

SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Actraphane. 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 β 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.

Marketing authorisation for this human insulin has been obtained for the treatment of patients with diabetes mellitus. The active substance of Actraphane is insulin human manufactured by recombinant DNA technology in *Saccharomyces cerevisiae*.

Actraphane is a biphasic formulation consisting of a premix of soluble fast acting insulin and isophane long acting insulin in the proportions:

Actraphane 10:	10% soluble and 90% isophane
Actraphane 20:	20% soluble and 80% isophane
Actraphane 30:	30% soluble and 70% isophane
Actraphane 40:	40% soluble and 60% isophane
Actraphane 50:	50% soluble and 50% isophane

Actraphane is intended for marketing in dose strengths of 40 IU/ml and 100 IU/ml in vials, Penfill, InnoLet, NovoLet and FlexPen presentations. Actraphane is presented in 10 ml vials, 3 ml cartridges (Penfill) and in multidose pre-filled 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 Penfill presentation is a cartridge that 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 pre-filled pen that 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 3 ml cartridge after a multiple use. The device is targeted for use by geriatric patients with impaired dexterity and/or vision.

The NovoLet presentation is a multidose pre-filled pen delivering 2-78 units per dose in increments of 2 units.

The FlexPen presentation is also a multidose pre-filled 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 dosage in single unit increments.

2. Part II: Chemical, pharmaceutical and biological aspects

Composition

The formulation, designed for dual action, is a premixed insulin product consisting of a mixture of fast acting insulin and long acting insulin (insulin formulated in a suspension where protamine and insulin are brought together in isophane proportions at a neutral pH. It contains insulin human (rDNA) as active ingredient and agents for functions as follows: protamine sulphate (protracting agent - forms isophane crystals with the insulin in the formulation), zinc (crystal formation), glycerol (isotonic) and phenol and metacresol (preservatives).

Actraphane is presented in vials, cartridges (Penfill) and in multidose pre-filled pens (Innolet, NovoLet and FlexPen).

The 10 ml vial is a glass container with a laminated isoprene/brombutyl 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/brombutyl rubber stopper and a brombutyl rubber plunger.

Innolet, NovoLet and FlexPen are multidose disposable pens made of a plastic injector device fitted with 3 ml Penfill cartridges.

Active substance

The active substance of Actraphane 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 developed by the applicant are fully described with relevant validations.

Development Genetics

Insulin human is produced using a genetically modified strain of *Saccharomyces cerevisiae*. The strain carries a plasmid which codes for the expression of a single amino acid chain insulin precursor attached to a pre-pro leader region of the yeast mating factor (MF α 1) gene.

The plasmid is constructed based on the yeast 2 μ 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 insulin human, 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 insulin human 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 the recommended storage temperature.

Other ingredients

All excipients comply with Ph.Eur. specifications apart from metacresol which is USP standard.

Product development and finished product

Development pharmaceuticals

The current formulation represents an accumulation of experience the applicant has gained with a wide variety of insulin products over the years. The first biphasic protamine insulin formulation 30/70 was developed based on the applicant's experience at that time with a soluble fast acting insulin and an insulin/protamine complex). The formulation was introduced in the early 1980's and fine-tuned later to improve overall product physical stability and to rationalise manufacture such that there was only one crystalline formulation for all protamine insulin products manufactured by the applicant.

Emphasis has been placed on correct insulin crystal size in the crystalline phase of 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.

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

Manufacturing process

Actraphane consists of a mixture of a suspension of crystalline human protamine insulin and dissolved insulin human. Crystalline and dissolved fractions are prepared separately and the formulated bulk made by sterile filtration of the dissolved fraction into the sterile tank containing the crystalline fraction.

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.

Formulae for the auxiliary solutions (HCl, NaOH and ZnCl₂) and preservative solution are provided.

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 over the years, 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 Actraphane products manufactured at various approved sites and in different batch sizes. Available data show a consistent, well-controlled manufacturing process.

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

1999:0854 Insulin Preparations, Injectables

1997:0832 Insulin Injection, Biphasic Isophane

In addition to monograph tests the products are tested by in-house methods for percentage of dissolved insulin, identity and content of preservative and for dose accuracy (pre-filled pen 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. All batches comply with their respective specifications.

Stability of the Product

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

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 insulin human, rDNA. These are peptone, beef extract and pepticase which are used in the preparation and storage of cell banks, L-threonine and trypsin- used in the purification process, and protamine sulphate used as excipient in the final product. L-threonine is sourced from avian feathers and porcine gelatine, trypsin from porcine pancreas and protamine sulphate from salmon.

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.

The risk of transmission of TSE from Actraphane 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 (EMA/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: Toxicopharmacological aspects

Insulin is a hormone which primarily regulates the glucose metabolism in the human body. It is normally synthesized in the beta cells of the pancreas, induces storage of ingested food by allowing glucose to enter muscles and adipose tissue, promoting glycogen synthesis in all sensitive tissues and facilitating fat synthesis. Insulin inhibits the breakdown of fat and protein in adipose tissue and muscle.

Reduced insulin levels permit breakdown of glycogen, fat and protein for energy production and for the maintenance of blood glucose levels between food intake. Insulin effects are counteracted by different hormones, (growth hormone, epinephrine, glucagon and cortisol), which play a part in negating the anabolic effects of insulin during starvation, facilitating provision of energy reserves and maintaining blood glucose levels.

Through its metabolic actions, insulin lowers blood glucose and is used in the treatment of diabetes mellitus.

The mixture between insulin human with a rapid-onset and the intermediate acting protamine-insulin human facilitates a twice daily dosage regimen. Biphasic human insulin (biphasic insulin human 30/70) is a premixed suspension of insulin containing the rapid acting, insulin human (30%), and the long acting insulin human-protamine complex (70%). Insulin human is a synonym for human insulin obtained by recombinant DNA technique.

The preclinical evaluation of the present product is based on the documentation for the active ingredients: insulin human and isophane insulin human, the protamine complex contained in Actraphane. The programme includes recent studies performed with the insulin analogue, insulin aspart. In several of these studies, insulin human was used as a reference substance. In addition, other recent studies concerned the mixture of the insulin analogue insulin aspart and protamin-insulin aspart 30/70. Once again in several of these studies, Actraphane 30 (biphasic insulin human 30) was used as a reference substance.

