CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-868

PHARMACOLOGY REVIEW

MEMORANDUM
Jan. 27, 2006
TO: File
FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-868

I concur that the pharmacology/toxicology sections of the product label for EXUBERA® Blister Insulin (Human) Inhalation Powder EXUBERA Inhaler is adequate and that the product may be approved based on n onclinical data.

_____
Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director for Pharmacology and Toxicology
Office of Drug Evaluations II & III

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/s/

Kenneth Hastings
1/27/2006 10:08:17 AM
PHARMACOLOGIST

4 Page(s) Withheld

8 § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(4) Draft Labeling

_____ § 552(b)(5) Deliberative Process

Withheld Track Number: Pharm/Tox-_____

NDA 21-868
Suggested revisions to Pharm/tox sections of sponsors proposed labeling

Carcinogenesis, Mutagenesis, Impairment of Fertility
Two-year carcinogenicity studies in animals have not been performed. Insulin was not mutagenic in the Ames bacterial reverse mutation test in the presence and absence of metabolic activation. Because recombinant human insulin is identical to the endogenous hormone, reproductive/ fertility studies were not performed in animals.

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

Nursing Mothers
Many drugs, including human insulin, are excreted in human milk.. For this reason, caution should be exercised when EXUBERA is administered to a nursing woman. Patients with diabetes who are lactating may require adjustments in EXUBERA dose, meal plan, or both.

Re: Carcinogenicity, Mutagenicity, Fertility section
The 6-month toxicology data recommended for deletion are not relevant to the categories listed, In addition, animal toxicology data are generally only described in the labeling when there are safety issues not adequately characterized by the clinical data, and then in a separate " Animal Toxicology" section.

Re: Nursing Mothers
It is contradictory to state " many drugs including human insulin are excreted in human milk" and then '

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/s/

Jeri El Hage
7/27/05 12:03:31 PM
PHARMACOLOGIST
pharm/tox labeling recommendations

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-868**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**12/27/2004**
PRODUCT:	**EXUBERA® (Inhaled insulin)**
INTENDED CLINICAL POPULATION:	**Diabetes**
SPONSOR:	**Pfizer**
DOCUMENTS REVIEWED:	**Pharmacology /Toxicology (e NDA)**
REVIEW DIVISION:	**Division of Metabolism and Endocrine Drug Products Drug Products (HFD-510)**
PHARM/TOX REVIEWER:	**Fred Alavi, Ph.D.**
PHARM/TOX SUPERVISOR:	**Jeri ElHage, Ph.D.**
DIVISION DIRECTOR:	**David Orloff, MD**
PROJECT MANAGER:	**Oluchi Elekwachi, PharmD.**

Date of review submission to Division File System (DFS): **July 27, 2005**

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Approval

Recommendation for nonclinical studies: The available non-clinical data supports the safety of the inhalation insulin, EXUBERA® in humans (0.15 mg/kg/day). Due to the extensive nonclinical and clinical experience with insulin, carcinogenicity and reproductive toxicology studies were not performed. No further studies are needed.

B. Recommendations on labeling:
Carcinogenesis, Mutagenesis, Impairment of Fertility
Carcinogenicity and fertility studies were not performed. Insulin was not mutagenic with or without metabolic activation in the Ames test with *Salmonella typhimurium* and *Escherichia coli.*

Pregnancy Category C
Animal reproductive studies have not been performed with EXUBERA®. Whether EXUBERA® can cause fetal harm when administered to pregnant women is unknown.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings:

EXUBERA® is a new recombinant human insulin inhalation (INH) formulation being developed by Pfizer for the treatment of diabetes. To enhance the bioavailability and stability of powder insulin for inhalation, several formulations had been tested using the excipients, mannitol, sodium citrate and glycine. Early in the development, the I-004 formulation containing 20% insulin (Lilly) was prepared and used in all the non-clinical studies. Later in the development, an [redacted] insulin (Aventis) was developed. The [redacted] formulation was used in the phase [redacted] clinical [redacted]-month bridging toxicity study in rats. This formulation [redacted] will be marketed commercially.

Due to extensive clinical experience with insulin, the scope of the nonclinical evaluations was limited to 6-month rat and monkey inhalation toxicity studies. The objectives of the toxicology studies were to evaluate the impact of the inhaled powder insulin on the lung tissue, pulmonary function and antibody formation. The carcinogenicity, reproductive toxicology and genotoxicity of INH were not tested. With hypoglycemia limiting the insulin dose, the 6 mg/kg/day dose in rats or 0.6 mg/kg/d in monkeys were considered the MTD, thus they were the highest dose tested in the respective species. As expected, administration of inhalation insulin generally produced a dose-related decrease in glucose and increase in serum insulin levels in both rats and monkeys. The doses used in the 6-month rat study of 0.9, 2.7 and 5.8 mg/kg/d via nose only were approximately 1, 3 and 6X the clinical dose of 0.15 mg/kg/day based on mg/m^2. In the 6-monkey study the evaluated doses of 0.29 and 0.64 mg/kg/d via dome were approximately 0.6 and 1.4X the clinical dose of 0.15 mg/kg/day based on mg/m^2.

Administration of INH to rats produced sporadic increases in lung weights. However, the increases in lung weights were not consistent across studies. In the 1-month rat study, the increase in lung weight occurred in males at low dose (1.1 mg/kg/d) and in females at high dose (6 mg/kg/d) while in the 6-month rat study there was an increase in lung weight in females at mid dose (2.7 mg/kg/d). There was no increase in lung weight in either the 1-month rat bridging study or in the 1-month monkey studies. In the 6-month monkey study slight increase in lung weight (27%) was noted in the low dose female (0.29 mg/kg/d) monkeys. When lung tissues were evaluated histologically, incidences of focal and multifocal inflammation and aggregation of alveolar histiocytes (foam cells) were seen in all groups in both 6-month rat and monkey studies. In the 1-month bridging study in rats there was minimal focal thickening of the interstitium (associated with alveolar inflammation) in 1 mid dose (3.2 mg/kg/d) and 1 high dose (5.9 mg/kg/d) female. Since alveolar inflammation is occasionally observed in healthy animals, the findings may be incidental. There was no evidence of insulin or placebo-related increase in lung cell proliferation in lung tissues from the 6-month rat and monkey studies. It appears that most of the respiratory tract findings may have been related to mechanical trauma due to alveolar lavage or the stress from the drug delivery method. Evaluation of the pulmonary function in the rat was unremarkable. There were no drug or excipient related change in respiratory rate, lung volumes and pulmonary compliance or lung resistance in rats. In the 6-month monkey study, there was a significant decrease in lung compliance in males at the high dose and an increase in minute volume in both sexes at high dose of 0.64 mg/kg/d (1.4X clinical dose, based on mg/m^2). The decrease in compliance in high dose males suggests there was some decrease in elasticity of the lung tissue in males but not in females. During the clinical observation, monkeys treated with excipient and insulin had frequent incidences of coughing and sneezing throughout the 6-month study.

Inhalation insulin had no effect on body weight, food intake or ophthalmic parameters in either rats or monkeys. There were no notable ECG abnormalities in either 1- or 6-month monkey studies. Blood pressure parameters were not measured in the chronic monkey studies. However, in dogs, administration of insulin by the intravenous route increased the heart rate and the systolic blood pressure which are in agreement with inotropic and chronotropic properties of acute insulin administration in the literature.

As a protein, the recombinant human insulin in EXUBERA® has the potential to elicit immunogenic response, i.e. antibody formation and consequent insulin neutralization. Analysis of serum insulin antibodies found slight increases in human insulin antibody concentrations in several serum samples in rats. Since human insulin sequence is different from rat insulin, the slight increases in antibody formation in rats is not unexpected. In the monkey, with insulin sequence similar to humans, no increase in antibody titer was detected. Lung lavage samples collected during the chronic rat and monkey studies found no measurable concentrations of antihuman insulin antibodies. There was no evidence of diminished insulin activity or serum insulin accumulation with repeated administration of inhalation insulin. However, one of the most consistent findings across all non-clinical studies was the variability in inhalation insulin relative bioavailability. The relative bioavailability was likely affected by insulin formulation, the delivery system, species variation and pulmonary insulin clearance. The mean

relative bioavailability of I-004 formulation was about 44% in rats and 12% in monkeys. The relative bioavailability of [redacted] formulation was about 7 and 10% in dogs and humans, respectively. This is not unexpected since insulin is rapidly degraded in the lungs and in the circulation.

Although there appeared to be more frequent coughing and sneezing in monkeys, occasional increases in lung weights in rats and sporadic alveolar inflammation in rats in the 1-month bridging study, no clear insulin related pulmonary toxicity was observed. Therefore the maximum dose in rats (5.8 mg/kg/d) and monkeys (0.64 mg/kg/d) were considered no observed adverse effect level (NOAEL). The safety margins based on the NOAEL dose in rats and monkeys were approximately 6X and 1.4 X the clinical dose of 0.15 mg/kg/d based on mg/m^2, respectively. The non-clinical studies provided significant safety margins for the excipients, mannitol, sodium citrate and glycine (Rats: 34, 40 and 20X, Monkeys: 8, 9 and 4X the clinical dose, based on mg/m^2, respectively).

B. Pharmacology:
Insulin is an endogenous protein released by the pancreatic β cells in response to increases in blood glucose concentrations. The primary function of insulin is to lower blood glucose by stimulating peripheral glucose uptake by skeletal muscle and fat, and by inhibiting hepatic glucose production. Other functions attributed to insulin include inhibition of lipolysis in the adipocyte, inhibition of proteolysis, and enhancement of protein synthesis. Insulin receptors are found in nearly all tissues. *In-vitro* studies have demonstrated presence of insulin receptors in rat alveolar Type II epithelial cells (Sugahara et al, 1984). Whether there are active insulin receptors in human lungs has not been well demonstrated.

C. Nonclinical safety issues relevant to clinical use:
The primary limitations of the toxicology studies with pharmacologically active proteins such as insulin have always been the inability to test high doses in the non-clinical setting. The potential utility of both rat and monkey toxicology studies were therefore limited by low insulin doses due to hypoglycemia and stressful mechanism of insulin delivery, low number of animals per treatment, limited duration and variability in insulin exposure. Whether higher doses and less traumatic delivery procedure would have clearly identified the lung as the target organ is unknown.
With extensive clinical experience with subcutaneous insulin, carcinogenicity and reproductive toxicology studies were not performed. It is highly unlikely that carcinogenicity studies would have resolved any pulmonary safety concern regarding chronic use of inhalation insulin in humans due to insulin delivery method and the potential for tumorigenic pulmonary response to proteins in rodents.

2.6 PHARMACOLOGY / TOXICOLOGY REVIEW

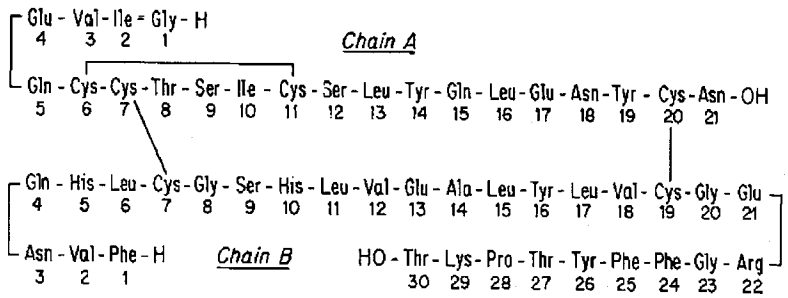
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-868
Review number: 1
Sequence number/date/type of submission: Dec 27, 2004
Information to sponsor: Yes () No (X)
Sponsor and/or agent: Pfizer Inc.
Manufacturer for drug substance: Pfizer will be using insulin manufactured by Aventis

Reviewer name: Fred K. Alavi
Division name: Division of Metabolic and Endocrine Drug Products
HFD #: 510
Review completion date: July 26, 2005

Drug:

Trade name: EXUBERA®
Generic name: INH, inhaled human insulin
Code name: CP-464,005 (aerosol insulin)
Chemical name: recombinant human insulin
CAS registry number: 11061-68-01
Molecular formula/molecular weight: $C_{257}H_{383}N_{65}O_{77}S_6$, MW 5808
Structure: synthetic 51 amino acid polypeptide consisting of 2 polypeptide chains



Relevant INDs/NDAs/DMFs: IND 43,313 (inhalation insulin, Pfizer)
Drug class: antidiabetic drug
Intended clinical population: Type 1 and type 2 diabetics
Clinical formulation:

Active ingredient: Insulin, 1 and 3 mg strength blister packs (Aventis insulin, / HMR4006, formulation

Inactive ingredients: Mannitol , glycine, sodium hydroxide, sodium citrate

Clinical Dose: starting dose of 0.15 mg/kg/day
Route of administration: pulmonary inhalation (aerosolized powder insulin)

Disclaimer: Tabular and text information are constructed by the reviewer except for the tabular or graphical images that appear slightly shaded that are derived from the sponsors' submission.

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA21-868 are owned by Pfizer or are data for which Pfizer has obtained a written right of reference. Any information or data necessary for approval of NDA21-868 that Pfizer does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Pfizer does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA21-868.

Studies reviewed within this submission:

Cardiovascular Safety pharmacology study in dogs
1-month bridging study in rats,
Serum and alveolar lavage analysis for insulin antibodies
Genotoxicity tests

Studies <u>not</u> reviewed within this submission (appendix, page 82):

1- and 6-month rat toxicology studies (reviewed under IND 43,313)
1- and 6-month monkey toxicology studies (reviewed under IND 43,313)

2.6.2 PHARMACOLOGY

Traditionally, insulin has been injected by subcutaneous route to control glucose and prevent hyperglycemia in type 1 and type 2 diabetic subjects. To make insulin administration less stressful to the patients, insulin has been formulated for delivery by pulmonary, oral, buccal and intranasal routes. The pulmonary system, with large surface area and high blood perfusion rate (5 liters of blood/min) and thin alveolar-capillary barrier is an ideal drug delivery route. In this application, Pfizer in partnership with Aventis (formerly Hoechst Marion Roussel) and Nektar (formerly Inhale Therapeutic Systems, Inc.) has developed a dry powder inhaled human insulin (INH) formulation with particle size less than [redacted] microns that allows insulin to be delivered by pulmonary route with sufficient bioavailability.

All the clinical and non-clinical studies prior to Feb 1999 used insulin from [redacted] Clinical studies after Feb 1999 used insulin from Aventis ([redacted] insulin, [redacted]/HMR4006). Aventis insulin ([redacted] was manufactured using recombinant DNA technology with E.coli strain K12. The amino acid sequence of [redacted] was identical to endogenous human insulin. Nearly all the toxicology studies were performed with I-004 insulin formulation with Lilly insulin (20%) containing a [redacted] (see table). The insulin [redacted] formulation used in the phase 1-3 studies, will be also marketed commercially with the trade name EXUBERA®. Since the to be marketed formulation was different than the non-clinical formulation, a 1-month rat bridging study with the clinical formulation ([redacted] was performed to confirm the toxicological equivalence of the two formulations (I-004 and [redacted]).

Formulations Used in clinical and non-clinical studies

Formulation Identity	I-033, Phase 1	I-004, pre-clinical & Phase 1-2	I-005, Phase 1	[redacted]/97060, Phase 1-3 & Commercial
% Insulin Component	[redacted]	[redacted]	[redacted]	[redacted]
Insulin	[redacted]	[redacted]	[redacted]	[redacted]
Mannitol	[redacted]	[redacted]	[redacted]	[redacted]
Glycine	[redacted]	[redacted]	[redacted]	[redacted]
Sodium Hydroxide	[redacted]	[redacted]	[redacted]	[redacted]
Sodium Citrate	[redacted]	[redacted]	[redacted]	[redacted]
Citric Acid	[redacted]	[redacted]	[redacted]	[redacted]
Total	100.00	100.0	100.00	100.00

INH (EXUBERA®) is delivered by a specialized instrument that pumps specific amount of dry powder insulin into the lungs during inhalation.

2.6.2.1 Brief summary

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

Insulin is an endogenous protein hormone released by the pancreatic β cells in response to increases in blood glucose concentrations. The primary function of insulin is to lower blood glucose by stimulating peripheral glucose uptake by skeletal muscle and fat, and by inhibiting hepatic glucose production. Other functions attributed to insulin include inhibition of lipolysis in the adipocyte, inhibition of proteolysis, and enhancement of protein synthesis. Since inhalant insulin is a powder formulation of recombinant human insulin, specific mechanistic studies

were not done nor are they necessary. Insulin receptors are found in most tissues including lungs. *In-vitro* studies have demonstrated presence of insulin receptors in rat alveolar Type II epithelial cells (Sugahara et al, 1984). Insulin receptors are thought to be more abundant on the basolateral side than on the apical side, and don't appear to mediate the directional transport of insulin. Whether these receptors bind to insulin in the lung in any appreciable amount is unknown. The lung tissue insulin receptor binding may play a critical role on the safety of the inhalation insulin since insulin is a growth factor and activation of insulin receptors has the potential to cause lung tissue hypertrophy *in-vivo*.

Drug activity related to proposed indication:
In an attempt to compare effectiveness and bioequivalence, inhalation insulin (INH) and SC human insulin were administered to rats, dogs and monkeys. Inhaled insulin was delivered as dry powder into the chamber air for inhalation by Sprague Dawley rats. Administration of inhaled insulin to rats produced a rapid increase in serum insulin (Tmax 16-21 min) followed by decrease in serum glucose levels.

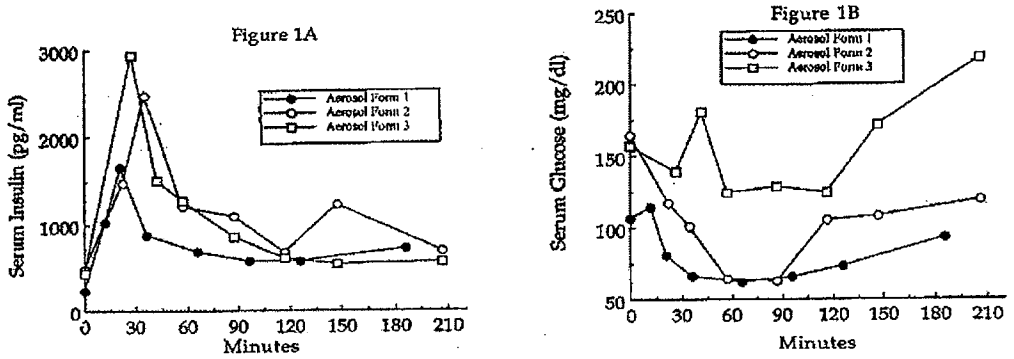


Figure 1. The Serum Insulin Concentration (Figure 1A) and Glucose Response (Figure 1B) in Rats Following Inhalation of Three* Different Dry Powder Formulations of Recombinant Human Insulin (Each Point Represents the Mean of 3 Animals)

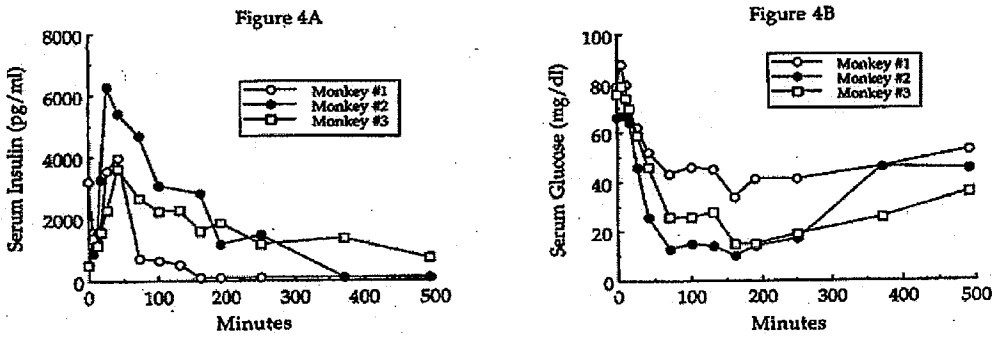
*Aerosol Form insulin, ; Aerosol Form 2: insulin, (mannitol, citrate; Aerosol Form 3: insulin, , citrate.

In another group of rats, different formulations of inhaled insulin (13-32 µg/rat) were compared to SC human insulin (96 µg/kg, up to 30 µg/rat). The relative bioavailability of inhaled insulin was approximately 10% to 40 % depending on the formulation (1, 2 or 3, as above).
The best relative bioavailability was achieved with Aerosol 2 formulation (I-004). Based on reduction in blood glucose and relative bioavailability, Aerosol 2 formulation was selected for future non-clinical testing.

A Comparison of Aerosol and Subcutaneous (SC) Insulin in Rats

	Aerosol Form 1	Aerosol Form 2	Aerosol Form 3	SC
Insulin T_{max}	16 min	21 min	17 min	15 min
Glucose T_{min}	31 min	43 min	37 min	30 min
Glucose Drop	42%	62%	21%	77%
Relative Bioavailability	10%	44%	14%	100%

The effectiveness of aerosol 2 ~ insulin, — mannitol, ~ citrate) at 489 µg insulin/animal was also tested in macaca fascicularis cynomolgus monkeys. Administration of aerosol insulin increased serum insulin within 30 min and decreased serum glucose (-73%) suggesting that administration of aerosol insulin by inhalation is effective in reducing blood glucose. To determine the bioavailability of the aerosol insulin relative to SC insulin, another group of monkeys were administered Humulin® insulin subcutaneously (100 U/ml, up to 27 µg of insulin /monkey). The bioavailability of aerosol insulin formulation relative to SC insulin was approximately 12%.

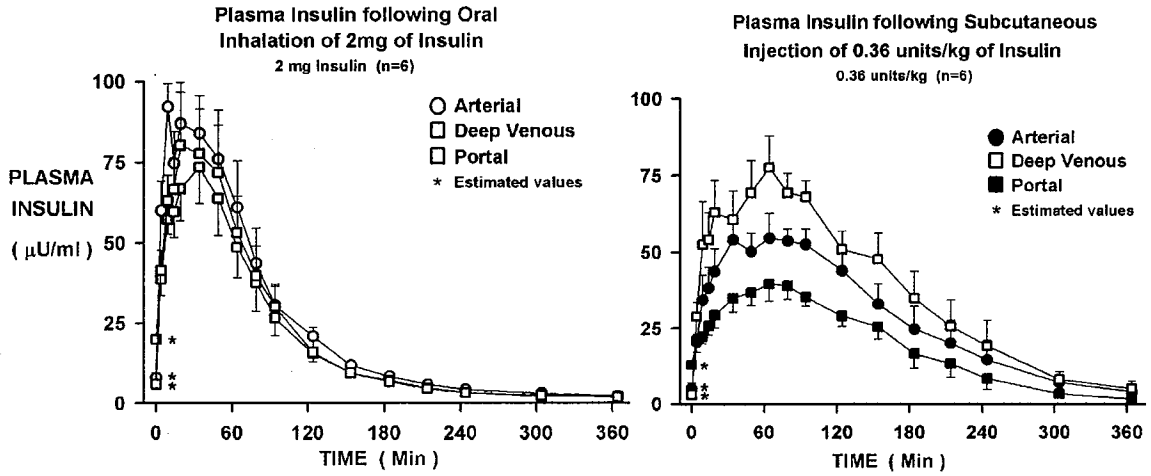


The Serum Insulin Concentration (Figure 4A) and Glucose Response (Figure 4B) in Three Cynomolgus Monkeys Following Inhalation of a Dry Powder Formulation* of Recombinant Human Insulin at 489 µg/Monkey

A Comparison of High Dose Aerosol and Subcutaneous (SC) Insulin in Cynomolgus Monkeys (The Results Represent the Mean of 3 and 4 Animals, Respectively)

	Aerosol	SC
Insulin T_{max} (min)	30 min	15 min
Glucose T_{min} (min)	45 min	45 min
Glucose Drop (%)	73%	45%
Relative Bioavailability (%)	12%	100%

The efficacy and relative bioavailability of the insulin formulation [redacted] for commercial development [redacted] blister packs) was evaluated in anesthetized dogs pre-treated with somatostatin to prevent endogenous release of insulin and glucagon. Aerosol insulin was delivered via endotracheal tube to anesthetized dogs instrumented with femoral arterial, portal and renal vein catheters. To prevent hypoglycemia, dogs were also given exogenous glucose infusion to maintain glucose at steady state. Administration of inhalation insulin produced a dose-related increase in arterial plasma insulin in anesthetized dogs.



2.6.2.3 Secondary pharmacodynamics: No specific studies were performed

2.6.2.4 Safety pharmacology

Cardiovascular effects:

The cardiovascular safety of insulin ([redacted] Hoechst Marion Roussel insulin) used in the [redacted] formulation for commercial development was evaluated in anesthetized (pentobarbitone, 32 mg/kg) beagle dogs. Anesthetized dogs were administered intravenously with 1.5 U/kg of aerosol insulin and systolic (Ps), diastolic (Pd) in brachial artery were measured. Heart rate was integrated from the Ps/Pd signal. Left ventricular pressure (LVP) and the mean pulmonary artery pressures were measured by a Millar tip catheter. Cardiac output was determined by thermodilution method. A comparator group of animals were also treated with Humulin® intravenously (1.5 U/kg, Lilly). To prevent the potential effect of insulin induced hypoglycemia, glucose levels were maintained constant and monitored every 5 min. Cardiovascular parameters were measured for 4 hrs post dose. [redacted] increased systolic blood pressure (SBP) as a consequence of an increase in dp/dtmax. The positive inotropic effect of insulin has been reported by other investigators. Similar effect was also noted with iv Humaninsulin® Normal 100 (1.5 U/kg, iv). The positive inotropic effect appeared within 3 to 5 min post injection and lasted through out the test. Previous published studies have shown insulin to produce inotropic and chronotropic effects, independent of hypoglycemic activity (Christensen et al, 1983).

Cardiovascular study in dogs (n = [redacted] 1.5 U/kg i.v., injection volume 0.5 ml/kg bolus 10 s) (mean ± sd)

Parameter	Time in min															
	0	1	3	5	10	20	30	40	50	60	90	120	150	180	210	240
systolic blood pressure	163	163	172	175	173	175	173	170	172	168	172	170	177	178	180	178
A. femoralis (mmHg)	31	31	33	31	28	31	34	26	28	20	18	20	15	18	15	3
diastolic blood pressure	103	103	107	110	107	108	105	102	102	103	100	102	103	108	108	107
A. femoralis (mmHg)	12	12	15	13	10	13	13	13	13	10	5	3	6	10	10	8
mean blood pressure	130	129	135	138	135	137	134	131	132	131	131	131	135	138	139	137
A. femoralis (mmHg)	18	19	23	21	17	20	22	17	18	13	9	9	9	12	11	5
left ventricular	140	140	148	152	150	153	150	147	148	145	147	147	150	152	160	155
blood pressure (mmHg)	23	23	26	24	23	25	26	23	20	18	15	15	15	18	17	5
dp/dt_{max}	2933	2967	3800	4233	4067	4200	4400	4533	4667	4600	4533	4233	4167	3800	3733	3800
(mmHg/s)	945	929	1217	874	987	1000	1200	1222	1137	1058	702	866	493	529	462	346
heart rate	140	140	142	145	155	165	170	170	173	173	175	165	163	160	153	153
(pulse/min)	26	26	26	23	23	25	25	25	26	23	18	15	13	10	13	10
mean blood pressure	17.7	18.0	18.7	19.0	19.2	19.3	19.5	19.5	19.3	19.2	19.5	19.3	19.7	19.0	19.2	19.2
A. pulmonalis (mmHg)	2.8	2.6	3.2	2.6	3.3	3.1	3.3	3.3	3.1	2.8	2.6	2.5	2.0	2.0	2.0	2.5
blood flow	157	157	163	168	163	173	182	178	170	168	155	148	133	115	107	97
A. femoralis (ml/min)	61	61	67	69	65	74	85	82	75	78	74	82	77	74	71	72
cardiac output	2.23	2.47	2.42	2.55	2.62	2.78	2.91	2.88	2.93	2.85	2.84	2.72	2.63	2.49	2.43	2.27
(l/min)	0.44	0.40	0.34	0.45	0.48	0.29	0.57	0.52	0.32	0.40	0.14	0.14	0.27	0.18	0.23	0.08
stroke volume	15.9	17.7	17.2	17.5	16.8	16.9	17.1	16.9	17.0	16.4	16.3	16.5	16.2	15.5	15.8	14.8
(ml)	1.1	2.0	0.9	1.1	0.6	0.9	1.5	1.3	1.0	0.3	1.0	0.7	1.7	0.7	0.2	0.5
peripheral resistance	58.2	52.4	55.5	54.4	52.1	49.2	46.4	45.8	45.0	46.3	46.0	48.2	51.3	55.7	57.6	60.5
(mmHg x min/l)	3.8	0.6	2.7	1.9	4.3	2.6	1.8	3.0	4.3	3.7	1.3	0.7	2.0	2.9	5.9	3.4

Cardiovascular study in dogs (n = 3. Humaninsulin® Normal 100 1.5 U/kg i.v.. Injection volume 0.5 ml/kg bolus 10 s) (mean ± sd)

Parameter	Time in min															
	0	1	3	5	10	20	30	40	50	60	90	120	150	180	210	240
systolic blood pressure	173	173	182	185	180	180	182	177	182	180	165	172	180	167	170	173
A. femoralis (mmHg)	3	3	6	9	10	9	10	6	13	13	13	3	10	15	5	15
diastolic blood pressure	118	118	123	123	118	117	117	112	117	117	108	112	118	113	113	115
A. femoralis (mmHg)	8	8	8	8	8	6	10	12	10	10	15	12	8	15	10	13
mean blood pressure	142	142	148	150	145	144	145	140	145	144	133	137	145	136	138	140
A. femoralis (mmHg)	3	4	3	3	5	4	5	4	8	9	14	6	5	13	5	13
left ventricular	152	153	158	160	157	155	158	160	162	157	150	148	157	150	147	150
blood pressure (mmHg)	3	3	3	5	8	5	10	9	8	13	20	3	15	5	10	13
dp/dt_{max}	2867	3067	3667	4133	4033	3933	4067	4267	4267	4200	3600	3767	3600	3333	3100	3133
(mmHg/s)	231	416	493	231	58	306	231	231	231	200	400	404	346	702	361	757
heart rate	143	143	142	143	142	148	150	153	157	155	153	155	152	150	148	147
(pulse/min)	25	25	23	25	28	28	23	25	23	23	25	25	20	28	26	24
mean blood pressure	16.2	16.3	16.7	16.8	16.7	17.2	17.3	17.3	17.3	17.2	16.2	16.7	16.8	16.7	15.5	17.7
A. pulmonalis (mmHg)	0.8	0.8	0.8	1.0	0.8	0.8	1.0	1.0	1.0	1.2	0.8	1.2	1.0	1.6	3.3	1.8
blood flow	157	162	177	175	163	160	167	163	163	157	150	150	143	120	110	108
A. femoralis (ml/min)	31	26	25	25	23	36	38	40	40	38	35	46	36	35	36	38
cardiac output	2.07	2.22	2.21	2.28	2.45	2.21	2.41	2.48	2.42	2.41	2.33	2.64	2.33	2.16	2.00	2.01
(l/min)	0.42	0.35	0.32	0.46	0.80	0.33	0.45	0.43	0.53	0.42	0.51	0.30	0.27	0.43	0.39	0.32
stroke volume	14.5	15.5	15.6	16.1	17.4	15.1	16.1	16.2	15.3	15.6	15.1	17.1	15.4	14.4	13.4	13.7
(ml)	2.0	1.4	1.4	3.2	5.5	2.6	2.5	1.9	1.9	1.6	1.5	0.8	0.7	1.4	0.4	0.7
peripheral resistance	70.9	65.3	68.4	67.6	63.8	66.2	62.0	57.5	62.5	61.4	58.9	52.4	52.8	64.2	70.5	70.4
(mmHg x min/l)	17.0	12.2	11.8	15.0	21.8	11.9	15.0	10.4	18.7	15.2	14.6	5.0	9.9	8.2	12.3	5.5

2.6.2.5 Pharmacodynamic drug interactions: No specific studies were performed.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.3.1. Pharmacology **Overview** **Test Article:** Dry Powder Human Insulin

Type of Study	Test System	Method of Administration	Dose (mg/kg)	Testing Facility	Report Number	CTD Location Vol.	CTD Location Section
Primary Pharmacodynamics							
Bioactivity of dry powder aerosolized human insulin	Sprague-Dawley Rats	Inhalation[a]	13-32 µg/rat (average)	Nektar (formally Inhale) Therapeutic, San Carlos, CA, USA	AA001		4.2.3.1
Bioactivity of subcutaneously injected insulin	Sprague-Dawley Rats	Subcutaneous[b]	96 µg/kg (up to 30 µg/rat)	Nektar (formally Inhale) Therapeutic, San Carlos, CA, USA	AA001		4.2.3.1
Bioactivity of dry powder aerosolized human insulin	Cynomolgus monkeys / *Macaca fascicularis*	Inhalation[c]	489 µg /monkey (average)	Nektar (formally Inhale) Therapeutic, San Carlos, CA, USA	AA001 SC930056		4.2.3.1
Bioactivity of subcutaneously injected insulin	Cynomolgus monkeys / *Macaca fascicularis*	Subcutaneous[d]	7.2 µg/kg (up to 27 µg/ monkey)	Nektar (formally Inhale) Therapeutic, San Carlos, CA, USA	AA001 SC930056		4.2.3.1
Bioactivity of dry powder aerosolized human insulin	Beagle Dogs	Inhalation[e]	1mg/dog, 2mg/dog	USA	NRA 217001		4.2.1.1
Bioactivity of dry powder aerosolized human insulin	Beagle Dogs	Inhalation[e], Intravenous[d], Subcutaneous[d]	1mg/dog, 1.7-2.1 U/dog, 0.18, 0.24 U/kg	USA	NRA 2171004		4.2.1.1

[a] Aerosol Form 1: [redacted] Aerosol Form 2: [redacted] insulin, [redacted] mannitol, [redacted] citrate; Aerosol Form 3: [redacted] insulin, [redacted] citrate.
[b] Subcutaneous formulation: [redacted] insulin, [redacted] mannitol, [redacted] citrate.
[c] Aerosol: [redacted] insulin, [redacted] mannitol, [redacted] citrate.
[d] Insulin used for subcutaneous injection and intravenous infusion: Humulin R (100 U/ml).
[e] Aerosol: [redacted] insulin, [redacted] mannitol, [redacted] citrate

2.6.3.1. Pharmacology **Overview - continued** **Test Article:** Dry Powder Human Insulin

Type of Study	Test System	Method of Administration	Dose (mg/kg)	Testing Facility	Report Number	CTD Location Vol.	CTD Location Section
Bioactivity of subcutaneously injected insulin	Beagle Dogs	Subcutaneous[f]	0.36 U/kg	[redacted]	NRA 217001		4.2.1.1
Comparison of blood glucose lowering potencies of recombinant human insulin and semisynthetic human insulin	Rabbits	Intravenous[g]	0.1 U/kg	Aventis, Frankfurt, Germany	015510		4.2.1.1
Comparison of blood glucose lowering potencies of recombinant human insulin and corresponding reference insulin formulations	Rats	Subcutaneous[h]	2 U/kg	Aventis, Frankfurt, Germany	014787		4.2.1.1
Comparison of blood glucose lowering potencies of recombinant human insulin and corresponding reference insulin formulations	Dogs	Subcutaneous[i]	0.3 U/kg	Aventis, Frankfurt, Germany	014790		4.2.1.1

[f] Subcutaneous formulation: Humulin R (100 U/ml), 0% mannitol.
[redacted] (recombinant human insulin) compared with Isuhuman® Rapid (semisynthetic human insulin).
[h] Insulin formulations containing recombinant human insulin [redacted] compared with corresponding reference depot formulations, Isuhuman® Rapid, Huminsulin Profil® III 100 and Insulin Hoechst®-Basal U-100.
[i] These studies were performed as part of the Toxicology program and are described in detail in Table 2.6.7.1

2.6.3.1. Pharmacology **Overview - continued** **Test Article:** Dry Powder Human Insulin

Type of Study	Test System	Method of Administration	Dose (mg/kg)	Testing Facility	Report Number	CTD Location Vol.	CTD Location Section
Secondary Pharmacodynamics[i]							
30-day repeat dose toxicity study	Sprague-Dawley Rats	Inhalation	0, 1.1, 3.2, 6.0 mg/kg		N001603C		4.2.3.2
1-month repeat dose toxicity study	Sprague-Dawley Rats	Inhalation	0 (air), 0 (placebo), 1, 3.2, 5.9 mg/kg		01-906-06		4.2.3.2
6-month repeat dose toxicity study	Sprague-Dawley Rats	Inhalation	0 (air), 0 (placebo), 0.9, 2.7, 5.8 mg/kg		N002448A		4.2.3.2
5-day repeat dose toxicity study	Cynomolgus Monkey	Inhalation	0, 0.13, 0.60 mg/kg		SC930055		4.2.3.2
30-day repeat dose toxicity study	Cynomolgus Monkey	Inhalation	0, 0.14, 0.58 mg/kg		N001603B		4.2.3.2
6-month repeat dose toxicity study	Cynomolgus Monkey	Inhalation	0, 0.29, 0.64 mg/kg		N002448B		4.2.3.2
Safety Pharmacology							
Cardiovascular effects of recombinant human insulin compared to semisynthetic human insulin	Beagle Dogs	Intravenous[j]	1.5 U/kg	Aventis, Frankfurt, Germany	016746		4.2.1.1

Pharmacodynamic Drug Interactions

None

(recombinant human insulin) compared with Huminsulin® Normal 100.

