CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21-081

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

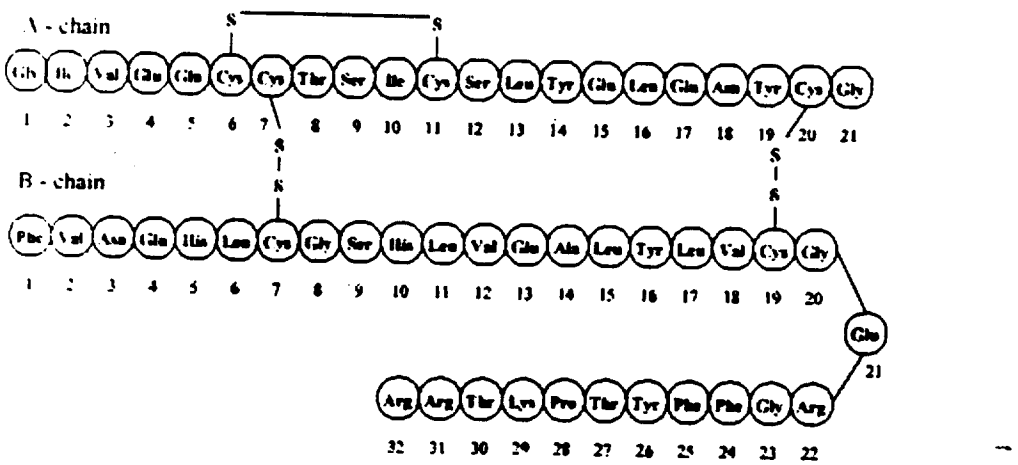
KEY WORDS: Insulin glargine: Lantus™, Diabetic complications, Insulin sensitivity and resistance, Glucose sensitivity and tolerance test, Insulin analogue

Reviewer Name: Herman Rhee, Ph.D., Pharmacology Reviewer
Division Name: Division of Metabolic and Endocrine Drug Products (DMEDP) HFD#510
Review Completion Date: January 19, 2000
Review number: 001

IND/NDA NUMBER: NDA21-081
Serial number/date/type of submission: 000/April 9, 1999/Initial NDA
Information to sponsor: Yes (x) No ( )
Sponsor (or agent): Hoechst Marion Roussel, Inc., Kansas City, MO(Dr. Patton: (816)966-5000)
Manufacturer for drug substance: Hoechst Marion Roussel Deutschland GmbH, Frankfurt, Germany

DRUG: Insulin glargine injection
Code Name: HOE 901
Generic Name: Insulin glargine injection
Trade Name: Lantus™
Chemical Name:21^{A}-Gly-30^{B}a-L-Arg-30^{B}b-L-Arg-human insulin
CAS Registry Number: 160337-95-1
Molecular Formula/ Molecular Weight: $C_{267}H_{404}N_{72}O_{78}S_6$/606.3

Structure: Human insulin was substituted with 2 arginines at positions 31 and 32 of the β-chain of human insulin and replacing the asparagine at position 21 of the α-chain with glycine.



Relevant INDs/NDAs/DMFs: IND —— (HOE901), IND [redacted] and IND [redacted]
Drug Class: Human Insulin Analog
Indication: Antidiabetic agent
Clinical formulation: Clear 5 and 10 ml vials, and 3 ml cartridges for pen injection device. Insulin concentration would be 3.6378 mg/ml with m-cresol, glycerol, zinc, and HCl.
Route of administration: Subcutaneous injection

Proposed clinical protocol or Use: It is hypothesized that insulin glargine will be neutralized in subcutaneous tissue due to its acidity, leading to formation of microprecipitates. Thus, it is expected that it will be continuously released for a prolonged period of time. This may allow once-daily dosing to meet a patient's basal insulin needs. Initial starting dose would be 10 IU per day.

[redacted]

Disclaimer -- use of sponsor's material: Relevant portions of the sponsor's IND submission may be used directly without any modification as noted.