Pharmacodynamics

- **Primary pharmacology programme.**

The programme includes studies performed in the eighties demonstrating the similarity between insulin human and semi-synthetic insulin human, later studies supplementing above studies and recent studies where insulin human 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 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 IGF-1-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, 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 dependent antidiabetic effect.

In a few studies, Actraphane 30, insulin human was used as reference substance in order to show the additional effect of dual-acting insulin aspart 30. The hypoglycaemic effect in the pig of SC injection of dual-acting insulin aspart 30 and Actraphane 30, insulin human was compared.

In this study the blood glucose lowering effect of Actraphane 30 was confirmed.

- **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 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 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 bromsulphthalein-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.

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

Pharmacokinetics

Single Dose Pharmacokinetics Studies The pharmacokinetic profile of biphasic insulin human, after a single doses was explored in several animals species (rats, dogs and pigs).

The pharmacokinetics properties of fast-acting insulin human were investigated after a single dose IV and SC in the rat. In these studies insulin human showed rapid uptake and slower elimination after SC than IV administration. Bioavailability from the SC injection was high. These experiments have all shown that insulin human has regular, predictable kinetics in the rat after SC injection of various high doses.

Fast-acting insulin human was given to dogs in a single dose 0.05, 0.1, or 0.2 U/kg IV and 0.25, 0.5 or 1 U/kg SC to three males and three female dogs in a cross-over experiment. Insulin human was completely absorbed from the SC injection site.

A single dose pharmacokinetic study in the pig where fast-acting insulin human was administered IV and SC showed that $T_{\max}$ equals 79 min [$T_{\max}$ is the time at which the highest drug concentration occurs following administration of an extravascular dose. $T_{\max}$ is expressed in min or hr].

Multiple Dose Pharmacokinetics Studies

The pharmacokinetic profile of biphasic insulin human given at repeated doses was explored in several animals species (rats, dogs and pigs).

A multiple SC dose kinetics study was performed in rats and compared the pharmacokinetic profile of insulin aspart and fast acting insulin human. The kinetics after multiple doses were basically similar to those after single doses injected SC.

The mean values of some of the basic pharmacokinetic parameters are summarised in Table 2 below. It should be noted that this study was designed to show equivalent kinetics between two different formulations of dual-acting insulin aspart. Thus the parameters of Actraphane do not reflect the actual kinetic profile due to the biphasic kinetics of the test substance.

Some basic human pharmacokinetic parameters are shown in Table 2 below. It should be noted that the parameters derived from the method used in the pig pharmacokinetics/ pharmacodynamic study above are not directly comparable to the results obtained by the euglycaemic clamp technique used in the human tests.

Table 2 Pharmacokinetic parameters of dual-acting insulin human and insulin human

		Actraphane 30		Fast acting insulin human			
Admini- stration	Endpoint	Man 031/UK (0.2 U/kg)	Pig NN970010 (0.15 U/kg)	Man 022/UK (0.1 U/kg)	Pig NN950475 (0.125U/kg)	Dog NN960548 (1 U/kg)	Rat NN960550 (6 U/kg)
SC	t _{1/2} (min)	449	174	122	121	57	23
	C _{max} (pM)	93.0	361	102	122	2871	18000
	T _{max} (min)	110	231	145	99	60	15
IV	Cl (l·min/kg)				0.021	0.048	0.058

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

Toxicokinetics

Toxicokinetic studies were done during the 52 weeks repeated dose toxicity studies in the rat and the dog and the Segment II test (teratogenicity studies) in the pregnant rabbit. They demonstrated linearity of the plasma levels of insulin human with the dose, the C_{max}, occurred 1-5 hours after administration of either type of insulin. The plasma levels and AUCs of insulin human remained directly related to dose throughout the 52 weeks of treatment and that the rate of elimination did not increase with time.

Toxicology

Some experiments where Actraphane 30 was used as reference substance for the testing of dual-acting insulin aspart have been performed. 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 subcutaneous at dosage up to 4000 U/kg. In higher dosage groups insulin human was compared to semi-synthetic insulin human. Apart from few sporadic hypoglycaemic reactions on the day of dosing, no treatment related signs were seen. No significant difference between insulin human and semi-synthetic insulin human was observed.

- **Repeated doses toxicity.**

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.

In the rat the highest dose of 200 IU/kg caused hypoglycaemic deaths. In surviving rats only a few signs were seen. In samples taken approximately 20 hours after administration, higher dosage of both insulins lowered plasma concentration of protein. A slightly lowered urea and increased blood glucose were observed as well. These findings are in accordance with other studies on insulin (Andersen *et al*, 1983 and Hansen *et al*, 1986). The observations in the dogs were attributed to the hypoglycaemic state of the animals and not due to toxic effect of insulin human.

human 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.

In the dog study, beagle dogs were given insulin human subcutaneous for 13 weeks. Both dogs given 1 U/kg daily and 3 U/kg daily showed peripheral vasodilatation. Ocular discharge and quiet behaviour were seen in dogs at 3 U/kg daily only. Body weight and food consumption were slightly increased. All these observations were attributed to the hypoglycaemic state of the animals and not due to any toxic effect of the drug.

Beagle dogs were given insulin human 1 U/kg twice daily SC for 12 months. General episodes of hypoglycaemia and one dead animal, led to a change in the dosing regimen. Besides one case of abnormal weight gain, there were no other important effects of the treatments.

- **Genotoxicity.**

The genotoxic potential of insulin human 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 was found non-mutagenic.

Insulin human 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 and an experimental insulin analogue. Dose response curves from seven studies were the same for insulin aspart and insulin human, 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 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 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 doses (200 U/kg) of insulin human 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 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 F₁ animals but no major effect was found.

Embryo-foetal development of rabbits born from pregnant females exposed to insulin human 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. 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. 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.**

Due to the life-long administration of insulin, evaluation of the local irritancy is of particular importance.