2.6.3.2. Primary Pharmacodynamics **Test Article:** Dry Powder Human Insulin

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses[a] (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report Number	CTD Location Vol. / Section
Serum	Rats/Sprague-Dawley	Inhalation	13-32 µg/rat (average)	Female 24/group (72 total)	Insulin appeared rapidly in the serum and resulted in decreases in serum glucose concentrations	No	AA001	/4.2.3.1
Serum	Rats/Sprague-Dawley	Subcutaneous	96 µg/kg (up to 30 µg/rat)	Sex not specified 24/group (24 total)	Insulin appeared rapidly in serum and produced the expected decrease in serum glucose concentrations	No	AA001	/4.2.3.1
Serum	Cynomolgus monkeys / *Macaca fascicularis*	Inhalation	489 µg /monkey (average)	Male 3/ group	Insulin appeared rapidly in the serum and resulted in decreases in serum glucose concentrations	No	AA001 SC930056	/4.2.3.1
Serum	Cynomolgus monkeys / *Macaca fascicularis*	Subcutaneous	7.2 µg/kg (up to 27 µg/ monkey)	Male 4 /group	Insulin appeared rapidly in serum and produced the expected decrease in serum glucose concentrations	No	AA001 SC930056	/4.2.3.1
Plasma	Dog/Beagle	Inhalation	1mg/dog, 2mg/dog	Male/Female 5/sex	Insulin appeared rapidly in the plasma and produced the expected pharmacodynamic response[b]	No	NRA 217001	/4.2.1.1
Plasma	Dog/Beagle	Subcutaneous	0.36 U/kg	Male/Female 3/sex	Insulin appeared rapidly in the plasma and produced the expected pharmacodynamic response[b]	No	NRA 217001	/4.2.1.1
Plasma	Dog/Beagle	Inhalation	1mg/dog	Male 14/group	Insulin appeared rapidly in the plasma and produced the expected pharmacodynamic response[c,d]	No	NRA 2171004	/4.2.1.1
Plasma	Dog/Beagle	Intravenous	1.7-2.1U/dog	Male 10/group	Insulin was infused to reproduce the arterial plasma insulin levels produced by inhalation of 1 mg insulin and produced the expected pharmacodynamic response[c]	No	NRA 2171004	/4.2.1.1
Plasma	Dog/Beagle	Subcutaneous	0.18, 0.24 U/kg	Male 6/group	Insulin appeared in the plasma and produced the expected pharmacodynamic response[c]	No	NRA 2171004	/4.2.1.1

[a] Single dose unless specified otherwise.
[b] The pharmacodynamic response was the glucose infusion rate required to maintain euglycemia.
[c] The pharmacodynamic response was the control of a glycemic rise due to an intraportal infusion of glucose
[d] The pharmacodymaic response was greater for the inhalation route than that produced by intravenous infusion or subcutaneous injection of insulin

2.6.3.2. Primary Pharmacodynamics **Test Article:** Dry Powder Human Insulin

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses[a] (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report Number	CTD Location Vol. / Section
Blood	Rabbit/ Chincilla New Zealand	Intravenous	0.1 U/kg	Male 6/group	Recombinant and semisynthetic human insulins were bioequivalent.	No	015510	/4.2.1.1
Blood	Rat/Wistar	Subcutaneous	2 U/kg	Male 14-15/group	Recombinant human insulin was bioequivalent to corresponding reference formulations.	No	014787	/4.2.1.1
Blood	Dog/Beagle	Subcutaneous	0.3 U/kg	Male 10-12/group	Recombinant human insulin was bioequivalent to corresponding reference formulations.	No	014790	/4.2.1.1

[a] Single dose unless specified otherwise.

Safety Pharmacology **Test Article:** Dry Powder Human Insulin

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses[a] (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report Number	CTD Location Vol. / Section
Heart and Cardiovascular system	Dog/Beagle	Intravenous[b]	1.5 U/kg	Female 3/group	Effects of recombinant human insulin did not differ from reference insulin formulation.	No	016746	/4.2.1.1

[a] Single dose unless specified otherwise.
[b] Exogenous glucose was infused to maintain euglycemia.

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ON ORIGINAL

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

No specific pharmacokinetic studies were performed in animals except for toxicokinetic (TK) evaluations and relative bioavailability studies. Most of the discussion will center on the human PK data and published literature. The bioavailability of inhaled insulin in healthy subjects has ranged between 4 to 9% relative to SC insulin. The relative bioavailability appears to be slightly better in type 2 diabetes patients (8-11%). Type 2 diabetic patients are generally heavier than type 1 diabetic patients. The improvement in the relative bioavailability of INH insulin in type 2 diabetics is suspected to be due to greater amount of subcutaneous adipose tissue and poor absorption of SC insulin. SC insulin is generally given as international units (U) rather than in mg. One mg of insulin is equal to 26 U of insulin.

2.6.4.2 Analytical Methods

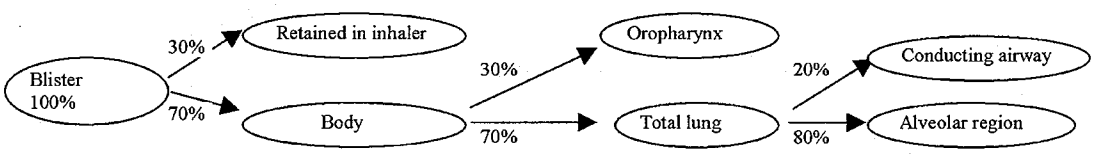
Plasma concentrations of insulin were measured by radioimmunoassay method (RIA). Non-validated Coat-A-Count RIA kit from Diagnostic Products Corporation and validated RIA kits from LINCO (Human insulin Kit, Linco Research Inc, St. Charles, MO) were used to measure serum and plasma insulin. Non-validated assays were used primarily in pharmacology and non-clinical pilot studies. These assays have been extensively used in research.

Summary of Analytical Assays

Study	Species	Matrix	Non-validated or Validated Assays	RIA Assay for Human Insulin and Source
AA001	Rat	Serum	Non-validated	Coat-A-Count RIA Kit, Diagnostic Products Corp.
AA001	Monkey	Serum	Non-validated	Coat-A-Count RIA Kit, Diagnostic Products Corp.
N001603A(1)	Rat	Serum	Non-validated	Coat-A-Count RIA Kit, Diagnostic Products Corp.
N001603A(2)	Monkey	Serum	Non-validated	Coat-A-Count RIA Kit, Diagnostic Products Corp.
NRA217001	Dog	Plasma	Non-validated	RIA (Morgan and Lazarow, 1963), Vanderbilt University Hormone Assay Core
N001603C	Rat – 1 month	Serum	Validated	LINCO Human Insulin Kit, LINCO Research INC
N002448A	Rat – 6 month	Serum	Validated	LINCO Human Insulin Kit, LINCO Research INC
01-906-06	Rat – 1 month	Serum	Validated	LINCO Human Insulin Kit, LINCO Research INC
N001603B	Monkey – 1 month	Serum	Validated	LINCO Human Insulin Kit, LINCO Research INC
N002448B	Monkey – 6 month	Serum	Validated	LINCO Human Insulin Kit, LINCO Research INC

2.6.4.3 Absorption

Published literature and the sponsors' data suggest that after the administration of powder inhalation insulin, each step in the process (device to lung structure) reduces the total availability of the contents of the insulin blister pack. It appears that after the pump device is triggered, approximately 30% of the insulin powder is not inhaled at all and remains in the blister/inhalant device. Only 40% of blister pack is actually available at the absorption site.



Percentage of Blister: 70% (x 70%) = 50% (x 80%) = 40%

Once insulin is deposited (40%) into the alveolar region, a portion of it is absorbed into the systemic circulation. A significant amount of insulin remaining in the alveolar region in the lungs undergoes degradation by phagocytes, alveolar macrophages, insulin specific and non-specific enzymatic degradation and lymphatic drainage. All these factors limit the bioavailability of the insulin delivered by inhalation. Powder insulin deposited in the lungs, has been presumed to cross alveolar epithelium by passive diffusion to blood stream thought extracellular tight membrane (paracellular transport). Insulin transported across a concentration gradient (high to low) is affected by particle size, charge, site of deposition and the condition of the lung tissue itself (smoking, fibrosis). Whether other transport systems such as transcytosis (from luminal airspace to blood stream) are involved is unknown. Large protein products (≥ 40 KDa) have been shown to be transported by transcytosis.
In clinical studies, powder insulin was delivered using pulmonary inhaler. In rats, the inhalation insulin was delivered by nose only while in monkeys via a head dome. In anesthetized dogs, powder insulin was delivered via endotracheal tube. These different methods/devices may have increased variability in insulin delivery. Thus is not surprising that the relative bioavailability in animals ranged from 10 to 90%. The mean relative bioavailability of I-004 formulation was about 44% in rats and 12% in monkeys. In dogs and humans, the relative bioavailability was 7 and 10% respectively. Accuracy of the pulmonary dose delivery (major factor), changes in the formulation and species difference appeared to account for most of the variability in the insulin bioavailability.

A Comparison of Aerosol and Subcutaneous (SC) Insulin in Rats

	Aerosol Form 1	Aerosol Form 2	Aerosol Form 3	SC
Insulin T_{max}	16 min	21 min	17 min	15 min
Glucose T_{min}	31 min	43 min	37 min	30 min
Glucose Drop	42%	62%	21%	77%
Relative Bioavailability	10%	44%	14%	100%

2.6.4.4 Distribution:

Multiple dose pre-clinical studies suggests that repeated administration of inhaled insulin does not lead to insulin accumulation. This is in part due to biological nature of the insulin. As a protein, insulin is rapidly degraded both locally in the lungs and in the circulation (i.e. liver). In healthy subjects Tmax for INH ranged from 41 to 89 min compared to 63 to 148 min for SC insulin (lispro). Tmax for INH in diabetics was between 38 and 78 min compared to SC insulin (83 to 258 min). Cmax of INH was comparable to SC injectable insulin.

2.6.4.5 Metabolism:

Published studies suggest that most of the insulin degradation takes place in the lungs by enzymes resident in the airway fluid and as it is absorbed across the air/blood barrier. Aminopeptidase is thought to be the primary enzyme responsible for the metabolism of insulin in the rat lungs. Insulin degrading enzyme (IDE), a metalloprotease generally localized in the cytosolic compartment of cells with multiple cellular function also plays an important role. Although rats are thought to have low levels of IDE in the lungs, theses enzymes in type 2 alveolar epithelial cells may account for 80% of insulin metabolism. Alveolar macrophages containing potent lytic enzymes also play an important role in degradation of insulin in the lungs. Studies with labeled insulin have shown that absorbed insulin enters the interstitial tissue of alveoli with in 5 -30 min and is cleared from the lungs by 6 hrs.

2.6.4.6 Excretion: Not performed

2.6.4.7 Pharmacokinetic drug interactions: Not performed

2.6.4.8 Other Pharmacokinetic Studies:
Absorption of early formulations of inhalation powder insulin was compared to SC insulin in rats (Report #AA001). Aerosol 2 with higher relative bioavailability (44%) was selected as the most suitable formulation for insulin delivery by inhalation route.

A comparison of aerosol and subcutaneous (SC) insulin in animals

	Rat SC	Rat Aerosol Form 1	Rat Aerosol Form 2	Rat Aerosol Form 3	Monkey SC	Monkey Aerosol Form 2
Insulin Max.*	15 min	16 min	21 min	17 min	15 min	30 min
Glucose Min.*	30 min	31 min	43 min	37 min	45 min	45 min
Glucose Drop	77%	42%	62%	21%	45%	73%
Rel Bioavail.	100%	10%**	44%**	14%**	100%	12%***

* T's measured from end of aerosol exposure period
Glucose min = time to >85% of maximal reduction
** Based on insulin recovered by lung lavage at end of aerosol exposure
***Based on insulin inhaled, includes losses in nasal passages and throat

Rat Test Articles: 3 Powdered Aerosol Insulin Formulations

PK in Multiple Dose Studies:

Pilot Pharmacokinetic study in rats
In a single dose pilot rat study (n=3/sex/dose), the PK parameters and relative bioavailability of I-004 insulin formulation insulin was compared to SC insulin (Lilly). The relative bioavailability of the inhaled insulin (I-004) was approximately 64% in the pilot study. The mean residence time (MRT), the time it takes for 63.2% of the administered dose to be eliminated from the body was 0.94 hrs in rats. The clinical signs of hypoglycemia in the fasted rats were marked by lethargy, hypoactivity and unresponsiveness. Based on observed hypoglycemia in the pilot study, 6 mg/kg/d was considered the maximum tolerated dose (MTD) in rats.

Serum Pharmacokinetics of Insulin in the Rat

Parameter	Inhalation (INH)	Subcutaneous (SC)[a]
Average Dose (μg) / rat	185.90	30.00
C_{max} (ng/ml)	72.50	14.20
T_{max} (h)	1.0	0.25
AUC_{2h} (ng-h/mL)	59.63	15.01
$AUMC_{2h}$ (ng-h^2/mL)	55.89	11.88
MRT (h)	0.94	0.79
% Rel. Bioavailability	64.13	NA

Pilot Pharmacokinetic study in Monkeys

In a single dose pilot study in monkeys (n=1/sex/dose), the PK parameters and relative bioavailability of I-004 insulin formulation was compared to SC insulin (Lilly). The relative bioavailability of inhaled insulin (I-004) was approximately 92% in the monkeys. The mean residence time (MRT), the time it takes for 63.2% of the administered dose to be eliminated from the body was 2.5 hr in monkeys. Hypoglycemia was the most frequent clinical signs. Hypoglycemia was marked by lethargy, hypoactivity and unresponsiveness. Due to hypoglycemia, the 0.6 mg/kg/d was considered the MTD in monkeys.

Serum Pharmacokinetics of Insulin in the Monkey

Parameter	Inhalation (INH) / Female	Subcutaneous (SC) / Male[a]
Dose (µg) / monkey	644.4	27.0
C_{max} (ng/ml)	80.82	2.39
T_{max} (h)	1.033	0.25
Ke (h^{-1})	0.3641 ± 0.0251	0.3062 ± 0.0272
T1/2 Ke (h)	1.91 ± 0.08	2.28 ± 0.12
AUC∞ (µg-equiv-h/mL)	125.33	5.68
AUMC∞ (µg-equiv-h^2/mL)	313.57	17.84
Cl_{tot}/f (L/h)	5.14	4.76
MRT (h)	2.50	3.14
% Rel. Bioavailability	92.51	NA

30-Day Toxicokinetic Monkey Study

Monkeys were administered aerosol insulin I-004 at doses of 135 (LD) and 583 µg/kg/day (HD) for 30 days. Plasma insulin concentrations were highly variable (large standard deviations).

Mean Serum Insulin and Glucose Levels for the 30-Day Inhalation Toxicity Study of Aerosol Insulin in Cynomolgus Monkeys

	Day				Day				Day			
	1		29		1		29		1		29	
Dose Group[1]	Sample Collection Time (min post-exposure)				Insulin (ng/mL)				Glucose (mg/dL)			
Males												
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1-Vehicle	predose				3.37	2.41	2.93	0.88	95	24	71	15
	5	0	6	2	2.31	0.57	3.59	1.44	81	27	67	17
	15	0	17	2	2.24	1.34	2.32	1.05	70	17	70	18
	30	0	30	0	1.33	0.78	1.70	1.02	67	6	70	11
2-Low-dose	predose				2.03	1.58	4.16	1.31	152	29	84	20
	6	2	5	1	5.08	1.49	19.41	4.30	85	14	57	17
	15	0	15	0	4.21	1.13	8.22	4.03	74	28	41	13
	30	0	30	0	3.45	0.96	4.39	0.89	60	20	29	6
3-High-dose	predose				2.33	1.09	4.68	1.53	116	96	100	17
	6	1	5	0	12.13	10.72	15.50	4.42	56	37	22	7
	16	1	16	3	8.62	3.22	16.75	8.92	48	25	20	6
	30	0	30	0	9.81	5.76	20.64	2.04	46	17	21	8
Females												
1-Vehicle	predose				2.30	1.22	36.55	38.29	82	39	71	20
	5	1	5	1	1.08	0.28	10.07	7.86	70	12	52	9
	15	0	15	1	1.55	0.44	11.14	3.72	87	12	60	5
	30	0	30	0	1.29	0.45	8.51	3.19	76	13	61	5
2-Low-dose	predose				1.69	0.72	4.12	1.64	107	36	111	50
	5	0	7	3	7.12	4.18	21.86	20.79	69	29	63	8
	15	0	15 (N=3)	1	7.11	4.41	9.84 (N=3)	2.49	61	28	46 (N=3)	13
	30	0	31	2	6.96	2.47	6.34	2.56	48	22	35	12
3-High-dose	predose				4.26	3.60	22.17	17.85	151	31	88	17
	6	1	5	0	12.91	3.93	33.00	9.83	28	12	9	6
	15	1	16	1	12.19	5.71	33.09**	23.07	27	20	10	8
	32	3	30	0	13.80	7.28	30.29	16.55	30	18	14 (N=3)	9

1. Dose Group 1: 60-min exposures to placebo. Dose Group 2: 12-min exposures to test article. Dose Group 3: 60-min exposures to test article.
**Dextrose given to Animal 354 after 15 minute sample.
No statistical comparisons were performed. N = 4 except as indicated.

30-Day Toxicokinetic Rat Study

In the 30-Day TK study, SD rats were exposed to 10, 30 and 60 min of 20% aerosol insulin (I-004). Control group was exposed to placebo for 60 min. Blood samples were collected from the retro-orbital plexus for RIA analysis, of glucose and human insulin (RIA, Linco Research). Pulmonary administration of insulin significantly increased serum insulin levels with accompanied decrease in serum glucose suggesting that pulmonary administered insulin is biologically active. The decrease in serum glucose, however, appeared to lag behind the increase in serum insulin levels. Although some serum insulin levels on Day 30 appeared to be higher than day 1, the overall variability prevented any kind of conclusion. It is not clear whether higher trend in serum insulin on Day 30 was due to rat acclimation to the procedure, or insulin accumulation. As a protein, insulin should be quickly degraded.

Mean Serum Insulin (ng/mL) and Glucose (mg/dL) Levels per Post-Exposure Timepoint in the Toxicokinetic Subgroups for the 30-Day Inhalation Toxicity Study of Aerosol Insulin in Rats

Inhaled Insulin Dosage (mg/kg/day)		Day of Study					
		1	30	1	30	1	30
		Actual Time (min)		Insulin (ng/mL)		Glucose (mg/dL)	
Males							
Satellite	Mean	3.7	5.3	10.12	10.25	141	121
	STD	0.6	0.6	15.53	17.67	8	9
	Mean	4.3	6.0	2.00	1.06	155	145
	STD	0.6	0.0	0.29	1.81	7	12
	Mean	7.0	5.0	1.00	0.29	161	134
	STD	0.0	0.0	0.51	0.41	4	10
1	Mean	2.3	3.7	30.10	62.42	179	142
	STD	0.6	0.6	20.46	42.55	7	9
	Mean	10.3	11.3	36.34	148.43	152	107
	STD	0.6	1.5	17.68	131.59	14	22
	Mean	30.3	30.3	20.20	30.12	140	100
	STD	0.6	0.6	3.75	16.11	10	33
3	Mean	2.3	3.7	58.02	77.72	115	120
	STD	0.6	1.2	11.81	74.41	20	7
	Mean	11.0	10.7	141.73	503.04	94	105
	STD	1.0	0.6	40.26	544.87	19	16
	Mean	30.3	30.7	82.99	101.23	124	92
	STD	0.6	0.6	40.95	84.96	21	14
6	Mean	3.3	3.3	291.11	76.59	88	99
	STD	0.6	0.6	206.46	11.83	4	9
	Mean	10.3	11.3	156.67	153.99	103	79
	STD	0.6	0.6	107.08	166.90	10	2
	Mean	29.3	30.3	47.45	46.19	107	78
	STD	0.6	0.6	12.29	25.48	27	9

Mean Serum Insulin (ng/mL) and Glucose (mg/dL) Levels per Post-Exposure Timepoint in the Toxicokinetic Subgroups for the 30-Day Inhalation Toxicity Study of Aerosol Insulin in Rats

Inhaled Insulin Dosage (mg/kg/day)		Day of Study					
		1	30	1	30	1	30
		Actual Time (min)		Insulin (ng/mL)		Glucose (mg/dL)	
Females							
Satellite	Mean	12.0	4.3	1.59	5.21	131	133
	STD	1.0	0.6	2.73	7.86	10	5
	Mean	5.7	4.3	4.93	3.99	136	127
	STD	0.6	0.6	3.12	5.95	11	2
	Mean	4.7	4.3	4.65	1.23	135	123
	STD	0.6	0.6	6.04	0.93	9	11
1	Mean	9.0	3.3	114.34	101.02	126	129
	STD	1.0	0.6	112.79	73.25	11	13
	Mean	12.3	11.0	31.21	69.47	102	92
	STD	0.6	1.0	14.91	86.45	8	10
	Mean	30.7	20.3	39.61	45.82	87	74
	STD	0.6	0.6	25.79	23.72	11	18
3	Mean	4.3	3.3	231.52	406.87	70	71
	STD	1.2	0.6	102.05	231.47	9	5
	Mean	10.7	10.3	279.19	208.63	66	56
	STD	0.6	0.6	218.14	70.91	18	4
	Mean	30.0	30.3	62.10	149.57	57	62
	STD	0.0	0.6	56.50	94.44	10	19
6	Mean	3.3	3.7	402.00	879.03	68	56
	STD	0.6	1.2	184.14	326.84	25	4
	Mean	11.0	10.7	519.33	145.59	49	52
	STD	1.0	0.6	586.82	92.54	9	33
	Mean	30.7	30.3	108.48	423.04	58	61
	STD	1.2	0.6	100.56	318.88	16	4

6-Month Toxicokinetic Rat Study

In the 6-month rat study, animals were administered insulin formulation I-004 for 10, 30 and 60 min (insulin concentration 0.2 mg/l). Placebo animals received the excipients in the formulation (see table). An air control group receiving only air stream was also included in the study. Serum insulin levels were determined on Day 1, 91 and 182. There was no apparent accumulation in serum insulin with repeated administration of aerosol insulin in rats. Variability in plasma insulin levels observed in rats after repeated administration of insulin by pulmonary route suggests that there are significant inter- and intra-animal differences in addition to differences in species.

Group Mean Excipient Exposure Levels (mg/kg/day)						
Exposure Group	Average Total Mass Exposure	Mannitol	Glycine	Citric Acid	Sodium Citrate	Safety Margin[1]
Air	0	0.0	0.0	0.0	0.0	0
Placebo	27.15	4.9	0.7	0.0	21.5	27[2]
Low dose	4.47	0.8	0.1	0.0	2.6	5
Mid dose	13.69	2.5	0.4	0.0	8.1	14
High dose	28.92	5.3	0.8	0.0	17.1	29

1. Safety margin of group mean rat exposure relative to maximum clinical exposure.
2. Except for [redacted] which was 36 times the maximum human exposure due to the replacement of [redacted] insulin with [redacted] in the placebo formulation.

Inhaled Insulin Dosage (mg/kg/day)

Exposure Group	Males	Females	Group Mean	Safety Margin[1]
Air	0	0	0	0
Placebo	0	0	0	0
Low Dose	0.83	0.96	0.90	5[2]
Mid Dose	2.55	2.92	2.74	14[2]
High Dose	5.40	6.17	5.79	29[2]

1. Safety margin of group mean rat exposure relative to maximum clinical exposure.
2. In the maximum clinical exposure in humans of 1 mg/kg/day total inhaled mass, insulin would comprise 0.2 mg/kg/day.

Mean Serum Insulin (ng/mL) Values for Toxicokinetic Subgroups per Post-Exposure Timepoint: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

a. N = 3 per timepoint.

		Day 1		Day 91		Day 182		Day 1		Day 91		Day 182	
Exposure Group[a]	Target Timepoint	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
		Males						Females					
Satellites for Low Dose	10 min	0.0	0.0	0.1	0.1	0.3	0.4	0.0	0.2	1.5	1.8	0.2	0.2
Satellites for Mid Dose	10 min	0.0	0.1	3.5	3.1	0.2	0.1	0.2	0.1	2.1	3.3	0.0	0.0
Satellites for High Dose	10 min	0.3	0.1	0.6	0.6	1.3	1.1	0.2	0.2	3.3	2.8	0.4	0.3
3-Low Dose	3 min	177.4	75.6	94.1	18.1	112.0	80.3	70.3	47.2	190.9	277.4	136.3	109.6
	10 min	155.4	59.7	82.2	45.5	49.6	47.1	69.8	59.7	106.0	77.0	75.9	69.4
	30 min	111.1	34.5	33.7	27.8	39.3	45.2	110.8	45.6	117.3	13.3	60.3	43.7
4-Mid Dose	3 min	883.4	666.4	364.1	431.5	215.1	190.2	278.2	322.8	177.8	141.6	141.9	88.7
	10 min	391.0	196.7	212.7	36.6	239.6	258.1	206.0	114.4	360.5	317.1	463.2	362.4
	30 min	226.4	119.6	91.5	53.8	108.7	74.5	69.8	43.6	92.2	25.3	121.0	54.3
5-High Dose	3 min	248.3	192.1	183.1	45.4	133.8	46.4	827.3	408.0	352.1	278.3	164.8	95.9
	10 min	734.4	109.6	1140.8	788.8	209.4	94.9	492.0	553.6	596.5	570.8	395.5	256.4
	30 min	418.2	237.0	650.6	668.0	313.4	160.3	351.3	270.3	232.9	138.7	565.0	543.7

6-Month Toxicokinetic Monkey Study

Monkeys were administered insulin formulation I-004 for 10, 30 and 60 min by inhalation route (insulin conc. 0.2 mg/l). Placebo animals received the excipients in the formulation. A separate air control group received only filtered air.

Inhaled Insulin Dosage (µg/kg/day)

Exposure Group	Males	Females	Group Mean	Safety Margin[1]
Air	0	0	0	0
Placebo	0	0	0	0
Low Dose	298	284	291	1.5[2]
High Dose	624	654	639	3.2[2]

1. Safety margin of group mean monkey exposure relative to maximum clinical exposure.
2. In the maximum clinical exposure in humans of 1000 µg/kg/day total inhaled mass, insulin would comprise 200 µg/kg/day.

Group Mean Excipient Exposure Levels (µg/kg/day)

Exposure Group	Average Total Mass Exposure	Mannitol	Glycine	Citric Acid	Sodium Citrate	Safety Margin[1]
Air	0	0	0	0	0	0
Placebo	2848	518.6	74.0	0.0	2255.2	2.9[2]
Low Dose	1454	264.8	37.8	0.9	859.9	1.5
High Dose	3193	581.5	83.0	1.9	1888.3	3.2

1. Safety margin of [redacted] mean monkey exposure relative to maximum clinical exposure.
2. Except for [redacted] which was 3.8 times the of maximum clinical exposure due to replacement of [redacted] insulin with [redacted] the placebo formulations.

Mean Serum Insulin Values (ng/mL): 6-Month Inhalation Toxicity Study of Aerosol Insulin in Cynomolgus Monkeys

Exposure Group	Target Time (min)	Day of Study 1 Mean	1 SD	91 Mean	91 SD	178 Mean	178 SD
Males							
1-Air	predose	1.5	0.3	2.6	2.2	3.5	1.9
	5	1.0	0.5	0.7	0.1	2.4	0.8
	15	0.8	0.2	1.0	0.4	1.4	0.8
	30	1.0	0.7	2.2	3.1	1.2	0.3
2-Placebo	predose	1.7	1.7	2.9	2.6	4.0	3.1
	5	0.7	0.5	1.2	0.8	2.7	0.9
	15	1.0	0.5	1.4	0.7	1.7	1.0
	30	1.1	0.2	0.9	0.8	1.5	1.7
3-Low Dose	predose	1.5	0.7	2.1	1.4	3.3	1.7
	5	6.8	3.0	6.4	2.9	7.3	1.9
	15	7.1	2.8	7.1	2.0	8.0	1.7
	30	6.6	1.5	5.5	1.0	6.0	0.6
4-High Dose	predose	1.6	0.8	1.2	0.2	3.3	0.5
	5	17.4 (N=3)	3.8	17.7	8.0	18.3	3.6
	15	23.0	5.3	17.7	7.7	19.0	4.8
	30	23.3	6.4	19.3	9.9	21.9	7.6
Females							
1-Air	predose	0.5	0.1	1.0	0.1	1.4	0.3
	5	0.7	0.4	0.9	0.4	1.5	0.8
	15	0.8	0.4	1.4	1.0	1.2	0.7
	30	1.0	0.7	0.9	0.5	1.1	0.5
2-Placebo	predose	1.4	0.7	1.3	0.5	1.9	0.7
	5	0.9	0.6	1.3	0.6	1.6	0.7
	15	1.3	1.0	0.9	0.2	1.9	1.3
	30	1.0	0.2	0.9	0.3	1.7	0.7
3-Low Dose	predose	0.8	0.4	1.8	0.9	4.0	1.3
	5	3.9	1.2	6.4	2.4	4.2	1.0
	15	4.1	0.9	6.5	2.4	4.7	1.5
	30	4.2	1.0	6.0	2.3	4.6	1.5
4-High Dose	predose	0.7	0.3	2.6	1.7	2.0	0.5
	5	8.4	5.5	23.0	26.1	12.3	7.0
	15	9.8	5.2	23.0	26.0	11.6	5.7
	30	10.6	4.7	17.8	17.8	12.6	4.9

Exposure Group 1: 60-minute exposure to air
Exposure Group 2: 60-minute exposure to placebo
Exposure Group 3: 18-minute exposure to aerosol insulin
Exposure Group 4: 60-minute exposure to aerosol insulin
N=4, except where noted.

Bioavailability study in dogs with the new formulation (60% insulin)

In an acute dog study, AUC for inhalation insulin (2 mg or 52 units) was compared to SC insulin (0.36 mg/kg). The relative bioavailability of inhalation insulin was 7% in dogs. The arterial $AUC_{364\ min}$ for inhalation and injectable insulin were 8600±1200 µU/ml and 10200 ±999 µU/ml. The variability in the AUC was slightly greater in the inhalation insulin. In addition to the differences in the exposure tissue (lungs vs. subcutaneous) tissue, the inflexibility of inhalation dose adjustment (2 mg of blister pack) to dogs with BW ranging from 8 to 10 kg was likely an important contributor to AUC variability in dogs.

Arterial, Deep Venous and Portal Vein Plasma Insulin Levels in Dogs Given 2 mg Insulin by Oral Inhalation

ARTERIAL PLASMA INSULIN µU/ml 2 mg INHALATION

	PRE	INJ																
TIME	-8	0	4	9	14	19	34	49	64	79	94	124	154	184	214	244	304	364
Dog #																		
210	1.5		48.8	77.2	88.5	106.3	111.0	127.5	119.9	89.9	56.0	30.6	16.9	9.3	6.8	4.7	2.6	1.1
211	22.3		63.0	94.1	94.4	100.1	88.4	92.3	62.4	47.1	38.1	20.1	10.9	7.4	5.1	2.9	1.8	1.2
250	4.4		86.2	121.1	83.6	116.8	106.7	76.1	54.3	36.9	24.4	20.3	9.7	7.7	4.7	2.9	3.1	1.4
251	3.9		50.7	75.4	45.6	45.6	51.8	40.5	39.7	27.8	22.0	18.7	12.7	9.3	6.2	5.3	3.8	3.3
252	0.2		79.8	113.6	87.3	97.2	94.8	83.4	64.0	40.1	29.8	24.4	13.6	11.3	8.0	6.9	4.6	4.5
285	1.6		33.0	72.1	49.7	56.3	51.2	36.8	27.0	20.8	13.7	11.9	7.7	5.3	4.4	3.3	2.9	1.7
AVG	5.7		60.2	92.3	74.8	87.0	84.0	76.1	61.2	43.8	30.7	21.0	11.9	8.4	5.9	4.3	3.1	2.2
SEM	3.7		9.0	9.4	9.6	12.9	11.8	15.2	14.3	10.9	6.6	2.8	1.4	0.9	0.6	0.7	0.4	0.6

DEEP VENOUS PLASMA INSULIN µU/ml

	BL	PRE	INJ																
TIME		-8	0	4	9	14	19	34	49	64	79	94	124	154	184	214	244	304	364
Dog #																			
210	3.1			47.3	68.3	84.5	103.2	114.7	127.2	92.9	77.7	55.9	29.3	14.0	10.4	5.9	4.9	2.1	1.2
211	7.6			54.6	68.0	67.5	74.0	62.0	76.0	60.6	39.8	30.6	13.5	10.5	6.7	4.2	2.1	1.4	1.4
250	6.4			54.1	85.2	91.8	132.9	104.5	71.9	55.9	31.4	23.9	14.0	7.6	5.2	3.4	2.0	2.9	1.8
251	2.5			30.4	48.7	43.8	35.7	42.7	50.0	29.8	26.4	24.6	13.8	9.4	7.2	5.6	4.4	2.5	2.3
252	50.0			44.3	72.5	72.9	90.6	95.9	77.4	59.2	44.2	33.4	18.7	10.7	7.2	5.6	4.8	4.1	3.3
285	1.9			19.0	36.3	39.6	45.6	46.6	28.6	21.3	19.8	13.6	6.8	4.8	3.4	2.2	1.8	1.2	1.3
AVG	11.9			41.6	63.2	66.7	80.3	77.7	71.8	53.3	39.9	30.3	16.0	9.5	6.7	4.5	3.3	2.4	1.9
SEM	8.4			6.3	7.9	9.5	16.3	13.9	14.8	11.4	9.2	6.4	3.4	1.4	1.0	0.7	0.7	0.5	0.4

** [ ] = missing - samples not drawn due to catheter malfunction. Values interpolated using Art & DV relationship at 14 min.

PORTAL PLASMA INSULIN µU/ml

	PRE	INJ																
TIME	-8	0	4	9	14	19	34	49	64	79	94	124	154	184	214	244	304	364
Dog #																		
210			53.5	62.9	65.9	78.8	95.2	98.3	82.8	74.0	47.7	24.4	13.5	9.4	5.7	4.3	1.8	1.2
211			41.8	65.0	64.0	73.9	73.0	69.0	53.6	39.4	30.8	14.9	9.5	7.3	5.2	2.1	1.8	2.7
250			47.6	68.2	71.1	89.4	94.7	66.4	44.6	33.1	22.2	14.5	6.4	5.5	2.7	2.1	1.4	0.6
251			32.4	51.1	40.0	41.4	44.3	42.6	30.0	25.8	20.6	13.7	9.9	6.6	5.2	4.2	2.9	2.8
252			35.7	55.6	82.0	81.6	92.5	79.8	57.4	38.6	28.0	17.8	11.4	10.2	6.4	5.3	3.6	4.3
285			21.7	41.7	35.6	36.2	41.5	27.4	23.6	15.6	11.2	7.8	6.0	3.9	3.3	1.6	1.5	0.8
AVG			38.8	57.4	59.8	66.9	73.5	63.9	48.7	37.7	26.7	15.5	9.4	7.1	4.7	3.3	2.2	2.1
SEM			5.1	4.5	8.1	10.0	11.2	11.4	9.5	8.9	5.5	2.4	1.3	1.1	0.6	0.7	0.4	0.6

** [ ] = Assay error - value interpolated using 214 & 304 minutes.

Arterial, Deep Venous and Portal Vein Plasma Insulin Levels in Dogs Given 0.36 units/kg Insulin by Subcutaneous Injection

ARTERIAL PLASMA INSULIN µU/ml 0.36 units/kg SUBCUTANEOUS

	BL	PRE	INJ																
TIME		-8	0	4	9	14	19	34	49	64	79	94	124	154	184	214	244	304	364
Dog #																			
205		1.8		21.7	24.0	26.5	29.0	39.4	35.3	36.3	50.0	51.8	47.8	44.9	51.2	43.1	31.6	14.7	9.4
207		3.0		16.1	32.0	30.5	31.2	43.8	47.9	48.5	43.6	38.0	55.1	49.7	39.5	39.1	33.5	18.8	11.0
212		0.0		15.3	30.3	37.1	41.6	70.0	59.7	61.2	55.1	52.9	36.5	25.1	13.7	8.6	4.8	1.6	0.8
216		1.3		30.4	71.0	69.0	75.0	82.2	72.6	87.8	69.5	65.4	34.9	12.5	5.3	2.7	0.5	0.4	0.4
208		8.1		18.2	25.8	33.7	44.3	42.1	47.3	47.5	53.9	65.6	61.1	42.1	19.7	12.2	7.6	2.6	0.4
214		1.3		21.0	23.3	32.9	40.8	46.8	38.5	46.9	50.3	42.0	28.9	23.5	18.7	14.7	9.9	4.5	2.2
AVG		2.6		20.5	34.4	38.3	43.7	54.1	50.2	54.7	53.7	52.6	44.0	33.0	24.7	20.1	14.6	7.1	4.0
SEM		1.3		2.5	8.2	6.9	7.4	7.9	6.2	8.1	3.9	5.1	5.6	6.6	7.7	7.5	6.4	3.4	2.2

** [ ] (205) = missing sample. Point interpolated by using 4 & 14 minute values.
(216) = missing samples because arterial line was pinched off and had to be reaccessed. Data interpolated based on DV value - 19.

DEEP VENOUS PLASMA INSULIN µU/ml

	BL	PRE	INJ																
TIME		-8	0	4	9	14	19	34	49	64	79	94	124	154	184	214	244	304	364
Dog #																			
205	21.5			41.2	28.6	38.4	32.8	42.3	54.4	64.8	70.5	80.1	71.7	77.1	63.0	58.8	25.3	15.9	8.4
207	13.9			15.6	28.4	37.9	56.5	52.4	59.2	52.8	53.3	49.0	39.4	60.2	54.1	38.0	54.6	15.2	14.8
212	3.7			35.0	95.4	62.1	87.9	99.8	105.4	105.8	70.6	74.7	47.9	39.6	29.8	12.5	11.4	5.6	2.3
216				30.2	89.6	87.8	94.2	68.1	92.9	104.4	81.0	69.0	41.7	22.8	11.3	7.7	2.3	1.1	1.1
208	5.3			35.0	42.1	40.3	55.7	47.8	52.5	78.5	87.0	76.9	64.2	48.9	20.4	18.8	9.3	3.9	1.6
214	4.2			16.6	31.1	58.3	50.7	54.0	52.3	59.0	54.5	58.1	40.8	37.8	30.7	18.3	11.8	6.9	2.4
AVG	9.7			28.9	52.5	54.1	63.0	60.7	69.5	77.6	69.5	68.0	50.9	47.7	34.9	25.7	19.1	8.1	5.1
SEM	3.9			4.7	14.0	8.8	10.5	9.4	10.5	10.3	6.1	5.4	6.1	8.5	8.9	8.6	8.5	2.7	2.4

PORTAL PLASMA INSULIN µU/ml

	BL	PRE	INJ																
TIME		-8	0	4	9	14	19	34	49	64	79	94	124	154	184	214	244	304	364
Dog #																			
205				10.8	15.9	20.2	19.2	24.7	28.7	27.2	34.5	35.7	31.6	33.2	32.2	24.9	11.0	9.0	4.0
207				30.3	19.7	22.5	22.8	33.1	32.6	31.7	31.5	31.1	27.6	35.4	27.1	25.7	23.3	4.0	2.0
212				13.0	16.0	14.0	19.0	26.0	27.0	27.0	20.0	22.0	14.0	16.0	5.0	3.0	2.0	1.0	1.0
216				19.7	30.0	38.2	46.6	50.7	55.2	62.3	57.9	41.3	27.6	11.6	4.4	1.9	1.0	0.5	1.1
208				15.1	19.5	23.2	30.2	27.5	32.5	38.2	40.0	43.2	42.4	28.9	14.4	10.0	6.4	1.7	1.1
214				33.4	26.8	24.0	27.0	30.3	29.2	34.7	30.5	25.4	20.3	23.5	11.6	10.9	5.9	4.1	1.6
AVG				20.4	21.3	23.7	27.5	32.0	34.2	36.9	35.7	33.1	27.3	24.8	15.8	12.7	8.3	3.4	1.8
SEM				4.2	2.6	3.6	4.6	4.3	4.7	5.9	5.7	3.8	4.4	4.3	5.2	4.6	3.7	1.4	0.5

** [ ] = Value interpolated based on Arterial & Deep Venous values due to missing samples.

PK in humans

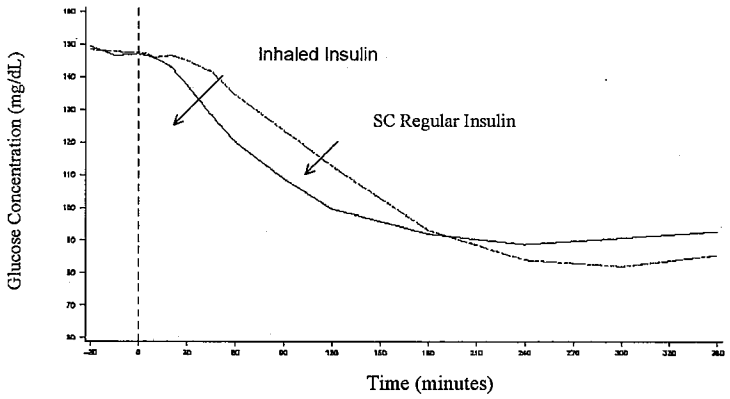
Pharmacokinetic parameters of INH in humans were not affected by age, gender or body mass index of the diabetic subjects. Mean glucose AUC concentrations after 4 mg INH in humans was similar to 12 Units of SC insulin. Some of the factors that may affect Pk parameters of INH are smoking and diabetes. Absorption of INH was greater in diabetic subjects than healthy subjects. Smoking increased the absorption rate and bioavailability of INH. It appears that smoking can increase the permeability of the lungs to aerosols. In smokers the Tmax was reduced to 20 to 30 min and Cmax increased by approximately 3 to 5 fold compared to non-smokers. The pk parameters of inhalation insulin in several clinical studies are shown in the table below.