INTRODUCTION AND DRUG HISTORY:

Long-acting insulin formulations have been demanded for long time because available preparations did not provide a constant and reliable 24-hour basal insulin supply. The main difficulties were the facts that duration of drug action was too short with once-daily injection (NPH insulin) or the rate of insulin absorption was not dependable. Daily multiple injections of insulin are certainly inconvenient for many diabetics. Studies also indicate that NPH insulin treatment often results in nocturnal hypoglycemic events due to unwanted plasma insulin peaks during the night. Consequently, the insulin dose cannot be increased as appropriate, and this results in elevated blood glucose levels in the morning.

In an effort to provide acceptable long-acting insulin preparation, the sponsor investigated insulin glargine (Lantus) in this NDA submission. Insulin glargine (HOE 901, 21 A -Gly-30 B a-L-Arg-30 B b-L-Arg-human insulin) is an analogue of human insulin produced by recombinant DNA technology. The structural modifications result in a shift of the isoelectric point reducing solubility at physiological pH [redacted] Insulin glargine has a delayed and prolonged absorption from the injection site following subcutaneous administration. Thus, it appears that insulin glargine might be released slowly for considerably long period of time.

Studies reviewed within this submission:
Study Title: HOE901-General pharmacological studies
Study Title: HOE901-Pharmacokinetic studies
Study Title: HOE901-Toxicological studies:
Study Title: HOE901-Single-dose subcutaneous toxicity study in mice
Study Title: HOE901-Single-dose subcutaneous toxicity study in rats(92.0383)
Study Title: HOE901-Single-dose subcutaneous toxicity study in rats(95.0427)

Study Title: HOE901-Single-dose subcutaneous toxicity study in dog
Study Title: HOE901-One month subcutaneous toxicity study in rats
Study Title: HOE901-3-Month subcutaneous toxicity study in rats
Study Title: HOE901-6 Month toxicity subcutaneous toxicity study in rats
Study Title: HOE901-Three month subcutaneous toxicity study in Beagle dogs
Study Title: HOE901-Six month subcutaneous toxicity study in Beagle dogs
Study Title: HOE901-2-Year Carcinogenicity Study in Mice
Study Title: HOE901-Carcinogenicity Study in Rats
Study Title: HOE901-Immunotoxicology
Study Title: HOE901-Reproductive pharmacology studies
Study Title: HOE901-Embryotoxicity and teratologic studies
Study Title: HOE901-Genetic toxicity studies

Studies not reviewed within this submission:
Report# 92.0456/Study#92.0374—Single dose toxicity study in rats, which was reviewed on 12/11/199 in original IND.

PHARMACOLOGY:

Mechanism of Action: The mechanism of blood glucose lowering action of Insulin glargine injection(HOE 901) is similar to human insulin since it is a human insulin analogue. However, it has been demonstrated that insulin glargine has a depot effect after subcutaneous injection in many animal models such as rats, rabbits and dogs. The mechanism of the prolonged action has to do with its ability to be neutralized in subcutaneous tissue due to its acidity, leading to formation of microprecipitates.

Drug Activity Related to Proposed Indication:
In an euglycemic clamp study in dogs with determination of plasma drug levels (PK/PD study), HOE 901 and human regular insulin were infused in acidic (pH 4.3) or neutral saline containing 0.5% dog serum, respectively, at a constant rate of 1.8 mU/min/kg for 3 hours. The criteria of an euglycemic clamp were met by both compounds. In the steady-state phase, HOE 901 and human insulin required similar glucose infusion rates of about 10 mg/kg. The effect of HOE 901 started slightly later and persisted somewhat longer. In the steady-state phase, the plasma concentration of HOE 901 was 1.8 times higher than that of human insulin, for a similar effect. After the end of infusion, the plasma concentrations of HOE 901 and human insulin declined monoexponentially, human insulin decreasing significantly faster. It is concluded that higher plasma drug concentrations of HOE 901 would be required for equivalent blood glucose lowering activity when compared with the human insulin after an i.v. infusion.