The local toxicity after subcutaneous injection has been studied in pigs. Pigs were given subcutaneous injections of different preparations including three dual-acting insulin aspart 30 formulations, Actraphane 30, long-acting protaphane recombinant insulin, 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 Actraphane 30 and long-acting protaphane insulin was similar. The saline and all media preparations caused almost no change.

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.

- **Immunotoxicity studies.**

The immunogenicity of different dual-acting insulin aspart 30 formulations has been compared to several well-known insulin products including Actraphane 30. The findings do not indicate any special risk of Actraphane 30 even after storage under severe conditions. The immunogenicity in this animal model is unfortunately a poor predictor of antibody occurrence in man. The ingredients in Actraphane 30 have however a well-established clinical use and no excessive reports on antibody formation have been received.

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.

In addition, the immunogenicity of insulin human has been studied in Rabbits. Freund's adjuvant and 20 U of respectively insulin human, semi-synthetic insulin human 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 human was found, whereas they both were demonstrated to be significantly less immunogenic than 5-times crystallized porcine insulin. It was concluded, that recombinant human insulin 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 recombinant 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.**

Actraphane 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 original studies for primary and secondary pharmacodynamics was to demonstrate the similarity between the new insulin human and marketed semi-synthetic variant. Newer studies support the conclusion from the original ones that insulin human and semi-synthetic insulin human have identical blood sugar lowering effect. Effects seen in the original and newer 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 human 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.

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 dual-acting insulin, but some experiments (local toxicity studies and an immunogenicity study) where Actraphane was used as a reference substance to the testing of dual-acting insulin aspart are reported. No differences in toxicity between the insulin products dual-acting insulin aspart 30 and Actraphane 30 were seen.

The noted effects on embryos and fetuses 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 months 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 insulin human. 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-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-acting and fast-acting insulins, the two insulin preparations may be mixed (by the patient or as ready-made pre-mixed 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. Although this regimen may not offer optimal glycaemic control, patient compliance is generally better for this simpler regimen than for the multiple injections regimens. Therefore, for some patients, the twice-daily regimen may be an acceptable alternative to the basal-bolus regimens.

Actraphane, insulin human consists of a suspension of fast-acting insulin human and long-acting insulin human. Actraphane, insulin human can be administered before a meal providing both the meal-related requirements of fast-acting insulin as well as the basal requirements in the post-absorptive phase. Clinical experience has demonstrated that in a considerable proportion of diabetic patients, adequate (although not optimal) glycaemic control can be obtained by twice daily injections of Actraphane, insulin human.

Clinical pharmacology

Pharmacodynamics in healthy subject

The pharmacodynamic properties of Actraphane 30, insulin human in healthy subjects were investigated in 3 clinical trials (031/UK, 032/UK, 033/D) involving 75 subjects. The purpose of these trials was to compare the effects of single dose dual-acting insulin aspart 30/70 and single dose Actraphane 30, insulin human on 24-hour serum glucose profiles (study 031/UK) as well as glucose infusion rates during euglycaemic clamp (study 033/D). All three studies were single dose, randomised, double-blind 2 periods crossover trials with a washout period of 4 to 28 days between each treatment. All studies involved healthy subjects from 21 to 41 years with glycosylated haemoglobin [HbA_{1c}] $\leq$ 6.1%, fasting blood glucose [FBG] $\leq$ 6.0 mmol and body mass index [BMI] $\leq$ 27 kg/m². Studies 031/UK and 033/D involved only male subjects (24 in both studies) whereas study 032/UK involved both men and women (27 in total).

Statistical analyses of all efficacy data (except $t_{\min}$, which was analysed by Wilcoxon signed ranks test) were analysed using an analysis of variance (ANOVA) after logarithmic transformation of data.

The **study 031/UK** was a crossover trial in which fasting test subjects received a single subcutaneous injection (in the abdominal wall) of either 0.2 U/kg of dual-acting insulin aspart 30/70 or 0.2 U/kg of Actraphane 30, insulin human. Serum glucose levels were monitored for 24 hours post injection. No food was allowed during this period. Based on the glucose profiles the following parameters were determined and used for comparison of the pharmacodynamic effects of dual-acting insulin aspart 30/70 and Actraphane 30, insulin human: $C_{\min}$ (minimal serum glucose concentration), $t_{\min}$ and AOC (area over the serum glucose concentration curve and below the baseline glucose level). These data demonstrate that the onset of action of Actraphane 30, insulin human is 30 minutes and that the peak action occurs approximately 3 hours after injection. The duration of action is difficult to estimate, but may be as long as 24 hours.

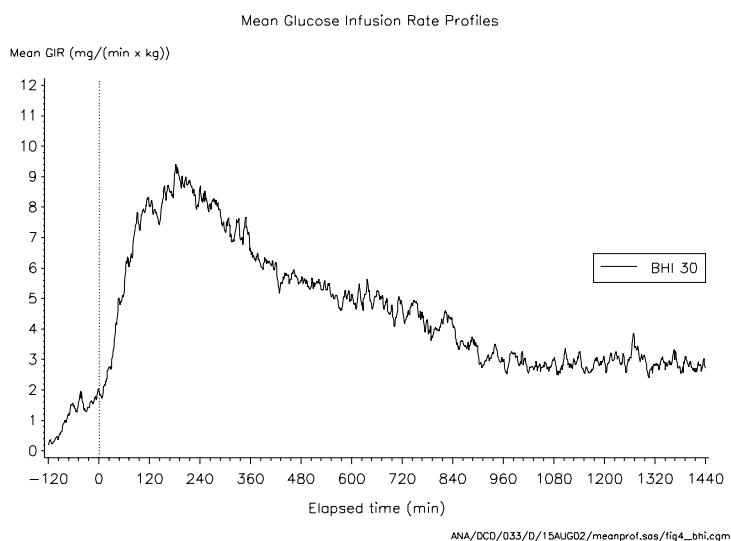
In **study 033/D**, the pharmacodynamic response to a single dose of dual-acting insulin aspart 30/70 or Actraphane 30, insulin human was evaluated during a euglycaemic clamp. In order to suppress the endogenous insulin secretion, each subject received a basal infusion of insulin throughout the study period. By intravenous infusion of glucose, venous blood glucose was clamped at 5 mmol/l. The glucose infusion rate necessary to maintain a constant blood glucose level (i.e. 5 mmol/l) was monitored and recorded for a period of 24 hours following a single subcutaneous injection of Actraphane 30, insulin human (0.3 U/kg). The results of the study are summarised in Table 3 below. The maximal glucose infusion rate (denoted by $GIR_{\max}$) for Actraphane 30, insulin human is shown in Fig. 4.