Effects of Age on the Pharmacokinetics of Inhaled Insulin

			Dose		$AUC_{0\text{-}360}$[a] (µU.min/mL)		Cmax[a] (µU/mL)		Tmax (min)		F
Study	Age (y) (mean)	N	INH (mg)	SC (U)	INH	SC	INH	SC	INH	SC	(%)
217-018[b]	6-11 (8.8)	10	1, 2, or 3	3, 6, or 9	3760[c]	4590[c]	27.1[c]	22.9[c]	68	134	8.4[d]
	12-17 (14.2)	12	2, 3, or 4	6, 9, or 12	5730[c]	6880[c]	32.8[c]	31.9[c]	78	107	8.8[d]
217-021	23-43 (32.4)	22	3, 4, or 6	9, 12, or 18	6140	7340	35.2	32.9	61	136	8.2-10.7[d]
A2171003[e]	43-68 (55.6)	14	6	18	6770[f]	6420[f]	49.2	26.5	60	258	11[f]
A2171004[g]	63-80 (71.6)	20	4	12	4700	4820	48.6	28.6	38	100	11[d]

The AUC for 4 mg (104 U) inhaled insulin appeared to be equivalent to 12 U of insulin by SC route. The relative bioavailability of the EXUBERA® (—— with 60% insulin) was about 10% in humans. Nearly 90% of the inhalation insulin is lost to complex delivery system, powder settlement in the device and degradation in the lungs. Clinical data also have shown significant variability in drug delivery. In addition to the factors identified in animals, insulin absorption in humans has been shown to be improved by smoking and decreased by emphysema.

Mean Glucose Concentrations After 4 mg Inhaled Insulin or 12 U Subcutaneous Regular Insulin to Fasting Subjects with Type 2 DM



2.6.4.9 Discussion and Conclusions

The bioequivalence of different inhalation insulin formulations were compared to subcutaneous insulin in SD rats, monkeys, dogs and humans. Several insulin formulations with different concentrations of excipients were formulated to enhance pulmonary absorption of insulin powder. The mean relative bioavailability of the I-004 aerosol insulin formulation (20% insulin and —> mannitol and —— citrate salt) was about 44% in rats and 12% in monkeys. In dogs and humans, the relative bioavailability of inhalation insulin (— was about 7 and 10%.

Native insulin in blood is quickly degraded by the liver. Insulin powder administered by inhalation is both metabolized locally by the lungs and by the liver after reaching the systemic circulation. In chronic nonclinical studies, repeated administration of inhalation insulin was not associated with any notable insulin accumulation. There was only a dose-related increase in serum insulin and decrease in serum glucose. The variability in the relative bioavailability was a consistent finding across all inhalation insulin formulations and all species tested. The contributing factors to variability in pulmonary insulin were likely due to the formulation, delivery method/device, species differences and pulmonary metabolism. Additional contributing factors in humans are lung health status (i.e. emphysema) and smoking condition. Smoking has been shown to increase absorption rate and bioavailability of INH. It appears that smoking can increase the permeability of the lungs to aerosols. In smokers the Tmax was reduced to 20 to 30 min and Cmax increased by approximately 3 to 5 fold higher than non-smokers.

2.6.4.10 Tables and figures to include comparative TK summary

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Summary of Nonclinical Pharmacokinetics

Study	**Species**	**Route of Administration**	**Formulation[a]**	**Tmax**	**Bioavailability Relative. To Sc (Basis For Calculation)**
AA001	Rat	Inhalation (5-20 min; MMAD[b]	(1) rhu-insulin, citrate salts	16 min	10% (relative to rhu-insulin recovered by lung lavage)
			(2) rhu-insulin, mannitol, citrate salts	21 min	44% (same basis as above)
			(3) rhu-insulin, raffinose, citrate salts	17 min	14% (same basis as above)
	Monkey	Inhalation (12 min; mean MMAD = GSD[b] = 2.0); exposure described in study SC930056	Formulation (2) above	30 min	12% (based on estimated rhu-insulin inhaled)
N001603A(1)	Rat	Inhalation (60 min; MMAD GSD =	I-004[a]	At end of exposure	64% (based on estimated respiratory tract deposited dose)
N001603A(2)	Monkey	Inhalation (60 min; MMAD GSD = 3.3)	I-004[a]	2 min after end of exposure	93% (same basis as above) *note:* based on n=1
NRA217001	Dog	Inhalation (single or two bolus inhalations from 1 mg blisters, size not determined)		14 min (arterial)	Not available

[a] Denotes recombinant human insulin (rhu-insulin) source from Eli Lilly
[b] MMAD = mean mass aerodynamic diameter; GSD = geometric standard mean
[c] Denotes rhu insulin source from Aventis.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:
The toxicology profile of injectable insulin has been well documented, thus the objectives of the nonclinical studies were to evaluate the impact of inhalation of powder insulin formulation, EXUBERA® on the respiratory tract, pulmonary histopathology and function. Most of the toxicology studies included inhalation of fixed concentration of insulin for 5 to 60 min. Studies also included an excipient control and a filtered air control group (6-month studies). The aerosol powder insulin and placebo treatments were delivered to rats via nose only and to monkeys by a nose dome. The pivotal nonclinical studies were performed with an earlier insulin formulation (I-004, Lilly insulin) containing approximately 20% insulin. The new insulin formulation (Aventis insulin) used in the phase 3 clinical trials which will be also be marketed commercially 1-month bridging study in rats to test toxicological bioequivalence of the new formulation .

Insulin Inhalation Powder Formulations

Formulation ID % Insulin Component	% w/w	I-004 % w/w
Insulin		
Mannitol		
Glycine		
Sodium Hydroxide		
Sodium Citrate		
Citric Acid		
Total	100.0	100.0

The non-clinical studies performed are listed in table below with full review in the appendix (page 82). In the pilot and dose range finding studies in rats and monkey, insulin induced hypoglycemia was considered the dose limiting factor. Therefore, 6 mg/kg/d and 0.6 mg/kg/d were considered the maximum tolerated dose (MTD) in rat and monkeys, respectively.

Nonclinical Safety Studies of Inhaled Insulin in Rats and Monkeys

Study Type	Species/ Strain	No./Sex/ Group[a]	Recombinant Human Insulin Source	Target Doses (mg/kg/day)	Achieved Doses[b] (mg/kg/day)
1-month	Rat/Sprague-Dawley	10	Lilly	0[c], 1, 3, 6	0, 1.1, 3.2, 6.0[d]
1-month	Rat/Sprague-Dawley	10	Aventis	0[c], 0[e], 1, 3, 6	0, 0, 1, 3.2, 5.9[d]
6-month	Rat/Sprague-Dawley	10	Lilly	0[c], 0[e], 1, 3, 6	0, 0, 0.9, 2.7, 5.8[d]
5-day	Monkey/Cynomolgus	3	Lilly	Not available	0, 0.12, 0.60[d]
1-month	Monkey/Cynomolgus	4	Lilly	0[c], 0.15, 0.6	0, 0.14, 0.58[d]
6-month	Monkey/Cynomolgus	4	Lilly	0[c], 0[e], 0.2, 0.6	0, 0, 0.29, 0.64[d]

[a] Number animals evaluated in core toxicology group
[b] Dose is based on exposure concentration, exposure duration, and assumed (for rats) and measured (for monkeys) respiratory minute volume. Doses were adjusted by changing exposure duration. Exposure duration ranged from 10 to 60 minutes for rats, and from 12 to 60 minutes for monkeys.
[c] Excipient control animals received an excipient powder formulation of mannitol, glycine, and sodium citrate.
[d] Doses are maximum tolerated doses based on the limiting effect of hypoglycemia.
[e] Air control group received only filtered room air.

In the initial 1-month rat study # N001603C (0, 1.1, 3.2 and 6 mg/kg/d, n=10/sex/group) there were no notable clinical signs except for the decrease in plasma glucose concentration at 3.2 and 6 mg/kg/d in males and at ≥ 1.1 mg/kg/d in females. The death one female at 3.2 mg/kg/d after blood collection was not determined but was unlikely to be drug related. There was a significant increase in absolute lung weights in males treated with 1.1 mg/kg/d and female at 6.0 mg/kg/d. The exact nature of the increase in lung weight was not clear since it occurred at low dose in males and at high dose in females. Furthermore, there were no associated histological findings in the respiratory system. Whether the variability in insulin bioavailability or individual sensitivity to exogenous insulin played regarding increases in lung weight is unknown. The exposure to inhalation insulin doses in rats (1.1, 3.2 and 6 mg/kg/d) were 1.2, 3.8 and 6.4 fold greater than clinical dose of 0.15 mg/kg/day based on mg/m^2.

In the 6-month rat study # N002448A, (0, 0, 1, 0.9, 2.7 and 5.8 mg/kg/d, n=10/sex/group), animals were treated via nose with 0.2 mg/L aerosol powder insulin for 10 min (low dose, M:0.83 and F: 0.96 mg/kg/d), 30 min (mid dose, M:2.55 and F: 2.92 mg/kg/d) and 60 min (high dose, M: 5.4 and F: 6.17 mg/kg/d). The total inhaled mass in the placebo, LD, MD and HD groups were 25, 4, 13 and 27 mg/kg/d in females and 29, 5, 15 and 31 mg/kg/d in male rats, respectively. The placebo and air controls were exposed to 60 min of excipients and stream of air, respectively. Additional animals (6-10/sex/group) were used for evaluation of pulmonary function and toxicokinetic parameters.
There were total of seven deaths (main study: 3MD, 1 HD, TK study: 1 MD, 2HD). The causes of deaths were attributed to accidental trauma (2 MD), hypoglycemia (1MD, 2HD) and pathological conditions unrelated to insulin such as hydronephrosis and cystitis (1MD, 1HD). There were excipient or insulin related changes in body weight, ophthalmic or pulmonary function. As expected aerosol insulin administration significantly reduced serum glucose levels in dose-related manner. Serum insulin levels generally increased with increase in insulin dose but the increase was not dose-proportional due to variability in insulin concentrations. Similar variability in serum insulin levels have been observed in other nonclinical and clinical studies. Evaluation of presence of antibodies found only slight increase above the assay detection limits in one rat treated with human insulin. Although majority of the antibody concentrations were below assay detection limits, rat data collectively suggest antibody formation against recombinant human insulin. A slight increase in lung weight in the females at 2.7 mg/kg/d was not seen at higher doses or in males at any dose. There were no notable drug related changes in macroscopic or microscopic evaluations. The sporadic increases in lung weights were also noted in the 1-month study with no specific histopathology or in dose-related manner. The most common histopath findings observed in the lungs were minimal to mild focal or multifocal chronic-active inflammation and aggregation of alveolar histiocytes (foam cells) in all groups including the excipient and air control groups.

Most Common Findings in Lungs of Rats from 6-Month Study

	Air Controls		Excipient Con.		Low Dose		Mid Dose		High Dose	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Inflammation, chronic-active	5	2	6	5	6	5	8	7	7	6
Infiltrating cell, histiocyte, alveolus	4	5	3	1	0	1	0	1	1	0

Retrospective analysis of lung tissue for cell proliferation found no evidence of insulin or placebo related cellular proliferation. Evaluation of the pulmonary function in rats found no significant drug-related change in pulmonary parameters.

Pulmonary Function Data Following 6 Months Daily Inhalation of Rhu- Insulin and Excipients in Rats (Study N002448A)

	Mean (Standard Error)						
Exposure Group	Tidal Volume (mL)	Respiratory Rate (breaths/min)	Minute Volume (mL/min)	PIF[a] (mL/sec)	PEF[b] (mL/sec)	Resistance (cm H_2O/mL/sec)	Compliance (mL/cm H_2O)
Air Control	0.95 (0.14)	125.5 (8.4)	115.1 (15.5)	5.4 (0.8)	7.0 (0.7)	0.276 (0.052)	0.33 (0.04)
Excipients	0.87 (0.10)	118.1 (8.0)	96.2 (9.7)	4.7 (0.5)	6.2 (0.5)	0.357 (0.064)	0.32 (0.03)
Low Dose	0.87 (0.13)	124.1 (11.3)	98.5 (15.6)	4.8 (0.7)	6.4 (0.9)	0.352 (0.059)	0.43 (0.04)
Mid Dose	0.82 (0.12)	125.2 (12.2)	92.2 (15.1)	4.5 (0.7)	6.0 (0.9)	0.306 (0.039)	0.38 (0.06)
High Dose	0.88 (0.10)	117.2 (7.1)	103.2 (13.0)	5.1 (0.7)	6.6 (0.6)	0.273 (0.058)	0.37 (0.04)

[a] Peak Inspiratory Flow Rate. [b] Peak Expiratory Flow Rate.

In the absence of any significant drug related finding, the maximum dose used in the 6-month rats study, 5.8 mg/kg/d was considered NOAEL. The exposure at NOAEL was approximately 6.2X the clinical dose of 0.15 mg/kg/d based on mg/m^2. The safety margins for rat NOAEL dose based in alveolar surface area was slightly lower (4 fold the clinical dose). The excipient concentrations in the 6-month study provided sufficient safety margins for the clinical dose. The safety margins for mannitol, Na citrate and glycine were 34, 40 and 20X the excipient concentrations in the clinical dose based on mg/m^2, respectively.

In the 1-month Cynomolgus monkey study # N001603B (placebo, 0.14 and 0.58 mg/kg/d, n=4/sex/dose), animals were treated with aerosol insulin (30 µg/L) for 12 min (LD) or 30 min (HD). As expected, human insulin aerosol treatment decreased plasma glucose and increased plasma insulin in a dose-related manner in monkeys. There was no notable change in body weight, clinical chemistry, hematology, ophthalmic or ECG parameters. Respiratory function parameters measured during the study were not significantly affected by the aerosol insulin administration. There were no antibodies against human insulin in serum suggesting that exogenous human insulin was not antigenic. Insulin levels increased with increase in the insulin dose. Serum insulin levels on Day 29 were higher than Day 1 in the high dose groups. It is not clear whether higher insulin levels were due to gradual accumulation of insulin in the lungs were acclimation of the animals to the inhalation procedure. Histopathological evaluation of the tissues revealed no major findings except for minimal subacute inflammation of the nasal and tracheal mucosa and subacute inflammation were noted sporadically in all groups in a non-dose dependent manner. The only adverse effects noted in the study were a few incidences of hypoglycemia in the high dose females which were resolved with dextrose administration also suggesting that the high dose was likely the maximum tolerated dose. The exposure at LD (0.14 mg/kg/d) and HD (0.58 mg/kg/d) were approximately 0.3 and 1.2X the clinical dose of 0.15 mg/kg/day, based on mg/m^2, respectively.

In the follow up 6-month monkey study # N002448B (0, 0, 0.29 and 0.64 mg/kg/d, n=4/sex/group), animals were exposed to human insulin aerosol (30 µg/L) for 18 min (LD, M:0.298, F:0.284 mg/kg/day insulin) and 60 min (HD, M: 0.624 and F: 0.654 mg/kg/day

insulin). Placebo (excipient) control and air controls were exposed for 60 min to excipients or filtered air, respectively. Human insulin aerosol treatment decreased plasma glucose and increased plasma insulin in a dose-related manner. Similar to the 1-month study, several incidences of hypoglycemia were noted in the high dose groups. Body weight gain in the insulin treated groups increased relative to air control. Respiratory difficulties were observed in some of the control and treatment groups in the first 2 to 3 weeks of the study, which were likely related to inhalation of the excipients. A low incidence of cough and sneezing were observed through out of the study with inhalation of both insulin and placebo powder in monkeys.

Respiratory Events That Limited a Daily Exposure in the 6-Month Monkey Study (Study N002448B)

Study Day	Group	Animal	Sex	Observation
13	High Dose	453	F	Cough, wheezing, gasping - exposure paused, re-started and then terminated for the day.
14	High Dose	453	F	Cough, wheezing, gasping - exposure terminated for the day.
16	High Dose	401	M	Cough, gasping – exposure terminated for the day.
16	Excipients	203	M	Labored respiration - exposure paused and then resumed.
35	Low Dose	303	M	Gasping, wheezing - exposure terminated for the day.
42	Low Dose	302	M	Labored respiration - exposure paused and then resumed.

Excipients = formulated powder containing mannitol, glycine and sodium citrate without rhu-insulin.

Examination of the lungs found no drug-related pathological abnormalities except for mature dense fibrous connective tissue in the periphery of the lung lobes representative of adhesion in one female at low dose. Acute inflammation was noted in one low dose male and epithelial ulcer in one low dose female. The remaining pulmonary, tracheal and larynx findings were seen in controls and treated groups thus were considered related to mechanical trauma due to bronchial alveolar lavage procedure.

Most Common Findings in the Lungs of Monkeys from 6-Month Study

	Air Controls		Excipient Con		Low Dose		High Dose	
	Male	Female	Male	Female	Male	Female	Male	Female
Infiltrating cell, histiocyte, alveolus	4	4	4	4	4	4	3	4
Inflammation, subacute	1	2	3	3	0	2	2	1

There were no notable treatment or excipient related changes in ophthalmic, hematologic, clinical chemistry, ECG, immunologic or pulmonary function. The insulin exposure at 0.14 and 0.64 mg/kg/d were approximately 0.6 and 1.4X the clinical dose of 0.15 mg/kg/day based on mg/m^2. With the lower concentrations of excipients in the clinical insulin formulation, the safety margins for the excipients were adequate. The safety margins for the mannitol, Na citrate and glycine were 8, 9 and 4X the excipients in the clinical dose based on mg/m^2, respectively. To assess the toxicological equivalence of the new clinical insulin formulation, [redacted], a 1-month bridging study was performed in rats (0, 0, 1, 3.2 and 5.9 mg/kg/day, n=10/sex/group,

study # 01-906-06). Similar to other studies, the dose level was adjusted by changing the duration of the inhalation of 333 µg/L of insulin (LD: 10 min, MD: 30 min and HD: 60 min). The new formulation, [redacted] was made with Aventis recombinant human insulin (60%) containing [redacted] portions of mannitol, Na citrate and glycine. In addition to TK group, the study also included a placebo (excipients) and air control (filtered air) groups similar to 6-month studies. There were total of 3 deaths (1 air control, 1 placebo and 1 HD) that occurred on Day 16. Deaths appear to be CO_2 anesthesia related. There were no treatment related changes in BW, ophthalmic or hematology parameters in the main study. However, as expected plasma glucose levels decreased and serum insulin levels increased in a linear manner. Glucose levels decreased within 30 min of MD and HD after insulin administration. Analysis of antibody against human insulin found at least 18 animals (air control=1, LD=3, MD=5, HD=9) with antibody levels above the assay detection limits suggesting that human insulin with dissimilar sequence to rat insulin is weakly immunogenic in rats. There were no drug-related changes in lung weight or any other organ weights, suggesting that sporadic increase in lung weight in rats is not consistent. Whether the inconsistent increase in the lung weight is due to the variability in bioavailability or lung insulin sensitivity is not clear. Histopathological analysis found minimal focal thickening of the lung interstitium in 1 MD and 1 HD female. In addition minimal hyperplasia of the bronchial epithelium was noted in 1 placebo control and 1 MD male. Common to all rats was the incidence of alveolar inflammation. Although there may have been a slight tendency for a treatment related effect on the lungs, collectively there was no concrete evidence of insulin related lung toxicity. Pharmacokinetic analysis found no accumulation in serum insulin with repeated administration of [redacted] in the 1 month bridging study in rats. The 1-month bridging study findings appeared to be in agreement with the results of the 1 and 6-month rats study with previous formulation (I-004). The NOAEL dose of 5.9 mg/kg/d was approximately 6.4 fold the clinical dose of 0.15 mg/kg/d, based on mg/m^2. The

Human safety margins for the clinical dose of 0.15 mg/kg/d was calculated based on body weight, body surface area and alveolar surface area.

Species	Dose, mg/kg/d	Dose, mg/m^2	Human safety margins (animal/human) based on different criteria		
			mg/kg body weight	mg/m^2 body surface area	mg/m^2 alveolar surface area
Rat, 1-month study	1.1	6.6	7	1	
	3.2	19.2	21	4	
	6	36	40	6	4
Monkey, 1- month study	0.14	1.68	1	0.3	
	0.58	7	4	1.2	1
Rat, 6-month study (NOAEL 5.8 mg/kg/d)	0.9	5.4	6	1	0.6
	2.7	16.2	18	3	2
	5.8	35	39	6	4.0
Monkey, 6-month study (NOAEL 0.64 mg/kg/d)	0.29	3.4	2	0.6	0.5
	0.64	7.7	4	1.4	1
Rat, 1 month bridging study	5.9	36	40	6	4
Clinical therapeutic dose, 0.15 mg/kg/d	0.15	5.6			

Since the safety margins based body surface area (1.4 to 6 fold) and alveolar surface area (1 to 4 fold) were in close agreement and in stark contrast to overstated safety margins based on mg/kg

body weight, the safety margins based on mg/m^2 were used throughout the review. As for the excipients, the higher proportion of mannitol, Na citrate and glycine in the I-004 formulation provided even greater safety margin than the "to be marketed" clinical formulation, The safety margins the excipients based on mg/m^2 and body weight for are listed in tables below.

Human Safety margin for excipients based on body surface area (mg/m^2)

Species	Excipient doses, mg/kg/day (mg/m^2)			Human safety margins (animal/human) based on body surface area, mg/m^2		
	Mannitol	Na Citrate	Glycine	mannitol	Na Citrate	Glycine
Rat, 6-month study	5.3 (31.8)	17.1 (102.6)	0.8 (4.8)	34	40	20
Monkey, 6-month study	0.582 (6.98)	1.89 (22.7)	0.083 (1)	7.5	9	4
Clinical dose of excipients, mg/kg/d (mg/m^2)	0.025 (0.93)	0.0687 (2.54)	0.0065 (0.24)			

Human Safety Margins for Inhaled Excipients in the Rhu-Insulin Inhalation Powder Formulation

		Mannitol		Sodium citrate		Glycine	
Species	Group	mg/kg/day inhaled	Safety margin	mg/kg/day inhaled	Safety margin	mg/kg/day inhaled	Safety margin
Human		0.025	n/a	0.0685	n/a	0.0065	n/a
Rat	High dose insulin	5.3	212X	17.1	249X	0.8	123X
	Excipients Control	4.9	196X	21.5	314X	0.7	107X
Monkey	High dose insulin	0.582	23X	1.89	27X	0.083	12X
	Excipients Control	0.519	21X	2.26	33X	0.074	11X

Genetic toxicology: The sponsor had conducted several genotoxicity studies for the insulin formulation. Under the assay conditions, insulin was not genotoxic.

Carcinogenicity: Insulin is an endogenous protein with long history of use in humans, thus required no specific carcinogenicity studies. The excipients used in the formulation are generally regarded as safe (GRAS).

Reproductive toxicology: No specific reproductive toxicity studies were done or required.

Special toxicology: NA

2.6.6.2 Single-dose toxicity.

Single dose studies were briefly reviewed but will not be discussed here due to limited toxicological value.

2.6.6.3 Repeat-dose toxicity

The 6-month rat and monkey studies were reviewed earlier by Dr. Herman Rhee. The reviews are attached to the appendix on page 73.

Study title: One month inhalation toxicity study with insulin powder to rats

Key study findings: In this 1-month rats study (333 µg/L of insulin for 10, 30 ad 60 min), animals were treated with the clinical formulation (——) containing Aventis insulin (60%). The study was designed to bridge previous pre-clinical studies with earlier formulation (I-004) containing 20% insulin and to of confirm the toxicological equivalence of the two formulations. As expected there was a significant dose-related decrease in blood glucose and increase in serum insulin levels. Although there appeared to be slight increase in incidence of focal thickening of the interstitium with alveolar inflammation in 1 MD and 1 HD female and hyperplasia of bronchial epithelium in 1 vehicle and 1 HD male, the pulmonary signals were not decisive. Analysis of insulin antibodies revealed a small but dose-related increase (3 LD, 5 MD and 9 HD) in insulin antibodies above the limits of quantification (3 µU/ml).

Study no.: 01-906-06 (—— # MN10214)
Volume #, and page # electronic submission, page 1-340
Conducting laboratory and location: ——
Date of study initiation: Aug 20, 2001
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: lot# 2330, — insulin (—— of powder)
Methods

EXPERIMENTAL DESIGN						
					Number of Rats	
Group Number	Target Exposure Time (min.)	Target Total Aerosol Mass Concentration (µg/L)	Target Aerosol Insulin Concentration (µg/L)	Estimated Inhaled Insulin Dose[a] (mg/kg)	Core Tox Subgroup	Toxicokinetic Subgroup
1-Air Control	60	0	0	0	10/sex	–
2-Vehicle	60	333	0	0	10/sex	10/sex
3-Low Dose	10	333	200	1	10/sex	10/sex
4-Mid Dose	30	333	200	3	10/sex	10/sex
5-High Dose	60	333	200	6	10/sex	10/sex
6-Satellite	NA[b]	NA	NA	NA	–	10/sex

a. Calculated on the basis of a 0.132 L minute volume and a 250 g rat. Actual values for aerosol concentration and body weights will be used in the calculation of the inhaled dose and may not reflect values listed in above table.
b. NA indicates not applicable.

Doses: 333 µg/L for 10, 30 ad 60 min for LD, MD, HD, respectively
Species/strain:—— CD® (SD) IGS BR, ——
Number/sex/group or time point (main study): 20/sex/dose
Route, formulation, volume, and infusion rate: inhalation
Satellite groups used for toxicokinetics or recovery: yes
Age: 6-8 weeks
Weight: 128.8 to 278.2 g
Sampling times: TK samples for analysis of glucose and insulin were collected at 3, 10 and 30 min post exposure from groups 2, 3, 4 and 5 on Day 1 and 30. Blood samples were also collected for human insulin antibody analysis on Day 31 at the time of necropsy.

Unique study design or methodology (if any): This study was designed to bridge the existing pre-clinical studies with old formulation (— insulin) to new clinical formulation containing — insulin. Animals were exposed by nose only to the dry insulin powder. Control animals were exposed to vehicle excipients in the insulin powder or air for 60 min. Histopathology of the entire respiratory tract (nasal turbinates, larynx, trachea and lungs with bronchial tree) was also examined. Aerosol samples were analyzed for spatial and temporal uniformity. Spatial uniformity is defined as the percent relative standard deviation (%RSD) of the mean aerosol concentration measurements collected from each of the different tiers. Chamber aerosol concentration spatial uniformity data indicated a uniform distribution (11 to 14 %RSD) of the aerosol within each exposure chamber. Temporal stability is defined as the percent relative standard deviation (%RSD) of the aerosol concentration from a series of aerosol filter samples collected at the reference port of the exposure system over the duration of the expected dose period. The results of the aerosol concentration temporal stability testing indicated stable aerosol concentrations (5 to B%RSD).

Pre-Study Aerosol Concentration Mean (µg/L) (and %RSD)			
Exposure System:		Vehicle	Test Article
Spatial Uniformity	Total Mass	375 (14%)	299 (13%)
	Insulin Mass	0.0 (--%)[a]	157 (11%)
Temporal Stability	Total Mass	411 (9%)	325 (8%)
	Insulin Mass	0.0 (--%)[a]	170 (5%)

a. Below the limit of quantitation or not detected.

Pre-Study Aerosol Particle Size Distribution			
	Total Mass		Insulin Mass
Exposure System:	Vehicle	Test Article	Test Article
MMAD (µm)			
GSD	1.7	1.8	1.5

Observations and times:
Mortality: daily observation
Clinical signs: twice daily
Body weights: weekly
Food consumption: not measured
Ophthalmoscopy: yes (before and near the end of the study)
EKG: not measured
Hematology: standard hematology
Clinical chemistry: standard clinical chemistry plus insulin and human insulin antibodies
Urinalysis: not measured
Gross pathology: standard tissue
Organ weights (specify organs weighed if not in histopath table): Adrenal glands, brain, heart, kidneys, liver, lungs, ovaries and testes

Histopathology: Adequate Battery: yes (X), no ( ) (Full standard tissue pathology list)
Peer review: yes (x), no ( )

Results

There was variability in the aerosol concentrations. Overall data suggest, the administered doses were close to target value of 333 µg/L.

Aerosol Variability Summary					
Dose Group	Dates	Aerosol Concentration (µg/L)			
		Mean	SD	%RSD	% Target
3	082001 to 091101	327	57	17%	98%
	091201 to 091901	376	214	57%	113%
	082001 to 091901	340	119	35%	102%
4	082001 to 091001	351	42	12%	105%
	091101 to 091901	350	129	37%	105%
	082001 to 091901	350	77	22%	105%
5	082001 to 090901	334	49	15%	100%
	091001 to 091901	286	119	42%	86%
	082001 to 091901	318	82	26%	95%

Mortality:

- 3 Males in the main study died prior to inhalation on Day 16 during clinical pathology blood collections (#107, air control, #203, 0 mg/kg insulin, #501, 6 mg/kg insulin). There were no notable clinical observations, injury or microscopic findings. Since animals were anesthetized by CO2 for blood collection, it is likely that there was too high of exposure to CO2.

Clinical signs:

- Nine males and 5 females had clinical signs (alopecia, physical restraint abrasions) however, the abnormal clinical signs were not insulin or excipient related.

Body weights:

- There were no insulin related changes in body weight. The BW of male non-exposed satellite group was higher than males in air control groups. This is likely due to less activity and stress.

Summary of Group Mean Body Weights (grams)

Target Inhaled Insulin Dosage (mg/kg/day)		Days						
		-5	1	8	15	22	29	31[a]
Males								
0 (Air)	(N)	10	10	10	10	9	9	9
	Mean	201.3	237.9	278.0	315.1	340.6	371.7	377.6
	SD	13.2	16.3	17.1	19.1	24.6	24.9	25.9
0 (Vehicle)	(N)	20	20	20	20	19	19	9
	Mean	201.9	236.2	272.0	309.4	342.7	369.4	378.2
	SD	14.3	18.1	21.8	25.5	26.1	27.6	25.5
1	(N)	20	20	20	20	20	20	10
	Mean	202.3	239.5	278.2	315.0	342.7	372.1	382.3
	SD	14.6	18.8	26.0	33.1	42.3	46.9	60.9
3	(N)	20	20	20	20	20	20	10
	Mean	202.1	237.1	275.8	311.6	339.2	367.2	361.0
	SD	14.3	19.7	25.6	30.3	34.4	40.8	25.4
6	(N)	20	20	20	20	19	19	9
	Mean	202.3	239.2	279.9	319.5	349.0	381.1	389.0
	SD	14.0	17.4	20.6	25.0	30.1	30.9	42.0
Satellite	(N)	10	10	10	10	10	10	--
	Mean	202.7	244.6	297.0	**347.4***	**392.5***	**430.5***	--
	SD	14.3	15.2	23.1	28.5	31.1	34.0	--

a. Toxicokinetic animals and satellite animal not listed here.

* Group mean was statistically significant from the air control at p≤0.05

Ophthalmoscopy: No insulin or excipient-related ophthalmic findings
Hematology: No notable finding

Clinical chemistry:
No notable insulin dose-related findings except for insulin, glucose and triglycerides.

- On Day 31, triglycerides in the HD males (6 mg/kg/day) increased by 64% relative to air control and 44% relative to vehicle control (no change in triglycerides on Day 16).
- On Day 1, glucose levels in LD, MD and HD males decreased by 11, 36 and 44% in males and 1in females by 7, 33 and 52%, respectively.
- On Day 30, glucose levels in LD, MD and HD males decreased by 9, 13 and 39% and in females by 24, 38 and 47%, respectively.
- Significant decrease in glucose levels in rats at MD and HD at 30 min post dose on Day 1 and Day 30 suggests insulin was getting absorbed through the lungs after aerosol nose delivery of powder insulin.

Group Mean Serum Glucose (mg/dL) in Toxicokinetic Animals within 30-Minutes Post-Exposure

Target Inhaled Insulin Dosage (mg/kg/day)		Study Days			
		1	30	1	30
		Males		Females	
Satellite	Mean	144	139	129	137
	SD	25	32	16	17
0 (Vehicle)	Mean	147	135	**144***	140
	SD	13	25	7	32
1	Mean	131	123	119	106
	SD	15	26	31	28
3	Mean	**94***	117	**97***	**87***
	SD	23	48	34	35
6	Mean	**82***	**83***	**69***	**74***
	SD	27	20	14	14

* Group mean was statistically significant from the control (satellite group) at $p \leq 0.05$. N=9

Analysis of human insulin antibody in rats
Since the aerosol insulin was a recombinant human insulin, the antibodies in rat serum against human insulin was evaluated.

- Majority of samples were below the insulin antibody assay limits of quantification (<3μU/ml).
- Few serum samples from air control (n=3), vehicle control (n=1) low dose (n=3), mid dose (n=5) and high dose (n=9) had insulin antibodies that were above the limits of quantification in the antibody assay (3μU/ml of serum).
- Overall, the insulin antibody formation appeared to increase with increase in recombinant human insulin dose suggesting that aerosol human insulin is weakly immunogenic.

Insulin Binding Capacity At or Above the Limit of Quantitation (LOQ)

Dose Group	Sex	Animal ID	Binding Capacity (uU/mL)
Air Control	F	153	3.8
	F	155	3.4
	F	158	4.3
Vehicle	M	202	5.3
Low-Dose	F	352	4.0
	F	355	58.6
	F	358	4.2
Mid-Dose	M	407	6.3
	F	451	3.0
	F	452	3.7
	F	456	3.5
	F	457	58.0
	F	459	8.3
High-Dose	M	504	8.0
	M	505	3.8
	M	506	3.1
	F	552	3.8
	F	554	4.2
	F	555	8.3
	F	556	4.9
	F	558	4.9
	F	559	4.5
LOQ = 3 uU/mL M = male; F = Female			

Gross pathology:
No drug or vehicle-related macroscopic findings. As noted earlier there were 3 unscheduled deaths. The deaths appeared to be due to blood collection with no respiratory tract pathology.

Organ weights: No drug or vehicle-related change in organ weight.
Histopathology:

- Minimal focal thickening of the interstitium (inflammation, interstitial) associated with alveolar inflammation 1 MD, 1 HD female. This is occasionally seen in healthy animals as well.
- Hyperplasia of bronchial epithelium in 1 male vehicle and 1 MD male
- Small epithelial polyp in bronchus of 1 air control male
- Isolated inflammatory foci in the nose and lungs of most rats including air control and vehicle control, likely related to route used in the study
- There were no clear relationship between lung observations and treatment. However, there appeared to be a trend to slightly higher incidence of interstitial thickening in vehicle and insulin treated groups.

Summary of Microscopic Observations

Controls from group(s): 1 (Air)	Sex:	Males					Females				
	Inhaled Insulin Dosage (mg/kg/day):	0 (Air)	0 (Vehicle)	1	3	6	0 (Air)	0 (Vehicle)	1	3	6
	Number in Group:	10	20	20	20	20	10	20	20	20	20
Tissues with Diagnoses	Number Necropsied:	10	10	10	10	10	10	10	10	10	10
NOSE/TURBINATES	Number examined:	10	10	10	10	10	10	10	10	10	10
Inflammation, Respiratory Epithelium		1	0	0	0	0	0	0	0	0	0
Disorganization, Olfactory Epithelium		0	1	0	0	0	0	1	0	0	0
Inflammation, Squamous Epithelium		0	0	0	0	1	1	0	1	0	1
TRACHEA	Number examined:	10	10	10	10	10	10	9	10	10	10
LARYNX	Number examined:	10	10	10	10	10	10	10	10	9	10
Inflammation, Subacute		0	0	0	0	0	1	0	0	0	0
LUNG	Number examined:	10	10	10	10	10	10	10	10	10	10
Inflammation, Alveolar		2	6	3	3	8	4	3	3	6	1
B-polyp, Bronchial Epithelium		1	0	0	0	0	0	0	0	0	0
Hyperplasia, Bronchial Epithelium		0	1	0	1	0	0	0	0	0	0
Inflammation, Interstitial		0	0	0	0	0	0	0	0	1	1
LN-BRONCHI	Number examined:	8	10	9	10	7	10	9	10	8	6

All Diagnoses; Phases: All; Death types: All; Date of death range: 04-Sep-01 To 20-Sep-01

Toxicokinetics:

- On Day 1, mean maximum insulin levels in rats treated with 1 (LD), 3 (MD) and 6 mg/kg (HD) insulin were 174, 582 and 394 ng/ml in males and 171, 323 and 274 ng/ml in females
- On Day 3, mean maximum insulin levels in rats treated with 1 (LD), 3 (MD) and 6 mg/kg (HD) insulin were 82, 200 and 456 ng/ml in males and 292, 326 and 280 ng/ml in females

Mean Serum Insulin, Serum Glucose and Blood Collection Times for the Toxicokinetic Groups

Inhaled Insulin Dose (mg/kg)		Day of Study					
		1	30	1	30	1	30
		Actual Time (min.)		Insulin (ng/mL)		Glucose (mg/mL)	
Males							
Satellite	Mean	6	7	0.332	0.258	142	146
	SD	0	0	0.094	0.044	5	33
	Mean	8	7	0.319	0.267	160	117
	SD	2	1	0.072	0.046	39	8
	Mean	5	7	0.567	0.332	128	154
	SD	0	0	0.259	0.196	10	42
0	Mean	3	3	0.251	0.201	148	120
	SD	1	0	0.021	0.032	6	7
	Mean	10	10	0.243	0.209	160	136
	SD	1	1	0.049	0.016	12	31
	Mean	30	30	0.245	0.154	134	148
	SD	0	0	0.059	0.033	3	31
1	Mean	3	3	174	52.7	146	127
	SD	0	0	112	26.4	6	28
	Mean	10	11	148	84	122	129
	SD	0	0	72	82	13	40
	Mean	30	30	140	67	125	112
	SD	0	1	48	60	12	10
3	Mean	3	3	221	124	105	172
	SD	0	0	195	39	18	36
	Mean	10	10	582	200	77	87
	SD	1	1	436	75	12	16
	Mean	30	30	122	120	102	92
	SD	0	0	85	82	30	30
6	Mean	3	3	314	421	110	88
	SD	0	1	415	632	26	16
	Mean	10	10	359	456	69	95
	SD	0	0	138	432	17	25
	Mean	30	30	394	410	66	66
	SD	1	0	293	273	6	6

Mean Serum Insulin, Serum Glucose and Blood Collection Times for the Toxicokinetic Groups

Inhaled Insulin Dose (mg/kg)		Day of Study					
		1	30	1	30	1	30
		Actual Time (min.)		Insulin (ng/mL)		Glucose (mg/mL)	
Females							
Satellite	Mean	7	5	0.92	0.316	129	135
	SD	1	1	0.46	0.127	13	9
	Mean	6	7	0.89	0.232	141	144
	SD	1	2	0.59	0.046	17	31
	Mean	5	6	0.315	10.5[a]	117	133
	SD	1	0	0.058	17.9[a]	10	5
0	Mean	3	3	0.376	0.230	146	124
	SD	0	0	0.058	0.070	8	14
	Mean	10	11	0.302	0.232	145	160
	SD	0	1	0.070	0.100	8	50
	Mean	30	30	0.277	0.286	141	135
	SD	1	1	0.078	0.068	6	16
1	Mean	3	3	116	97	152	135
	SD	0	0	37	47	26	33
	Mean	10	10	42.2	187	105	91
	SD	0	1	29.1	108	22	7
	Mean	30	30	171	297	100	93
	SD	0	0	142	224	13	5
3	Mean	3	3	218	111	122	124
	SD	0	0	102	53	22	25
	Mean	10	10	323	326	65	79
	SD	0	1	174	206	9	33
	Mean	31	30	208	118	103	59
	SD	1	1	127	117	38	4
6	Mean	3	3	255	280	76	72
	SD	1	1	72	45	21	6
	Mean	10	10	230	149	64	69
	SD	0	0	19	23	12	10
	Mean	30	30	274	126	67	82
	SD	0	0	165	43	11	22

a. Mean and standard deviation are 0.198 and 0.019, respectively, if the value for animal #658, outlier, is not utilized.

