic control.

The patients enrolled in the study consisted of patients with type 2 diabetes being unsatisfactorily controlled (i.e. with fasting blood glucose [FBG] >8.5 mmol) by an oral hypoglycaemic agent administered at the maximum, with a body mass index (BMI <35kg/m^2) and an age between 30 and 80 years old. Patients with any insulin therapy 6 months before inclusion, with severe renal or hepatic failure or cardiac disease or with rapidly progressive retinopathy could not be enrolled in this study.

Following a run-in period of 8 weeks where patients continued their oral hypoglycaemic therapy, they were randomised to either treatment (Protaphane, insulin human (rDNA) or zinc insulin) for a period of 6 months. Starting dose was for both treatments 0.3 IU/kg daily (and subsequently adjusted to achieve a fasting blood glucose FBG of 4-7 mmol/l), divided equally between a morning and an evening dose (Protaphane, insulin human (rDNA)) or administered as a single evening dose (zinc insulin). After 6 months of treatment patients were switched to the other insulin therapy.

A sample size of 80 patients was calculated to detect a true treatment difference of 25% in the number of hypoglycaemic episodes, with a power of 80% assuming that the lowest incidence of hypoglycaemic episodes was 40%.

2. Primary endpoints

The primary endpoint was measured by the total number of hypoglycaemic reactions during each treatment period. The secondary efficacy endpoints were measured by the total insulin dose administered to the patient, weight, quality of life questionnaire and overall treatment satisfaction score in addition to different biological parameters such as HbA_{1c}, glycaemia 7- and 4-point profile, HDL and LDL cholesterol, triglycerides, fasting insulin, fasting C-peptide.

3. Statistical analysis

Treatment comparisons in the number of reactions per month were tested using the Wilcoxon Signed Rank Test. The sequence effect was tested using the Wilcoxon Rank Sum Test. For efficacy variables data results were analysed using analysis of variance appropriate to cross-over design.

4. Study population

A total of 73 patients were randomised and received study medication. Of these, 68 patients provided some follow-up data and could be included in the safety analysis. Sixty patients completed the study and were included in the efficacy analysis. Thirteen patients withdrew from the study, 2 patients experienced adverse reactions (unlikely related to study medication), 6 patients withdrew because of ineffective therapy (all were treated with insulin NPH at the time), the remaining patients withdrew for other reasons.

A total of 33 patients were treated with the sequence zinc insulin/ Protaphane, insulin human (rDNA) (28 of them completed the study), and 38 were treated with the sequence Protaphane, insulin human (rDNA)/zinc insulin (32 completed the study).

5. Efficacy results

On an efficacy point of view, insulin doses increased with time irrespective the treatment sequence. The mean dose of Protaphane, insulin human (rDNA) was greater than the zinc insulin dose at the end of the treatment periods (difference of 4.9 IU). Data on HbA_{1c} were available for 26 patients from the group treated successively with zinc insulin and Protaphane, insulin human (rDNA) and for 32 patients from the other treatment sequence (Protaphane, insulin human (rDNA) and zinc insulin). Decreases in HbA_{1c} were observed for both groups. A significant difference of 0.72% in favour of Protaphane, insulin human (rDNA) has been observed (normally HbA_{1c} should be inferior to 6.0% of total haemoglobin). Finally, blood glucose control generally was slightly better achieved with Protaphane, insulin human (rDNA).

- **Study NN304-1038**

The study **NN304-1038** has been performed in type 1 diabetes patients and was aimed at comparing the blood glucose lowering effect of an insulin analogue with Protaphane, insulin human (rDNA).

1. Description of the study

This trial was an open multi-centre randomised cross-over trial. After a 2-weeks run-in period the two treatment periods of 6 weeks each followed. Both insulin analogue and Protaphane, insulin human (rDNA) were to be administered in the evening.

Adult patients with type 1 diabetes mellitus for at least 2 years and being treated according to the basal/bolus regimen were enrolled in this study. The non inclusion criteria were the following: patients with proliferative retinopathy, patients requiring Protaphane, insulin human (rDNA) dosages > 40 IU/day, impaired hepatic or renal function, NYHA class III and IV congestive heart failure, unstable angina pectoris or recent myocardial infarction, uncontrolled hypertension, pregnancy. Patients self-monitored their blood glucose. Sixty subjects were to be included.