Table 3: Pharmacodynamic response – healthy subjects – Euglycaemic clamp trial 033/D

Glucose	Actraphane 30		
Endpoint	N	mean	(SD/Q1-Q3 ^b)
AUC _{GIR, 0-90 min} (g/kg)	23	0.39	(0.18)
AUC _{GIR, 6-24h} (g/kg)	23	4.23	(1.26)
GIR _{max} (mg/min×kg)	23	11.2	(2.0)
tGIR _{max} ^a (min)	23	198.5	(129.0-269.0)

a. tGIR_{max} is given as a median value followed by 1st and 3rd quartile

b. Ratios are presented for AUC_{gir} and GIR_{max}; Differences between medians is presented for t GIR_{max}

Figure 1: Mean glucose infusion rate profiles – Healthy subjects 033/D

Pharmacodynamics in diabetic patients

The **Study 046/NL/UK** is a randomised double-blind crossover study in diabetic patients comparing the pharmacokinetic and pharmacodynamic properties of dual-acting insulin aspart 30/70 to Actraphane 30, insulin human. Thirteen patients with type 2 diabetes (as defined by WHO) for at least 12 months (both females and males) were enrolled. Additional inclusion criteria included age, body mass index (< 39 kg/m²) and glycated haemoglobin (HbA_{1c}) <12%. Patients were randomised to receive 2 weeks of treatment with Actraphane 30, insulin human. The product was administered subcutaneously (in the anterior abdominal wall) twice daily (immediately before breakfast and dinner) at dose identical to what the patient had used previously. At the end of each 2 weeks treatment phase, 24-hour serum insulin and serum glucose profiles (starting at dinner) were obtained during treatment with both insulins and ingestion of standardised meals. Patients were allowed to vary their insulin dose according to their need. However, in the treatment phase, initial dose had to be identical to the dose used immediately before that treatment phase. Pharmacodynamic parameters included various serum glucose profiles, the maximal glucose concentration and the time to maximal serum glucose concentration following each meal. Statistical analysis was performed by ANOVA following logarithmic transformation of data.

Table 4 : Pharmacodynamic response – Type 2 diabetic patients – Study 046/NL, UK

Actraphane 30			
Parameter	N	mean	SD
AVE _(0-24h)	13	10.6	2.8
After dinner			
EXC _(0-4h, dinner) (mmol/l×min)	13	13.0	6.4
C _{max} (glu, dinner) (mmol/l)	13	15.0	3.4
t _{max} (glu, dinner) (min)	13	118	46
After breakfast			
EXC _(0-4h, breakfast) (mmol/l·min)	13	23.6	5.5
C _{max} (glu, breakfast) (mmol/l)	13	17.4	3.5
t _{max} (glu, breakfast) (min)	13	133	31
After lunch			
EXC _(19-23h, lunch) (mmol/l×min)	13	16.9	8.5
C _{max} (glu, lunch) (mmol/l)	13	14.9	3.5
t _{max} (glu, lunch) (min)	13	128	40

The pharmacodynamic parameters are listed in Table 4.

In conclusion, the mode of action and pharmacodynamic effects of insulin are well known. Following binding to insulin receptors on muscle and fat cells, insulin facilitates uptake of glucose. The present pharmacodynamic studies demonstrate that Actraphane 30 has biphasic hypoglycaemic effect in healthy subjects: a fairly rapid pronounced effect followed by a more protracted effect. Onset of action is approximately 30 minutes after injection. The maximum effect is reached 2 to 8 hours after injection and duration of action is at least 24 hours. Data presented also indicates that the different proportions of fast-acting insulin human have the expected effect on the time action profile of Actraphane. Finally, uncontrolled data presented suggests that Actraphane has both immediate and protracted glucose-lowering effect in patients with type 2 diabetes.

Pharmacokinetics

The pharmacodynamic studies (031/UK, 033/D and 046/NL, UK) also involved collection of pharmacokinetic data. In all 3 studies pharmacokinetic parameters, based on 24-hour insulin concentration profiles, were determined using standard methods.

Table 5: Pharmacokinetics of Actraphane 30 - Healthy subjects - Singledose

Parameter		Trial ^a	Actraphane 30 Mean or Median [95 % CI]
AUC _(0-90 min)	(mU/l·h)	031/UK	12.5±3.2
		033/D ^b	26.3±7.6
C _{max}	(mU/l)	031/UK	15.5±3.7
		033/D ^b	29.9±8.1
t _{max}	(min)	031/UK	110 (90-180)
		033/D ^b	165 (120-225)
AUC _(6-24h)	(mU/l·h)	031/UK	71.3±12.1
		033/D ^b	261±41
t _{1/2} (min) ^c		031/UK	449 (363-844)

^a All doses were administered s.c. into the abdominal wall
031/UK N=23, dose=0.20 U/kg; 033/D, N=24, dose=0.30 U/kg.

^b euglycaemic hyperinsulinaemic clamp trial

^c t_{1/2} based on the period from 10-24 hours

Pharmacokinetic studies performed in healthy subjects

- **Absorption and bioavailability**

The respective absorption of single dose dual-acting insulin aspart 30/70 and Actraphane 30, insulin human was compared in studies 031/UK, 032/UK and 033/D. (In study 033/D subjects were given a higher dose of Actraphane 30, insulin human than in 031/UK (0.3 U/kg versus 0.2 U/kg). In addition, as part of the clamp design, subjects in study 033/D also received a basal infusion of insulin (to suppress endogenous insulin secretion). Therefore the results from study 033/D are not directly comparable to the results from study 031/UK).