APPEARS THIS WAY ON ORIGINAL

Supplemental Toxicity studies:

Study Title: Assessment of cellular proliferation indices in the lungs of Cynomolgus monkeys and SD rats (Study #01-906-05)

The potential for recombinant insulin administered by pulmonary route (inhalation) to induce proliferation of lung cellular indices were evaluated from subgroup of animals in the 6-month toxicology studies. Lung samples fixed in formalin from a subgroup of monkeys (air and vehicle controls, 06 mg/kg/d, n=4/sex/grup) and rats (air and vehicle controls, 6 mg/kg/d, n=10/sex/group) were used the immunohistochemistry staining with Ki-67 and PCNA, markers of cell proliferation. Ki-67 is rabbit polyclonal antibody used in labeling proliferating cells in variety of tissue types and works well with formalin fixed tissue samples. PCNA is a mouse IgG2a antibody used primarily to stain cell nuclei at different stage of cell division (S phase, G1 and G2 phases).

Results:

The lung tissues from monkeys and rats were successfully stained with Ki-67 and PCNA antibodies. The positive control, peribronchiolar lymphoid tissues from all animals had distinct yellow-brown staining of nuclei suggesting the staining procedure was reliable. There was no statistical difference in control and insulin treated groups for bronchiole or alveolar cell labeling indices.

In monkeys the mean labeling indices in the terminal/respiratory bronchiolar epithelium for all groups were less than 2% (range: 0.97-1.99%). Mean alveolar labeling indices for monkeys were less than 1% (range: 0.5 1-0.95%). In rats the mean labeling indices for bronchioles were less than 1% (range:0.20-0.63%) and the mean alveolar labeling indices were slightly lower (range: 0.15-0.49%). In both of these 6-month inhalation studies there was no evidence of a treatment-related increase in cell proliferation in the lungs of either species. The absence of lung cell proliferation evaluated by immunohistochemistry supports lack of histopathology reported in the 6-month rat and monkey toxicology studies.

ASSESSMENT OF CELLULAR PROLIFERATION INDICES IN THE LUNGS OF CYNOMOLGUS MONKEYS AND SPRAGUE-DAWLEY RATS

Study No. 01-906-05 **LABELING INDICES**

			AIR CONT.	VEHICLE CONTROL	HIGH DOSE
Monkey (Study N002448B)					
Males (n=4/group)	Bronchioles	Mean (Std.)	**1.99% (1.30%)**	**1.20% (0.39%)**	**1.78% (0.60%)**
		Min Max	1.21% 3.93%	0.71% 1.66%	1.40% 2.67%
	Alveoli	Mean (Std.)	**0.95% (0.36%)**	**0.85% (0.26%)**	**0.77% (0.24%)**
		Min Max	0.64% 1.43%	0.51% 1.06%	0.46% 1.03%
Females (n=4/group)	Bronchioles	Mean (Std.)	**1.60% (0.73%)**	**0.97% (0.48%)**	**0.98% (0.17%)**
		Min Max	0.96% 2.63%	0.40% 1.48%	0.79% 1.19%
	Alveoli	Mean (Std.)	**0.88% (0.19%)**	**0.51% (0.24%)**	**0.78% (0.25%)**
		Min Max	0.60% 1.03%	0.37% 0.88%	0.46% 1.01%
Rat (Study N002448A)					
Males (n=10/group)	Bronchioles	Mean (Std.)	**0.25% (0.22%)**	**0.20% (0.19%)**	**0.38% (0.27%)**
		Min Max	0% 0.79%	0% 0.59%	0% 0.80%
	Alveoli	Mean (Std.)	**0.16% (0.16%)**	**0.15% (0.10%)**	**0.32% (0.29%)**
		Min Max	0% 0.40%	0% 0.30%	0% 0.87%
Females (n=10/group)	Bronchioles	Mean (Std.)	**0.33% (0.27%)**	**0.38% (0.31%)**	**0.63% (0.32%)**
		Min Max	0% 0.79%	0.10% 1.00%	0.10% 1.08%
	Alveoli	Mean (Std.)	**0.23% (0.30%)**	**0.25% (0.17%)**	**0.49% (0.43%)**
		Min Max	0% 0.90%	0.10% 0.60%	0% 1.20%

All entries are rounded to two decimal places.

Title: Radioligand binding assay for insulin antibodies in serum and bronchial alveolar lavage samples from 6-month inhalation toxicity study of aerosol insulin in monkeys (01-906-02)

To examine the potential neutralization of recombinant human insulin, the presence of antibody to human insulin in the 6-month monkey study was evaluated using enzyme-linked immunosorbent assay (ELISA, Study # N002448B). The samples were also measured using a validated monkey anti-human insulin antibody radioligand binding assay (RLB) assay.

Results:
There were no detectable insulin antibodies in serum samples collected pre-dose and on Day 183 post dose and in bronchial alveolar lavage fluid (BAL) samples collected on Day 183 from monkeys. The samples were also evaluated using RLB. The RLB assay is a very sensitive assay for measuring insulin-specific antibodies the samples. Using this RLB assay, insulin antibodies were not detected in most of the stored serum and BAL. A few serum and BAL samples from both control and insulin-treated monkeys had measurable antibodies slightly above the limit of quantitation (LOQ). Overall the findings suggest that inhaled insulin did not result in notable insulin antibody formation in monkeys. It should be noted that the degradation of antibodies during storage (approximately 3.5 years) could have affected the sample integrity.

Samples with Insulin Binding Capacities Above the LOQ*

Sample Type	Exposure Group	sample collection day	Animal ID	Sex	binding capacity (uU/mL)
Serum	NA	Pretest	304**	M	4.1
	Air	30	154	F	4.3
	Air	59	104	M	5.4
	Placebo	30	202	M	6.3
	Placebo	30	251	F	3.4
	Placebo	59	203	M	3.2
	High dose	30	454	F	6.0
	High dose	59	404	M	4.5
	High dose	59	451	F	3.4
	High dose	120	401	M	8.4
	High dose	183	452	F	6.6
BAL	Air	183	102	M	3.3
	Placebo	183	204	M	4.8
	Low dose	183	303	M	3.3

*LOQ: Limit of quantitation
**Pre-test animal # 66-753
NA = not applicable; M = male; F = female

Title: Radioligand binding assay for insulin antibodies in serum samples from 6-month inhalation toxicity study of aerosol insulin in rats (01-906-03).

To examine the potential for neutralization of recombinant human, the presence of antibody to human insulin in the 6-month rat study was evaluated using a validated rat anti-human insulin antibody radioligand binding assay (RLB) assay.

Results:
Except for one rat treated with 3 mg/kg/d of inhaled insulin (3.6μU/ml), the antibody levels in rats were below the assay detection limit of 3 μU/ml.

2.6.6.4 Genetic toxicology

Study title: Bacterial Reverse Mutation Test

Key findings: The genotoxic potential of Aventis insulin ([redacted], batch # [redacted], was tested using Salmonella strains TA100, TA1535, TA1537 and TA98 and E.coli WP2uvrA at insulin concentration ranging from 4 to 5000 µg/plate in the presence and absence of metabolic activation (S9 mix). [redacted] was not mutagenic under the assay conditions described.

Study no.: 98.0020
Volume #, and page #: electronic submission
Conducting laboratory and location: Hoechst Marion Roussel, Frankfurt, Germany
Date of study initiation: January 28, 1998 /Jan 30, 1998
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Bach # [redacted] human insulin

Methods
Strains/species/cell line:

Tester bacterial strains	*his/trp* mutation	Additional mutation		Plasmid
		Repair	Lipopolysaccharide	
Salmonella typhimurium				
TA98 (frameshift)	*his*D3052	uvrB	*rfa*	pKM101
TA100 (base shift)	*his*G46	uvrB	*rfa*	pKM101
TA 1535 (base shift)	*his*G46	uvrB	*rfa*	-
TA 1537 (frame shift)	*his*C3076	uvrB	*rfa*	-
Escherichia coli WP2	*trp*	uvrA,	-	pKM101

Doses used in definitive study:
Without metabolic activation: 4, 20, 100, 500, 2500 and 5000 µg/plate
With metabolic activation: 4, 20, 100, 500, 2500 and 5000 µg/plate

Basis of dose selection: Standard limit dose is 5000 µg/plate. In the dose ranging study used to determine toxicity and dose selection for the definitive test, the maximum insulin concentration was 5000 µg/kg/day.

Negative controls: Untreated and vehicle control (dH20), DMSO was for positive controls
Positive controls:

positive controls:

a: **without** metabolic activation:
sodium-azide for strain TA 100 and TA 1535
9-aminoacridine for strain TA 1537
2-nitrofluorene for strain TA 98
MNNG for strain WP2uvrA

b: **with** metabolic activation:
2-aminoanthracene for all strains

Incubation and sampling times:
For metabolic activation, liver microsomes from rats treated with Aroclor (500 mg/kg, IP) were used. Triplicate plates were prepared for each concentration including positive and negative controls and solvent vehicle. For mutagenicity testing top agar was prepared for the Salmonella strains by mixing 100 ml agar (0.6 % (w/v) agar, 0.5 % (w/v) NaCl) with 10 ml of a 0.5 mM histidine-biotin solution. With E. coli, histidine was replaced by tryptophan (2.5 ml, 0.5 mM). The following ingredients were added to 2 ml of molten top agar:

0.5 ml S9-mix (if required) or buffer
0.1 ml of an overnight nutrient broth culture of the bacterial tester strain
0.1 ml test compound suspension (suspended in double-distilled water)

After mixing, the liquid was poured into a petri dish containing a 25 ml layer of minimal agar (1.5 % *(wlv)* agar, Vogel-Banner E medium with 2 % *(wlv)* glucose). After incubation for approximately 48 hours at approximately 37 °C in the dark, colonies (his and trp revertants) were counted with an automatic colony counter

Analysis:

No. slides/plates/replicates/animals analyzed: 3 plate/ dose
Cytotoxic endpoints: prevention of normal growth of bacteria (antibacterial toxic effect)
Genetic toxicity endpoints/results: revertant mutation
Statistical methods: No specific statistical methods were used except for mean ± SD.

Results

Study validity:
The assay is considered valid if the foil owing criteria are met:

- the solvent control data are within the laboratory's normal control range for the spontaneous mutant frequency
- the positive controls induced increases in the mutation frequency which were both statistically significant and within the laboratory's normal range

Criteria for a positive response: A test compound is classified as mutagenic if it has either of the following effects:

- it produces at least a 2-fold increase in the mean number of revertants per plate of at least one of the tester strains over the mean number of revertants per plate of the appropriate vehicle control at complete bacterial background lawn
- it induces a dose-related increase in the mean number of revertants per plate of at least one of the tester strains over the mean number of revertants per plate of the appropriate vehicle control in at least two to three concentrations of the test compound at complete bacterial background lawn.
- If the test substance does not achieve either of the above criteria, it is considered to show no evidence of mutagenic activity in this system.
- The test results must be reproducible.

Study outcome:
Insulin batch [redacted] was dissolve in sterile distilled water and tested at up to maximum acceptable concentration of 5000 µg/plate. Insulin did not produce an increase in reverse mutations in any of the bacterial strains tested. Positive controls produced significant mutation in the bacterial strains suggesting that the study was valid and acceptable. Under the conditions of the assay insulin [redacted] from Aventis was not mutagenic in the presence or absence of metabolic activation in the *in-vitro* bacterial reverse mutation assay.

TEST

STUDY ZR0485 TEST 00 SPONSOR DIVISION L DATE TESTED 28/01/98
DATE COUNTED 30/01/98

COMPOUND L00469/001/001 [redacted]

Batch: [redacted]

COMMENTS: VOTOX 98.0020
ALL STERILITY CONTROL PLATES WERE STERILE

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 100	+S9	0.	133.0	4.0			129	137	133
		4.	129.0	2.6	1.0		130	126	131
		20.	135.3	4.0	1.0		131	136	139
		100.	142.7	11.1	1.1		153	144	131
		500.	130.0	4.6	1.0		129	126	135
		2500.	124.7	10.1	0.9		134	114	126
		5000.	133.7	18.5	1.0		155	123	123
TA 100	-S9	0.	126.0	2.0			124	126	128
		4.	128.0	18.7	1.0		122	149	113
		20.	137.0	7.0	1.1		137	130	144
		100.	130.3	13.6	1.0		132	116	143
		500.	126.3	13.5	1.0		126	140	113
		2500.	135.0	10.4	1.1		142	140	123
		5000.	133.7	6.0	1.1		128	133	140
TA 1535	+S9	0.	9.0	0.0			9	9	9
		4.	11.0	3.6	1.2		8	15	10
		20.	10.7	4.2	1.2		12	6	14
		100.	11.3	2.5	1.3		14	9	11
		500.	9.0	2.6	1.0		7	12	8
		2500.	8.0	3.0	0.9		11	5	8
		5000.	11.0	1.0	1.2		10	12	11
TA 1535	-S9	0.	9.0	1.7			8	11	8
		4.	10.3	5.1	1.1		9	16	6
		20.	12.0	1.0	1.3		11	13	12
		100.	9.3	0.6	1.0		9	10	9
		500.	6.0	1.7	0.7		8	5	5
		2500.	5.7	2.3	0.6		3	7	7
		5000.	10.0	2.0	1.1		8	12	10

STUDY ZR0485 TEST 00 SPONSOR DIVISION L DATE TESTED 28/01/98
DATE COUNTED 30/01/98

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1537	+S9	0.	7.0	1.7			8	5	8
		4.	7.3	2.3	1.0		10	6	6
		20.	4.0	1.7	0.6		3	6	3
		100.	6.7	0.6	1.0		7	6	7
		500.	9.0	3.0	1.3		6	9	12
		2500.	9.7	3.1	1.4		7	13	9
		5000.	7.3	2.1	1.0		9	5	8
TA 1537	-S9	0.	10.0	1.0			11	9	10
		4.	12.0	1.7	1.2		10	13	13
		20.	8.3	3.8	0.8		10	11	4
		100.	6.7	3.2	0.7		3	9	8
		500.	9.3	1.2	0.9		10	10	8
		2500.	8.0	1.0	0.8		8	7	9
		5000.	9.0	2.0	0.9		11	9	7
TA 98	+S9	0.	29.7	3.8			27	34	28
		4.	28.7	2.1	1.0		31	28	27
		20.	33.7	4.0	1.1		36	29	36
		100.	30.7	4.5	1.0		31	35	26
		500.	27.0	1.0	0.9		28	26	27
		2500.	30.0	6.2	1.0		23	35	32
		5000.	27.3	4.0	0.9		25	25	32
TA 98	-S9	0.	23.3	3.2			21	22	27
		4.	25.3	4.9	1.1		22	23	31
		20.	20.3	0.6	0.9		20	21	20
		100.	23.7	1.5	1.0		25	24	22
		500.	25.3	3.5	1.1		29	22	25
		2500.	27.0	1.7	1.2		26	29	26
		5000.	25.7	4.6	1.1		23	31	23
WP2uvrA	+S9	0.	32.7	2.9			31	31	36
		4.	30.3	4.7	0.9		32	25	34
		20.	35.3	1.5	1.1		35	34	37
		100.	30.3	5.0	0.9		31	35	25
		500.	33.3	5.7	1.0		35	38	27
		2500.	33.0	5.2	1.0		39	30	30
		5000.	30.3	6.8	0.9		25	28	38

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
WP2uvrA	-S9	0.	32.7	3.2			29	34	35
		4.	32.7	4.2	1.0		34	28	36
		20.	35.0	2.6	1.1		36	37	32
		100.	29.3	6.7	0.9		25	37	26
		500.	29.0	3.0	0.9		26	32	29
		2500.	26.7	6.4	0.8		34	23	23
		5000.	27.3	4.9	0.8		33	25	24

TEST

STUDY ZR0485 TEST 01 SPONSOR DIVISION L DATE TESTED 28/01/98
DATE COUNTED 30/01/98

COMPOUND L00469/001/001

Batch: [redacted]

COMMENTS: VOTOX 98.0020
ALL STERILITY CONTROL PLATES WERE STERILE.

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 100	+S9	0.	170.3	6.4			174	174	163
		4.	168.3	15.3	1.0		180	174	151
		20.	159.7	2.9	0.9		163	158	158
		100.	154.0	11.5	0.9		167	145	150
		500.	153.3	28.6	0.9		178	160	122
		2500.	164.3	7.8	1.0		158	173	162
		5000.	156.7	13.5	0.9		157	170	143
TA 100	-S9	0.	133.3	2.5			133	131	136
		4.	141.3	2.5	1.1		141	144	139
		20.	134.7	9.1	1.0		143	136	125
		100.	133.7	3.1	1.0		133	131	137
		500.	138.3	5.7	1.0		140	143	132
		2500.	133.3	10.7	1.0		124	145	131
		5000.	141.7	19.1	1.1		124	162	139
TA 1535	+S9	0.	6.3	1.5			8	5	6
		4.	8.0	1.0	1.3		8	9	7
		20.	11.3	4.6	1.8		6	14	14
		100.	7.0	2.6	1.1		10	5	6
		500.	11.7	0.6	1.9		12	12	11
		2500.	7.3	1.2	1.2		8	6	8
		5000.	8.7	1.5	1.4		7	10	9
TA 1535	-S9	0.	9.3	0.6			9	9	10
		4.	9.0	2.6	1.0		8	12	7
		20.	8.0	3.5	0.9		6	12	6
		100.	11.3	1.5	1.2		10	11	13
		500.	7.3	0.6	0.8		8	7	7
		2500.	7.3	2.5	0.8		7	5	10
		5000.	7.7	0.6	0.8		8	8	7

STUDY ZR0485 TEST 01 SPONSOR DIVISION L DATE TESTED 28/01/98
DATE COUNTED 30/01/98

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1537	+S9	0.	4.3	0.6			5	4	4
		4.	6.7	1.5	1.6		7	5	8
		20.	5.3	1.5	1.2		4	7	5
		100.	4.3	1.5	1.0		6	3	4
		500.	4.7	2.1	1.1		4	7	3
		2500.	4.3	2.5	1.0		7	4	2
		5000.	5.0	1.0	1.2		6	4	5
TA 1537	-S9	0.	6.3	2.5			9	6	4
		4.	9.0	1.0	1.4		10	9	8
		20.	7.3	2.1	1.2		8	9	5
		100.	6.7	2.3	1.1		8	8	4
		500.	6.0	1.0	1.0		5	6	7
		2500.	6.3	1.2	1.0		7	7	5
		5000.	8.7	2.1	1.4		11	7	8
TA 98	+S9	0.	26.0	5.0			31	21	26
		4.	27.0	5.3	1.0		29	31	21
		20.	28.0	4.6	1.1		29	32	23
		100.	23.3	2.5	0.9		26	21	23
		500.	31.7	5.0	1.2		31	27	37
		2500.	21.0	7.5	0.8		20	29	14
		5000.	26.3	4.0	1.0		30	27	22
TA 98	-S9	0.	22.7	6.0			17	22	29
		4.	24.0	6.0	1.1		30	18	24
		20.	22.0	3.6	1.0		25	18	23
		100.	19.0	4.4	0.8		21	22	14
		500.	23.7	0.6	1.0		24	24	23
		2500.	21.3	1.2	0.9		22	20	22
		5000.	23.0	1.0	1.0		23	24	22
WP2uvrA	+S9	0.	14.7	2.3			12	16	16
		4.	16.7	6.0	1.1		11	16	23
		20.	15.0	1.7	1.0		16	13	16
		100.	15.7	4.2	1.1		19	11	17
		500.	19.3	2.1	1.3		21	17	20
		2500.	17.0	0.0	1.2		17	17	17
		5000.	19.0	1.0	1.3		20	19	18

STUDY ZR0485 TEST 01 SPONSOR DIVISION L DATE TESTED 28/01/98
DATE COUNTED 30/01/98

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
WP2uvrA	-S9	0.	15.7	1.5			14	16	17
		4.	14.7	3.1	0.9		12	18	14
		20.	18.0	2.6	1.1		19	15	20
		100.	17.0	6.1	1.1		14	24	13
		500.	14.0	2.0	0.9		16	14	12
		2500.	16.7	3.5	1.1		20	17	13
		5000.	18.3	2.1	1.2		19	20	16

STUDY ZR0485 TEST 00 ***CONTROLS*** DATE TESTED 28/01/98
DATE COUNTED 30/01/98

POSITIVE CONTROLS: QN0059/64 SOLVENT CONTROLS: QN0058/40 NEGATIVE CONTROLS: QN0062/28

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 100	+S9	SOLVENT CONTROLS	133.0	4.0			129	137	133
		NEGATIVE CONTROLS	134.0	3.5	1.0		130	136	136
		POSITIVE CONTROLS		P00001/001/001		2-AMINOANTHRACENE			
		0.5	2323.3	91.8	17.5		2366	2218	2386
TA 100	-S9	SOLVENT CONTROLS	126.0	2.0			124	126	128
		NEGATIVE CONTROLS	114.0	5.6	0.9		119	115	108
		POSITIVE CONTROLS		P00002/001/001		SODIUM-AZIDE			
		1.	560.3	24.5	4.4		536	585	560

STUDY ZR0485 TEST 00 SPONSOR DIVISION L DATE TESTED 28/01/98
DATE COUNTED 30/01/98

POSITIVE CONTROLS: QN0059/64 SOLVENT CONTROLS: QN0058/40 NEGATIVE CONTROLS: QN0062/28

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1535	+S9	SOLVENT CONTROLS	9.0	0.0			9	9	9
		NEGATIVE CONTROLS	9.3	1.5	1.0		9	11	8
		POSITIVE CONTROLS		P00001/001/001		2-AMINOANTHRACENE			
		1.0	279.3	7.0	31.0		286	280	272
TA 1535	-S9	SOLVENT CONTROLS	9.0	1.7			8	11	8
		NEGATIVE CONTROLS	8.3	2.5	0.9		8	11	6
		POSITIVE CONTROLS		P00002/001/001		SODIUM-AZIDE			
		1.	413.0	16.8	45.9		407	400	432
TA 1537	+S9	SOLVENT CONTROLS	7.0	1.7			8	5	8
		NEGATIVE CONTROLS	5.7	0.6	0.8		6	6	5
		POSITIVE CONTROLS		P00001/001/001		2-AMINOANTHRACENE			
		1.	400.3	23.1	57.2		417	410	374

STUDY ZR0485 TEST 00 SPONSOR DIVISION L DATE TESTED 28/01/98
DATE COUNTED 30/01/98

POSITIVE CONTROLS: QN0059/64 SOLVENT CONTROLS: QN0058/40 NEGATIVE CONTROLS: QN0062/28

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1537	-S9	SOLVENT CONTROLS	10.0	1.0			11	9	10
		NEGATIVE CONTROLS	7.7	4.0	0.8		10	10	3
		POSITIVE CONTROLS		P00003/001/001		9-AMINOACRIDINE			
		50.	143.0	23.0	14.3		166	120	143
TA 98	+S9	SOLVENT CONTROLS	29.7	3.8			27	34	28
		NEGATIVE CONTROLS	26.0	2.6	0.9		29	25	24
		POSITIVE CONTROLS		P00001/001/001		2-AMINOANTHRACENE			
		0.5	1840.3	25.7	62.0		1870	1827	1824
TA 98	-S9	SOLVENT CONTROLS	23.3	3.2			21	22	27
		NEGATIVE CONTROLS	26.3	3.1	1.1		23	27	29
		POSITIVE CONTROLS		P00004/001/001		2-NITROFLUORENE			
		2.5	490.0	30.0	21.0		461	488	521

Study title: Bacterial Reverse Mutation Test

Key findings: The genotoxic potential of insulin —— batch ——— was tested using Salmonella strains TA100, TA1535, TA1537 and TA98 and E.coli WP2uvrA at insulin concentration ranging from 4 to 5000 µg/plate in the presence and absence of metabolic activation (S9 mix). ——— was not mutagenic under the assay conditions described.

Study no.: 98.0073
Volume #, and page #: electronic submission
Conducting laboratory and location: Hoechst Marion Roussel, Frankfurt, Germany
Date of study initiation: January 28, 1998 /Jan 30, 1998
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Bach —— ——— human insulin ——

Methods
Strains/species/cell line:

Tester bacterial strains	*his/trp* mutation	Additional mutation		Plasmid
		Repair	Lipopolysaccharide	
Salmonella typhimurium				
TA98 (frameshift)	*his*D3052	uvrB	*rfa*	pKM101
TA100 (base shift)	*his*G46	uvrB	*rfa*	pKM101
TA 1535 (base shift)	*his*G46	uvrB	*rfa*	-
TA 1537 (frame shift)	*his*C3076	uvrB	*rfa*	-
Escherichia coli WP2	*trp*	uvrA,	-	pKM101

Doses used in definitive study:
Without metabolic activation: 4, 20, 100, 500, 2500 and 5000 µg/plate
With metabolic activation: 4, 20, 100, 500, 2500 and 5000 µg/plate

Basis of dose selection: Standard limit dose is 5000 µg/plate. In the dose ranging study used to determine toxicity and dose selection for the definitive test, the maximum insulin concentration was 5000 µg/kg/day.

Negative controls: Untreated and vehicle control (dH20),
DMSO was used for positive controls

Positive controls:

positive controls:

a: **without** metabolic activation:
sodium-azide for strain TA 100 and TA 1535
9-aminoacridine for strain TA 1537
2-nitrofluorene for strain TA 98
MNNG for strain WP2uvrA

b: **with** metabolic activation:
2-aminoanthracene for all strains

Incubation and sampling times:
For metabolic activation, liver microsomes from rats treated with Aroclor (500 mg/kg, IP) were used. Triplicate plates were prepared for each concentration including positive and negative controls and solvent vehicle. For mutagenicity testing top agar was prepared for the Salmonella strains by mixing 100 ml agar (0.6 % (w/v) agar, 0.5 % (w/v) NaCl) with 10 ml of a 0.5 mM histidine-biotin solution. With E. coli, histidine was replaced by tryptophan (2.5 ml, 0.5 mM). The following ingredients were added to 2 ml of molten top agar:

0.5 ml S9-mix (if required) or buffer
0.1 ml of an overnight nutrient broth culture of the bacterial tester strain
0.1 ml test compound suspension (suspended in double-distilled water)

After mixing, the liquid was poured into a petri dish containing a 25 ml layer of minimal agar (1.5 % *(wlv)* agar, Vogel-Banner E medium with 2 % *(wlv)* glucose). After incubation for approximately 48 hours at approximately 37 °C in the dark, colonies (his and trp revertants) were counted with an automatic colony counter

ANALYSIS:

No. slides/plates/replicates/animals analyzed: 3 plate/ dose
Cytotoxic endpoints: prevention of normal growth of bacteria (antibacterial toxic effect)
Genetic toxicity endpoints/results: revertant mutation
Statistical methods: No specific statistical methods were used except for mean ± SD.

Results
Study validity:
The assay is considered valid if the foil owing criteria are met:

- the solvent control data are within the laboratory's normal control range for the spontaneous mutant frequency
- the positive controls induced increases in the mutation frequency which were both statistically significant and within the laboratory's normal range

Criteria for a positive response: A test compound is classified as mutagenic if it has either of the following effects:

- it produces at least a 2-fold increase in the mean number of revertants per plate of at least one of the tester strains over the mean number of revertants per plate of the appropriate vehicle control at complete bacterial background lawn
- it induces a dose-related increase in the mean number of revertants per plate of at least one of the tester strains over the mean number of revertants per plate of the appropriate vehicle control in at least two to three concentrations of the test compound at complete bacterial background lawn.
- If the test substance does not achieve either of the above criteria, it is considered to show no evidence of mutagenic activity in this system.
- The test results must be reproducible.

Study outcome:
Insulin batch ——— at concentrations up to 5000 µg/plate was tested for genotoxicity using *in-vitro* reverse bacterial mutation assay. Insulin did not produce a reverse an increase in mutations in any of the bacterial strains tested. Positive controls produced significant mutation in the bacterial strains suggesting the study was valid and acceptable. Under the conditions of the assay insulin ——— (batch ——— was not mutagenic in the presence or absence of metabolic activation in the bacterial reverse mutation assay.

TEST

STUDY ZR0486 TEST 00 SPONSOR DIVISION L DATE TESTED 28/01/98
DATE COUNTED 30/01/98

COMPOUND L00469/002/001 [redacted]

Batch: [redacted]

COMMENTS: VOTOX 98.0021
ALL STERILITY CONTROL PLATES WERE STERILE

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 100	+S9	0.	131.0	13.0			123	124	146
		4.	121.0	8.2	0.9		130	114	119
		20.	128.0	5.3	1.0		130	122	132
		100.	123.7	8.3	0.9		121	117	133
		500.	132.0	11.3	1.0		126	125	145
		2500.	130.3	17.2	1.0		144	136	111
		5000.	134.0	11.0	1.0		134	145	123
TA 100	-S9	0.	126.7	24.0			154	117	109
		4.	123.3	6.7	1.0		125	116	129
		20.	132.3	5.9	1.0		128	139	130
		100.	128.7	11.9	1.0		125	142	119
		500.	131.3	6.5	1.0		138	131	125
		2500.	123.3	2.3	1.0		126	122	122
		5000.	124.3	4.6	1.0		127	119	127
TA 1535	+S9	0.	10.3	2.1			11	12	8
		4.	11.3	2.5	1.1		11	14	9
		20.	10.3	2.5	1.0		8	10	13
		100.	6.3	2.1	0.6		7	4	8
		500.	9.7	2.3	0.9		11	11	7
		2500.	13.0	3.6	1.3		16	14	9
		5000.	8.0	2.0	0.8		6	10	8
TA 1535	-S9	0.	9.3	2.1			11	7	10
		4.	8.0	1.7	0.9		7	10	7
		20.	7.7	2.9	0.8		11	6	6
		100.	9.0	2.0	1.0		7	11	9
		500.	11.0	3.0	1.2		8	14	11
		2500.	10.0	3.5	1.1		14	8	8
		5000.	10.0	3.5	1.1		8	8	14

STUDY ZR0486 TEST 00 SPONSOR DIVISION L DATE TESTED 28/01/98
DATE COUNTED 30/01/98

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1537	+S9	0.	6.0	3.6			10	5	3
		4.	5.0	0.0	0.8		5	5	5
		20.	10.3	4.7	1.7		12	5	14
		100.	6.7	3.1	1.1		6	4	10
		500.	9.0	4.4	1.5		4	12	11
		2500.	7.3	1.2	1.2		6	8	8
		5000.	9.0	1.7	1.5		8	8	11
TA 1537	-S9	0.	10.0	1.0			10	11	9
		4.	6.7	1.2	0.7		8	6	6
		20.	5.0	2.6	0.5		3	8	4
		100.	6.7	1.2	0.7		8	6	6
		500.	6.3	3.1	0.6		9	3	7
		2500.	5.3	1.5	0.5		7	4	5
		5000.	6.7	2.1	0.7		9	6	5
TA 98	+S9	0.	32.3	5.1			38	31	28
		4.	26.0	3.6	0.8		27	22	29
		20.	28.7	1.5	0.9		27	30	29
		100.	33.7	5.0	1.0		39	33	29
		500.	33.7	0.6	1.0		34	33	34
		2500.	30.7	5.1	1.0		35	32	25
		5000.	28.0	2.6	0.9		27	31	26
TA 98	-S9	0.	25.0	4.6			30	24	21
		4.	28.0	2.6	1.1		30	29	25
		20.	22.0	6.1	0.9		29	19	18
		100.	24.3	3.5	1.0		24	28	21
		500.	23.7	5.5	0.9		29	24	18
		2500.	24.0	5.6	1.0		23	30	19
		5000.	25.7	4.2	1.0		27	21	29
WP2uvrA	+S9	0.	35.3	4.5			31	35	40
		4.	32.0	6.6	0.9		31	26	39
		20.	33.7	4.9	1.0		28	36	37
		100.	30.3	6.7	0.9		26	38	27
		500.	30.3	3.2	0.9		34	28	29
		2500.	36.0	10.8	1.0		27	33	48
		5000.	31.0	5.6	0.9		25	32	36

STUDY ZR0486 TEST 00 SPONSOR DIVISION L DATE TESTED 28/01/98
DATE COUNTED 30/01/98

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
WP2uvrA	-S9	0.	28.7	2.5			31	26	29
		4.	33.3	6.7	1.2		39	35	26
		20.	31.0	7.0	1.1		39	28	26
		100.	34.3	4.2	1.2		33	31	39
		500.	33.0	0.0	1.1		33	33	33
		2500.	33.0	4.6	1.1		37	28	34
		5000.	32.0	1.0	1.1		33	31	32

TEST

STUDY ZR0486 TEST 01 SPONSOR DIVISION L DATE TESTED 28/01/98
DATE COUNTED 30/01/98

COMPOUND L00469/002/001 [redacted]

Batch: [redacted]

COMMENTS: VOTOX 98.0021
ALL STERILITY CONTROL PLATES WERE STERILE

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 100	+S9	0.	151.7	14.4			146	168	141
		4.	138.3	10.2	0.9		134	150	131
		20.	149.7	28.1	1.0		153	176	120
		100.	139.7	5.0	0.9		145	139	135
		500.	159.0	26.5	1.0		158	186	133
		2500.	143.7	11.7	0.9		139	157	135
		5000.	159.3	10.0	1.1		163	148	167
TA 100	-S9	0.	134.0	14.7			125	151	126
		4.	130.3	6.5	1.0		124	130	137
		20.	137.3	11.0	1.0		125	141	146
		100.	130.3	19.9	1.0		137	146	108
		500.	143.7	19.5	1.1		135	166	130
		2500.	142.7	9.5	1.1		146	150	132
		5000.	148.0	6.6	1.1		147	142	155
TA 1535	+S9	0.	8.3	1.5			10	7	8
		4.	7.0	3.0	0.8		4	7	10
		20.	10.0	2.0	1.2		10	8	12
		100.	6.7	1.5	0.8		5	7	8
		500.	9.3	0.6	1.1		10	9	9
		2500.	9.7	2.1	1.2		8	12	9
		5000.	11.0	3.0	1.3		8	14	11
TA 1535	-S9	0.	8.3	5.9			6	4	15
		4.	8.7	1.2	1.0		8	10	8
		20.	9.3	0.6	1.1		9	9	10
		100.	10.7	2.1	1.3		13	9	10
		500.	7.0	2.0	0.8		5	7	9
		2500.	7.0	1.0	0.8		6	7	8
		5000.	8.7	4.2	1.0		10	4	12

STUDY ZR0486 TEST 01 SPONSOR DIVISION L DATE TESTED 28/01/98
DATE COUNTED 30/01/98

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1537	+S9	0.	5.0	2.0			3	7	5
		4.	7.3	4.5	1.5		7	3	12
		20.	4.7	2.1	0.9		4	3	7
		100.	8.3	0.6	1.7		8	8	9
		500.	5.7	2.5	1.1		6	3	8
		2500.	8.0	1.0	1.6		8	7	9
		5000.	5.3	0.6	1.1		6	5	5
TA 1537	-S9	0.	5.0	1.0			5	4	6
		4.	5.7	1.5	1.1		4	7	6
		20.	7.0	1.7	1.4		5	8	8
		100.	6.0	1.7	1.2		4	7	7
		500.	9.3	1.5	1.9		9	8	11
		2500.	6.3	1.2	1.3		7	7	5
		5000.	8.0	2.6	1.6		6	11	7
TA 98	+S9	0.	28.3	1.2			29	27	29
		4.	24.7	8.5	0.9		16	25	33
		20.	31.0	7.5	1.1		38	32	23
		100.	25.7	6.0	0.9		25	32	20
		500.	30.0	2.6	1.1		31	27	32
		2500.	26.7	2.9	0.9		25	30	25
		5000.	25.3	1.2	0.9		24	26	26
TA 98	-S9	0.	27.3	3.1			30	24	28
		4.	29.3	5.1	1.1		28	25	35
		20.	27.3	5.7	1.0		21	32	29
		100.	28.3	2.5	1.0		26	28	31
		500.	29.3	0.6	1.1		29	30	29
		2500.	27.3	2.9	1.0		24	29	29
		5000.	26.7	6.7	1.0		21	34	25
WP2uvrA	+S9	0.	17.0	2.6			14	19	18
		4.	17.3	6.5	1.0		24	17	11
		20.	16.0	5.3	0.9		10	20	18
		100.	18.0	3.6	1.1		15	22	17
		500.	17.7	4.9	1.0		21	20	12
		2500.	23.7	4.2	1.4		27	19	25
		5000.	17.7	5.1	1.0		19	12	22

STUDY ZR0486 TEST 01 SPONSOR DIVISION L DATE TESTED 28/01/98
DATE COUNTED 30/01/98

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
WP2uvrA	-S9	0.	16.0	1.0			17	16	15
		4.	17.3	4.0	1.1		21	13	18
		20.	12.0	1.0	0.8		13	12	11
		100.	16.0	2.0	1.0		14	16	18
		500.	15.3	2.5	1.0		13	15	18
		2500.	14.3	2.1	0.9		12	15	16
		5000.	16.7	2.1	1.0		19	15	16

CONTROLS

STUDY ZR0486 TEST 00 SPONSOR DIVISION L DATE TESTED 28/01/98 DATE COUNTED 30/01/98

POSITIVE CONTROLS: QN0059/64 SOLVENT CONTROLS: QN0058/41 NEGATIVE CONTROLS: QN0062/29

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 100	+S9	SOLVENT CONTROLS	131.0	13.0			123	124	146
		NEGATIVE CONTROLS	133.7	9.5	1.0		141	137	123
		POSITIVE CONTROLS	P00001/001/001			2-AMINOANTHRACENE			
		0.5	2323.3	91.8	17.7		2366	2218	2386
TA 100	-S9	SOLVENT CONTROLS	126.7	24.0			154	117	109
		NEGATIVE CONTROLS	113.7	2.3	0.9		115	111	115
		POSITIVE CONTROLS	P00002/001/001			SODIUM-AZIDE			
		1.	560.3	24.5	4.4		536	585	560

STUDY ZR0486 TEST 00 SPONSOR DIVISION L DATE TESTED 28/01/98 DATE COUNTED 30/01/98

POSITIVE CONTROLS: QN0059/64 SOLVENT CONTROLS: QN0058/41 NEGATIVE CONTROLS: QN0062/29

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1535	+S9	SOLVENT CONTROLS	10.3	2.1			11	12	8
		NEGATIVE CONTROLS	8.0	1.7	0.8		6	9	9
		POSITIVE CONTROLS	P00001/001/001			2-AMINOANTHRACENE			
		1.0	279.3	7.0	27.1		286	280	272
TA 1535	-S9	SOLVENT CONTROLS	9.3	2.1			11	7	10
		NEGATIVE CONTROLS	11.7	1.2	1.3		11	13	11
		POSITIVE CONTROLS	P00002/001/001			SODIUM-AZIDE			
		1.	413.0	16.8	44.4		407	400	432
TA 1537	+S9	SOLVENT CONTROLS	6.0	3.6			10	5	3
		NEGATIVE CONTROLS	8.0	3.5	1.3		6	6	12
		POSITIVE CONTROLS	P00001/001/001			2-AMINOANTHRACENE			
		1.	400.3	23.1	66.7		417	410	374

POSITIVE CONTROLS: QN0059/64 SOLVENT CONTROLS: QN0058/41 NEGATIVE CONTROLS: QN0062/29

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1537	-S9	SOLVENT CONTROLS	10.0	1.0			10	11	9
		NEGATIVE CONTROLS	5.0	2.0	0.5		3	7	5
		POSITIVE CONTROLS	P00003/001/001			9-AMINOACRIDINE			
		50.	143.0	23.0	14.3		166	120	143
TA 98	+S9	SOLVENT CONTROLS	32.3	5.1			38	31	28
		NEGATIVE CONTROLS	26.0	8.2	0.8		24	19	35
		POSITIVE CONTROLS	P00001/001/001			2-AMINOANTHRACENE			
		0.5	1840.3	25.7	57.0		1870	1827	1824
TA 98	-S9	SOLVENT CONTROLS	25.0	4.6			30	24	21
		NEGATIVE CONTROLS	20.3	4.5	0.8		25	20	16
		POSITIVE CONTROLS	P00004/001/001			2-NITROFLUORENE			
		2.5	490.0	30.0	19.6		461	488	521

2.6.7.1 Toxicology

Overview – Inhalation – continued

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Achieved Doses (mg/kg)[a] as insulin	GLP Compliance	Testing Facility	Study Number	CTD Location Vol./ Section
	Monkey / Cynomolgus	Inh	30 days	0, 0.14 and 0.58	Yes		N001603B	/4.2.3.2
	Monkey / Cynomolgus	Inh	6 months	0 (air), 0 (placebo), 0.29 and 0.64	Yes		N002448B	/4.2.3.2
Other Toxicity Studies (Pivotal)	Rat / Sprague-Dawley; Monkey / Cynomolgus	-	-	0 (air), 0 (placebo), and 5.8 for rat and 0(air), 0 (placebo), and 0.64 for monkey	Yes		01-906-05	/4.2.3.2.1
	Rat / Sprague-Dawley	-	-	0 (air), 0 (placebo), 0.9, 2.7, 5.8	No		01-906-03	/4.2.3.2.1
	Monkey / Cynomolgus	-	-	0 (air), 0 (placebo), 0.29 and 0.64	No		01-906-02	/4.2.3.2.1

[a] Unless otherwise specified, for repeat-dose toxicity, the highest NOAEL (No Observed Adverse Effect Level) is underlined.