2. Primary endpoints

The primary efficacy endpoint was the area under the serum glucose concentration-time curve between 23:00 and 8:00 (denoted by $AUC_{glu,\ 23\text{-}8}$). Secondary endpoints were the difference between the maximum and the minimum serum glucose between 23:00 and 8:00 (denoted by delta $_{glu,\ 23\text{-}8}$), the shape of the serum glucose profile in the same time interval [parallelism of the curves], the area under the glucose concentration-time curve measured between different period of time: 8:00 and 12:00 ($AUC_{glu,\ 8\text{-}12}$) and 12:00 and 22:00 ($AUC_{glu,\ 12\text{-}22}$). Other secondary endpoints were C_{max} (glu, 8-12), fructosamine at visits 1,2,5, and 8, home monitored fasting blood glucose (denoted by FBG) on the last 4 days of each treatment period, dose of basal insulin

The safety profile of the products was assessed with the following endpoints: the frequency of hypoglycaemic episodes, number of adverse events, the presence of antibodies, haematological and biochemical parameters, vital signs, electrocardiogram (ECG), fundoscopy and physical examination.

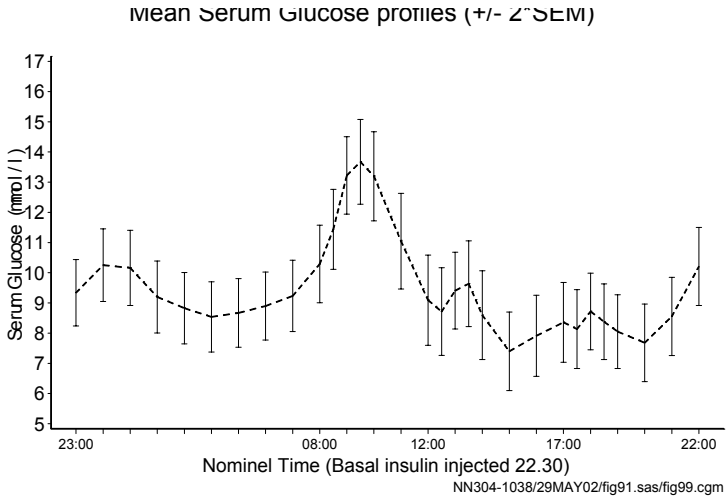
3. Study population

Fifty-nine subjects were randomised in the trial and 56 subjects completed the trial. The reasons for premature discontinuation were that 2 cases had a C-peptide level above the inclusion criteria.

4. Efficacy results

The serum glucose profiles obtained with Protaphane, insulin human (rDNA) are depicted in Figure 6 (see below).

Figure 6 Serum Glucose profile



The daily dose of Protaphane, insulin human (rDNA) in the last seven days of each treatment phase was 0.28 IU/kg (standard deviation 0.09). The total bolus insulin dose of fast-acting insulin human in the last seven days of each treatment phase was 0.42 IU/kg.

Protaphane, insulin human (rDNA) was tested against an insulin analogue. The trial was not blinded but it helped to characterise the glucose profile obtained by the basal/bolus treatment with Protaphane, insulin human (rDNA) given once daily to cover the basal insulin requirements and fast-acting insulin human given to the meals.

- **Additional studies**

Some additional studies have been conducted with Protaphane, insulin human (rDNA). Study **NN304/DCD/006/D** was a multicentre open and uncontrolled titration study of a insulin analogue in a basal/bolus regimen in type 1 diabetic patients. The objective of this trial was to find out how to switch patients on a basal/bolus regimen from Protaphane, insulin human (rDNA) once or twice daily to an insulin analogue once daily. During the 2-week run-in period patients were treated with Protaphane, insulin human (rDNA). This trial confirmed that the serum glucose profile obtained with a treatment with Protaphane, insulin human (rDNA) was comparably shaped to the glucose profile in trial observed in trial NN304-1038.

- **Other phase III studies (devices).**

Study	Population (Number of subjects)	Design	Objective
Innolet-1207	Type 2 diabetic patients(112 patients)	Multicentre open randomised comparative cross-over study.	Comparison of the metabolic control control by means of HbA1c and 4-point blood glucose profiles using Innolet and vial/syringe in type 2 diabetic patients.
MS216-1278	Type 1 and type 2 diabetic patients (113 patients).	Multicentre open randomised comparative cross-over trial with two 6-weeks treatment periods.	Compare the fructosamine level using FlexPen and Novolet treatment in patients with type 1 and type 2 diabetes.

The objective of study **Innolet-1207** was to compare the metabolic control obtained by the administration of different types of insulins administered either with InnoLet or with a syringe. This study was an open comparative randomised crossover trial conducted in elderly type 2 diabetic patients. A total of 112 type 2 diabetes patients were included and 101 completed the study. The treatment duration was 6 weeks with each device. The insulins used in this trial were eitherfast-acting insulin human (rDNA), Protaphane, insulin human (rDNA) or biphasic dual-acting insulin human (rDNA) 30. The metabolic control was measured by means of HbA_{1c} and 4-point blood glucose profiles. Both treatments were estimated to be comparable with respect to HbA_{1c} and 4-point blood glucose measurements.
The incidence of severe adverse reactions was higher among vial/syringe users than in InnoLet users (7% vs. 2%). There was no difference regarding the incidence and severity of hypoglycaemic events.