Assessment of the absorption of the soluble fraction with the fast-acting insulin of Actraphane 30 was based on C_{max} , t_{max} and AUC_{ins} , 0-90 min (area under the insulin curve from 0 to 90 minutes). Assessment of the absorption of the protamine bound fraction (long-acting insulin) of Actraphane 30, insulin human was based area under the serum insulin curve from 6 to 24 hours (AUC_{ins} , 6-24h). Results are listed in Table 5. As previously mentioned the results from studies 031/UK and 033/D are not directly comparable but the pattern was identical.

As it is known that the elimination half-life of intravenously injected insulin is fairly short, the time-concentration curves are primarily determined by the rate of absorption. Thus, the presented data indicates that Actraphane 30 has a biphasic absorption. The fraction with fast acting insulin is absorbed quickly with a t_{max} of 2 to 3 hours. The long acting, protamine bound fraction has a more prolonged absorption. Study 011/US demonstrates that for Actraphane 50 C_{max} is reached slightly faster than for Actraphane 30. In addition, AUC during the first 4 hours after injection is higher for Actraphane 50 than for Actraphane 30 (see Table 5)

Table 5: Pharmacokinetic results in study 011/US

Parameter	Actraphane 50 N=(31)	Actraphane 30 (N=31)
$AUC_{(0-4)}$	37433.5 (11177.1)*	304½5.0 (9708.3)
$AUC_{(4-8)}$	25668.4 (9061.0)	23517.1 (8481.2)
$AUC_{(8-12)}$	11484.2 (6088.3)	12910.6 (5094.4)
$AUC_{(0-12)}$	84435.8 (22503.2)	76291.8 (21815.8)
C_{max}	220.8 (57.3)*	178.5 (57.1)
T_{max} (min)	184.8 (84.5)	202.3 (108.7)

* Significantly different between the 2 treatment groups at 0.05 level

- **Bioavailability**

No studies of absolute bioavailability could be performed as biphasic insulin human cannot be injected intravenously.

- **Distribution**

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

- **Elimination**

Metabolism

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

Excretion

Actraphane, insulin human cannot be administered intravenously, thus clearance after IV administration could not be studied. As the half-life of intravenously injected fast-acting insulin human is relatively short, the terminal half-life of insulin human following subcutaneous injection of Actraphane, insulin human is a measure of the terminal absorption rather than the elimination of insulin from plasma *per se*. The terminal half-life ($t_{1/2}$) of insulin human following SC injection of Actraphane, insulin human was 7 to 8 hours (Table 4), indicating a delayed absorption of the long-acting protamine bound fraction.

- **Pharmacokinetics in the target population**

Diabetic patients

In study 046/NL, UK the pharmacokinetics of Actraphane 30, insulin human in patients with type 2 diabetes was assessed. Actraphane 30, insulin human was administered as subcutaneous injections twice daily immediately before breakfast and dinner. At the end of each treatment period 24-hour serum insulin concentration profiles were obtained during continued treatment with Actraphane 30, insulin human and ingestion of standardised meals. The pharmacokinetic are listed in Table 5.

Table:6: Pharmacokinetics in patients with type 2 diabetes – study 046/NL, UK.

Parameter	Actraphane 30 ^a
AUC _(0-2 h, dinner) (mU/l·h)	114±66
AUC _{0-2 h, breakfast)} (mU/l·h)	102±55
t _{max} (after dinner) (min)	124 (95-125)
t _{max} (after breakfast) (min)	155 (126-182)
C _{max} (after dinner) (mU/l)	79±43
C _{max} (after breakfast) (mU/l)	81±45
AUC _{0-24 h} (mU/l·h)	1095±627

N=13, dose individualised

^a, total serum insulin;

- **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. It is known that the liver, the kidneys and the muscles are primary sites of insulin degradation. Renal and hepatic impairment may reduce insulin degradation and thus reduce insulin requirements.

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. There are no literature reports of direct pharmacokinetic interactions between insulin and other products. The products which interfere with glucose metabolism through various mechanisms are well identified.

- **Conclusion on pharmacokinetic studies.**

As it is known that the elimination half-life of intravenously injected insulin is fairly short, the time-concentration curves are primarily determined by the rate of absorption. Thus, the presented data indicates that Actraphane 30, insulin human has a biphasic absorption.

The soluble fraction is absorbed quickly with a $t_{\max}$ of 2 to 3 hours. The protamine bound fraction has a more prolonged absorption. Study 011/US demonstrates that for Actraphane 50, insulin human $C_{\max}$ is reached slightly faster than for Actraphane 30, insulin human.

No formal distribution studies were performed with Actraphane, insulin human. It is established that human insulin is not bound to plasma proteins unless circulating insulin antibodies are present.

Metabolism of Actraphane, insulin human was not formally investigated as well. From previously published data it is known that human insulin is degraded by various proteases. The degradation products are not active. Presumably, these degradation products are broken down into amino acids.

The terminal half-life ($t_{1/2}$) Actraphane, insulin human following SC injection was 7 to 8 hours, indicating a delayed absorption of the protamine bound fraction.

The applicant has not submitted any data on the pharmacokinetics in patients with impaired renal/hepatic function. It is known that the liver, the kidneys and the muscles are primary sites of insulin degradation. Renal and hepatic impairment may reduce insulin degradation and thus reduce insulin requirements.

There are no formal studies of the effect of gender, age or race on the pharmacokinetics of Actraphane, insulin human. As insulin doses are titrated individually, the lack of these studies is considered acceptable.

Clinical efficacy

Main study (phase III = therapeutic confirmatory trials).

Table 7: Phase III trials

Studies	Population (Number of patients)	Design	Objectives
038/D, UK	Type 1 and type 2 diabetic patients. (294 patients were randomised in the study and 268 completed the trial).	Multicentre parallel groups open-label randomised active controlled.	Compare the efficacy of dual-acting insulin aspart 30/70 with Actraphane 30, insulin human on the glycaemic control evaluated by the glycosylated haemoglobin [HbA _{1c}] after 12 weeks of treatment.

The documentation for the clinical efficacy of dual-acting insulin aspart (30/70) consists of a single multinational (multicentre parallel groups open-label randomised active controlled) phase III trial [038/D, UK].