2.6.7.2 Toxicology

Overview – Non-Inhalation

Test Article: Recombinant Human Insulin or extracts of various pulmonary inhaler components

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)[a] as insulin	GLP Compliance	Testing Facility	Study Number	CTD Location Vol./ Section
Single-Dose Toxicity	Mice / NMRI	Subcutaneous (SC)	Single	1000 IU/kg	Yes		96-0175	/4.2.3.7.7
	Rat / WISKf	SC	Single	1000IU/kg	Yes		96-0176	/4.2.3.7.7
	Dog / Beagle	SC	Single	5, 15IU/kg	Yes		97012	/4.2.3.7.7
Repeat-Dose Toxicity	Rat/Sprague-Dawley	SC	4 weeks	0, 0.45, 0.90, and 1.8 and 1.8 for Humacart® R	Yes		SBL 78-90	/4.2.3.7.7
Genotoxicity	S. typhimurium TA 100, TA 1535, TA 1537 and TA 98, E. coli WP2uvrA	In vitro		0, 4, 20, 100, 500, 2500, and 5000 µg/plate	Yes		980020 and 980021	/4.2.3.3
Local Tolerance	Rabbit / New Zealand	SC and Intramuscular (IM)	Single	0.1 ml SC and 0.5 ml IM	Yes		96-0174	/4.2.3.7.7
	Rabbit / New Zealand	Intravenous (IV) and Paravenous (PV)	Single	0.1 ml PV and 0.5 ml IV	Yes		96-0370	/4.2.3.7.7

[a] Unless otherwise specified, for repeat-dose toxicity, the highest NOAEL (No Observed Adverse Effect Level) is underlined.

2.6.7.2 Toxicology

Overview – Non-Inhalation - continued

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)[a] as insulin	GLP Compliance	Testing Facility	Study Number	CTD Location Vol./ Section
Other Toxicity Studies:								
Immunogenicity	Swine / Deutsches Landschwein	SC	approximately 13 weeks	0.0625, 0.125, 0.25, 0.50 and 1 IU/kg	No	Hoechst, Frankfurt Germany	016711	/4.2.3.7.2
Antigenicity	Guinea pig / Hartley	SC for immunization; IV for challenge	approximately 29 days	20 µg	Yes	[redacted]	184-03	/4.2.3.7.1
Antigenicity	Guinea pig / Hartley; Mice BALBc and C3H/He; and Rat / Crj:CD (SD)	SC Intraperitoneal Intravenous challenge animals	approximately 22 days approximately 22 days	10 IU/kg 20 IU/kg	Yes	[redacted]	I-1136	/4.2.3.7.1
Binding Affinity	Human Osteosarcoma B10 cells	In Vitro	Single dose	-	No	[redacted]	016750	/4.2.3.7.7
Pyrogenicity	Rabbit / Japanese White	IV	Single dose	50 and 100 IU/kg	Yes		I-1137	/4.2.3.7.7

[a] Unless otherwise specified, for repeat-dose toxicity, the highest NOAEL (No Observed Adverse Effect Level) is underlined.

2.6.7.2 Toxicology

Overview – Non-Inhalation - continued

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)[a] as insulin	GLP Compliance	Testing Facility	Study Number	CTD Location Vol./ Section
Other Toxicity Studies:								
Biocompatibility Cytotoxicity	Mouse fibroblast cells, L-929	In Vitro	Single Dose	NA to insulin: 2 ml extract of inhaler components	Yes	[redacted]	120 0634R 120 0530R 120 0715R.01 120 0836R 120 0217R 120 0214R.01 120 0391R	/4.2.3.7.7 /4.2.3.7.7 /4.2.3.7.7 /4.2.3.7.7 /4.2.3.7.7 /4.2.3.7.7 /4.2.3.7.7
Sensitization	Guinea Pig/Crl:(HA) BR	Intradermal; Dermal Patch	Single Dose	NA to insulin; 0.1 ml extract of inhaler components for intradermal; and 0.3 ml extract of inhaler components for dermal patch	Yes		120 0713R.02 120 0531R 120 0718R.01 120 0834R	/4.2.3.7.7 /4.2.3.7.7 /4.2.3.7.7 /4.2.3.7.7
Irritation	Rabbit/New Zealand White	Intra-Vaginal	5 consecutive days	NA to insulin: 2 ml extract of inhaler components	Yes	[redacted]	120 0720R.01 120 0714R.02 120 0532R.01 120 0722R.01 120 0717R.01 120 0835R	/4.2.3.7.7 /4.2.3.7.7 /4.2.3.7.7 /4.2.3.7.7 /4.2.3.7.7 /4.2.3.7.7

[a] Unless otherwise specified, for repeat-dose toxicity, the highest NOAEL (No Observed Adverse Effect Level) is underlined. NA – Not Applicable

2.6.7.2 Toxicology **Overview – Non-Inhalation - continued**

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)[a] as insulin	GLP Compliance	Testing Facility	Study Number	CTD Location Vol./ Section
Other Toxicity Studies: Biocompatibility								
Systemic toxicity	Mouse/Crl:CF -1 BR	Intravenous; Intraperitoneal	Single Dose	NA to insulin; 50 ml/kg of extract of inhaler components	Yes	→	120 0721R.02 120 0716R 120 0533R 120 0833R	/4.2.3.7.7 /4.2.3.7.7 /4.2.3.7.7 /4.2.3.7.7

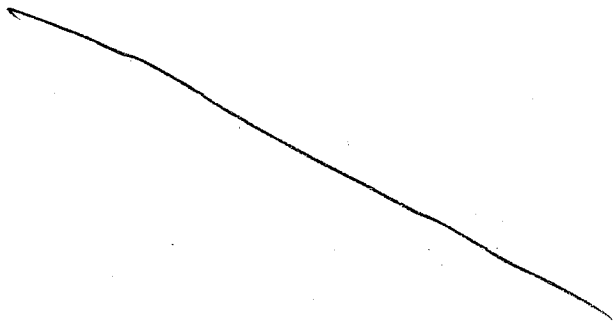
[a] Unless otherwise specified, for repeat-dose toxicity, the highest NOAEL (No Observed Adverse Effect Level) is underlined. NA – Not Applicable

APPEARS THIS WAY ON ORIGINAL

2.6.7.5 Toxicology

Analytical Data for Drug Substance and Drug Product Used in Toxicology Studies

Test Article: Drug Substance is recombinant human insulin; Drug Product consists of recombinant human insulin with mannitol, glycine and sodium citrate/citric acid



2.6.7.6 Single-Dose Inhalation Toxicity

Nonpivotal Studies

Test Article: Insulin Inhalation Powder

Species/Strain	Method of Administration (Vehicle/ Formulation)	Dose (mg/kg) as insulin	Gender and No. per Group	Observed Maximum Non-Lethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Rat/Sprague-Dawley	Inhalation-nose only with Formulation [redacted] containing recombinant human insulin in a mixture of mannitol, glycine and sodium citrate excipients. No vehicle used	Single exposure (using total mass concentration of about 1 mg/L) for up to 1 hour resulted in an approximate dose of 6.8 mg/kg	3 F/ group	Approximately 6.8 based on potential hypoglycemia	Not Determined	Clinical signs of hypoglycemia (lethargy, hypoactivity, unresponsiveness, comatose state and seizure activity) were observed in only rats fasted overnight prior to exposure. Dextrose administration resulted in recovery. No mortality or body weight changes reported. Glucose concentration decreased with an increase in insulin concentration. Maximum insulin levels observed at end of exposure. The data from this study aided in the design of the pivotal 1- month rat study (N001603C) with a target maximum dose of 6 mg/kg using fed animals to help control hypoglycemia.	N001603A(1)	/4.2.3.1

2.6.7.6 Single-Dose Inhalation Toxicity **Nonpivotal Studies** **Test Article:** Recombinant Human Insulin

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (µg/kg) as insulin	Gender and No. per Group	Observed Maximum Non-Lethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Monkeys/ Cynomolgus	Inhalation and Subcutaneous	Inhalation doses from 54 to 176 µg/kg; Subcutaneous dose 7.2 µg/kg	3-4 M	Not Determined	Not Determined	No abnormal clinical observations following subcutaneous and inhalation exposure. Serum samples collected and results are reported in study AA001.	SC930056	/4.2.3.1

2.6.7.6 Single-Dose Inhalation Toxicity **Nonpivotal Studies** **Test Article:** Recombinant Human Insulin

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg) as insulin	Gender and No. per Group	Observed Maximum Non-Lethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Rat / Sprague-Dawley	Inhalation powder administration with 3 formulations for rats: 1) insulin, and citrate ; 2) insulin, mannitol, and citrate; 3) insulin, raffinose, and citrate.	No specific doses reported	21-24, sex not specified	Not determined	Not determined	In both rat and monkey, powdered insulin aerosols provided rapid acting insulin to the systemic circulation. Time to insulin peak and glucose minimum was similar to subcutaneous injection	AA001	/4.2.3.1
Monkey / Cynomolgus	As stated in study SC930056	As stated in study SC930056	As stated in study SC930056	As stated in study SC930056	As stated in study SC930056			

2.6.7.7 Repeat-Dose Inhalation Toxicity **Non-Pivotal Studies** **Test Article:** Insulin Inhalation Powder

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg) as insulin	Gender and No. per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Monkey/ Cynomolgus	Head-only inhalation with recombinant human insulin and the excipients sodium citrate, mannitol, citric acid and water	5 days	0, and approximately 0.12 and 0.60	3M, 3F	0.60	At 0.60 mg/kg, lethargy, unresponsive and comatose state consistent with symptoms of the pharmacological response of hypoglycemia. All recovered with administration of dextrose. Clinical pathology and histopathological findings were unremarkable as were body weight and ophthalmology findings.	SC930055	/4.2.3.2

[a] No Observed Adverse Effect Level.

2.6.7.8A Repeat-Dose Inhalation Toxicity **Report Title:** 30-Day Inhalation Toxicity Study of Aerosol Insulin in Rats **Test Article:** Insulin Inhalation Powder

Species/Strain: Rat/Sprague-Dawley
Initial Age: Approximate 8 weeks
Date of First Dose: 22 Oct 1995
Duration of Dosing: 30 Days
Duration of Postdose: None
Method of Administration: Inhalation - nose only
Vehicle/Formulation: Dry powder placebo of excipients of
Study No. N001603C
Location in CTD: Vol. / Section 4.2.3.2
GLP Compliance: Yes

Special Features:
- Baseline serum insulin and glucose were determined in unexposed satellite toxicokinetic (TK) group of 10/rats/sex/group on Days 1 and 30 of study. Serum and glucose were determined in satellite low, mid and high dose groups (10/rats/sex/group) on Days 1 and 30. Blood samples were taken from the retro-orbital plexus under light carbon/dioxide/oxygen anesthesia at approximately 3, 10, and 30 minutes post inhalation exposure.
- Ophthalmology was performed before treatment initiation and during Week 4 of study.
- Animals had access to feed ad libitum except during the inhalation exposure period and immediately (30-60 min.) post exposure.

No Observed Adverse-Effect Level: 6 mg/kg/day

	Satellite Toxicokinetic Group									
Daily Dose (mg/kg) as Insulin	0 (Unexposed)		0 (Placebo Control)		1.1		3.2		6.0	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Mean Serum Insulin (ng/mL)[a]										
Day 1 within 30 min. post-dose	4.4	3.7	ND	ND	28.9	61.7	94.2	190.9	165.1	343.3
Day 30 within 30 min. post-dose	3.9	3.5	ND	ND	80.3	72.1	227.3	255.0	92.3	482.6
Mean Serum Glucose (mg/dL)[a]										
Day 1 within 30 min. post-dose	152	134	ND	ND	157	105*	111*	65*	99*	58*
Day 30 within 30 min. post-dose	133	128	ND	ND	116	99*	106*	63*	85*	56*
Died or Sacrificed Moribund	0	0	0	0	0	0	0	1[b]	0	0
	Core Toxicology Group									
Noteworthy findings										
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0	0	0
Body Weight	ND	ND	-	-	-	-	-	-	-	-
Food Consumption	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

*Statistical significance according to Dunnett's Test at p≤0.05.
- No noteworthy findings / ND: Not determined
[a] Descriptive statistical analyses (mean and standard deviation) were calculated for serum insulin data only. N=9
[b] One 3.2 mg/kg female from the satellite toxicokinetic group was found dead on Day 1, approximately 1.5 hours after exposure and blood collection.

	Core Toxicology Group							
Daily Dose (mg/kg)	0 (Placebo Control)		1.1		3.2		6.0	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Clinical Observations	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum Chemistry	-	-	-	-	-	-	-	-
Urinalysis	ND	ND	ND	ND	ND	ND	ND	ND
Absolute Organ Weights[c] **(%)**								
Lung[d]	1.85	1.29	+ 14*	- 0.7	+ 4	+ 5	+ 6	+ 14*
Relative Organ Weights[e] **(%)**								
Lung[d]	0.423	0.515	+ 12*	+ 0.6	+ 3	+ 2	+ 3	+ 12*
Gross Pathology	-	-	-	-	-	-	-	-
Histopathology[f]								
Lung								
Alveolar Histiocytosis	2	0	0	0	0	0	1	0
Inflammation – Mixed cells, Alveoli	1	0	0	0	1	0	1	1
Hemorrhage	2	0	0	0	0	0	1	0
Larynx								
Inflammation – Mixed Cells, Mucosa	3	1	2	1	1	0	1	0

*Statistical significance according to Dunnett's Test at p≤0.05.
- No noteworthy findings / ND: Not determined
[c] For controls, group means are shown. For treated groups, number indicates percent differences from the control weight in grams. Statistical significance is based on actual data (not on the percent differences).
[d] The increase in mean absolute and relative lung weights for low dose males and high dose females were not considered insulin related since the differences were of low incidence, not consistent in corresponding treatment groups, nor dose related.
[e] For controls, group means are shown and are calculated as follows: absolute organ weight x 100)/ terminal body weight. For treated groups, number indicates percent differences from the control relative body weight. Statistical significance is based on actual data (not on the percent differences).
[f] All findings were considered to be incidental in nature and not the result of inhalation exposure to either insulin or the excipients.

2.6.7.8B Repeat-Dose Inhalation Toxicity **Report Title:** One Month Inhalation Toxicity Study with Insulin Powder in Rats **Test Article:** Insulin Inhalation Powder

Species/Strain: Rat/Sprague-Dawley
Initial Age: approximate 6-8 weeks
Date of First Dose:
August 20, 2001 for males
August 21, 2001 for females

Duration of Dosing: 30 Days
Duration of Postdose: None
Method of Administration: Inhalation - nose only
Vehicle/Formulation: Dry powder placebo of excipients consisting of

Study No. 01-906-06
LocationinCTD: Vol. / Section 4.2.3.2
GLP Compliance: Yes

Special Features:

- Baseline serum insulin and glucose were determined for 0 (Air Control) and 0 (Placebo Control) satellite toxicokinetic (TK) groups of 10/rats/sex/group on Days 1 and 30 of study. Serum insulin and glucose were determined for low, mid, and high dose satellite groups of 10/rats/sex/group on Days 1 and 30. Blood samples in general were taken from the retro-orbital plexus under light carbon/dioxide/oxygen anesthesia at approximately 3, 10, and 30 minutes post inhalation exposure. Additional samples were collected in the same manner from the core toxicology animals on Day 31 for insulin antibody analysis.
- Ophthalmology was performed before treatment initiation and during Week 4 of study.
- Animals had access to feed ad libitum except during the inhalation exposure period and immediately (30 min.) post exposure.
- Two control groups 10/sex were maintained on this study. Groups were exposed to either filtered air or aerosolized placebo powder to achieve a total mass equivalent to the high-dose insulin group.

No Observed Adverse-Effect Level: 5.9 mg/kg/day

	Satellite Toxicokinetic Group									
Daily Dose (mg/kg) as Insulin	0 (Air Control)		0 (Placebo Control)		1		3.2		5.9	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Mean Serum Insulin (ng/mL)[a]										
Day 1 within 30 min. post-dose	0.4	0.71	0.25	0.3	154	110	308	250	356	253
Day 30 within 30 min. post-dose	0.3	0.2	0.19	0.2	68	194	148	185	429	185
Mean Serum Glucose (mg/dL)										
Day 1 within 30 min. post-dose	144	129	147	144*	131	119	94*	97*	82*	69*
Day 30 within 30 min. post-dose	139	137	135	140	123	106	117	87*	83*	74*

*Statistical significance according to Dunnett's Test at $p \leq 0.05$.
[a] Descriptive statistical analyses (mean and standard deviation) were calculated for serum insulin data only. Mean values were calculated from mean data of the three timepoints collected during that day. (Statistical significance from satellite group shown). N=9

2.6.7.8B Repeat-Dose Inhalation Toxicity **Study No. 01-906-06 (Continued)**

	Core Toxicology Group									
Daily Dose (mg/kg) as Insulin	0 (Air Control)		0 (Placebo Control)		1		3.2		5.9	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Noteworthy findings										
Died or Sacrificed Moribund	1[b]	0	1[b]	0	0	0	0	0	1[b]	0
Body Weight	-	-			-	-	-	-	-	-
Food Consumption	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Clinical Observations	-	-	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-	-	-
Serum Chemistry	-	-	-	-	-	-	-	-	-	-
Urinalysis	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Absolute Organ Weights	-	-	-	-	-	-	-	-	-	-
Relative Organ Weights	-	-	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-	-	-
Histopathology										
Lung										
Inflammation, Alveolar	2	4	6	3	3	3	3	6	8	1
Hyperplasia, Bronchial Epithelium	0	0	1	0	0	0	1	0	0	0
Inflammation, Interstitial	0	0	0	0	0	0	0	1	0	1
Larynx										
Inflammation, Subacute	0	1	0	0	0	0	0	0	0	0
Additional Examinations										
Insulin antibody analysis	-	-	-	-	-	-	-	-	-	-

- No noteworthy findings / ND: Not determined
[b] Unscheduled death determined to be an overdose of carbon dioxide during blood collection process on Day 16.

2.6.7.8C Repeat-Dose Inhalation Toxicity | **Report Title:** Six-Month Inhalation Toxicity Study of Aerosol Insulin in Rats | **Test Article:** Insulin Inhalation Powder

Species/Strain: Rat/Sprague-Dawley | **Duration of Dosing:** 182 Days | **Study No.** N002448A

Initial Age: 8 weeks | **Duration of Postdose:** None | **Location in CTD:** Vol. / Section 4.2.3.2

Date of First Dose: 19(M) & 20 (F) Aug. 1997 | **Method of Administration:** Inhalation – nose only | **GLP Compliance:** Yes

Vehicle/Formulation: Dry powder placebo of excipients consisting of [redacted] and [redacted]

- Baseline serum insulin and glucose were determined in an unexposed satellite group of 10/rats/sex/group on Days 1, 91, and 182 of study. Serum insulin and glucose were determined in satellite low, mid, and high dose groups (10/rats/sex/group) on Days 1, 91, and 182. Blood samples were taken from the retro-orbital plexus under light carbon/dioxide/oxygen anesthesia at approximately 3, 10, and 30 minutes post inhalation exposure.
- Ophthalmology was performed before treatment initiation and during the last week of study.
- Animals had access to feed ad libitum except during the inhalation exposure period. Toxicokinetic satellite animals had feed withheld until after blood samples were collected.
- Two control groups (16/sex) were maintained on this study (unexposed satellite TK groups of 10/sex/group were used for baseline serum glucose and insulin measurements; 6/sex/group were used for pulmonary function evaluation). Groups were exposed to either filtered air or aerosolized placebo powder to achieve a total mass equivalent to the high-dose insulin group.

No Observed Adverse-Effect Level: 5.8 mg/kg/day

	Satellite Toxicokinetic Group							
Daily Dose (mg/kg) as Insulin	0		0.9		2.7		5.8	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Mean Serum Insulin (ng/mL)[a]								
Day 1 within 30 min. post-dose	0.1	0.1	148	84	500	185	467	557
Day 91 within 30 min. post-dose	1.4	2.3	70	138	223	210	658	394
Day 182 within 30 min. post-dose	0.6	0.2	67	91	188	242	219	375
Mean Serum Glucose (mg/dL)[a]								
Day 1 within 30 min. post-dose	137	131	120	123	79*	68*	60*	52*
Day 91 within 30 min. post-dose	127	124	115	100*	99*	96*	95*	64*
Day 182 within 30 min. post-dose	130	114	105*	103	99*	83*	73*	54*
Died or Sacrificed Moribund	0	0	0	0	1	0	1	1

*Statistical significance according to Dunnett's Test at $p \leq 0.05$.

[a] Descriptive statistical analyses (mean and standard deviation) were calculated for serum and glucose insulin data. Statistical significance from satellite animals represented. N=9

2.6.7.8C Repeat-Dose Inhalation Toxicity **Study No. N002448A (Continued)**

	Core Toxicology Group									
Daily Dose (mg/kg) as Insulin	0 (Air Control)		0 (Placebo Control)		0.9		2.7		5.8	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Noteworthy findings										
Died or Sacrificed Moribund	0	0	0	0	0	0	2	1	1	0
Body Weight	-	-	-	-	-	-	-	-	-	-
Food Consumption	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Clinical Observations										
Hypoglycemia	0	0	0	0	0	0	0	0	1	0
Ophthalmoscopy	-	-	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-	-	-
Serum Chemistry	-	-	-	-	-	-	-	-	-	-
Urinalysis	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

- No noteworthy findings / ND: Not determined

2.6.7.8C Repeat-Dose Inhalation Toxicity **Study No. N002448A (Continued)**

	Core Toxicology Group									
Daily Dose (mg/kg) as Insulin	0 (Air Control)		0 (Placebo Control)		0.9		2.7		5.8	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Absolute Organ Weights[b] (%)										
Lung[c]	2.12	1.64	2.15	1.67	-0.5	+1	+4	+18*	+9	+5
Relative Organ Weights	-	-			-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-	-	-
Histopathology[d]										
Lung										
Inflammation, chronic-active	5	2	6	5	6	5	8	7	7	6
Infiltrating cell, histiocyte, alveolus	4	5	3	1	0	1	0	1	1	0
Hemorrhage	0	0	0	0	0	1	0	0	0	0
Additional Examinations										
Pulmonary function	-	-	-	-	-	-	-	-	-	-

*Statistical significance according to Dunnett's Test at $p \leq 0.05$.
- No noteworthy findings
[b] For controls, group means are shown. For treated groups, number indicates percent differences from the air control weight in grams. Statistical significance is based on actual data (not on the percent differences).
[c] The increase in mean absolute weights were not considered insulin related since the differences were of low incidence, not consistent in corresponding treatment groups, nor dose related.
[d] All findings were considered to be incidental in nature and not the result of inhalation exposure to either insulin or the excipients.

2.6.7.8D Repeat-Dose Inhalation Toxicity

Report Title: 30-Day Inhalation Toxicity Study of Aerosol Insulin in Monkey

Test Article: Insulin Inhalation Powder

Species/Strain: Monkey/Cynomolgus
Initial Age: At least 18 months old at first exposure
Date of First Dose: 31 October 1995

Duration of Dosing: 30 Days
Duration of Postdose: None
Method of Administration: Inhalation – head only
Vehicle/Formulation: Dry powder placebo of excipients consisting of [redacted]

Study No. N001603B
Location in CTD: Vol. / Section 4.2.3.2
GLP Compliance: Yes

Special Features:

- Serum concentrations of insulin and glucose were measured pre-dose on Days 1 and 29 and at approximately 5, 15, and 30 min. post exposure. Feed was withheld (approximately 30 min.) on sampling days. Additional samples were collected for serum anti-human insulin antibody titer prior to treatment initiation and on Days 1, 7, 15, 21, and 31.
- Animals had access to feed twice daily (morning and afternoon) with morning feeding occurring prior to exposure to help prevent the development of hypoglycemia.
- Ophthalmology was performed before treatment initiation and during the last week of study on sedated animals.
- Electrocardiograms were collected on sedated animals, prior to treatment initiation and on Day 31.
- Respiratory parameters were measured three times per week throughout the study. The parameters measured were: respiratory rate, minute volume, tidal volume, and accumulated inhaled volume.
- Bronchial alveolar lavage samples were collected prior to necropsy on Day 31. Animals were anesthetized and a pediatric fiberoptic bronchoscope was guided into the lung for sample collection.

No Observed Adverse-Effect Level: 0.58 mg/kg/day

Daily Dose (mg/kg) as Insulin	0 (Placebo Control)		0.14		0.58	
Number of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Mean serum insulin (ng/ml)[a]						
Pre-dose Day 1	3.37	2.30	2.03	1.69	2.33	4.26
Day 1 within 30 min. post-dose	1.96	1.31	4.25	7.06	10.19	12.97
Pre-dose Day 29	2.93	36.55	4.16	4.12	4.68	22.17
Day 29 within 30 min. post-dose	2.54	9.91	10.67	12.68	17.63	32.13

[a] Descriptive statistical analyses (mean and standard deviation) were calculated for serum insulin and glucose data only. Mean values were calculated from mean data of the three timepoints collected during that day. N= 4 except: Day 29 (0.14 mg/kg female) n=3 for ~15 min. collection and Day 29 (0.58 mg/kg female) n=3 for approximately 30 min. collection of serum glucose only.

2.6.7.8D Repeat-Dose Inhalation Toxicity **Study No. N001603B (Continued)**

Daily Dose (mg/kg) as Insulin	0 (Placebo Control)		0.14		0.58	
Number of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Mean serum glucose (mg/dL)[b]						
Pre-dose Day 1	95	82	152	107	116	151
Day 1 within 30 min. post-dose	73	78	73	59	50	28
Pre-dose Day 29	71	71	84	111	100	88
Day 29 within 30 min. post-dose	69	58	42	48	21	11
Noteworthy findings						
Died or Sacrificed Moribund	0	0	0	0	0	0
Body Weight (kg)	-	-	-	-	-	-
Food Consumption	ND	ND	ND	ND	ND	ND
Clinical Observations						
Lethargy / prostration related to hypoglycemia	0	0	0	0	0	3
Ophthalmoscopy	-	-	-	-	-	-
Electrocardiography	-	-	-	-	-	-
Hematology	-	-		-	-	-
Serum Chemistry	-	-		-	-	-
Urinalysis	-	-	-	-	-	-
Absolute Organ Weights	-	-	-	-	-	-
Relative Organ Weights	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-
Histopathology						

- No noteworthy findings / ND: Not determined

[b] Glucose was administered as necessary to counteract any signs of hypoglycemia. Descriptive statistical analyses (mean and standard deviation) were calculated for serum insulin and glucose data only. Mean values were calculated from mean data of the three timepoints collected during that day. N= 4 except: Day 29 (0.14 mg/kg female) n=3 for approximately 15 min. collection and Day 29 (0.58 mg/kg female) n=3 for approximately 30 min. collection of serum glucose only.

2.6.7.8D Repeat-Dose Inhalation Toxicity **Study No. N001603B (Continued)**

Daily Dose (mg/kg) as Insulin	0 (Air Control)		0.14		0.58	
Number of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Lung						
Subacute Inflammation, Bronchus	0	1	0	0	0	0
Subacute Inflammation, Alveoli	0	1	0	0	0	0
Trachea						
Subacute Inflammation	0	0	0	1	0	1
Additional Examinations						
Serum and Pulmonary lavage anti-human insulin antibody assay	-	-	-	-	-	-
Pulmonary function	-	-	-	-	-	-

- No noteworthy findings

2.6.7.8E Repeat-Dose Inhalation Toxicity | **Report Title:** Six-Month Inhalation Toxicity Study of Aerosol Insulin in Monkeys | **Test Article:** Insulin Inhalation Powder

Species/Strain: Monkey/ Cynomolgus
Initial Age: At least 17 months old at first exposure
Date of First Dose: 26(M) 27(F) August 1997

Duration of Dosing: 182 Days
Duration of Postdose: None
Method of Administration: Inhalation – head only
Vehicle/Formulation: Dry powder placebo of excipients consisting of [redacted] and [redacted]

Study No. N002448B
Location in CTD: Vol. / Section 4.2.3.2
GLP Compliance: Yes

Special Features:

- Serum concentrations of insulin and glucose were measured pre-dose on Days 1, 91, and 178 and at approximately 5, 15, and 30 min. post exposure. Feed was withheld (approximately 30 min.) on sampling days. Additional samples were collected for serum anti-human insulin antibody titer prior to treatment initiation and on Days 30, 59, 120, and 183.
- Animals had access to feed twice daily (morning and afternoon) with morning feeding occurring prior to exposure to help prevent the development of hypoglycemia.
- Ophthalmology was performed before treatment initiation and during the last week of study on sedated animals.
- Electrocardiograms were collected on sedated animals, prior to treatment initiation and on Day 180.
- Respiratory parameters were measured one time per week throughout the study. The parameters measured were: respiratory rate, minute volume, tidal volume, and accumulated inhaled volume.
- Pulmonary function evaluations were performed on sedated animals, pre-study and on Day 180. A cuffed endotracheal tube was placed and inflated in the esophagus and advanced into the thorax until the maximum pressure signal was obtained. The parameters measured were: peak inspiratory flow, peak expiratory flow, minute volume, tidal volume, frequency, resistance and compliance.
- Bronchoalveolar lavage samples for antibody analysis were collected prior to necropsy on Day 183. Animals were anesthetized and a pediatric fiberoptic bronchoscope was guided into the lung for sample collection.

No Observed Adverse-Effect Level: 0.64 mg/kg/day

2.6.7.8E Repeat-Dose Inhalation Toxicity **Study No. N002448B (Continued)**

Daily Dose (mg/kg) as Insulin	0 (Air Control)[a]		0 (Placebo Control)[a]		0.29		0.64	
Number of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Mean serum insulin (ng/ml)[b]								
Pre-dose Day 1	1.5	0.5	1.7	1.4	1.5	0.8	1.6	0.7
Day 1 within 30 min. post-dose	0.9	0.8	0.9	1.1	6.8	4.1	21.2	9.6
Pre-dose Day 91	2.6	1.0	2.9	1.3	2.1	1.8	1.2	2.6
Day 91 within 30 min. post-dose	1.3	1.1	1.2	1.0	6.3	6.3	18.2	21.3
Pre-dose Day 178	3.5	1.4	4.0	1.9	3.3	4.0	3.3	2.0
Day 178 within 30 min. post-dose	1.7	1.3	2.0	1.7	7.1	4.5	19.7	12.2
Mean serum glucose (mg/dL)[b]								
Pre-dose Day 1	74	94	78	78	85	76	81	81
Day 1 within 30 min. post-dose	75	116	109	80	44	42	24	27
Pre-dose Day 91	66	71	55	61	71	66	76	86
Day 91 within 30 min. post-dose	79	93	96	79	45	40	33	36
Pre-dose Day 178	62	65	64	59	60	57	80	68
Day 178 within 30 min. post-dose	72	82	89	85	48	47	32	34

[a] Control groups were exposed to either filtered air or aerosolized placebo powder to achieve a total mass equivalent to the high-dose insulin group.
[b] Descriptive statistical analyses (mean and standard deviation) were calculated for serum insulin and glucose data only. Mean values were calculated from mean data of the three timepoints collected during that day. N= 4 except: Day 1 (0.64 mg/kg male) n=3 for approximately 5 min. collection.

2.6.7.8E Repeat-Dose Inhalation Toxicity **Study No. N002448B (Continued)**

Daily Dose (mg/kg) as Insulin	0 (Air Control)[a]		0 (Placebo Control)[a]		0.29		0.64	
Number of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Noteworthy findings								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Body Weight (kg)	-	-	-	-	-	-	-	-
Food Consumption	ND	ND	ND	ND	ND	ND	ND	ND
Clinical Observations								
lethargy /unresponsive/disoriented related to hypoglycemia	0	0	0	0	0	0	1	2
coughing	0	1	4	4	3	2	4	4
labored respiration/ rales	0	0	2	0	1	0	0	1
sneezing	1	2	4	4	1	2	4	3
Ophthalmoscopy	-	-	-	-	-	-	-	-
Electrocardiography	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Plasma Chemistry	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-
Absolute Organ Weights[c] (%)								
Lung[d]	9.81	8.68	-13	+10	+19	+27*	0	+12
Relative Organ Weights	-	-	-	-	-	-	-	-

*Statistical significance according to Dunnett's Test at $p \leq 0.05$.
- No noteworthy findings / ND: Not determined
[a] Control groups were exposed to either filtered air or aerosolized placebo powder to achieve a total mass equivalent to the high-dose insulin group.
[c] For controls, group means are shown. For treated groups, number indicates percent differences from the air control weight in grams. Statistical significance is based on actual data (not on the percent differences).
[d] The increase in mean absolute weights were not considered insulin related since the differences were of low incidence, not consistent in corresponding treatment groups, nor dose related.

2.6.7.8E Repeat-Dose Inhalation Toxicity **Study No. N002448B (Continued)**

Daily Dose (mg/kg) as Insulin	0 (Air Control)[a]		0 (Placebo Control)[a]		0.29		0.64	
Number of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Gross Pathology	-	-	-	-	-	-	-	-
Histopathology[e] Lung								
Infiltrating cell, histiocyte, alveolus	4	4	4	4	4	4	3	4
Inflammation, subacute	1	2	3	3	0	2	2	1
Hemorrhage	0	0	0	1	0	0	0	0
Additional Examinations								
Serum and Pulmonary lavage anti-human insulin antibody assay	-	-	-	-	-	-	-	-
Pulmonary function	-	-	-	-	-	-	-	-

- No noteworthy findings
[a] Control groups were exposed to either filtered air or aerosolized placebo powder to achieve a total mass equivalent to the high-dose insulin group.
[e] All findings were considered to be incidental in nature and not the result of inhalation exposure to either insulin or the excipients.