Finally, the study **MS216** (a randomised cross-over open trial) compared the metabolic control and the safety achieved with the use of FlexPen vs. NovoLet in type 1 and 2 diabetic patients. The efficacy of the different treatments was measured with the fructosamine level, HbA_{1c}, and 4-point blood glucose profiles. The insulins used in this study were fast-acting insulin human (rDNA), Protaphane, insulin human (rDNA), dual-acting insulin human (rDNA) 30 or rapid-acting insulin analogues. A total of 103 patients were randomised in the trial and 99 completed the trial. There was no significant difference of metabolic control between the different devices. Hypoglycaemic events and adverse reactions were comparable for both devices.

Discussion on clinical efficacy

The present data indicates that acceptable glycaemic control can be obtained by the administration of Protaphane, insulin human (rDNA) either in a twice-daily regimen or in a basal-bolus regimen. The data did not indicate a different metabolic control when using the various devices that may be used for ease of administration in comparison to vial/syringe.

Clinical safety

Patient exposure

Four hundred and five patients with type 2 diabetes participated in the study **Bi-Asp-1069** and 403 actually received the study medication. Three hundred and ninety two patients completed the trial. The study **INS/UK/002/UK** has also been conducted with non-insulin dependent diabetic patients, a total of 73 patients were randomised in this trial and received the study medication. Of these, 68 patients provided some follow-up data and could be included in the safety analysis. Sixty patients completed the study and were included in the efficacy analysis. Finally, the study **NN304-1038** has been performed in type 1 diabetes patients and 59 subjects were included in this trial. Fifty-six patients completed the trial.

Adverse events and serious adverse event/deaths

Thirty six percent and 38% of patients who participated in the study **Bi-Asp-1069** (and treated respectively with biphasic dualacting insulin aspart and with Protaphane, insulin human (rDNA)) reported one or more adverse events in the protocol Bi-Asp-1069. Most of the adverse events were considered unlikely to be related to treatment. Four patients in the Protaphane treatment group experienced adverse reactions with a possible or probable causal relationship to trial medication. The majority of adverse events were mild to moderate. The most frequently reported adverse events were headache and influenza like symptoms. One subject in the Protaphane, insulin human (rDNA) group was withdrawn due to allergy to protamine. One treated with Protaphane were withdrawn due to myocardial infarction; in both cases relation to trial medication was considered unlikely. No deaths were reported during the trial. Patients experienced 10 serious adverse reactions while treated by Protaphane, insulin human (rDNA). A few major hypoglycaemic episodes were reported.34% of subjects in the Protaphane, insulin human (rDNA) group experienced minor hypoglycaemic episodes.

Overall, the trials revealed no new safety concerns than what is known on insulin.

The study **INS/UK/002/UK** had a cross-over design consequently all the patients randomised in this trial received alternatively Protaphane, insulin human (rDNA) and zinc insulin (or the contrary). Twenty patients experienced a higher number of hypoglycaemic reactions with Protaphane, insulin human (rDNA) than with zinc insulin, while on the other hand 34 patients experienced more hypoglycaemic reactions with zinc insulin than with Protaphane, insulin human (rDNA). This difference was, however, not statistically significant.

A total of 220 hypoglycaemic reactions were reported in patients treated with zinc insulin and 171 in patients treated with Protaphane, insulin human (rDNA). More severe hypoglycaemic reactions (defined as reactions requiring a third party intervention) were observed for zinc insulin (14) than for Protaphane, insulin human (rDNA) (only one). Finally, a total of 63 patients experienced 208 adverse reactions while receiving zinc insulin compared to 51 patients who experienced 162 adverse reactions while treated with Protaphane, insulin human (rDNA).

Finally 51 subjects included in the study **NN304-1038** and treated with Protaphane, insulin human (rDNA) experienced 556 minor hypoglycaemic episodes. Seven subjects experienced eleven major hypoglycaemic episodes. Fifteen patients experienced nineteen serious adverse reactions in the Protaphane, insulin human (rDNA) treatment periods, nine of these were respiratory events. No serious adverse reaction has been observed with the Protaphane, insulin human (rDNA) treatment.

Post-marketing experience

An extensive marketing experience with human insulins has been gathered since human insulins have been marketed for almost ten years. The periodic safety update reports submitted since 1993 that between 1 March 1993 to 31 August 1998 a total of 21 million person years representing at least 4 million individual patients have been exposed to human insulin. In addition, between 1 September

1998 and 30 June 2000 an estimated total of 3.7 million person years representing approximately 2 million individual patients may have been exposed to this product.