1. Description of the study

The objective was to compare the efficacy of dual-acting insulin aspart 30/70 with Actraphane 30, insulin human on the glycaemic control evaluated by the glycosylated haemoglobin [HbA_{1c}] after 12 weeks of treatment.

The patients enrolled in the study consisted of male and female patients with type 1 or 2 diabetes mellitus for at least 24 months. All subjects had been on Actraphane 30, insulin human for at least 12 months prior to inclusion into the trial. Additional inclusion criteria included a body mass index (denoted by BMI) ≤ 35.0 kg/m², and glycosylated haemoglobin (HbA_{1c}) ≤ 11.0 %. Patients with renal and hepatic insufficiency and patients with severe cardiac insufficiency were excluded from the study.

Patients were randomised to receive either dual-acting insulin aspart 30/70 or Actraphane 30, insulin human administered SC twice daily. Actraphane 30, insulin human was injected 30 minutes before breakfast and dinner. Dosing was individual and adjustments during the trials were allowed. Actraphane 30, insulin human was injected in the anterior abdominal wall or the thigh according to local practice.

2. Primary endpoints/assays

The primary efficacy endpoint was the glycosylated haemoglobin (HbA_{1c}) measured after 12 weeks of treatment. Secondary parameters were derived from 8-point blood glucose (BG) profiles obtained after 12 weeks treatment: These parameters were firstly, the prandial blood glucose increment defined as the mean difference between the blood glucose value 90 minutes after the meal and the blood glucose value just before the meal over three meals, the average of the 8 blood glucose values for each subject and finally the blood glucose range of the 8 blood glucose values for each subject.

The assessment of the safety profile of the products was based on the collection of all adverse events and the measurement of different parameters (including haematology, biochemistry, lipid profile, antibodies directed against insulin, vital signs, physical examination and electrocardiogram).

3. Statistical analysis

All efficacy analyses were based on the intention to treat (ITT) population (described in ICH E9 as being the patients who were followed-up, assessed and the data analysed irrespective of their compliance to the planned course of treatment). For the primary efficacy parameter, a per-protocol (PP) analysis (defined in ICH E9 as an analysis performed on the data obtained from patients who complied sufficiently with the protocol to ensure that the data obtained with these patients reflected the effects of treatment) was also performed. For the primary endpoint (HbA_{1c}) the comparison between biphasic insulin aspart 30/70 and biphasic insulin human 30/70 was based on a non-inferiority criterion. The non-inferiority threshold was set at an absolute difference of 0.6%. In other words, non-inferiority was claimed to be established if the upper limit of the 95% confidence interval estimating the difference between the two treatments [i.e. biphasic insulin aspart 30/70 and biphasic insulin human 30/70] was less than 0.6%. Primary and secondary endpoints were analysed using different ANOVA models.

RESULTS

4. Study population

A total of 294 patients were randomised in the study and 268 completed the trial. Baseline demographic characteristics are listed in tables 8 and 9 respectively. Approximately one third of the patients had a type 1 diabetes (the others had type 2 diabetes). Average duration of disease was 15 and 15.3 years, respectively. Baseline HbA_{1c} was 8.24% in the Actraphane 30, insulin human group.

Table 8: Patients – Study 038/D, UK

	Actraphane 30
Randomized	151
Exposed	151 (100%)
Withdrawals in Treatment Period	
Adverse Event	3 (2.0%)
Ineffective Therapy	
Non-compliance	3 (2.0%)
Other	3 (2.0%)
Total	9 (6.0%)
Completed trial	142 (94%)
Efficacy Populations	
Intention to treat	145 (96%)
Per-Protocol	136 (90%)

Table 9: Baseline demographic characteristics – Study 038/D, UK

Characteristic	Actraphane 30
Number of subjects exposed	151
Age (years) Mean (SD)	58.1(12.9)
BMI (kg/m/m)Mean (SD)	27.3 (3.7)
Sex (males/females)	53%/47%
Race (Caucas/Black/Other)	99%/<1%/<1%
Smoker (yes/no)	19%/81%

5. Efficacy results

The data concerning the primary efficacy parameter (HbA_{1c}) were analysed by ANOVA. After adjustment for baseline HbA_{1c} and for centre, the level of HbA_{1c} measured at 12 weeks was 8.15% in Actraphane 30, insulin human treated patients (ITT analysis).

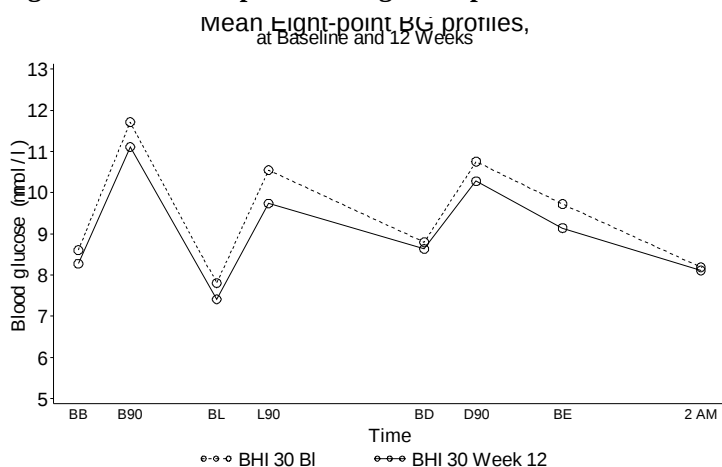
Table 10: Efficacy results for study 038/D, UK

	Actraphane 30		
	N	Mean	(SEM)
$HbA_{1c}(\%)$	143	8.15	(0.06)
Prandial			
BG Increment(mmol/l)	141	2.34	(0.19)
BG Range(mmol/l)	141	8.06	(0.29)
BG Average(mmol/l)	141	9.12	(0.19)
Total Biphasic			
Insulin Dose (U/kg)	145	0.62	(0.01)

All adjusted for baseline value and centre, (ITT population)

The statistical analysis of the results concerning HbA_{1c} (using an analysis of variance – ANOVA) showed that, with regard to treatment effect, there was no significant difference between type 1 and type 2 diabetic patients. Demographic and prognostic variables had a significant effect on HbA_{1c} levels at 12 weeks but there was no treatment interaction. ANOVA analysis with adjustment for rate of hypoglycaemic events was in agreement with the results of the primary analysis. No statistically significant treatment interaction was observed.