2.6.7.9 Other Toxicity Studies – Cellular Proliferation Evaluation of Lung of Rat and Monkey **Test Article:** Insulin Inhalation Powder

Species/Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) as Insulin	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Rat / Sprague-Dawley	Inhalation	6 month dosing Lung cell proliferation evaluation done on formalin-fixed tissue from study N002448A	0 (air), 0 (placebo), and 5.8 mg/kg high dose	10/sex/group	PCNA staining of lung tissue (alveoli and bronchioles) consisting of approximately 1000 cells each for evaluation. Peribronchiolar lymphoid tissue served as a positive control. No differences in nuclei staining were noted in the lung tissue of control and high dose rats. In conclusion, no proliferative response in lung was observed.	01-906-05	/4.2.3.2.1
Monkey / Cynomolgus	Inhalation	6 month dosing Lung cell proliferation evaluation done on formalin-fixed tissue form study N002448B	0 (air), 0 (placebo), and 0.64 mg/kg high dose	4/sex/group	Ki-67 staining of formalin-fixed lung tissue (alveoli and bronchioles) consisting of approximately 1000 cells each for evaluation. Peribronchiolar lymphoid tissue served as a positive control. No differences in nuclei staining were noted in the lung tissue of control and high dose monkeys. In conclusion, no proliferative response in lung was observed.		/4.2.3.2.1

2.6.7.9 Other Toxicity Studies – Radioligand Binding Assay (RLB) For Insulin Antibodies **Test Article:** Insulin Inhalation Powder

Species/Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) as Insulin	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Monkey / Cynomolgus	Inhalation ELISA (enzyme linked immuno-absorbant assay) showed no insulin antibodies when serum and brochioalveolar lavage (BAL) fluid was first analyzed following study termination.	6-month. Samples from study N002448B. Serum and BAL antibodies measured on stored, frozen samples using RLB. These samples had been stored for approximately 3.5 years.	0 (air), 0 (placebo), 0.29 and 0.64 mg/kg	4/sex/group	Using the RLB assay with potential increased sensitivity, no insulin antibodies were observed in serum and BAL. Thus, the RLB assay confirmed the original results from ELISA. Recombinant human insulin is not immunogenic in the monkey.	01-906-02	/4.2.3.2.1

2.6.7.9 Other Toxicity Studies – Radioligand Binding Assay (RLB) For Insulin Antibodies **Test Article:** Insulin Inhalation Powder

Species/Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) as Insulin	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Rat / Sprague-Dawley	Inhalation	6-month. Samples from study N002448A. Serum antibodies measured on stored, frozen samples using RLB. These samples had been stored for approximately 3.5 years.	0 (air), 0 (placebo), 0.9, 2.7, 5.8 mg/kg	9/sex/group	Using the RLB assay with potential increased sensitivity versus the ELISA, most serum samples were below the limit of quantitation. One mid-dose group rat had insulin binding capacity slightly above the limit of quantitation. The RLB assay results on the stored samples indicated that recombinant human insulin is not immunogenic in the rat. The possibility of antibody degradation with long term storage is not assured.	01-906-03	/4.2.3.2.1

2.6.7.10A Genotoxicity: In Vitro **Report Title:** [redacted] Mutation Test) Bacterial Reverse **Test Article:** Recombinant Human Insulin; Batch Numbe [redacted]

Test for Induction of: Reverse mutation in bacterial cells.
Strains: Escherichia. coli strain WP2uvrA; Salmonella typhimurium strains TA 98,TA 100,TA1535 and TA 1537
Metabolizing System: Liver S9 fraction from Arochlor 1254-induced rats
Vehicles: For Test Article: Double distilled water
Treatment: Incubation for 48 hours
Cytotoxic Effects: None reported
Genotoxic Effects: None

No. of Independent Assays: 2
No. of Replicate Cultures: 3
No. of Cells Analyzed/Culture: not applicable
For Positive Controls: Double distilled water and DMSO

Study No. 980020
Location in CTD: Vol. / Section 4.2.3.3
GLP Compliance: Yes
Date of Treatment: January 28 to January 30, 1998

Preliminary Cytotoxicity Test

Metabolic Activation	Test Article	Dose Level (mg/plate)	Revertant Colony Count (Mean ± SD)
			TA 100
Without Activation	Distilled water	-	183.0 (38.2)
	Insulin	0.004	179.0 (32.9)
		0.02	169.7(27.6)
		0.1	177.0(29.5)
		0.5	188.3(30.9)
		2.5	204.3(30.1)
		5.0	163.0(6.2)
With Activation	Distilled water	-	198.7 (30.9)
	Insulin	0.004	237.0(29.1)
		0.02	226.7(66.4)
		0.1	196.7(25.5)
		0.5	173.0(32.2)
		2.5	210.3(13.0)
		5.0	207.3(10.4)

Definitive Mutation Assay #1

Metabolic Activation	Test Article	Dose Level (mg/plate)	Revertant Colony Count (Mean ± SD)				
			WP2uvrA	TA 98	TA 100	TA 1535	TA 1537
Without Activation - S9	Distilled water	-	32.7(3.2)	20.3(3.2)	126.0 (2.0)	9.0(1.7)	10.0(1.0)
	Insulin	0.004	32.7(4.2)	25.3(4.9)	128.0 (18.7)	10.3(5.1)	12.0(1.7)
		0.02	35.0(2.6)	20.3(0.6)	137.0(7.0)	12.0(1.0)	8.3(3.8)
		0.1	29.3(6.7)	23.7(1.5)	130.3 (13.6)	9.3(0.6)	6.7(3.2)
		0.5	29.0(3.0)	25.3(3.5)	126.3 (13.5)	6.0(1.7)	9.3(1.2)
		2.5	26.7(6.4)	27.0(1.7)	135.0 (10.4)	5.7(2.3)	8.0(1.0)
		5.0	27.3(4.9)	25.7(4.6)	133.7(6.0)	10.0(2.0)	9.0(2.0)
	Sodium azide	0.001	-	-	560.3(24.5)	413.0(16.8)	-
	9-aminoacridine	0.05	-	-	-	-	143.0(23.0)
	2-nitrofluorene	0.025	-	490.0(30.0)	-	-	-
	MNNG	0.025	137.7(15.6)	-	-	-	-
With Activation + S9	Distilled water	-	32.7(2.9)	29.7(3.8)	133.0 (4.0)	9.0(0.0)	7.0(1.7)
	Insulin	0.004	30.3(4.7)	28.7(2.1)	129.0(2.6)	11.0(3.6)	7.3(2.3)
		0.02	35.0(1.5)	33.7(4.0)	135.3(4.0)	10.7(4.2)	4.0(1.7)
		0.1	30.3(5.0)	30.7(4.5)	142.7(11.1)	11.3(2.5)	6.7(0.6)
		0.5	33.3(5.7)	27.0(1.0)	130.0 (4.6)	9.0(2.6)	9.0(3.0)
		2.5	33.0(5.2)	30.0(6.2)	124.7 (10.1)	8.0(3.0)	9.7(3.1)
		5.0	30.3(6.8)	27.3(4.0)	133.7(18.5)	11.0(1.0)	7.3(2.1)
	2-aminoanthracene		209.0(12.3)	1840.3(25.7)	2323.3(91.8)	279.3(7.0)	400.3(23.1)

2.6.7.10A Genotoxicity: In Vitro **Study No. 980020 (Continued)**

Definitive Mutation Assay #2

Metabolic Activation	Test Article	Dose Level (mg/plate)	Revertant Colony Count (Mean ± SD)				
			WP2uvrA	TA 98	TA 100	TA 1535	TA 1537
Without Activation	Distilled water	-	15.7(1.5)	22.7(6.0)	133.3(2.5)	9.3(0.6)	6.3(2.5)
	Insulin	0.004	14.7(3.1)	24.0(6.0)	141.3(2.5)	9.0(2.6)	9.0(1.0)
		0.02	18.0(2.6)	22.0(3.6)	134.7(9.1)	8.0(3.5)	7.3(2.1)
		0.1	17.0(6.1)	19.0(4.4)	133.7(3.1)	11.3(1.5)	6.7(2.3)
		0.5	14.0(2.0)	23.7(0.6)	138.3(5.7)	7.3(0.6)	6.0(1.0)
		2.5	16.7(3.5)	21.3(1.2)	133.3(10.7)	7.3(2.5)	6.3(1.2)
		5.0	18.3(2.1)	23.0(1.0)	141.7(19.1)	7.7(0.6)	8.7(2.1)
	Sodium azide	1.0	-	-	540.7(52.5)	337.7(15.9)	-
	9-aminoacridine		-	-	-	-	173.0(16.6)
	2-nitrofluorene		-	394.7(33.5)	-	-	-
	MNNG		73.7(0.6)	-	-	-	-
With Activation	Distilled water	-	14.7(2.3)	26.0(5.0)	170.3(6.4)	6.3(1.5)	4.3(0.6)
	Insulin	0.004	16.7(6.0)	27.0(5.3)	168.3(15.3)	8.0(1.0)	6.7(1.5)
		0.02	15.0(1.7)	28.0(4.6)	159.7(2.9)	11.3(4.6)	5.3(1.5)
		0.1	15.7(4.2)	23.3(2.5)	154.0(11.5)	7.0(2.6)	4.3(1.5)
		0.5	19.3(2.1)	31.7(5.0)	153.3(28.6)	11.7(0.6)	4.7(2.1)
		2.5	17.0(0.0)	21.0(7.5)	164.3(7.8)	7.3(1.2)	4.3(2.5)
		5.0	19.0(1.0)	26.3(4.0)	156.7(13.5)	8.7(1.5)	5.0(1.0)
	2-aminoanthracene		103.0(14.8)	1686.3(141.7)	2104.7(47.7)	199.0(24.1)	351.7(15.4)

2.6.7.10B Genotoxicity: In Vitro **Report Title:** [redacted] Bacterial Reverse Mutation Test **Test Article:** [redacted] Recombinant Human Insulin; Batch Number [redacted]

Test for Induction of: Reverse mutation in bacterial cells.
Strains: Escherichia. coli strain WP2uvrA; Salmonella typhimurium strains TA 98,TA 100,TA1535 and TA 1537
Metabolizing System: Liver S9 fraction from Arochlor 1254-induced rats
Vehicles: For Test Article: Double distilled water
Treatment: Incubation for 48 hours
Cytotoxic Effects: None reported
Genotoxic Effects: None

No. of Independent Assays: 2
No. of Replicate Cultures: 3
No. of Cells Analyzed/Culture: not applicable
For Positive Controls: Double distilled water and DMSO

Study No. 980021
Location in CTD: Vol. / Section 4.2.3.3
GLP Compliance: Yes
Date of Treatment: January 28 to January 30, 1998

Preliminary Cytotoxicity Test

Metabolic Activation	Test Article	Dose Level (mg/plate)	Revertant Colony Count (Mean ± SD)
			TA 100
Without Activation	Distilled water	-	172.7(2.9)
	Insulin	0.004	155.0(11.1)
		0.02	153.0(17.1)
		0.1	164.3(7.5)
		0.5	173.7(17.6)
		2.5	156.3(5.5)
		5.0	154.7(5.8)
With Activation	Distilled water	-	217.3(28.6)
	Insulin	0.004	233.0(40.3)
		0.02	217.7(42.6)
		0.1	211.3(47.6)
		0.5	220.3(47.1)
		2.5	208.3(35.1)
		5.0	222.0(38.4)

2.6.7.10B Genotoxicity: In Vitro **Study No. 980021(Continued)**

Definitive Mutation Assay #1

Metabolic Activation	Test Article	Dose Level (mg/plate)	Revertant Colony Count (Mean ± SD)				
			WP2uvrA	TA 98	TA 100	TA 1535	TA 1537
Without Activation - S9	Distilled water	-	28.7(2.5)	25.0(4.6)	126.7(24.0)	9.3(2.1)	10.0(1.0)
	Insulin	0.004	33.3(6.7)	28.0(2.6)	123.3(6.7)	8.0(1.7)	6.7(1.2)
		0.02	31.0(7.0)	22.0(6.1)	132.3(5.9)	7.7(2.9)	5.0(2.6)
		0.1	34.3(4.2)	24.3(3.5)	128.7(11.9)	9.0(2.0)	6.7(1.2)
		0.5	33.0(0.0)	23.7(5.5)	131.3(6.5)	11.0(3.0)	6.3(3.1)
		2.5	33.0(4.6)	24.0(5.6)	123.3(2.3)	10.0(3.5)	5.3(1.5)
		5.0	32.0(1.0)	25.7(4.2)	124.3(4.6)	10.0(3.5)	6.7(2.1)
	Sodium azide	0.001	-	-	560.3(24.5)	413.0(16.8)	-
	9-aminoacridine	0.05	-	-	-	-	143.0(23.0)
	2-nitrofluorene	0.025	-	490.0(30.0)	-	-	-
	MNNG	0.025	137.7(15.6)	-	-	-	-
With Activation + S9	Distilled water	-	35.3(4.5)	32.3(5.1)	131.0(13.0)	10.3(2.1)	6.0(3.6)
	Insulin	0.004	32.0(6.6)	26.0(3.6)	121.0(8.2)	11.3(2.5)	5.0(0.0)
		0.02	33.7(4.9)	28.7(1.5)	128.0(5.3)	10.3(2.5)	10.3(4.7)
		0.1	30.3(6.7)	33.7(5.0)	123.7(5.3)	6.3(2.1)	6.7(3.1)
		0.5	30.3(3.2)	33.7(0.6)	132.0(11.3)	9.7(2.3)	9.0(4.4)
		2.5	36.0(10.8)	30.7(5.1)	130.3(17.2)	13.0(3.6)	7.3(1.2)
		5.0	31.0(5.6)	28.0(2.6)	134.0(11.0)	8.0(2.0)	9.0(1.7)
	2-aminoanthracene		209.0(12.3)	1840.3(25.7)	2323.3(91.8)	279.3(7.0)	400.3(23.1)

2.6.7.10B Genotoxicity: In Vitro **Study No. 980021(Continued)**

Definitive Mutation Assay #2

Metabolic Activation	Test Article	Dose Level (mg/plate)	Revertant Colony Count (Mean ± SD)				
			WP2uvrA	TA 98	TA 100	TA 1535	TA 1537
Without Activation	Distilled water	-	16.0(1.0)	27.3(3.1)	134.0(14.7)	8.3(5.9)	5.0(1.0)
	Insulin	0.004	17.3(4.0)	29.3(5.1)	130.3(6.5)	8.7(1.2)	5.7(1.5)
		0.02	12.0(1.3)	27.3(5.7)	137.3(11.0)	9.3(0.6)	7.0(1.7)
		0.1	15.0(2.0)	28.3(2.5)	130.3(19.9)	10.7(2.1)	6.0(1.7)
		0.5	15.3(2.5)	29.3(0.6)	143.7(19.5)	7.0(2.0)	9.3(1.5)
		2.5	14.3(2.1)	27.3(2.9)	142.7(9.5)	7.0(1.0)	6.3(1.2)
		5.0	16.7(2.1)	26.7(6.7)	148.0(6.6)	8.7(4.2)	8.0(2.6)
	Sodium azide	1.0	-	-	540.7(52.5)	337.7(15.9)	-
	9-aminoacridine		-	-	-	-	173.0(16.6)
	2-nitrofluorene		-	394.7(33.5)	-	-	-
	MNNG		73.7(0.6)	-	-	-	-
With Activation	Distilled water	-	17.0(2.6)	28.3(1.2)	151.7(14.4)	8.3(1.5)	5.0(2.0)
	Insulin	0.004	17.3(6.5)	24.7(8.5)	138.3(10.2)	7.0(3.0)	7.3(4.5)
		0.02	16.0(5.3)	31.0(7.5)	149.7(28.1)	10.0(2.0)	4.7(2.1)
		0.1	18.0(3.6)	25.7(6.0)	139.7(5.0)	6.7(1.5)	8.3(0.6)
		0.5	17.7(4.9)	30.0(2.6)	159.0(26.5)	9.3(0.6)	5.7(2.5)
		2.5	23.7(4.2)	26.7(2.9)	143.7(11.7)	9.7(2.1)	8.0(1.0)
		5.0	17.7(5.1)	25.3(1.2)	159.3(10.0)	11.0(3.0)	5.3(0.6)
	2-aminoanthracene		103.0(14.8)	1686.3(141.7)	2104.7(47.7)	199.0(24.1)	351.7(15.4)

2.6.7.11 Single-Dose Subcutaneous Toxicity **Ancillary Studies** **Test Article:** —— Recombinant Human Insulin

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg) as Insulin	Gender and No. per Group	Observed Maximum Non-Lethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Mouse/ Shoe:NMRI	Subcutaneous; vehicle of m-cresol, sodium phosphate and glycerin	1000 IU/kg	2M, 2F	> 1000 IU/kg	> 1000 IU/kg	All animals survived. One animal had reduced motility approximately 4 hours after injection. On study Day 2, this animal was free of symptoms. Body weight was normal. No macroscopic changes noted. The maximum tolerated dose: > 1000 IU/kg	96-0175	/4.2.3.7.7

2.6.7.11 Single-Dose Subcutaneous Toxicity **Ancillary Studies** **Test Article:** /—— Recombinant Human Insulin

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg) as Insulin	Gender and No. per Group	Observed Maximum Non-Lethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Rat/Hoe:WIS Kf (SPF71)	Subcutaneous; vehicle of m-cresol, sodium phosphate and glycerin	1000 IU/kg	2M, 2F	> 1000 IU/kg	> 1000 IU/kg	All animals survived. No signs of toxicity were observed. Body weight was normal. No macroscopic changes were noted. The maximum tolerated dose: >1000 IU/kg	96-0176	/4.2.3.7.7

2.6.7.11 Single-Dose Subcutaneous Toxicity **Ancillary Studies** **Test Article** —— Recombinant Human Insulin

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg) as Insulin	Gender and No. per Group	Observed Maximum Non-Lethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Dog/Beagle	Subcutaneous; vehicle of m-cresol, sodium phosphate and glycerin	5 and 15 IU/kg	2 Males / group	>15 IU/kg	>15 IU/kg	No deaths observed. One dog at 15 IU/kg noted in prone abdominal position and with sedation, hypersalivation, tremor, clonic convulsions, dyspnea, deep respiration, and vomiting. Decreased locomotor activity was noted in the other animal at 15 IU/kg. Vomiting was noted in one animal at 5 IU/kg. Body weight was normal in all dogs except decreased in one animal at 15 IU/kg. Hematology was normal in all dogs and blood glucose decreased starting at 1-hour post administration for both groups. Total bilirubin and phospholipids increased for both dose groups whereas alkaline phosphates and total cholesterol was increased only at 15 IU/kg.	97012	/4.2.3.7.7

2.6.7.12 Repeat-Dose Subcutaneous Toxicity — **Ancillary Studies** — **Test Article:** Lilly and Aventis Recombinant Human Insulin

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg) as Insulin	Gender and No. per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Rat/Crj:CD (SD)IGS	Subcutaneous; vehicle of m-cresol, sodium phosphate and glycerin	4 Weeks	0,12.5, 25, and 50 IU/kg; equivalent to approximately 0, 0.45, 0.9, 1.8 mg/kg of [redacted] (Aventis recombinant human insulin).	For Aventis insulin 16 M, 16 F/ group (6/sex group for toxicokinetic [TK] evaluation), except for high dose with 22 M / 22 F /group (6/sex/group for TK and 6/sex/group dosed for only 2 weeks	1.8 for both Lilly and Aventis insulins	In comparison, Aventis's [redacted] and Lilly's Humacart® recombinant human insulins were well tolerated with no clinical signs. Mortality (one for Aventis [redacted] high dose and two for Lilly's Humacart® high dose) was related to low blood glucose. No effects were observed in food consumption and body weight values. Various changes noted in urinalysis, hematology and blood chemistry values were considered incidental or within background incidence and not related to Aventis and Lilly insulins. Gross pathological, organ weight and histopathological changes were considered incidental or within background incidence and not related to Aventis and Lilly insulins.	SBL 78-90	/4.2.3.7.7

[a] No Observed Adverse-Effect Level.

2.6.7.12 Repeat-Dose Subcutaneous Toxicity — **Ancillary Studies** — **Test Article:** Lilly and Aventis Recombinant Human Insulin

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study Number	CTD Location Vol./ Section
			50 IU/kg; equivalent to 1.8 mg/kg of Lilly Humacart® (Lilly recombinant human insulin for comparison)	For [redacted] insulin, 22 M 22 F / group (6/sex/group for TK and 6/sex/group dosed for only 2 weeks)		Toxicokinetic values and blood glucose and insulin antibody concentrations were similar for the comparative groups. As no significant toxicological or pharmacological or immunological differences were observed in the Lilly and Aventis high dose comparative insulin groups, it was concluded that the non-toxic dose of Aventis's [redacted] and Lilly's Humacart® recombinant human insulin was 50 IU/kg (about 1.8 mg/kg/day).	SBL 78-90 -continued	/4.2.3.7.7

[a] No Observed Adverse-Effect Level.

2.6.7.13 Local Tolerance Toxicity **Ancillary Studies** **Test Article:** Aventis——— Recombinant Human Insulin

——— A Single–dose local tolerance study in rabbits (intramuscular / subcutaneous)

Species/Strain	Method of Administration	Doses (mg/kg) as Insulin	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Rabbits/New Zealand mature pure-bred	Subcutaneous under the flank skin	Concentration of 100 IU/ml equivalent to about 3.6 mg insulin/ml. Dose injected 0.1 ml in right side. Saline (9 mg/ml) control dose of 0.1 ml injected in left side.	4 F	Two rabbits were killed at 24 hours and remaining 2 were killed at 120 hours post injection. The subcutaneously injected areas were dissected and examined histologically. Microscopically, Aventis insulin was well tolerated in all animals injected subcutaneously.	96-0174	/4.2.3.7.7
Rabbits/New Zealand mature pure-bred	Intramuscular in dorsal muscle	Concentration of 100 IU/ml equivalent to about 3.6 mg insulin/ml. Dose injected 0.5 ml on right side. Saline (9 mg/ml) control dose 0.5 ml injected in left side.	4 F	Two rabbits were killed at 24 hours and remaining 2 were killed at 120 hours post injection. The intramuscular injected areas were dissected and examined histologically. Microscopically, Aventis insulin was well tolerated in all animals injected intramuscularly.	96-0174	/4.2.3.7.7

2.6.7.13 Local Tolerance Toxicity **Ancillary Studies** **Test Article:** Aventis——— Recombinant Human Insulin

——— Single-dose local tolerance study in rabbits (intravenous / paravenous)

Species/Strain	Method of Administration	Doses (mg/kg) as Insulin	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Rabbits/New Zealand mature pure-bred	Intravenous in marginal ear vein	Concentration of 100 IU/ml equivalent to about 3.6 mg insulin/ml. Dose injected 0.5 ml in right ear vein. Saline (9 mg/ml) control dose of 0.5 ml injected in left ear vein.	4 F	Two rabbits were killed at 24 hours and remaining 2 were killed at 120 hours post injection. The intravenous injected areas were dissected and examined histologically. Microscopically, Aventis insulin was well tolerated in all animals injected intravenously.	96-0370	/4.2.3.7.7
Rabbits/New Zealand mature pure-bred	Paravenous beside the marginal vein	Concentration of 100 IU/ml equivalent to about 3.6 mg insulin/ml. Dose injected 0.1 ml beside the right vein. Saline (9 mg/ml) control dose of 0.1 ml injected beside the left ear vein of animal.	4 F	Two rabbits were killed at 24 hours and remaining 2 were killed at 120 hours post injection. The paravenous injected areas were dissected and examined histologically. Microscopically Aventis insulin was well tolerated in all animals injected paravenously.	96-0370	/4.2.3.7.7

2.6.7.14 Other Toxicity Studies – Ancillary Studies

Evaluation of the potential immunogenicity of recombinant human insulin and its pre-insulin derivative Pre in the pig

Test Article: Aventis recombinant human insulin, side product from manufacturing processing.
Reference insulins: (recombinant human insulin) and human insulin (from semi-synthetic conversion of porcine insulin)

Species/Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) as Insulin	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Swine / Deutsches Landschwein	Subcutaneous	13 weeks For immunization, each test and reference insulin with Freund's complete adjuvant (FCA) starting at 1 IU/kg (then 0.5, 0.25, 0.125 and 0.0625 IU/kg) injected at 1-week intervals (total 5 weeks). Following the 5 weekly injections, each test and reference insulin (without FCA) at 0.2 IU/kg injected 8 times at 1 week intervals for challenge	0.0625, 0.125, 0.25, 0.50 and 1 IU/kg for each test and reference insulin		Aventis recombinant human insulin compared to the other insulins had equal or lower antibody titers. For Pre-I compared to the other 3 insulins, there was a tendency to higher antibody titers but lower than insulin. Immunogenicity of Aventis was equal or lower than the reference insulins. Immunogenicity of Pre- was lower than bovine insulin. Since Pre was below the limit of analytical detection in Aventis insulin, it was concluded that Aventis insulin appears to have no major risk for immunogenicity.	016711	/4.2.3.7.2

2.6.7.14 Other Toxicity Studies – Ancillary Studies

Antigenicity Study with Recombinant Human Insulin in the Guinea Pig

Test Article: Aventis Recombinant Human Insulin

Species/Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) as Insulin	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section
Guinea Pig/Hartley	Subcutaneous for immunization; IV for challenge	Approximately 29 days	20 µg total dose/animal On Days 1, 8, and 15 for immunization, 3 animals were administered either 20 µg Aventis recombinant human insulin in 0.5 ml mock preparation (contains non insulin-related impurities of Aventis insulin) plus 0.15 ml Freund's complete adjuvant (FCA); and the other 3 animals received the mock preparation and FCA but no insulin. On day 29, animals were challenged with either 0.5 ml mock preparation or 20 µg insulin in 0.5 ml mock preparation.	6M, 6F/group	No adverse reactions were observed in either group. There was no indication for the antigenic potential of either Aventis insulin or the mock preparation with impurities and additives related to the formulated insulin product	184-03	/4.2.3.7.1

2.6.7.14 Other Toxicity Studies – Ancillary Studies **Antigenicity Study with Recombinant Human Insulin in the Guinea Pig** **Test Article:** Aventis Recombinant Human Insulin

Species/Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) as Insulin	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section.
Guinea pig / Hartley	Subcutaneous	Approximately 22 days	10 IU/kg of Aventis recombinant insulin Guinea pigs sensitized 1 time/week to either 10 IU/kg insulin or insulin plus Freund's complete adjuvant (FCA) or to MCP (host-derived protein) plus FCA. After 12 days, serum collected for passive cutaneous anaphylaxis (PCA). After 14 days, active cutaneous anaphylaxis (ACA) done using 10 IU/kg insulin or MCP.	10/group except 5/group that received ovalbumin plus FCA	For animals sensitized to insulin or insulin plus FCA when challenged by insulin, positive ACA. For animals sensitized to MCP plus FCA when challenged by insulin or MCP, negative ACA. PCA reaction similar to ACA indicating antibody titers to insulin and insulin plus FCA	I-1136	/4.2.3.7.1

2.6.7.14 Other Toxicity Studies – Ancillary Studies **Antigenicity Study with Recombinant Human Insulin in the Guinea Pig** **Test Article:** Aventis Recombinant Human Insulin

Species/Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) as Insulin	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section.
Mice/BALB/c and C3H/He Rat/Crj: CD(SD)	Intraperitoneal	Approximately 22 days	20 IU/kg Aventis recombinant human insulin. Mice sensitized 1 time /week for 3 weeks to mixture of insulin plus aluminum hydroxide gel (Alum) or to MCP plus Alum. After 14 days, serum collected for PCA. Serum was injected subcutaneously to rats Crj: CD(SD). Then rats received intravenous provoking to insulin or MCP.	6 each strain/group	PCA reaction using serum from 2 strains of mice was negative in rats indicating little if any antibody production.	I-1136 - continued	/4.2.3.7.1

2.6.7.14 Other Toxicity Studies – Ancillary Studies

Binding Affinity – Study to Determine the Relative Affinities of Differently Produced Human Insulins to the IGF-1 Receptor

Test Article: Aventis recombinant human insulin; (side product from manufacturing processing); (recombinant human insulin); and (semi-synthetic conversion insulin from porcine insulin)

Species/Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) as Insulin	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section.
Human Osteosarcoma B10 cells	In vitro cell culturing; plated in wells	Single exposure; overnight	7.6 picoM to 32 µM final concentrations for Aventis recombinant human insulin; Pre (side product from processing); (recombinant human insulin); and (semi-synthetic conversion insulin from porcine insulin)	Not applicable	The human osteosarcoma derived cell line B10 that expresses many IGF-1 but only a few insulin receptors was used to compare IGF-1 receptor binding affinities of various human insulins with the binding capacity of IGF-1. Relative affinities were determined in a competition assay using ^{125}I labeled IGF-1 as tracer. The recombinant human insulins and are identical in their sequence with the exception of the last one, and were prepared in different ways. The presented study shows that human IGF-1 binds to its receptor at nanomolar concentrations. In contrast, each of the investigated insulins bind at least 5000-fold weaker than the native ligand to the IGF-1 receptor. The various kinds of insulin preparations did not affect the affinities of the products to the IGF-1 receptor.	016750	/4.2.3.7.7

2.6.7.14 Other Toxicity Studies – Ancillary Studies

Pyrogenicity – An Intravenous Pyrogen Test of in Rabbits

Test Article: Recombinant Human Insulin

Species/Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) as Insulin	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section.
Rabbit / Japanese White	Intravenous	Single dose	50 and 100 IU/kg injected into auricular vein	3 M / group	Both groups showed signs of hypoglycemia. No animals showed 0.6°C or greater increase in body temperature. The sum of all body temperature increases did not exceed 1.4°C, the criteria for judgment of pyrogenicity. insulin was not pyrogenic.	I-1137	/4.2.3.7.7

2.6.7.15 Other Toxicity Studies **Biocompatibility Studies** **Test Article: Pulmonary Inhaler Components as indicated**

Components Evaluated	Study Title	Study Conditions	Species/Strain	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section.
	Cytotoxicity Using the ISO Elution Method (1X MEM Extract)	A	L-929, mouse fibroblast cells	Not Applicable (N/A)	No cell lysis or toxicity		/ 4.2.3.7.7
	Cytotoxicity Using the ISO Elution Method (1X MEM Extract)	A	L-929, mouse fibroblast cells	N/A	No cell lysis or toxicity		/ 4.2.3.7.7
	Cytotoxicity Using the ISO Elution Method (1X MEM Extract)	A	L-929, mouse fibroblast cells	N/A	No cell lysis or toxicity		/ 4.2.3.7.7
	Cytotoxicity Using the ISO Elution Method (1X MEM Extract)	A	L-929, mouse fibroblast cells	N/A	No cell lysis or toxicity		/ 4.2.3.7.7
	Cytotoxicity Using the ISO Elution Method (1X MEM Extract)	A	L-929, mouse fibroblast cells	N/A	No cell lysis or toxicity		/ 4.2.3.7.7
	Cytotoxicity Using the ISO Elution Method (1X MEM Extract)	A	L-929, mouse fibroblast cells	N/A	No cell lysis or toxicity		/ 4.2.3.7.7

Study Title	Study Conditions	Species/Strain	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section.
Cytotoxicity Using the ISO Elution Method (1X MEM Extract)	A	L-929, mouse fibroblast cells	N/A	No cell lysis or toxicity		/ 4.2.3.7.7
ISO Maximization Sensitization Study	B, C	Guinea Pig / Crl:(HA) BR	Female / 10 Test and 5 Control	No erythema or edema; no evidence of delayed dermal contact sensitization		/ 4.2.3.7.7
ISO Sensitization Study	B, D	Guinea Pig / Crl:(HA) BR	Female / 10 Test and 5 Control	No erythema or edema; no evidence of delayed dermal contact sensitization		/ 4.2.3.7.7
ISO Maximization Sensitization Study	B, C	Guinea Pig / Crl:(HA) BR	Female / 10 Test and 5 Control	No erythema or edema; no evidence of delayed dermal contact sensitization		/ 4.2.3.7.7
ISO Maximization Sensitization Study	B, C	Guinea Pig / Crl:(HA) BR	Female / 10 Test and 5 Control	No erythema or edema; no evidence of delayed dermal contact sensitization		/ 4.2.3.7.7
ISO Vaginal Irritation Study	E	Rabbit / New Zealand White	Female / 3 Test and 3 Control	Minimal Irritant		/ 4.2.3.7.7
ISO Vaginal Irritation Study	F	Rabbit / New Zealand White	Female / 3 Test and 3 Control	Nonirritant		/ 4.2.3.7.7

Components Evaluated	Study Title	Study Conditions	Species/Strain	Gender and No. per Group	Noteworthy Findings	Study Number	CTD Location Vol./ Section.
	ISO Vaginal Irritation Study in the Rabbit	E	Rabbit / New Zealand White	Female / 3 Test and 3 Control	Nonirritant		/ 4.2.3.7.7
	ISO Vaginal Irritation Study	F	Rabbit / New Zealand White	Female / 3 Test and 3 Control	Nonirritant		/ 4.2.3.7.7
	ISO Vaginal Irritation Study	E, F	Rabbit / New Zealand White	Female / 3 Test and 3 Control	Nonirritant to Minimal Irritant		/ 4.2.3.7.7
	ISO Vaginal Irritation Study	E, F	Rabbit / New Zealand White	Female / 3 Test and 3 Control	Nonirritant		/ 4.2.3.7.7
	USP and ISO Systemic Toxicity Study	G, H	Mouse / Crl:CF-1 BR	Male / 5 Test and 5 Control	No mortality or evidence of systemic toxicity		/ 4.2.3.7.7
	USP and ISO Systemic Toxicity Study	G, H	Mouse / Crl:CF-1 BR	Male / 5 Test and 5 Control	No mortality or evidence of systemic toxicity		/ 4.2.3.7.7
	ISO Acute Systemic Toxicity Study	G, I	Mouse / Crl:CF-1 BR	Male / 5 Test and 5 Control	No mortality or evidence of systemic toxicity		/ 4.2.3.7.7
	USP and ISO Systemic Toxicity Study	G, H	Mouse / Crl:CF-1 BR	Male / 5 Test and 5 Control	No mortality or evidence of systemic toxicity		/ 4.2.3.7.7

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:
EXUBERA® is new recombinant human inhalation insulin formulation being developed by Pfizer for the treatment of diabetes. This new powder inhalation insulin is formulated with the excipients mannitol, sodium citrate and glycine. To enhance bioavailability and stability of the powder insulin for inhalation, several formulations had been tested. Early in the development, the I-004 formulation containing 20% insulin from Lilly was prepared. The sponsor used the I-004 insulin formulation in all the non-clinical studies as well as in some clinical studies. Later in development, the new formulation, [redacted] containing 60% insulin from Aventis [redacted] was used in the phase 3 clinical trails as well as in the 1-month bridging toxicity study in rats. This formulation will be also marketed commercially.

Administration of inhalation insulin powder produced a dose-related decrease in glucose and increase in serum insulin in rodent and monkey toxicology studies. With hypoglycemia limiting the insulin dose, 6 mg/kg/d in rats or 0.6 mg/kg/d in monkeys were considered the MTD. Due to extensive clinical experience with injectable insulin, the scope of the nonclinical studies was limited to the 6-month rat and monkey studies with no carcinogenicity or reproductive toxicology studies. The objectives of the rat and monkey studies were to evaluate the impact of the inhalation insulin on lung morphology and function and the potential for formation of antibodies. Administration of INH to rats up for to 6-months via nose produced non-dose dependent and sporadic increases in lung weight. Since the increase in lung weight occurred either at low dose (1.1 mkd) in males or high dose (6 mkd) in female in the 1-month study or at mid dose (2.7 mkd) in the 6-month study and was absent at any dose in the 1-month bridging study, it was not clear whether the increase in lung weight were coincidental or drug related. There was no apparent correlation between increased lung weight and serum insulin concentrations or any specific lung pathology in rats. The incidences of inflammation and aggregation of alveolar histiocytes (foam cells) were seen in all groups in the 6-month rats study and retrospective analysis of lung tissue found no treatment related increase in cell proliferation. Furthermore, pulmonary function was unaffected with inhalation insulin in rats, suggesting that rat toxicology study was unable to detect any significant drug related lung toxicity.

Inhalation insulin administered to monkeys for up to 6-months has no specific effect on pulmonary function or lung weights. Monkeys treated with placebo or insulin had a greater incidence of cough and sneezing throughout the investigation. Histological examination did not identify any drug-related pathology except for mature dense fibrous connective tissue in the periphery of the lungs lobes representative of adhesion in the low dose females. Acute inflammation was also noted in one low dose male. Most of the findings in the respiratory tract appeared to be related to mechanical trauma due to alveolar lavage or possible the drug delivery method. Inhalation insulin up to 0.64 mg/kg/d had no effect on ECG, ophthalmic or pulmonary function in monkeys. Safety pharmacology study with insulin reported an increase in systolic blood pressure in dogs. This finding is in agreement with published literature with injectable insulin. Insulin has been shown to have both chronotropic and inotropic effects on heart. It seems that inotropic and chronotropic effects take place at much higher dose than the 0.6 mg/kg/d used in the 6-month monkey study.

Since insulin used in the formulation is recombinant human insulin, the potential for formation of antibodies and neutralization were evaluated in both rats and monkey. There was no evidence of antibody formation or decrease in insulin activity in monkeys. Lung lavage analysis found no measurable antibody presence in either rat or monkey. However, in rats, serum analysis found several samples to have antibodies against human insulin slightly above the assay limits of quantification. Since, human insulin sequence is different from rat, the weak antibody response in not unexpected. There was no evidence that insulin activity was diminished with repeated exposure in either rats or monkeys. The toxicokinetic evaluation found no insulin accumulation with repeated administration of inhalation insulin in either rats or monkeys. This is not unexpected since insulin is a protein and rapidly degraded in the lungs and in the circulation. It should be noted that variability in the bioavailability of inhalation insulin and serum insulin concentration made it very difficult to correlate insulin exposure to occasional toxicological findings. Whether the absence of any specific dose-related findings were related to variability in bioavailability, dose limitation, or real absence of toxicological findings is not clear. In absence of any specific pulmonary finding, the NOAEL dose was 5.8 mg/kg/d in rats and 0.64 mg/kg/d in monkeys. The safety margins based on the NOAEL dose in rats and monkeys were approximately 6 and 1.4 fold the clinical dose of 0.15 mg/kg/d based on mg/m^2, respectively. The non-clinical studies provided significant safety margins for the excipients, mannitol, sodium citrate and glycine (Rats: 34, 40 and 20X, Monkeys: 8, 9 and 4X the clinical dose, based on mg/m^2, respectively). Although there may have been a slight tendency for a treatment related effect on the lungs, collectively there was no concrete evidence of insulin related lung toxicity. The potential value of both rat and monkey toxicology studies were limited by low insulin dose due to hypoglycemia and stressful mechanism of drug delivery, low number of animals per treatment and limited duration of exposure. Considering the limitations of the nonclinical studies, there were no specific and consistent pulmonary findings in rats and monkeys to suggest that administration of inhalation insulin would be pose a safety concern to humans.

Human Safety margin for inhalation insulin:

Species	Dose, mg/kg/d	Dose, mg/m^2	Human safety margins (animal/human) based on different criteria		
			mg/kg body weight	mg/m^2 body surface area	mg/m^2 alveolar surface area
Rat, 1-month study	1.1	6.6	7	1.2	
	3.2	19	21	4	
	6	36	40	6	4
Monkey, 1- month study	0.14	1.7	1	0.3	
	0.58	7	4	1	1
Rat, 6-month study (NOAEL 5.8 mg/kg/d)	0.9	5.4	6	1	0.6
	2.7	16	18	3	2
	5.8	35	39	6	4
Monkey, 6-month study (NOAEL 0.64 mg/kg/d)	0.29	3.4	2	0.6	0.5
	0.64	7.7	4	1.4	1
Rat, 1 month bridging study	5.9	36	40	6	4
Clinical therapeutic dose, 0.15 mg/kg/d	0.15	5.6			

Recommendations: Approval

Suggested labeling:

Carcinogenesis, Mutagenesis, Impairment of Fertility
Carcinogenicity and fertility studies were not performed. Insulin was not mutagenic or clastogenic with or without metabolic activation in the Ames test with *Salmonella typhimurium* and *Escherichia coli.*

Pregnancy Category C
Animal reproductive studies have not been performed with EXUBERA®. Whether EXUBERA® can cause fetal harm when administered to pregnant women is unknown.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

STUDY: 30-DAY INHALATION TOXICITY STUDY OF AEROSOL INSULIN IN RATS

Objective: To characterize the toxicity profile of aerosol insulin administered to male and female rats via inhalation for 30 days.
Test article: Lot

Methods:
A total of 80 male and 80 female Sprague-Dawley rats were used for the 30-day nose-only inhalation study. The rats were assigned to the various study groups as shown below. The satellite group rats were used for determination of baseline endogenous insulin and glucose.