Since the report from Teuscher and Berger (Hypoglycaemia unawareness in diabetics transferred from beef/porcine insulin to human insulin. Lancet 1987, ii.382-5) there had been focus on diminished awareness of hypoglycaemia after changing from animal insulin to human insulin. A review of clinical and epidemiological studies prepared by the applicant could not support this hypothesis, an update of this paper including literature research up to may 1997 could either.

The most common reactions reported in the Periodic Safety Update Reports were hyper- and hypoglycaemia, injection site reaction and –pain, therapeutic response decreased, allergic reaction and rash or pruritus.

Serious and unexpected cases of optic atrophy, on limb malformation in a new-born baby (born without limbs and with cardiac problems, subsequently dying) and of thrombocythaemia have been reported. Twenty two cases reported in Japan (11 of these were classified as serious) of impaired liver function were received by the company. No such reports were received from other countries. According to evidence from some studies liver enzyme increases are most likely related to diabetes mellitus non insulin-dependent/treatment with oral antidiabetic agents but not to insulin. In addition, the hypothesis of an idiosyncratic reaction was abandoned since no other signs of hypersensitivity were observed and no eosinophile granulocytes were found in biopsies.

In this period, two changes have been made in the summary of product characteristics for safety reasons: a more detailed description of the symptoms of hypo- and hyperglycaemia and a more detailed description of possible generalised hypersensitivity reactions. Apart from these amendments, no regulatory or manufacturer actions have been taken for safety reasons.

Discussion on clinical safety

Based on the review of the safety data from the vast post marketing experience, no new safety issues were identified. The most frequent adverse reactions are hypo-or hyperglycaemia. The safety profile of Protaphane, insulin human (rDNA) seems to be well characterised.

5. Overall conclusions, benefit/risk assessment and recommendation

Quality

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

Viral Safety and Batch to batch consistency has been documented and the relevant test will be performed according to the agreed specifications

Preclinical pharmacology and toxicology

The toxic effects seen in the single dose and repeated dose toxicity studies were attributed to the hypoglycaemic activity and thus an exaggerated pharmacological effect caused by the high doses of the insulin. Increased weight, depressed activity, convulsions and death were some of these effects. No specific studies were conducted on toxicity of Protaphane (containing recombinant human insulin), as the active component is similar to Actrapid (containing recombinant human insulin). An increase in the number of benign mammary adenomas and fibroadenomas has been shown in Sprague Dawley rats. In one 12 months study, there was a statistically significant increase of female animals bearing benign and malign mammary gland tumours at the highest dose. It is concluded that the increased incidence of mammary tumours seen in rats is probably caused by mitogenic and growth-promoting action via the insulin receptor, but is probably also related to the fact that Sprague Dawley rats are especially sensitive and were given large doses. It is concluded that newer studies conducted since the original marketing authorisation for insulin human support the older documentation and do not give reason for new safety concerns.

Efficacy

Protaphane is a long-acting human insulin. It is a suspension of crystalline protamine insulin. The treatment of diabetes mellitus with insulin has been established for many decades. It is a life saving treatment for patients with type 1 diabetes and is required by many patients with type 2 diabetes. A number of different insulin regimens have been proposed for treatment of diabetes. It is generally accepted that the so-called basal-bolus insulin regime (one or two injections of NPH insulin covering basal insulin requirements in combination with three injections of fast-acting insulin to cover meal-related insulin requirements) generally yields the best glycaemic control in diabetes. However a number of patients, especially patients with type 2 diabetes can be adequately regulated by twice daily injections of NPH insulin with or without concomitant injection of soluble insulin. The present data indicates that acceptable glycaemic control can be obtained by the administration of Protaphane either in a twice-daily regimen or in a basal-bolus regimen. The data did not indicate a different metabolic control when using the various devices that may be used for ease of administration in comparison to vial/syringe.

Safety

Based on the review of the safety data from the vast post marketing experience, no new safety issues were revealed that should be included in the present summary of product characteristics. The most frequent adverse reactions are hypo-or hyperglycaemia. The safety profile of Protaphane is well described and acceptable.

Benefit/risk assessment

Based on the submitted documentation on pharmacodynamic, pharmacokinetic and clinical data as well as the well-established use of Protaphane, the efficacy and safety of Protaphane is considered adequately demonstrated.

Recommendation

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of Protaphane was favourable in the treatment of diabetes mellitus, the initial stabilisation of diabetes, during treatment of diabetic ketoacidosis and the hyperosmolar non ketotic syndrome, and during stress situations such as severe infections and major surgery in hyperglycaemic patients.