The efficacy of Actraphane, insulin human has been assessed with the following secondary endpoints: Mean 8-point blood glucose profiles at baseline and after 12 weeks of treatment with Actraphane 30, insulin human. The results are shown in Figure 2. An analysis (ANOVA) on the measurements of the blood pressure measurements at 12 weeks is shown in Table 11.

Figure 2: Mean 8 point blood glucose profiles at baseline and after 12 weeks – Study 038/D, UK

Note: BI = Baseline

ANA/DCD/038/D-UK/15AUG02/fig6_bhi.sas/fig6_bhi.cgm

BB = before breakfast, B90 = 90 min after breakfast,
 BL = before lunch, L90 = 90 min after lunch,
 BD = before dinner, D90 = 90 min after dinner,
 BE = bedtime, and 2 AM = 2 a.m.

Table 11: Results of the statistical analysis (ANOVA) performed blood glucose levels taken at 8 different periods after 12 weeks – study 038/D, UK

Time of Day	Actraphane 30		
	N	Mean	(SEM)
Before breakfast	143	8.24	(0.27)
Breakfast + 90 min.	142	11.4	(0.36)
Before lunch	143	7.57	(0.27)
Lunch + 90 min.	142	9.97	(0.27)
Before dinner	143	8.72	(0.29)
Dinner + 90 min.	142	10.2	(0.32)
Bedtime	142	9.10	(0.30)
2 am	135	8.12	(0.25)

Discussion on clinical efficacy

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 long-acting isophane 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 (in certain subgroups of patients even better glycaemic control can be obtained by administering insulin as a continuous subcutaneous infusion in combination with subcutaneous boluses at meals). However a number of patients, especially patients with type 2 diabetes can be adequately regulated by twice daily injections of long-acting insulin with or without concomitant injection of fast-acting insulin. For these patients ready-mixed long-acting insulin and fast-acting insulin such as Actraphane is a convenient alternative. The present data indicates that in certain type 1 and type 2 diabetic patients previously having a favourable response to twice daily biphasic insulin human, acceptable glycaemic control can be maintained by twice daily injections of Actraphane.

Clinical safety

Patient exposure

The pharmacodynamic properties of Actraphane 30, human insulin in healthy subjects were investigated in 3 clinical trials (031/UK, 032/UK, 033/D) involving 75 subjects. A total of 294 patients were randomised and 268 completed the multinational phase III trial [038/D, UK].

An important marketing experience with human insulins has been gathered since human insulins have been marketed for almost ten years. (see paragraph post-marketing experience below).

Adverse events and serious adverse event/deaths

In the single dose trial in fasting subjects (study 031/UK and 032/UK), 40% of all subjects exposed to Actraphane 30, insulin human reported at least one adverse reaction. The majority of the adverse reactions were mild and related to hypoglycaemia. The high frequency of reactions in study 031/UK compared to the study 033/D was probably due to the fact that both studies 031/UK and 032/UK involved prolonged fasting without glucose infusion whereas in study 033/D glucose was infused to maintain euglycaemia.

In the phase II trial 7 patients out of 13 reported at least one reaction while on Actraphane 30, insulin human.

In the phase III trial, amongst the Actraphane 30, insulin human treated patients 75 patients out of 151 reported 174 adverse reactions. Table 12 summarises the AEs reported in study 038/D/UK.

Table 12: Adverse events reported in study 038/D/UK

	Healthy Subjects Actraphane		
	N	%	E
<i>Number of subjects exposed</i>	151		
<i>All Adverse Events</i>	75	(50%)	174
<i>Trial product relation:</i>			
Probable	2	(1%)	5
Possible	6	(4%)	8
Unlikely	69	(46%)	141
Unknown	7	(5%)	20
<i>Severity:</i>			
Mild adverse events	58	(38%)	115
Moderate adverse events	32	(21%)	52
Severe adverse events	6	(4%)	7
<i>SOCs in which AEs were reported in >5% of subjects exposed:</i>			
Centr & periph nervous system disorders	22	(15%)	34
Headache	14	(9%)	24
Respiratory system disorders	23	(15%)	33
Upper resp tract infection	7	(5%)	8
Pharyngitis	5	(3%)	5
Gastro-intestinal system disorders	24	(16%)	31
Diarrhoea	5	(3%)	5
Body as a whole – general disorders	10	(7%)	12
Influenza-like symptoms	5	(3%)	5
Musculo-skeletal system disorders	8	(5%)	9
Skin and appendages disorders	7	(5%)	8
Resistance mechanism disorders	3	(2%)	3

N = Number of subjects with event

% = Proportion of exposed subjects having the event

E = Number of adverse events

SOC = System Organ Class

The majority of the reported events were mild to moderate in severity and considered unlikely to be related to treatment.

Serious adverse reactions

An adverse reaction (or event) is considered serious when at any dose it results in death, requires inpatient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability/incapacity or is life-threatening. No such serious adverse reactions were reported in phase I and II trials. In the Actraphane 30, insulin human treated group, 8 patients reported a total of 9 serious experiences (hypoglycaemia (twice in the same patient), urinary tract infection, bundle branch block, cranial nerve lesion, pancreatic adenocarcinoma, neuropathy, uterine carcinoma, angina pectoris). In all of the cases, causal association with Actraphane 30, insulin human treatment was considered to be unlikely related to the product.

No deaths were reported in Actraphane 30, insulin human treated patients.

Among all subjects exposed to Actraphane 30, insulin human, three cancers were reported (pancreatic adenocarcinoma, uterine carcinoma, renal carcinoma). In all of the cases, causal relation to treatment was considered unlikely.

Discontinuation due to adverse events

In the phase I and II trials, three patients were withdrawn due to the occurrence of an adverse reaction. In the phase III trial, three subjects in the Actraphane 30, insulin human group were withdrawn due to a reaction to the treatment. The reactions leading to withdrawal were abdominal pain, nausea, pain in one patient and rash and neuropathy in the two other patients.