Experimental Group	Aerosol Conc. (mg/L)	Exposure Duration(min)	Numbers of rats/sex
Control	1	60	10
Low dose	1	10	20
Mid-dose	1	30	20
High dose	1	60	20
Satellite	None	None	10

Results:
Inhaled daily dose determination: The mean total inhaled mass dosages for the female animals were 34.18, 5.71, 16.91, and 31.79 mg total inhaled mass/kg body weight/day for vehicle control, low, mid, and high dose groups, respectively. The mean inhaled insulin dosages for the male animals were 0, 1.02, 3.00, and 5.68 mg inhaled insulin/kg body weight/day for control, low, mid, and high dose groups, respectively.

Clinical observations: There were no test article related effects, although there were signs that might have been related to physical trauma caused by restraint in nose-only inhalation tubes.

Toxicokinetics:
The mean serum glucose levels for male rats in the mid- and high dose groups and all treated females were lower than means for satellite group rats on Day 1 and Day 30. There was general increase in serum insulin levels in treated rats, which was preceded the reduction of serum glucose.

Clinical Pathology:
Sporadic increased or decreased pathology values were noted, which were not dose-dependent. There were no apparent test article related differences in organ weights, histopathology, and necropsy findings.

Conclusions:
Aerosol insulin was biologically active in the rat as indicated by the statistically significant decrease in mean serum glucose levels at Day 1 and Day 30. The time to maximum serum insulin concentrations was encompassed by the 30-minute post exposure blood collection periods. There was no clear indication of toxicological effects of aerosol insulin.

Study: 30-DAY INHALATION TOXICITY STUDY OF AEROSOL INSULIN IN CYNOMOLGUS MONKEYS (N001603B).

This study was conducted b[redacted] under GLP conditions.

Objective: to characterize the toxicity profile of aerosol insulin administered to male and female monkeys via inhalation for 30 days.

Test Article ([redacted]) contains: [redacted] recombinant human zinc insulin, [redacted] sodium citrate[redacted] mannitol, [redacted] glycine [redacted] citric acid.

Methods:
A total of 12 male and 12 female cynomolgus monkeys (Macaca fasicularis) were used. The animals were treated by head-only inhalation exposure to an aerosol of the test article or vehicle. The exposure groups and exposure levels were:

Exposure Group	Animal # and Sex	Aerosol Conc(μg/L)	Insulin Conc(μg/L)	Exposure Time(min)
Control	4M + 4F	150	0	60
Low Dose	4M + 4F	150	30	12
High Dose	4M + 4F	150	30	60

Results:
1) Dose estimation: The validation trials indicated that the delivery system was operated as designed. The following daily dosages were delivered. The expected maximum clinical dosage is 154 μg/kg/day and the high dose was sufficient to produce hypoglycemia in the female monkeys.

Inhaled Insulin Dosage (μg/kg/day)			
Group	Males	Females	Group Mean
Control	0	0	0
Low-dose	136	133	135
High-dose	521	645	583

Clinical observations:
There were no male animals that had abnormal clinical symptoms attributed to exposure to the test article. In female animals, there were several observations that were considered to be a result of the test article, which were hunched posture, lethargy, prostration, and dilated pupils. The signs were judged to be caused by hypoglycemia produced by exposure of aerosolized insulin because the signs were disappeared within 1 hour after dextrose injection.
Body weights:
There were no changes in the parameter attributed to exposure to the test article.
Serum insulin and glucose: According to the sponsor's radioimmunoassay data, insulin absorption was in insulin dose-dependent. In males, low and high doses groups had 4.39 and 20.64 ng/ml, respectively

29 days after treatment. In females, the concentrations were 6.34 and 30.29 ng/ml, respectively. It appeared that the decrease in serum glucose follows a trend similar to the elevations in serum insulin.
Clinical pathology:
There were no remarkable observations attributed to exposure to the test article.

Immunologic evaluation:
Bronchial alveolar lavage samples were assayed for anti-human insulin antibody levels. There were no positive titers in the samples.

Conclusions:
Twenty-four monkeys were exposed to aerosol insulin for 30 days, which delivered insulin in drug-dose dependently. There were several incidences of hypoglycemia, which were rescued by dextrose administration. There were no abnormal findings or changes as a result to exposure to aerosol insulin in major parameters.

RECOMMENDATIONS: (Letter to the sponsor)

Please consider to perform 3- to 6-month toxicology study to support long-term clinical studies. And please provide this Division experimental data or published findings as to the safety of 18% mannitol in respiratory tracts.

6-MONTH INHALATION TOXICITY STUDY OF AEROSOL INSULIN IN RATS (——Study ——

Objective: To evaluate the toxicity profile of aerosol insulin administered to male and female rats via inhalation for 6-months under GLP condition. The study was conducted by [redacted]

Test article: —, rh- zinc insulin with —— sodium citrate, —— mannitol, —glycine and —— citric acid (Placebo Lot: ——). Insulin Lot # was —— and ——. The particle size was ranged from —— and the flow rate was maintained at one liter per minute.

Study Design: Ten SD rats/sex/group were administered air or placebo as control, or insulin by oral inhalation at dose of I mg/l with 10-, 30- or 60-minute exposure. The rats were necropsied one day after the last dose administration. The satellite group and remaining rats were designated toxicokinetic/pulmonary function subgroup rats and were euthanized without necropsy after final toxicokinetic blood collection or pulmonary function measurement on Day 183 as shown below.

Study Design for 6-Month Toxicity Study in Sprague-Dawley Rats(#N002448A)				
Animal group	Treatment group	Aerosol dose(mg/L)	Exposure duration(min)	Number of rats/sex
1	Air Control	0	60	16
2	Placebo	1	60	16
3	Low dose	1	10	20
4	Mid dose	1	30	20
5	High dose	1	60	20
6	Satellite	None	None	10

Biological measurements included mortality, body weight (weekly), clinical observations (twice a day), ophthalmology (core toxicology group only), pulmonary function (TK and pulmonary function group only) and clinical chemistry (core toxicology group only). Post-exposure serum insulin and glucose levels were determined on Days 1, 91 and 182 (TK and pulmonary function groups 3, 4, and 5 and satellite groups only). Postmortem studies for the core toxicology subgroup rats included gross necropsy, organ weights (adrenals, brain, ovaries, heart, kidneys, liver, lungs, and testes), and microscopic examination of selected tissues (nasal turbinates, larynx, trachea, and lungs with bronchial tree and bronchial lymph nodes of animals in core toxicology subgroups).

Toxicokinetic Study: Two blood samples from the retro-orbital plexus were collected in tubes without anticoagulant from the 3 toxicokinetic dose groups (Group 3, 4, and 5) and satellite group (Group 6) for the analysis of glucose and insulin after exposure on Days 1, 91 and 182. The target time points for the toxicokinetic subgroups were 3, 10, and 30 minutes post-exposure.

Pulmonary function evaluations were performed in the anesthetized rats with Ketamine (80-100 mg/kg ip) and diazepam (2 mg/kg ip). Respiratory rate, tidal volume, minute volume, peak inspiratory flow, peak expiratory flow, resistance, and compliance were recorded.

RESULTS

Mortality and Clinical signs: There were seven unscheduled deaths during the study, which were due to accidental trauma (2 deaths), urinary tract infections (2 deaths) and hypoglycemia (3 deaths) as an extension of pharmacodynamic effects of inhaled insulin as shown below. There were no insulin or excipient-related effects indicated by clinical observations throughout the study except for isolated

instances of the expected physiological response of hypoglycemia after insulin inhalation as shown below. The observation of labored respiration and convulsive behavior for low dose male 318 occurred on Days 146 and 147. Clinical signs were also summarized in a table below.

Unscheduled Animal Deaths: 6-Month Inhalation Toxicity Study of Aerosol Insulin

Exposure Group	Animal No.	Date of Death	Study Day	Cause of Death
Males				
4-Mid-Dose (Core Tox)	402	12-Jan-98	147	Accidental trauma-thoracic trauma resulting in lung rupture and hemorrhage
4-Mid-Dose (Core Tox)	408	17-Jan-98	152	Found dead-Prostatic infection with associated urocystitis and hydronephrosis
4-Mid-Dose (TK/ Pulmonary Function)	411	20-Jan-98	155	Found dead-possible hypoglycemia
5-High-Dose (Core Tox)	510	15-Sep-97	28	Found dead-possible hypoglycemia
5-High-Dose (TK/ Pulmonary Function)	511	20-Nov-97	94	Euthanasia-urinary tract infection (cystitis)
Females				
4-Mid-Dose (Core Tox)	459	15-Sep-97	27	Euthanasia-accidental trauma fractured tibia and fibula
5-High-Dose (TK/ Pulmonary Function)	569	17-Feb-98	182	Euthanasia-possible hypoglycemia

Summary of Abnormal Clinical Observations: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

Exposure Group	Category	Subcategory	No. of Animals Affected	Mean First Day Observed	Mean Last Day Observed	Total No. of Observations
Males						
2-Placebo	ABRASION/LESION	BODY DORSAL	1	136	154	38
2-Placebo	ABRASION/LESION	BODY VENTRAL	1	136	154	38
3-Low Dose	ABRASION/LESION	HEAD	2	107	131	100
3 Low Dose	ABRASION/LESION	NECK	1	11	105	189
2-Placebo	ALOPECIA	BODY DORSAL	1	136	154	38
3-Low Dose	ALOPECIA	BODY DORSAL	1	172	183	23
2-Placebo	ALOPECIA	BODY VENTRAL	1	136	183	95
3-Low Dose	ALOPECIA	HEAD	2	109	148	160
3-Low Dose	ALOPECIA	LEG	1	57	105	98
5-High Dose	ALOPECIA	LEG	1	29	183	309
3-Low Dose	ALOPECIA	NECK	1	11	105	189
4-Mid Dose	DIGESTIVE SYSTEM	DIARRHEA	1	151	151	1
5-High Dose	DIGESTIVE SYSTEM	DIARRHEA	1	94	94	2
5-High Dose	DIGESTIVE SYSTEM	FECES-REDDENED	1	94	94	1
5-High Dose	DIGESTIVE SYSTEM	MALOCCLUSION	1	151	170	40
4-Mid Dose	DIGESTIVE SYSTEM	SOILED ANAL REGION	1	151	151	2
5-High Dose	EYES/EARS	CORNEAL OPACITY	1	148	183	71
4-Mid Dose	GENERAL APPEARANCE	LETHARGIC	1	151	151	1
5-High Dose	GENERAL APPEARANCE	LETHARGIC	1	94	94	3
3-Low Dose	GENERAL APPEARANCE	ROUGH COAT	1	172	183	23
4-Mid Dose	GENERAL APPEARANCE	ROUGH COAT	1	151	151	1
5-High Dose	GENERAL APPEARANCE	ROUGH COAT	1	94	94	3
5-High Dose	GENERAL APPEARANCE	UNRESPONSIVE	1	94	94	2
3-Low Dose	NEURO/MUSCULOSKELETAL	CONVULSIVE	1	146	147	4
3-Low Dose	RESPIRATORY SYSTEM	LABORED RESPIRATION	1	146	147	4
4-Mid Dose	RESPIRATORY SYSTEM	LABORED RESPIRATION	1	151	151	1
5-High Dose	RESPIRATORY SYSTEM	LABORED RESPIRATION	1	94	94	2
4-Mid Dose	RESPIRATORY SYSTEM	NASAL DISCHARGE-RED	1	150	150	1
1-Air Control	SWELLING	FOOT	1	155	168	28
1-Air Control	SWELLING	LEG	1	155	168	28
3-Low Dose	TISSUE MASS	BODY VENTRAL	1	34	183	299

Inhaled Aerosol Insulin Dosage and Clinical Ratio as Safety Margin:

In this 6-month rat study, therapeutic exposure ratio was calculated based on the expected maximum clinical dosage of 0.2 mg/kg/day (3 mg/blisters and 3 blisters/day) as summarized below in a table.

Treatment Group	Aerosol Dose (mg/L)	Exposure Duration (min)	Inhaled Insulin (mg/kg/day)*		Clinical Ratio@	
			Male	Female	Male	Female
Air Control	0	60	0	0	0	0
Placebo	1	60	0	0	0	0
Low Dose	1	10	1	1	4	5
Mid Dose	1	30	3	3	13	15
High Dose	1	60	5	6	27	31

*Calculated by multiplying aerosol conc. by exposure time, — mass fraction of insulin, and minute volume per kg body weight. @Based on the expected maximum clinical dosage of 0.2 mg/kg/day(3 mg/blister x 3 blisters/day /50 kg)

The starting clinical dose is 0.15 mg/kg/day.

Body Weights: There was no insulin or excipient-related effects indicated by weekly mean body weights. An exception would be mean body weights in satellite group, which had stress of blood sampling via eyes as shown below.

Group Mean Body Weights (g): 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

Exposure Group		Day of Study								
		127	134	141	148	155	162	169	176	183
Males										
1-Air Control	N	16	16	16	16	16	16	16	16	16
	Mean	554.84	563.94	569.38	575.36	582.11	582.99	591.68	604.26	612.58
	STD	78.09	80.69	83.81	86.30	90.35	92.68	96.59	100.72	104.60
2-Placebo	N	16	16	16	16	16	16	16	16	16
	Mean	545.26	552.44	558.42	564.29	566.74	565.77	576.47	584.54	592.51
	STD	56.38	57.36	58.88	60.80	61.87	62.98	63.06	64.93	67.62
3-Low Dose	N	20	20	20	20	20	20	20	20	20
	Mean	547.80	554.75	562.60	566.85	574.66	574.46	579.26	587.78	597.07
	STD	30.73	32.25	33.00	33.56	34.96	36.30	36.61	37.98	39.22
4-Mid Dose	N	20	20	20	19	17	17	17	17	17
	Mean	563.20	570.01	574.81	581.98	588.42	593.50	602.41	611.60	619.04
	STD	62.59	63.54	64.86	66.04	74.68	76.73	77.03	77.76	74.70
5-High Dose	N	18	18	18	18	18	18	18	18	18
	Mean	537.98	544.05	548.76	554.08	561.48	564.16	576.38	582.98	588.21
	STD	49.55	51.74	53.51	57.62	59.51	62.85	66.37	67.53	70.11
6-Satellite	N	10	10	10	10	10	10	10	10	10
	Mean	627.91*	642.18*	648.15*	659.08*	671.18*	671.58*	686.43*	698.25*	702.13*
	STD	43.77	47.88	48.52	45.03	49.52	54.68	54.94	58.52	56.13
Females										
1-Air Control	N	16	16	16	16	16	16	16	16	16
	Mean	298.53	301.42	303.92	306.80	309.73	308.71	314.26	315.53	317.09
	STD	31.38	33.24	36.98	36.99	37.07	38.00	40.50	41.42	41.79
2-Placebo	N	16	16	16	16	16	16	16	16	16
	Mean	311.22	313.09	315.71	321.45	323.16	323.14	325.64	327.73	328.93
	STD	24.83	23.94	24.01	26.42	27.57	28.72	30.42	34.02	31.09
3-Low Dose	N	20	20	20	20	20	20	20	20	20
	Mean	304.18	304.00	308.20	310.18	312.76	313.93	318.64	321.19	320.17
	STD	26.21	26.67	26.63	26.12	29.19	29.76	29.48	30.34	28.98
4-Mid Dose	N	19	19	19	19	19	19	19	19	19
	Mean	320.03	322.19	326.72	329.57	332.97	336.02	341.94	343.51	346.42
	STD	25.02	25.01	26.75	27.60	29.36	30.88	34.19	36.79	37.41
5-High Dose	N	20	20	20	20	20	20	20	20	19
	Mean	306.86	308.62	314.33	316.51	319.90	320.16	327.52	330.44	330.04
	STD	25.37	25.15	26.74	26.20	25.94	25.20	28.97	30.60	32.73
6-Satellite	N	10	10	10	10	10	10	10	10	10
	Mean	380.57*	387.58*	394.88*	397.42*	405.22*	411.53*	420.13*	425.60*	423.93*
	STD	43.30	43.76	43.99	43.68	44.39	46.96	49.71	49.12	51.58

Ophthalmology: There were no treatment-related ophthalmologic abnormalities as shown below.

. Summary of Ophthalmologic Examinations:
6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

Exposure Group	Week 26
Males	
1 - Air Control	0
2 - Placebo	0
3 - Low Dose	0
4 - Mid Dose	0
5 - High Dose	1
Females	
1 - Air Control	0
2 - Placebo	2
3 - Low Dose	0
4 - Mid Dose	0
5 - High Dose	0

All animals were normal during Week -1 examination

Animal 505, male, Group 5, had a corneal scar, right eye

Animal 254, female, Group 2, had a collapsed globe, right eye

Animal 256, female, Group 2, had a collapsed globe, right eye

Pulmonary Resistance: There was no insulin or excipient-related effects indicated by resistance or compliance measurements as shown below. PIF and PEP stand for peak inspiratory and expiratory flow, respectively.

Group Mean Pulmonary Function Values for Pulmonary Functions Subgroups:
6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

Exposure Group	Mean (Standard Error)[a]						
	Tidal Volume (mL)	Respiratory Frequency (breaths/min)	Minute Volume (mL/min)	PIF (mL/sec)	PEF (mL/sec)	Resistance (cm H_2O/mL/sec)	Compliance (mL/cm H_2O)
Males							
1-Air Control	1.29 (0.12)	121.5 (7.8)	153.8 (10.0)	7.3 (0.8)	8.6 (0.2)	0.253 (0.028)	0.37 (0.08)
2-Placebo	1.06 (0.10)	106.2 (4.8)	111.0 (7.2)	5.5 (0.1)	6.8 (0.5)	0.319 (0.059)	0.35 (0.04)
3-Low Dose	1.18 (0.08)	112.9 (12.5)	131.8 (18.1)	6.4 (0.8)	8.2 (0.9)	0.271 (0.030)	0.52 (0.06)
4-Mid Dose	1.07 (0.05)	120.8 (16.4)	127.9 (16.4)	6.0 (0.7)	7.9 (1.1)	0.253 (0.032)	0.48 (0.08)
5-High Dose	1.11 (0.10)	117.6 (11.8)	130.9 (17.0)	6.7 (0.8)	7.5 (0.8)	0.191 (0.034)	0.45 (0.05)
Females							
1-Air Control	0.62 (0.13)	129.6 (15.7)	76.4 (15.0)	3.6 (0.7)	5.3 (1.0)	0.298 (0.105)	0.29 (0.04)
2-Placebo	0.68 (0.14)	130.1 (14.0)	81.5 (16.2)	3.9 (0.8)	5.5 (0.9)	0.394 (0.120)	0.30 (0.04)
3-Low Dose	0.56 (0.16)	135.3 (18.9)	65.3 (14.6)	3.1 (0.6)	4.6 (1.0)	0.433 (0.101)	0.34 (0.04)
4-Mid Dose	0.56 (0.17)	129.5 (19.8)	56.5 (10.9)	3.0 (0.7)	4.2 (0.8)	0.360 (0.067)	0.28 (0.06)
5-High Dose	0.65 (0.07)	116.7 (9.5)	75.5 (9.1)	3.6 (0.5)	5.6 (0.7)	0.376 (0.110)	0.26 (0.03)
All Animals							
1-Air Control	0.95 (0.14)	125.5 (8.4)	115.1 (15.5)	5.4 (0.8)	7.0 (0.7)	0.276 (0.052)	0.33 (0.04)
2-Placebo	0.87 (0.10)	118.1 (8.0)	96.2 (9.7)	4.7 (0.5)	6.2 (0.5)	0.357 (0.064)	0.32 (0.03)
3-Low Dose	0.87 (0.13)	124.1 (11.3)	98.5 (15.6)	4.8 (0.7)	6.4 (0.9)	0.352 (0.059)	0.43 (0.04)
4-Mid Dose	0.82 (0.12)	125.2 (12.2)	92.2 (15.1)	4.5 (0.7)	6.0 (0.9)	0.306 (0.039)	0.38 (0.06)
5-High Dose	0.88 (0.10)	117.2 (7.1)	103.2 (13.0)	5.1 (0.7)	6.6 (0.6)	0.273 (0.058)	0.37 (0.04)

Note: No significant differences were observed across dose groups at the 0.05 level for these parameters (based on an F-test within a parametric two-way analysis of variance or, when parametric methods were considered not appropriate, on a Kruskal-Wallace nonparametric test).

a. All statistics in this table are based on data for five animals per sex, except that data for four females were available in Group 5 for resistance and compliance.

Toxicokinetics: The aerosol insulin was active in the rat as indicated by significant decreases in mean serum glucose levels on Days 1, 91, and 182 in the toxicokinetic subgroups as shown below. There were dose-dependent increases in serum insulin levels in both male and female treated rats, although the values fluctuated markedly as shown below.

Mean Serum Insulin (ng/mL) Values for Toxicokinetic Subgroups per Post-Exposure Timepoint: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

		Day 1		Day 91		Day 182		Day 1		Day 91		Day 182	
Exposure Group[a]	Target Timepoint	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
		Males						Females					
Satellites for Low Dose	10 min	0.0	0.0	0.1	0.1	0.3	0.4	0.0	0.2	1.5	1.8	0.2	0.2
Satellites for Mid Dose	10 min	0.0	0.1	3.5	3.1	0.2	0.1	0.2	0.1	2.1	3.3	0.0	0.0
Satellites for High Dose	10 min	0.3	0.1	0.6	0.6	1.3	1.1	0.2	0.2	3.3	2.8	0.4	0.3
3-Low Dose	3 min	177.4	75.6	94.1	18.1	112.0	80.3	70.3	47.2	190.9	277.4	136.3	109.6
	10 min	155.4	59.7	82.2	45.5	49.6	47.1	69.8	59.7	106.0	77.0	75.9	69.4
	30 min	111.1	34.5	33.7	27.8	39.3	45.2	110.8	45.6	117.3	13.3	60.3	43.7
4-Mid Dose	3 min	883.4	666.4	364.1	431.5	215.1	190.2	278.2	322.8	177.8	141.6	141.9	88.7
	10 min	391.0	196.7	212.7	36.6	239.6	258.1	206.0	114.4	360.5	317.1	463.2	362.4
	30 min	226.4	119.6	91.5	53.8	108.7	74.5	69.8	43.6	92.2	25.3	121.0	54.3
5-High Dose	3 min	248.3	192.1	183.1	45.4	133.8	46.4	827.3	408.0	352.1	278.3	164.8	95.9
	10 min	734.4	109.6	1140.8	788.8	209.4	94.9	492.0	553.6	596.5	570.8	395.5	256.4
	30 min	418.2	237.0	650.6	668.0	313.4	160.3	351.3	270.3	232.9	138.7	565.0	543.7

a. N = 3 per timepoint.

Clinical chemistry: Isolated instances of significant difference ($P<0.05$) were seen in hematology and clinical pathology parameters in the main core toxicology rats on Days 87 and 183 as shown below. However, insulin dosage effects were not apparent. None of the clinical pathology results were interpreted to indicate insulin or excipient effects.

Group Mean Hematology Values for Core Toxicology Subgroups: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

		Day of Study													
Exposure Group		87	183	87	183	87	183	87	183	87	183	87	183	87	183
		Males													
		Neutrophils (10^3/UL)		Lymphocytes (10^3/UL)		Monocytes (10^3/UL)		Eosinophils (10^3/UL)		Basophils (10^3/UL)		White Blood Cells (10^3/UL)		Red Blood Cells (10^6/UL)	
1-Air Control	Mean	0.96	1.66	8.95	9.40	0.27	0.63	0.12	0.14	0.02	0.02	10.3	11.9	8.20	8.67
	STD	0.26	0.49	2.63	3.08	0.22	0.37	0.05	0.05	0.02	0.03	3.0	3.6	0.52	0.59
2-Placebo	Mean	1.14	2.16	9.80	9.89	0.23	0.36	0.12	0.16	0.01	0.01	11.3	12.6	7.96	8.21
	STD	0.49	1.68	1.71	1.94	0.17	0.30	0.06	0.05	0.01	0.01	1.8	2.7	0.42	0.52
3-Low Dose	Mean	1.14	1.89	9.75	9.53	0.27	0.43	0.10	0.14	0.02	0.01	11.3	12.0	7.84	8.30
	STD	0.29	0.85	2.40	2.16	0.19	0.50	0.06	0.06	0.01	0.01	2.7	2.4	0.53	0.41
4-Mid Dose	Mean	0.89	1.66	10.86	10.04	0.28	0.46	0.15	0.19	0.02	0.01	12.2	12.4	8.21	8.38
	STD	0.29	0.39	2.50	2.13	0.19	0.39	0.05	0.08	0.01	0.01	2.4	1.9	0.36	0.36
5-High Dose	Mean	1.10	2.22	10.24	9.84	0.31	0.55	0.13	0.14	0.01	0.02	11.8	12.8	7.89	8.29
	STD	0.38	1.15	1.85	2.39	0.19	0.38	0.06	0.05	0.01	0.02	1.7	2.3	0.28	0.29
		Hemoglobin (g/dL)		Hematocrit (%)		Mean Corpuscular Volume (FL)		Mean Corpuscular Hemoglobin (PG)		Mean Corpuscular Hemoglobin Concentration (g/dL)		Platelets (K/UL)		Reticulocytes (%)	
1-Air Control	Mean	15.5	15.5	46.8	47.8	57.2	55.2	18.9	17.9	33.1	32.3	1016	1107	0.6	0.6
	STD	0.5	0.7	1.8	2.4	2.0	1.9	0.9	0.7	0.7	0.5	126	86	0.4	0.3
2-Placebo	Mean	15.3	14.9	45.7	45.5	57.4	55.5	19.2	18.1	33.5	32.7	1016	1142	0.7	0.7
	STD	0.9	0.7	2.6	2.2	1.1	1.5	0.4	0.6	0.5	0.4	83	113	0.6	0.3
3-Low Dose	Mean	14.6*	14.7	44.4	45.2*	56.6	54.5	18.7	17.8	33.0	32.6	986	1161	0.7	0.7
	STD	0.9	0.6	3.3	2.2	0.9	0.9	0.6	0.3	0.8	0.4	191	161	0.5	0.3
4-Mid Dose	Mean	15.5	15.1	47.2	46.1	57.5	55.1	18.9	18.1	32.8	32.8	1022	1048	0.4	0.8
	STD	0.7	0.7	1.9	2.1	1.7	2.3	0.7	0.9	0.4	0.5	202	243	0.2	0.4
5-High Dose	Mean	15.1	15.0	45.1	46.0	57.2	55.4	19.1	18.1	33.4	32.7	997	1095	0.7	0.8
	STD	0.7	0.8	1.6	2.3	1.7	1.7	0.9	0.7	0.7	0.5	103	194	0.4	0.3

*Statistically significant differences ($p \leq 0.05$) in group mean compared to the air control group (N=10).

Group Mean Serum Chemistry Values for Core Toxicology Subgroups: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

Exposure Group		Day of Study													
		87	183	87	183	87	183	87	183	87	183	87	183	87	183
Males															
		Alkaline Phosphatase (U/L)		**Aspartate Aminotransferase (U/L)**		**Alanine Aminotransferase (U/L)**		**Total Bilirubin (mg/dL)**		**Total Protein (g/dL)**		**Albumin (g/dL)**		**Glucose (mg/dL)**	
1-Air Control	Mean	164	157	83	126	58	98	0.2	0.2	6.9	6.9	4.7	4.6	135	121
	STD	23	33	18	63	18	63	0.0	0.0	0.5	0.3	0.3	0.2	31	10
2-Placebo	Mean	190	166(N=11)	98	108(N=11)	58	82(N=11)	0.3	0.3	7.0	7.0(N=11)	4.8	4.7	136	116
	STD	52	56	44	44	18	40	0.0	0.1	0.2	0.4	0.1	0.3	29	9
3-Low Dose	Mean	178	168	97	118	58	91	0.3	0.3	6.9	6.9	4.6	4.7	133	120
	STD	46	61	31	57	7	40	0.0	0.0	0.4	0.4	0.2	0.3	25	12
4-Mid Dose	Mean	195	160	88	92	55	63	0.3	0.3	6.8	6.8	4.7	4.6	130	127
	STD	32	37	23	16	8	11	0.1	0.0	0.4	0.2	0.3	0.1	27	12
5-High Dose	Mean	193	177	90	80*	58	61	0.3	0.3	6.7	6.7	4.6	4.5	154	127
	STD	32	38	29	9	12	7	0.1	0.1	0.3	0.3	0.2	0.2	54	13
		Blood Urea Nitrogen (mg/dL)		**Creatinine (mg/dL)**		**Calcium (mg/dL)**		**Phosphorus (mg/dL)**		**Creatine Kinase (U/L)**		**Lactate Dehydrogenase (U/L)**		**Triglycerides (mg/dL)**	
1-Air Control	Mean	19	17	0.60	0.55	11.2	10.7	7.8	6.3	308	230	319	133	146	191
	STD	2	2	0.05	0.05	0.6	0.4	0.6	0.6	226	76	279	63	47	108
2-Placebo	Mean	19	17	0.64	0.55	11.0	10.7	7.6	6.2	284	386(N=11)	429	180(N=11)	181	228(N=11)
	STD	2	1	0.05	0.05	0.4	0.4	0.8	0.7	185	456	340	160	46	103
3-Low Dose	Mean	20	17	0.68*	0.61	11.0	10.7	8.1	6.2	619	186	657	149	153	212
	STD	2	2	0.04	0.03	0.7	0.4	1.0	0.7	632	82	550	112	37	74
4-Mid Dose	Mean	18	18	0.64	0.65*	11.1	10.8	8.0	6.5	408	341	532	256	185	238
	STD	2	2	0.05	0.08	0.8	0.2	0.9	0.7	402	242	539	378	59	49
5-High Dose	Mean	18	16	0.63	0.55	11.0	10.6	8.5	6.3	312	243	405	101	190	228
	STD	1	2	0.05	0.05	0.7	0.3	1.4	0.4	190	118	352	54	51	58
		Cholesterol (mg/dL)		**Sodium (mEq/L)**		**Potassium (mEq/L)**		**Chloride (mEq/L)**		**Globulin (g/dL)**		**A/G Ratio**			
1-Air Control	Mean	85	91	148	146	6.3	6.4	102	105	2.2	2.3	2.12	2.00		
	STD	16	22	2	1	0.3	0.5	3	1	0.2	0.2	0.17	0.12		
2-Placebo	Mean	81	91	149	145(N=11)	6.3	6.3(N=11)	104	105(N=11)	2.2	2.3	2.18	2.07		
	STD	22	28	2	2	0.4	0.4	3	2	0.1	0.2	0.10	0.10		
3-Low Dose	Mean	90	108	148	146	6.5	6.1	105	105	2.2	2.3	2.11	2.11		
	STD	11	11	2	1	0.4	0.3	2	2	0.3	0.3	0.28	0.29		
4-Mid Dose	Mean	79	88	149	147	6.6	6.5	105	107	2.1	2.2	2.23	2.09		
	STD	14	17	3	1	0.3	0.4	3	2	0.1	0.2	0.13	0.22		
5-High Dose	Mean	77	84	149	146	6.2	6.2	105	106	2.1	2.2	2.17	2.11		
	STD	16	19	3	1	0.4	0.5	3	1	0.2	0.2	0.22	0.22		

*Statistically significant differences ($p \leq 0.05$) in group mean compared to the air control group (N=10 except where noted).

Macroscopic pathology finding: There were 7 unscheduled deaths as indicated under Mortality Section. The causes of some deaths are as follows: male#408: prostatic and urinary infection with hydronephrosis; female #459: fractured tibia; male #402: thoracic trauma resulting in lung rupture and hemorrhage; and male #511: urinary tract infection (cystitis). In male rats #411 and 510, there were signs of moderate neuronal degeneration and edema involving portions of the cerebrum and cerebellum, which might be related to prolonged hypoglycemia as a result of pharmacodynamic action of treated substance. There were no gross or microscopic lesions that were noted for female rat #569. But, serum glucose levels for this animal were very low. Some of gross observations in the study were summarized in two tables below.

Summary of Gross Observations: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

Scheduled Sacrificed Animals	Air Ctls	-- Males -- 2	3	4	5	6	Air Ctls	-- Females -- 2	3	4	5	6
Number in group:	16	16	20	17	18	10	16	16	20	19	19	10
Number Necropsied in Core Tox group:	10	10	10	10	10	0	10	10	10	10	10	0
BONE												
SWELLING	0	0	0	0	0	0	0	1	0	0	0	0
CLITORAL GLAND												
NODULE	0	0	0	0	0	0	0	0	1	0	0	0
EPIDIDYMIS												
MASS	1	0	0	0	0	0	0	0	0	0	0	0
EYE												
DISCOLORATION	0	0	0	0	0	0	0	1	0	0	0	0
SMALL	0	0	0	0	0	0	0	1	0	0	0	0
KIDNEY												
FOCUS	0	0	0	0	0	0	0	1	0	0	0	0
CYST	0	0	0	0	0	0	0	1	0	0	0	0
LIVER												
ENLARGED	0	0	0	0	0	0	0	1	0	0	0	0
FOCUS	0	0	0	0	0	0	0	1	0	0	0	0
LUNG												
FOCUS	0	0	0	0	0	0	0	0	1	0	0	0
MAMMARY GLAND												
ENLARGED	0	0	0	0	0	0	0	0	0	1	0	0
PITUITARY GLAND												
ENLARGED	0	0	0	0	0	0	0	1	2	3	2	0
SKIN												
CRUST	0	1	0	0	0	0	0	0	0	0	0	0
ABRASION	0	0	0	0	0	0	0	0	0	1	0	0
UTERUS												
DILATATION	0	0	0	0	0	0	0	2	1	0	0	0
ANIMAL NOTE												
NO LESIONS FOUND AT NECROPSY	9	9	10	10	10	0	10	6	7	6	8	0

Summary of Gross Observations: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

Unscheduled Sacrificed Animals	Air	-- Males --					Air	-- Females --				
	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Number in group:	0	0	0	3	2	0	0	0	0	1	1	0
BONE												
FRACTURE	0	0	0	1	0	0	0	0	0	0	0	0
BROKEN	0	0	0	0	0	0	0	0	0	1	0	0
CAVITY, ABDOMINAL												
FLUID	0	0	0	0	1	0	0	0	0	0	0	0
CAVITY, THORACIC												
FLUID/BLOOD	0	0	0	1	0	0	0	0	0	0	0	0
FLUID	0	0	0	1	0	0	0	0	0	0	0	0
CECUM												
DISCOLORATION	0	0	0	0	1	0	0	0	0	0	0	0
FOCUS	0	0	0	0	1	0	0	0	0	0	0	0
THICK	0	0	0	0	1	0	0	0	0	0	0	0
COLON												
RED CONTENTS	0	0	0	0	1	0	0	0	0	0	0	0
KIDNEY												
DILITATION	0	0	0	1	0	0	0	0	0	0	0	0
DISCOLORATION	0	0	0	1	1	0	0	0	0	0	0	0
LUNG												
DISCOLORATION	0	0	0	2	0	0	0	0	0	0	0	0
RUPTURE/HEMORRHAGE	0	0	0	1	0	0	0	0	0	0	0	0
LN-MEDIASTINAL												
DISCOLORATION	0	0	0	1	0	0	0	0	0	0	0	0
PROSTATE												
ENLARGED	0	0	0	1	1	0	0	0	0	0	0	0
RECTUM												
RED CONTENTS	0	0	0	0	1	0	0	0	0	0	0	0
SEMINAL VESICLE												
DISCOLORATION	0	0	0	0	1	0	0	0	0	0	0	0
URINARY BLADDER												
DILATATION	0	0	0	1	1	0	0	0	0	0	0	0
ANIMAL NOTE												
NO LESIONS FOUND AT NECROPSY	0	0	0	0	1	0	0	0	0	0	1	0

Organ Weights: There were no apparent insulin or excipient-related differences seen in mean organ weight data for core toxicology subgroup animals collected one day after final inhalation exposure. Significant increases in mean absolute lung weights and lung-to-brain weights for females in the mid dose group were not dose related since there was no such effect at the HD group. Similar observation was noted in the reduction of male kidney-to-body weight ratios as summarized in a table below including findings from the females.

Group Mean Organ Weights for Core Toxicology Subgroups: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

Exposure Group		Brain	Heart	Kidney	Liver	Lung	Testes	Adrenal Gland
Males								
Absolute Weights (grams)								
1-Air Control	Mean	2.171	1.664	3.751	20.162	2.120	3.580	0.064
	STD	0.078	0.154	0.425	4.059	0.284	0.230	0.011
2-Placebo	Mean	2.113	1.619	3.442	19.251	2.156	3.343	0.058
	STD	0.115	0.163	0.431	3.975	0.155	0.287	0.008
3-Low Dose	Mean	2.184	1.566	3.373	20.133	2.109	3.428	0.060
	STD	0.097	0.116	0.321	2.943	0.138	0.201	0.008
4-Mid Dose	Mean	2.168	1.718	3.495	20.099	2.213	3.407	0.064
	STD	0.076	0.157	0.323	2.134	0.163	0.269	0.013
5-High Dose	Mean	2.151	1.589	3.515	18.969	2.304	3.444	0.066
	STD	0.154	0.224	0.414	4.396	0.373	0.379	0.014
Organ-to-Body Weight (g/100 g)								
1-Air Control	Mean	0.363	0.277	0.623	3.305	0.351	0.600	0.011
	STD	0.049	0.036	0.078	0.427	0.044	0.103	0.003
2-Placebo	Mean	0.362	0.276	0.584	3.238	0.368	0.571	0.010
	STD	0.045	0.026	0.031	0.337	0.034	0.063	0.001
3-Low Dose	Mean	0.362	0.259	0.558*	3.325	0.349	0.568	0.010
	STD	0.020	0.016	0.038	0.372	0.021	0.037	0.002
4-Mid Dose	Mean	0.349	0.275	0.558*	3.212	0.356	0.548	0.010
	STD	0.038	0.032	0.041	0.319	0.046	0.077	0.002
5-High Dose	Mean	0.369	0.272	0.598	3.183	0.392	0.590	0.011
	STD	0.049	0.042	0.038	0.349	0.054	0.088	0.003
Organ-to-Brain Weight (g/100 g)								
1-Air Control	Mean	100.00	76.62	172.56	927.61	97.51	165.13	2.96
	STD	0.00	6.12	15.27	177.25	11.29	12.48	0.47
2-Placebo	Mean	100.00	76.72	163.01	912.61	102.29	158.33	2.74
	STD	0.00	7.36	18.69	190.11	8.83	12.06	0.40
3-Low Dose	Mean	100.00	71.76	154.46*	920.99	96.70	157.17	2.76
	STD	0.00	5.12	13.14	122.62	6.99	10.38	0.46
4-Mid Dose	Mean	100.00	79.20	161.07	926.88	102.08	157.43	2.93
	STD	0.00	6.54	11.32	91.51	6.59	15.32	0.51
5-High Dose	Mean	100.00	73.77	163.44	880.83	106.92	160.11	3.08
	STD	0.00	7.31	15.39	182.83	13.25	12.90	0.61

*Statistically significant differences ($p \leq 0.05$) in group mean compared to the air control group (N=10).