Hypoglycaemic episodes

Major hypoglycaemic events were defined as hypoglycaemic events with severely impaired consciousness that require either third party assistance (Major type A) or intervention with IV glucose or glucagon (Major type B). Episodes with symptoms of hypoglycaemia (actual blood glucose measurement not required), which did not fulfil the definition of major hypoglycaemic events, were classified as minor hypoglycaemic events.

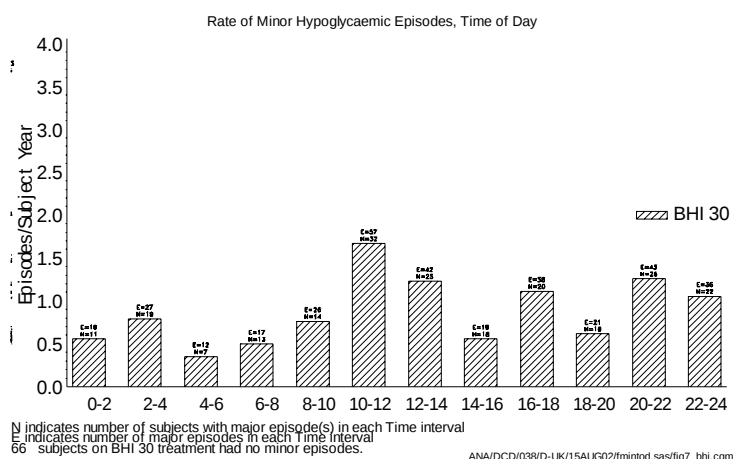
- **Major hypoglycaemic episodes**

Seventeen patients treated with Actraphane 30, insulin human reported a total of 36 major hypoglycaemic events (28 type A and 8 type B).

- **Minor hypoglycaemic episodes**

Eighty six patients in the Actraphane 30, insulin human group reported 361 minor hypoglycaemic events. The diurnal distribution of minor hypoglycaemic events is depicted in Figure 3.

Figure 3: Rate of minor hypoglycaemic episodes during the day



Insulin antibodies

Levels of insulin antibodies specific to human insulin remained unchanged throughout the 12-week treatment period..

Increase in cross-reactive antibody levels did not influence change in glycosylated haemoglobin (HbA_{1c}) nor did it correlate with dose of insulin, indicating that the increase in levels of cross-reactive insulin antibodies did not have a negative effect on efficacy. The increase in antibody levels did not result in any adverse events (including allergic reactions) that could be specifically linked to the antibody. Two cases of potentially hypersensitivity reactions with probable or possible relation to treatment were reported (the two patients treated with biphasic insulin human 30/70 experienced a rash and an injection site reaction).

Laboratory findings

Thirteen laboratory test abnormalities (five in the Actraphane 30, insulin human group) were reported. None of these were considered clinically relevant and primarily involved small increases in serum triglycerides or serum cholesterol levels.

Post-marketing experience

An extensive post-marketing experience (more than 31 million patient years of exposure) has been gathered with human insulin since 1988 when the first genetically engineered human insulin was marketed. Two periodic safety update reports (PSURs) covering the period from March 1993 to end of June 2000 have been assessed and no new concern has been raised following assessment of postmarketing experience.

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 has 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, neither could an update of this paper including literature research up to May 1997.

The most common reactions were hyper- and hypoglycaemia, injection site reaction and pain, therapeutic response decreased, allergic reaction and rash or pruritus.

A total of 29 serious adverse reaction reports associated with fast-acting administration were classified as serious unlisted. Of these a total of three cases of toxic epidermal necrolysis/Stevens Johnson syndrome have been reported. As of 30 August 2000, a total of 6 cases of epidermal necrolysis/Stevens Johnson syndrome/erythema multiforme have been reported in association with insulin human. In 5 of these cases concomitant medication provided a more likely explanation than insulin human.

In the last PSUR (1999-2000) concerning fast-acting insulin, the Company received 20 reports of impaired liver function (9 of these being serious). All these cases occurred in Japan. No reports on impaired liver function were received from other countries. Such reports have already been published (especially increased liver enzymes in patients with non-insulin dependant diabetes mellitus) and they are generally associated with overweight. According to evidence from three studies liver enzyme increases are most likely related to diabetes mellitus non insulin-dependent/treatment with oral antidiabetic agents but not to insulin. Many of the reports involved semisynthetic, - but not genetically engineered insulin. The hypothesis of an idiosyncratic reaction was ruled out by the Company since no other signs of hypersensitivity were observed and no eosinophile granulocytes were found in the biopsies.

During the reporting 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.

Based on the review of the safety data from the extensive post marketing experience, no new safety issue to be included in the product information was identified. The most frequent adverse reactions are hypo- or hyperglycaemia. The safety profile of Actraphane is 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

As the active component of biphasic insulin human is similar to Actraphane HM(ge), no new specific pharmacodynamic studies in laboratory animals have been conducted for this application. Effects seen in the original and newer safety pharmacology studies can all be related to hypoglycaemia.

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. There was no increase of mammary gland hyperplasia or tumours in the 12-month dog study. The newer studies conducted since the original marketing authorisation for insulin human do not give reason for new safety concerns.

Efficacy

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 (in certain subgroups of patients even better glycaemic control can be obtained by administering insulin as a continuous subcutaneous infusion in combination with subcutaneous boluses at meals). 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. For these patients ready-mixed NPH insulin and soluble insulin such as Actraphane is a convenient alternative. The present data indicates that in certain type 1 and type 2 diabetic patients previously having a favourable response to twice daily BHI, acceptable glycaemic control can be maintained by twice daily injections of Actraphane.

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 Actraphane is well-described and acceptable.

Benefit/risk assessment

Based on the submitted documentation on the pharmacodynamics and pharmacokinetics of Actraphane, it is considered adequately demonstrated that Actraphane has the proposed dual action. Based on the clinical data as well as the well-established use of biphasic HI, the efficacy and safety of Actraphane 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 Actraphane 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.