Group Mean Organ Weights for Core Toxicology Subgroups: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

Exposure Group		Brain	Heart	Kidney	Liver	Lung	Ovary	Adrenal Gland
Females								
Absolute Weights (grams)								
1-Air Control	Mean	1.988	1.053	2.161	11.516	1.642	0.078	0.075
	STD	0.135	0.151	0.317	2.669	0.135	0.016	0.016
2-Placebo	Mean	1.945	1.109	2.391	13.041	1.671	0.087	0.076
	STD	0.068	0.128	0.771	3.297	0.156	0.036	0.014
3-Low Dose	Mean	1.909	1.093	2.094	11.313	1.662	0.080	0.069
	STD	0.052	0.097	0.137	1.451	0.144	0.021	0.009
4-Mid Dose	Mean	1.980	1.154	2.285	12.616	1.931*	0.073	0.077
	STD	0.079	0.190	0.302	1.907	0.326	0.020	0.010
5-High Dose	Mean	1.914	1.149	2.186	12.182	1.727	0.077	0.081
	STD	0.083	0.134	0.193	1.789	0.146	0.020	0.019
Organ-to-Body Weight (g/100 g)								
1-Air Control	Mean	0.632	0.331	0.682	3.599	0.521	0.025	0.024
	STD	0.058	0.016	0.060	0.336	0.046	0.007	0.005
2-Placebo	Mean	0.601	0.340	0.736	3.983	0.513	0.027	0.023
	STD	0.063	0.033	0.236	0.863	0.038	0.011	0.005
3-Low Dose	Mean	0.612	0.349	0.671	3.615	0.531	0.026	0.022
	STD	0.050	0.024	0.072	0.476	0.046	0.008	0.004
4-Mid Dose	Mean	0.588	0.338	0.671	3.702	0.568	0.022	0.023
	STD	0.075	0.037	0.065	0.390	0.086	0.007	0.003
5-High Dose	Mean	0.586	0.351	0.666	3.682	0.528	0.024	0.025
	STD	0.073	0.054	0.066	0.314	0.073	0.008	0.007
Organ-to-Brain Weight (g/100 g)								
1-Air Control	Mean	100.00	52.79	108.37	574.97	82.71	3.93	3.76
	STD	0.00	4.86	10.54	90.07	6.55	0.86	0.72
2-Placebo	Mean	100.00	57.15	123.83	674.55	86.09	4.47	3.93
	STD	0.00	7.60	43.66	187.69	9.45	1.90	0.83
3-Low Dose	Mean	100.00	57.27	109.68	593.27	87.03	4.16	3.62
	STD	0.00	4.87	6.29	80.42	6.66	1.06	0.53
4-Mid Dose	Mean	100.00	58.46	115.80	638.08	97.51*	3.68	3.91
	STD	0.00	10.39	17.84	100.75	15.68	1.01	0.61
5-High Dose	Mean	100.00	60.02	114.29	638.76	90.57	4.02	4.20
	STD	0.00	6.49	9.69	104.60	10.87	1.01	0.96

*Statistically significant differences ($p \leq 0.05$) in group mean compared to the air control group (N=10).

Histopathology: The most common changes in rats from all study group were focal or multifocal chronic-active inflammation in the lungs and aggregations of alveolar histiocytes (foam cells). It appears that the inflammation was random foci of interstitial mononuclear cells or clusters of intra-alveolar polymorphonuclear leukocytes with mononuclear cells. The changes were observed in rats from all dose groups including the air controls. Thus, it appears that the observation was incidental as the result of exposure to forced air, rather than the insulin or excipients. The microscopic findings are summarized in 4 tables below.

Summary of Microscopic Observations: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

Notes: Animals = unscheduled dead for study days 1-184
Air Controls from group(s): 1

Tissues With Diagnoses	Animal sex:	Air Ctls	-- Animals -- Males -- 2	3	4	5	6	Air Ctls	Affected -- Females -- 2	3	4	5	6
	Dosage group:												
	No. in group:	0	0	0	3	2	0	0	0	0	1	1	0
ADRENAL GLD ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
AORTA ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
BONE ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
BRAIN ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
NEURONAL DEGENERATION/EDEMA		0	0	0	1	1	0	0	0	0	0	0	0
CECUM ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
COLON ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
DUODENUM ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
EPIDIDYMIDES ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
ESOPHAGUS ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
FORESTOMACH ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
GLAND, STOMACH ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
HEART ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
ILEUM ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
JENUMUM ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
KIDNEY ..... Number examined:		0	0	0	1	1	0	0	0	0	0	1	0
INFLAMMATION, CHRONIC-ACTIVE		0	0	0	0	0	0	0	0	0	0	1	0
LARYNX ..... Number examined:		0	0	0	3	1	0	0	0	0	1	1	0
LIVER ..... Number examined:		0	0	0	1	1	0	0	0	0	0	1	0
HYPERPLASIA, BILE DUCT		0	0	0	0	0	0	0	0	0	0	1	0

Summary of Microscopic Observations: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

Notes: Animals = unscheduled dead for study days 1-184
Air Controls from group(s): 1

Tissues With Diagnoses	Animal sex:	Air Ctls	-- Animals -- Males -- 2	3	4	5	6	Air Ctls	Affected -- Females -- 2	3	4	5	6
	Dosage group:												
	No. in group:	0	0	0	3	2	0	0	0	0	1	1	0
TESTES ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
THYMUS ..... Number examined:		0	0	0	1	1	0	0	0	0	0	1	0
HEMORRHAGE		0	0	0	1	0	0	0	0	0	0	0	0
THYROID GLD ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
TRACHEA ..... Number examined:		0	0	0	3	1	0	0	0	0	1	1	0
URINARY BLADDER ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
UTERUS ..... Number examined:		0	0	0	0	0	0	0	0	0	0	0	0

Summary of Microscopic Observations: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

Notes: Animals = unscheduled dead for study days 1-184
Air Controls from group(s): 1

Tissues With Diagnoses	Animal sex:	-- Animals Affected --											
		Air	-- Males --					Air	-- Females --				
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
	No. in group:	0	0	0	3	2	0	0	0	0	1	1	0
LUNG ..... Number examined:		0	0	0	3	1	0	0	0	0	1	1	0
INFLAMMATION, CHRONIC-ACTIVE		0	0	0	1	0	0	0	0	0	1	1	0
INFILTRATING CELL, HISTIOCYTE (FOAM CELL), ALVEOLUS		0	0	0	0	0	0	0	0	0	0	0	0
FOREIGN BODY (HAIR), BRONCHUS		0	0	0	0	0	0	0	0	0	0	0	0
HEMORRHAGE		0	0	0	2	1	0	0	0	0	0	0	0
CONGESTION		0	0	0	2	1	0	0	0	0	0	0	0
LN-BRONCHI ..... Number examined:		0	0	0	3	1	0	0	0	0	1	1	0
HEMORRHAGE, SINUSOIDAL		0	0	0	1	0	0	0	0	0	0	0	0
MESENTERIC LN ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
MANDIBULAR LN ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
MAMMARY GLD ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
NOSE/TURBINATES ..... Number examined:		0	0	0	3	1	0	0	0	0	1	1	0
INFLAMMATION, CHRONIC, MAXILLA		0	0	0	0	0	0	0	0	0	0	0	0
OVARIES ..... Number examined:		0	0	0	0	0	0	0	0	0	0	0	0
PANCREAS ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
PARATHYROID GLD ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
PITUITARY GLD ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
PROSTATE GLD ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
RECTUM ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
SALIVARY GLD ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
SCIATIC NERVE ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
SEMINAL VESICLES ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
SKIN ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
SPINAL CORD ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0
SPLEEN ..... Number examined:		0	0	0	1	1	0	0	0	0	0	0	0

Postmortem data for the rats revealed no insulin or excipient-related lesions at scheduled necropsy on Day 183. Histopathologic examination of the respiratory tract and bronchial lymph nodes indicated no clear insulin or excipient-related abnormalities as summarized below.

Summary of Microscopic Observations: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Rats

Notes: Animals = all scheduled sacrificed animal
Air Controls from group(s): 1

Tissues With Diagnoses		Animals Affected -- Males: Air Ctls	2	3	4	5	6	Animals Affected -- Females: Air Ctls	2	3	4	5	6
	Animal sex:												
	Dosage group:												
	No. in group:	16	16	20	17	18	10	16	16	20	19	19	10
	No. Necropsied in Core Tox group:	10	10	10	10	10	0	10	10	10	10	10	0
LARYNX	Number examined:	10	10	10	10	10	0	10	10	10	10	10	0
LUNG	Number examined:	10	10	10	10	10	0	10	10	10	10	10	0
INFLAMMATION, CHRONIC-ACTIVE		5	6	6	8	7	0	2	5	5	7	6	0
INFILTRATING CELL, HISTIOCYTE (FOAM CELL), ALVEOLUS		4	3	0	0	1	0	5	1	1	1	0	0
FOREIGN BODY (HAIR), BRONCHUS		0	0	0	0	1	0	0	0	0	0	0	0
HEMORRHAGE		0	0	0	0	0	0	0	0	1	0	0	0
CONGESTION		0	0	0	0	0	0	0	0	0	0	0	0
LN-BRONCHI	Number examined:	5	10	7	6	7	0	8	8	7	7	8	0
HEMORRHAGE, SINUSOIDAL		0	0	0	0	0	0	0	0	0	0	0	0
NOSE/TURBINATES	Number examined:	10	10	10	10	10	0	10	10	10	10	10	0
INFLAMMATION, CHRONIC, MAXILLA		0	0	0	0	1	0	0	0	0	0	0	0
TRACHEA	Number examined:	10	10	10	10	10	0	10	10	10	10	10	0

Summary and Conclusions:

A total of 204 SD rats were used to characterize the toxicity profile of nose-only inhalation of aerosol insulin for 6-months. The inhaled insulin concentration was 1000 μg/l. The exposure duration was 10, 30, and 60 minutes for low, mid, and high dose groups, respectively. Group mean inhaled insulin dosages of 1, 3, and 5 mg/kg/day were achieved for males and dosages of 1, 3, and 6 mg/kg/day were achieved for females, respectively for the low, mid, and high dose groups. According to toxicokinetic data, there were marked decreases in serum glucose levels in both male and female rats during the 30-minute post exposure period.

There were seven unscheduled deaths during the six-month study period. Two deaths were associated with accidental trauma and two were with pathologic conditions unrelated to insulin treatment. Three remaining deaths were associated in part with possible hypoglycemia-induced by the aerosol insulin. There was no indication of toxicological effects of aerosol insulin except for three animals whose deaths might have been related to hypoglycemia after the aerosol insulin administration.

Mean body weights for male and female rats in the non-exposed satellite group were higher than those for rats in the air control group due to possible repeated blood sampling stress. There were no treatment effects indicated by ophthalmology examinations and pulmonary function assessment after the aerosol treatment.

6-month inhalation toxicity study of aerosol insulin in monkeys

1. Objective: To evaluate the toxicity profile of aerosol insulin administered to male and female monkeys via inhalation for 6-months under GLP condition. The study was conducted at .

2. Methods:
Test article: rh- zinc insulin with sodium citrate, mannitol, glycine and citric acid (Placebo Lot). Insulin Lot number used were and . The particle size ranged from and the flow rate was maintained at one liter per minute.

Animals: A total 36 purpose bred cynomolgus monkeys (Macaca fascicularis) at least 17 months of age were used. Average weights of animals at Day 1 were 2.3 kg and 2.2 kg for males and females, respectively.

Study Design: Four Cynomolgus monkeys/sex/group were administered air or placebo as control or insulin by oral inhalation at dose of 30 μg/l with 18- or 60-minute exposure. The low and high doses were the same, but the exposure duration was different as shown below.

Animal Group	Treatment	Aerosol Conc(μg/L)	Exposure duration(min)
1	Air control	0	60
2	Placebo	0	60
3	Low dose	30	18
4	High dose	30	60

Biological measurements included mortality, body weight (weekly), and clinical observations (twice a day). Ophthalmologic and electrocardiographic evaluations were performed before study start and prior to necropsy. Toxicokinetic evaluations of serum insulin and glucose were performed at 5, 15 and 30 minutes after treatments during the study (Days 1, 91 and 178). Evaluations of hematology, clinical chemistry and urinalysis were performed 3 times during the study (prior to treatment initiation and on Days 87 and 183). Immunogenicity of inhaled insulin was evaluated by indirect ELISA tests for the presence of anti-human insulin antibodies in the serum (prior to treatment and Day 183) and in the lungs of the test subjects during the study on Day 183. All animals were euthanized and submitted for complete necropsy. The lung and upper respiratory structures (nasal turbinates, larynx, trachea, lungs with bronchial tree) were evaluated for histopathologic changes.

RESULTS

Inhaled Dose Estimation: Inhaled doses of insulin or excipients were estimated based on weekly dosimetry measurements. That is, the fraction of insulin that was absorbed from the total insulin estimated from the products of total inhaled volume and average aerosol concentration. Inhaled doses of insulin were corrected for animal body weight, which were summarized below.

Group Mean Insulin and Excipient Exposure Levels (μg/kg/day) in Monkeys@						
Group	Insulin	Mannitol	Glycine	Citric Acid	Sodium Citrate	Satety Margin*
Air	0	0	0	0	0	0
Placebo	0	519	74	0	2255	2.9^
Low Dose	291	265	38	0.9	860	1.5
High Dose	639	582	83	1.9	1888	3.2

@ Values were calculated based on respired volume x concentration x absorption fractions) / Body Weight; *Safety margin of Group Mean Exposure relative to maximum clinical exposure was based on absorption after the maximum clinical exposure in 50 kg subject (0.2 mg/kg/day). ^Except for sodium citrate which was 3.8 times the maximum clinical exposure due to replacement of insulin with sodium citrate in the placebo formulations.

Mortality and Clinical signs: On three separate occasions, animals were observed with symptoms of hypoglycemia: females 451 and 452, and male 403, were lethargic or found unresponsive to activity approximately 2 to 3 hours post exposure. The typical hypoglycemic signs were removed quickly upon either glucose injection or oral administration of fluid with sugar. Minor coughing, sneezing, and wheezing occurred in placebo, low and high dose animals sporadically throughout the entire study. There were no clinical observations that were considered to be a result of exposure to aerosol insulin or placebo.

Body Weights: Across the study there were some time periods of increased rates of weight gain observed in insulin treated animals as shown below. Occasionally these weight gains were significant relative to the air control group, which may be considered to be the result of the test article in young, growing animals (Textbook of Med. Physiol. 9th Ed., Guyton & Hall, W.B. Saunders, Philadelphia, PA 1996).

Hematology: It appeared that none of the hematologic results were interpreted to indicate treatment effects.

Group Mean Absolute Body Weight (kg) Gains Referenced to Day 1: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Cynomolgus Monkeys

Exposure Group		Day of Study								
		8	15	22	29	36	43	50	57	64
Males										
1-Air	Mean	0.11	0.13	0.18	0.19	0.15	0.20	0.23	0.26	0.28
	SD	0.07	0.07	0.08	0.06	0.07	0.07	0.08	0.12	0.13
2-Placebo	Mean	0.03	0.09	0.14	0.15	0.09	0.19	0.20	0.24	0.25
	SD	0.05	0.06	0.08	0.07	0.06	0.10	0.10	0.10	0.07
3-Low Dose	Mean	0.06	0.14	0.22	0.25	0.29	0.30	0.38	0.37	0.46
	SD	0.05	0.07	0.08	0.10	0.10	0.12	0.14	0.12	0.17
4-High Dose	Mean	0.11	0.18	0.23	0.28	0.26	0.29	0.34	0.37	0.44
	SD	0.04	0.07	0.06	0.08	0.08	0.12	0.15	0.12	0.14
Females										
1-Air	Mean	0.05	0.11	0.14	0.17	0.19	0.22	0.27	0.27	0.32
	SD	0.02	0.05	0.05	0.05	0.05	0.06	0.07	0.05	0.07
2-Placebo	Mean	0.06	0.06	0.12	0.15	0.17	0.23	0.25	0.28	0.29
	SD	0.01	0.03	0.01	0.02	0.02	0.03	0.01	0.03	0.01
3-Low Dose	Mean	0.08	0.13	0.18	0.22	0.25	0.31	0.33	0.34	0.36
	SD	0.04	0.06	0.04	0.05	0.06	0.09	0.11	0.09	0.08
4-High Dose	Mean	0.14*	0.20*	0.22*	0.25	0.28	0.36*	0.37	0.39*	0.44
	SD	0.04	0.04	0.05	0.06	0.07	0.04	0.06	0.06	0.08

Exposure Group 1: 60-minute exposure to air
Exposure Group 2: 60-minute exposure to placebo
Exposure Group 3: 18-minute exposure to aerosol insulin
Exposure Group 4: 60-minute exposure to aerosol insulin
* Statistically significant differences ($p \leq 0.05$) in group means compared to the air control group (N=4).

Clinical Chemistry: Toxicokinetic mean serum insulin and glucose sampled on Days 1, 91 and 178 as measured at 5, 15, and 30 minutes after the end of dosing decreased after the test article treatment as shown below. The air and placebo dose group did not have any post-exposure changes in serum glucose concentrations. There were no other clinical chemistry results that were interpreted to indicate specific treatment effects except a few sporadic changes in chloride or potassium concentrations as attached below.

Mean Serum Insulin Values (ng/mL): 6-Month Inhalation Toxicity Study of Aerosol Insulin in Cynomolgus Monkeys

Exposure Group	Target Time (min)	Day of Study 1 Mean	1 SD	91 Mean	91 SD	178 Mean	178 SD
Males							
1-Air	predose	1.5	0.3	2.6	2.2	3.5	1.9
	5	1.0	0.5	0.7	0.1	2.4	0.8
	15	0.8	0.2	1.0	0.4	1.4	0.8
	30	1.0	0.7	2.2	3.1	1.2	0.3
2-Placebo	predose	1.7	1.7	2.9	2.6	4.0	3.1
	5	0.7	0.5	1.2	0.8	2.7	0.9
	15	1.0	0.5	1.4	0.7	1.7	1.0
	30	1.1	0.2	0.9	0.8	1.5	1.7
3-Low Dose	predose	1.5	0.7	2.1	1.4	3.3	1.7
	5	6.8	3.0	6.4	2.9	7.3	1.9
	15	7.1	2.8	7.1	2.0	8.0	1.7
	30	6.6	1.5	5.5	1.0	6.0	0.6
4-High Dose	predose	1.6	0.8	1.2	0.2	3.3	0.5
	5	17.4 (N=3)	3.8	17.7	8.0	18.3	3.6
	15	23.0	5.3	17.7	7.7	19.0	4.8
	30	23.3	6.4	19.3	9.9	21.9	7.6
Females							
1-Air	predose	0.5	0.1	1.0	0.1	1.4	0.3
	5	0.7	0.4	0.9	0.4	1.5	0.8
	15	0.8	0.4	1.4	1.0	1.2	0.7
	30	1.0	0.7	0.9	0.5	1.1	0.5
2-Placebo	predose	1.4	0.7	1.3	0.5	1.9	0.7
	5	0.9	0.6	1.3	0.6	1.6	0.7
	15	1.3	1.0	0.9	0.2	1.9	1.3
	30	1.0	0.2	0.9	0.3	1.7	0.7
3-Low Dose	predose	0.8	0.4	1.8	0.9	4.0	1.3
	5	3.9	1.2	6.4	2.4	4.2	1.0
	15	4.1	0.9	6.5	2.4	4.7	1.5
	30	4.2	1.0	6.0	2.3	4.6	1.5
4-High Dose	predose	0.7	0.3	2.6	1.7	2.0	0.5
	5	8.4	5.5	23.0	26.1	12.3	7.0
	15	9.8	5.2	23.0	26.0	11.6	5.7
	30	10.6	4.7	17.8	17.8	12.6	4.9

Exposure Group 1: 60-minute exposure to air
Exposure Group 2: 60-minute exposure to placebo
Exposure Group 3: 18-minute exposure to aerosol insulin
Exposure Group 4: 60-minute exposure to aerosol insulin
N=4, except where noted.

Mean Serum Glucose Values (mg/dL): 6-Month Inhalation Toxicity Study of Aerosol Insulin in Cynomolgus Monkeys

Exposure Group	Target Time (min)	Day of Study					
		1		91		178	
		Mean	SD	Mean	SD	Mean	SD
Males							
1-Air	predose	74	17	66	7	62	11
	5	72	10	75	18	65	9
	15	76	10	75	13	65	17
	30	76	22	88	15	85	26
2-Placebo	predose	78	12	55	5	64	6
	5	89	17	85	17	80	15
	15	113	35	96	28	89	30
	30	124	30	108	32	98	31
3-Low Dose	predose	85	12	71	5	60	8
	5	53	26	53	11	63	7
	15	42	30	40	10	46	12
	30	37	28	43	9	36	8
4-High Dose	predose	81	10	76	8	80	14
	5	32 (N=3)	3	38	10	38	12
	15	24	5	28	6	30	8
	30	17	5	32	10	29	9
Females							
1-Air	predose	94	23	71	14	65	10
	5	87	10	78	12	71	6
	15	124	38	94	13	86	11
	30	137	59	106	21	90	11
2-Placebo	predose	78	8	61	2	59	9
	5	71	7	74	16	76	13
	15	85	17	78	18	88	22
	30	84	14	84	24	92	21
3-Low Dose	predose	76	23	66	3	57	16
	5	50	26	47	15	56	15
	15	41	24	40	19	45	9
	30	34	16	33	10	40	10
4-High Dose	predose	81	9	86	7	68	5
	5	30	16	37	5	32	13
	15	27	15	37	10	33	15
	30	25	15	34	13	36	11

Exposure Group 1: 60-minute exposure to air
Exposure Group 2: 60-minute exposure to placebo
Exposure Group 3: 18-minute exposure to aerosol insulin
Exposure Group 4: 60-minute exposure to aerosol insulin
N=4, except where noted.

Urinalysis: None of the urinalysis results were interpreted to indicate treatment effects. However, there was a significant decrease in urine pH in the females in aerosol treated groups on Day 87, which might be incidental since there were no such incidences in other time period as shown below.

Group Mean Urinalysis Values: 6-Month Inhalation Study of Aerosol Insulin in Cynomolgus Monkeys

Exposure Group		Day of Study														
		-7/-8	87	183	-7/-8	87	183	-7/-8	87	183	-7/-8	87	183	-7/-8	87	183
		Glucose[1]			Protein[1]			pH[1]			Ketones[1]			Occult Blood[1]		
Males																
1-Air	Mean	0	0	0	0	0	0	7.8	8.3	9.0	0	0	0	0	0	0
	SD	0	0	0	0	0	1	1.0	0.5	0.0	0	0	0	0	0	0
2-Placebo	Mean	0	0	0	0	0	0	7.5	7.5	9.0	0	0	0	0	0	0
	SD	0	0	1	0	0	1	1.3	0.6	0.0	0	0	0	0	1	0
3-Low Dose	Mean	0	0	0	0	0	0	7.3	8.3	9.0	0	0	0	0	0	0
	SD	1	0	0	0	0	1	1.3	1.0	0.0	0	0	0	0	1	0
4-High Dose	Mean	0	0	0	0	0	0	8.5	8.8	9.0	0	0	0	0	1	1
	SD	0	0	1	0	1	0	0.6	0.5	0.0	0	0	0	0	1	1
Females																
1-Air	Mean	0	0	0	0	0	0	8.3	9.0	9.0	0	0	0	2	0	0
	SD	0	0	0	0	0	0	0.5	0.0	0.0	0	0	0	2	0	0
2-Placebo	Mean	0	0	0	0	0	0	8.5	9.0	9.0	0	0	0	0	0	0
	SD	0	0	0	1	0	0	0.6	0.0	0.0	1	0	0	1	1	0
3-Low Dose	Mean	0	0	0	0	0	0	7.5	9.0	8.3*	0	0	0	0	1	0
	SD	0	0	0	0	0	0	0.6	0.0	0.5	0	0	0	0	1	1
4-High Dose	Mean	0	0	0	0	0	0	7.5	8.5	8.3*	0	0	0	0	1	2*
	SD	0	0	0	1	0	1	1.0	0.6	0.5	0	0	0	0	1	2

1. Qualitative determination
Exposure Group 1: 60-minute exposure to air
Exposure Group 2: 60-minute exposure to placebo
Exposure Group 3: 18-minute exposure to aerosol insulin
Exposure Group 4: 60-minute exposure to aerosol insulin
* Statistically significant differences ($p \leq 0.05$) in group means compared to the air control group (N=4).

Ophthalmic Examination: All animals were found to be normal during the ophthalmologic examinations performed on Day 183. Animal 104 had pale pigmentation of retina, which was a pre-existing condition.

ECG Analysis: ECG tracings were assessed by Robert L. Hamlin, D.V.M., Ph.D. All ECGs were within normal limits.

Pulmonary function: Lung resistance measurements indicate the resistance to air flow primarily in the large airways while compliance measurements indicate the elasticity of the lung tissue. There were statistically significant increases in minute volume in the high dose groups of both sexes when the data were compared with those in air control or placebo group. At the same time, there was a decrease in lung compliance in the high dose males, compared to the value in the air control or placebo group, based on Dunnett's test. The sporadic significant differences between dose groups observed both pre-study and at the end of study for tidal volume, peak expiratory flow rate (PEF) and respiratory rate appeared to be due to individual animal differences because these findings were also observed in placebo group as shown below.

Pulmonary Function Data: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Cynomolgus Monkeys

Exposure Group	Mean (Standard Error)[a]						
	Tidal Volume (mL)	Respiratory Rate (breaths/minute)	Minute Volume (mL/minute)	PIF[b] (mL/second)	PEF[c] (mL/second)	Resistance (cm H_2O/mL/sec)	Compliance (mL/cm H_2O)
PRE-STUDY							
Males							
1	19.8 (3.2)	29.6 (5.7)	541.7 (77.9)	29.7 (2.6)	52.6 (2.3)	0.0478 (0.0107)	3.62 (0.53)
2	11.7 (2.1)	52.9 (11.1)	545.5 (42.2)	26.3 (1.4)	40.9 (3.4)	0.0434 (0.0055)	4.00 (0.71)
3	17.3 (1.4)	30.6 (5.1)	516.9 (69.4)	29.1 (3.9)	47.6 (2.8)	0.0337 (0.0035)	4.08 (1.10)
4	12.9 (0.8)	40.4 (5.2)	504.0 (52.8)	25.6 (3.5)	40.5 (1.8)	0.0500 (0.0084)	2.76 (0.54)
Females							
1	14.2 (0.5)	32.2 (2.4)	456.0 (42.8)	26.2 (1.1)	41.3 (1.1)	0.0440 (0.0025)	2.91 (0.67)
2	12.7 (1.4)	43.7 (5.8)	531.1 (45.5)	26.6 (2.1)	40.3 (1.6)	0.0458 (0.0054)	3.56 (0.63)
3	15.4 (1.5)	28.2 (4.3)	409.5 (28.7)	24.2 (2.0)	41.0 (2.7)	0.0553 (0.0051)	3.50 (1.10)
4	12.9 (2.0)	37.3 (3.3)	468.6 (58.0)	23.9 (2.2)	40.2 (3.8)	0.0455 (0.0020)	2.98 (0.83)
All Animals							
1	17.0 (1.8)	30.9 (2.9)	498.9 (44.2)	28.0 (1.5)	47.0 (2.4)	0.0459 (0.0051)	3.26 (0.42)
2	12.2* (1.2)	48.3* (6.0)	538.3 (28.9)	26.4 (1.2)	40.6 (1.7)	0.0446 (0.0036)	3.78 (0.45)
3	16.3 (1.0)	29.4 (3.1)	463.2 (40.3)	26.6 (2.2)	44.3 (2.2)	0.0445 (0.0050)	3.79 (0.73)
4	12.9 (1.0)	38.8 (2.9)	486.3 (36.9)	24.7 (1.9)	40.3* (2.0)	0.0477 (0.0041)	2.87 (0.46)
END OF STUDY							
Males							
1	19.6 (1.6)	24.8 (3.6)	469.6 (50.3)	27.8 (2.0)	55.0 (3.0)	0.0438 (0.0059)	5.08 (1.75)
2	12.7 (1.8)	44.8 (10.9)	509.0 (67.7)	27.9 (3.5)	46.0 (4.6)	0.0281 (0.0085)	3.85 (1.58)
3	17.6 (2.1)	29.3 (5.9)	483.7 (71.1)	28.2 (4.1)	55.0 (3.7)	0.0281 (0.0018)	3.29 (0.55)
4	13.9 (2.1)	45.4 (9.3)	573.9 (67.4)	31.0 (3.3)	55.6 (2.5)	0.0259 (0.0028)	0.96 (1.84)
Females							
1	19.4 (1.9)	24.2 (1.3)	464.7 (58.6)	25.7 (2.2)	52.7 (2.1)	0.0237 (0.0057)	4.45 (1.08)
2	14.4 (1.1)	34.4 (2.9)	474.5 (11.7)	25.7 (0.8)	47.5 (4.2)	0.0203 (0.0032)	3.30 (0.38)
3	18.0 (2.4)	26.8 (4.0)	450.3 (47.6)	25.8 (1.6)	59.4 (8.2)	0.0180 (0.0018)	4.96 (0.72)
4	14.3 (0.7)	41.4 (2.5)	581.5 (47.9)	30.8 (2.8)	55.6 (7.1)	0.0191 (0.0056)	4.21 (0.47)
All Animals							
1	19.5 (1.2)	24.5 (1.8)	467.2 (35.8)	26.8 (1.4)	53.9 (1.8)	0.0352 (0.0056)	4.81 (1.03)
2	13.5* (1.0)	39.6* (5.6)	491.8 (32.4)	26.8 (1.7)	46.8 (2.9)	0.0242 (0.0044)	3.58 (0.76)
3	17.8 (1.5)	28.0 (3.3)	467.0 (40.1)	27.0 (2.1)	57.2 (4.3)	0.0223 (0.0024)	4.24 (0.55)
4	14.1* (1.0)	43.4* (4.5)	577.7 (38.3)	30.9 (2.0)	55.6 (3.5)	0.0225 (0.0032)	2.39 (1.07)

a. All statistics in this table are based on data for four animals per sex
b. Peak Inspiratory Flow rate.
c. Peak Expiratory Flow rate.
Exposure Group 1: 60-minute exposure to air
Exposure Group 2: 60-minute exposure to placebo
Exposure Group 3: 18-minute exposure to aerosol insulin
Exposure Group 4: 60-minute exposure to aerosol insulin
* Significantly different from the air control group at the 0.05 level by Dunnett's test.

Anti-human Insulin Antibody: There were no positive titers in any of the samples, which indicates the absence of an immunologic response to insulin in the lungs.

Gross pathology and Organ weights: Group 3 (LD insulin) females 352 and 353 had small, pale nodules on their spleens at necropsy. No microscopic correlation for the nodules was examined. Female 353 also had pulmonary adhesions involving the right apical and diaphragmatic lobes, which were between the lobes and the pleural wall. The group mean absolute, percent organ-to-body weight ratio and per cent organ-to-brain weight ratio were summarized in a table below. Significant differences in mean weights of thyroid, lung and liver were noted between treated groups from the air control animals.

Group Mean Absolute Organ Weights (grams): 6-Month Inhalation Toxicity Study of Aerosol Insulin in Cynomolgus Monkeys

Exposure Group		Adrenal Gland	Brain	Heart	Kidney	Liver	Lung	Pituitary Gland	Ovary	Testis	Thymus	Thyroid Gland
Males												
1-Air	Mean	0.54	73.88	11.19	14.81	60.20	9.81	0.043	--	1.47	5.41	0.25
	SD	0.10	5.13	1.29	1.24	5.24	0.79	0.014	--	0.42	1.02	0.04
2-Placebo	Mean	0.56	73.51	11.28	13.77	63.15	8.50	0.051	--	1.24	6.29	0.34
	SD	0.03	6.12	0.70	1.63	9.31	0.73	0.007	--	0.13	0.96	0.02
3-Low Dose	Mean	0.66	73.04	14.32	17.76	76.89	11.72	0.059	--	10.83	6.23	0.40*
	SD	0.05	9.17	3.26	2.79	18.01	2.64	0.018	--	18.17	3.11	0.08
4-High Dose	Mean	0.67	71.60	13.25	15.10	73.80	9.81	0.063	--	2.85	7.44	0.41*
	SD	0.12	8.00	1.65	1.22	7.08	1.10	0.023	--	1.45	2.14	0.11
Females												
1-Air	Mean	0.66	67.91	10.19	14.03	63.11	8.68	0.038	0.25	--	4.52	0.36
	SD	0.03	2.19	1.44	2.10	9.63	1.00	0.011	0.09	--	0.53	0.11
2-Placebo	Mean	0.65	71.72	10.77	13.07	68.36	9.59	0.048	0.50	--	4.27	0.39
	SD	0.11	3.68	2.05	1.22	4.29	0.94	0.020	0.24	--	0.86	0.12
3-Low Dose	Mean	0.66	66.34	11.62	14.61	72.97	11.03*	0.036	0.38	--	3.66	0.32
	SD	0.14	4.54	1.88	1.61	6.08	0.74	0.017	0.16	--	0.80	0.09
4-High Dose	Mean	0.77	64.25	10.71	15.03	77.62*	9.70	0.050	0.40	--	5.27	0.37
	SD	0.14	4.66	2.69	2.23	8.12	1.43	0.003	0.13	--	1.87	0.10

Exposure Group 1: 60-minute exposure to air
Exposure Group 2: 60-minute exposure to placebo
Exposure Group 3: 18-minute exposure to aerosol insulin
Exposure Group 4: 60-minute exposure to aerosol insulin
* Statistically significant differences ($p \leq 0.05$) in group means compared to the air control group (N=4).

Microscopic Pathology: The most frequently observed changes consisted of focal to multifocal inflammation and minute aggregations of a few alveolar histiocytes in the lungs, which were observed in all monkeys including air and placebo controls as shown below. Other lesions consisted of pulmonary alveolar hemorrhage in group 2 female #252, and ulceration of the tracheal epithelium in group 3 female #354. Focal, acute inflammation involving the epithelium of the external nares was observed in group 3 male #301, which appears to be incidental in nature because of similar morphology and the presence in monkeys from all groups as shown.

Summary of Microscopic Observations: 6-Month Inhalation Toxicity Study of Aerosol Insulin in Cynomolgus Monkeys

Notes: Animals = all dead animals
Controls from group(s): 1

Tissues With Diagnoses		Animal sex:	-- Males --				-- Females --			
		Dosage group:	Ctls	2	3	4	Ctls	2	3	4
		No. in group:	4	4	4	4	4	4	4	4
LARYNX	Number examined:		4	4	4	4	4	4	4	4
INFLAMMATION, ACUTE			1	3	1	3	0	2	0	1
EROSION, EPITHELIUM			3	3	4	4	2	2	3	2
LUNG	Number examined:		4	4	4	4	4	4	4	4
INFLAMMATION, SUBACUTE			1	3	0	2	2	3	2	1
ADHESION			0	0	0	0	0	0	1	0
INFILTRATING CELL, HISTIOCYTE, ALVEOLUS			4	4	4	3	4	4	4	4
HEMMORRHAGE			0	0	0	0	0	1	0	0
BRONCHIAL LN	Number examined:		4	4	4	4	4	4	4	4
NOSE/TURBINATES	Number examined:		4	4	4	4	4	4	4	4
INFLAMMATION, ACUTE			0	0	1	0	0	0	0	0
TRACHEA	Number examined:		4	4	4	4	4	4	4	4
ULCER, EPITHELIUM			0	0	0	0	0	0	1	0
INFLAMMATION, ACUTE			1	1	1	2	4	3	2	0
EROSION, EPITHELIUM			2	3	3	2	3	3	2	3

Summary and Conclusions:
Thirty-two cynomolgus monkeys were exposed to air, placebo (vehicle) or aerosol insulin by oral inhalation for up to one hour per day for 182 days. The inhaled insulin concentration was 30 μg/l. The exposure duration was 18 minutes for low dose group while it was 60 minutes for high dose group. Group mean insulin doses were 291 and 639 μg/kg/day, respectively, for the low and high dose groups. There was no significant difference between genders.

The main effects of aerosol insulin were the expected elevations in serum insulin as verified by pharmacokinetic data and the concomitant decreases in serum glucose in the low and high dose groups. The magnitude of the high dose exposure was manifested by the few animals that experienced symptoms of profound hypoglycemia, which was dissipated with oral dextrose.

Several animals from placebo and insulin exposure groups experienced respiratory difficulties during inhalation exposure that required premature removal from exposure. It was noted that these incidents occurred primarily during the second and third weeks of exposure without such incidents in air control group. This may suggest that adaptive process was needed to the materials in the placebo and aerosol preparations. At the same time there were sporadic observations of minor coughing and sneezing in the dose groups.
There was no clear evidence of physiologic and toxicological changes in ophthalmologic, electrocardiographic, hematologic, and immunologic parameters that could be attributed to the aerosol insulin or excipients. There were also no gross or histopathologic abnormalities that related to the treatment articles.

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/s/

Fred Alavi
7/27/05 10:58:39 AM
PHARMACOLOGIST
Final review of the Exubera NDA - Pharmtox section
Exubera NDA review

Jeri El Hage
7/27/05 11:07:11 AM
PHARMACOLOGIST

NDA Filing Meeting Checklist

NDA #: 21-868 DRUG: Exubera

Sponsor: Pfizer

NONCLINICAL PHARMACOLOGY/TOXICOLOGY

ITEM	YES	NO	COMMENT
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	X		
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	X		
3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	X		
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? (Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA - e.g., safety pharm, genotox, reprotox, chronic tox, carcinogenicity)	x		

ITEM	YES	NO	COMMENT
5) Were the studies adequately designed (ie., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?	x		
6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (ie., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	X		
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	X		
8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m2 or comparative serum/plasma AUC levels?	X		The sponsor used mg/m2 in labeling section, although they used mg/kg in many experiments for inhalation studies.

ITEM	YES	NO	COMMENT
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	X		
10) Reasons for refusal to file:			

Herman Rhee, Ph.D.
Reviewing Pharmacologist

_____
Supervisory Pharmacologist

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/s/

Herman Rhee
2/16/05 12:23:45 PM
PHARMACOLOGIST

Jeri El Hage
2/16/05 12:43:22 PM
PHARMACOLOGIST