In a similar euglycemic clamp study in dogs by i.v. infusion at a rate of 1.2 mU/min/kg for 3 hours, HOE 901 and human insulin also exhibited similar total hypoglycemic activity as demonstrated by glucose consumption during the entire study and in the steady-state phase. Again, the glucose infusion rates revealed a delayed onset of the effect of HOE 901 in the first 90 minutes and a similarly slightly delayed disappearance of the hypoglycemic effect at the end of the study.

Ancillary Pharmacology Studies:

To examine divergent behavior in vitro, HOE 901 and human insulin were compared in intact human hepatoma cells (Hep G2) and primary cultures from human skeletal muscle. The pH dependency of binding to isolated human insulin receptors was also examined. The association and dissociation kinetics were studied in rat fibroblasts over-expressing the human insulin receptor.

Binding studies on isolated receptors from human placenta showed that HOE 901 had an approximate 3-fold reduced affinity relative to human insulin at pH 8.0 and an about 2-fold reduced affinity at pH 7.0. The relative binding affinity of HOE 901, human insulin and some analogues were also assessed in the human hepatoma Hep G2 cell line. Differences between binding affinities of human insulin and all tested analogues to the insulin receptor were found in these cells. HOE 901 had a 40% reduction in binding affinity versus human insulin.

As a means of assessment of HOE901 potential for mitogenicity, comparative studies for HOE901 and human insulin were performed to quantitate relative IGF-1 binding and ^{3}H-thymidine incorporation into protein. The human osteosarcoma derived cell line B10 expressed at least 30 times more IGF-1 binding sites than insulin receptors. Binding affinities of human and porcine insulin were quite similar, bovine insulin had an about 2.5- to 3-fold reduced affinity. HOE 901 and Asp(B10) insulin exhibited a 3.5 to 7.6 times higher affinity for the IGF-1 receptor than human insulin. In different studies with the same cells HOE 901 displayed a higher affinity (IC_{50} =0.5 mM) for the IGF-1 receptor than human insulin (IC_{50} =7 mM), whereas Gly(A21) insulin and Gly(A21), des(B30) insulin had slightly lower affinities.

H9C2 cardiomyoblasts with no insulin receptors detectable were also used as a suitable model to study IGF-1 receptor binding. HOE 901 showed a 1.4-fold higher steady-state affinity for the IGF-1 receptor relative to human insulin (IC_{50} of 70 and 101 nM, respectively), whereas Asp(B10) insulin had a significantly higher IGF-1 receptor binding (IC_{50} : 44nM). In cultured human skeletal muscle cells, HOE 901 also had only slightly higher IGF-1 receptor affinity in the steady state than human insulin. However, this difference was seen only at very high, nonphysiological concentrations. The following table shows a comparison of insulin analogues on IGF-1 receptor binding.

IC_{50} Values(nM) of IGF-1 Receptor Binding in Several Different Cellular Assay Systems

Test Substances	Osteosarcoma Cells		Skeletal Muscle Cells		Cardiomyo-Blasts
	Expt. 2	Expt. 3	Nondiabetic	NIDDM	
Human Insulin	1100	6600	Not calculable		101
HOE901	140	470	133	431	70
Asp(B10) Insulin	140				140
Gly(A21) Insulin		7100			
Gly(A21)des(B30)*		8500			
IGF-1	0.67	0.20	0.25	0.34	–

*Indicate Gly(A21)des(B30) Insulin which is HOE901 metabolite II.

The stimulation of cell growth by insulin or insulin analogues results in an increased rate of DNA synthesis. Therefore, the incorporation of thymidine into DNA during a specified time period can be used as a direct measure for the proliferative activity. In B-10 osteosarcoma cells, the comparison of concentrations needed for 50% of the maximum stimulation (ED_{50}) was summarized below.

Test Substance	ED_{50} in Nanomolar		Relative Mitogenicity (%)	
	Exp.1(n=1)	Exp.2(n=3)	Exp.1(n=1)	Exp.2(n=3)
Human insulin	14	3.0 ± 2.1	100	100
Porcine insulin	14		100	
Bovine insulin	53		26	
HOE901	4.8	0.61 ± 0.17	291	492
Arg(^{B}31)Arg(^{B}32)	1.8		777	
Asp(^{B}10)		0.78 ± 0.44		384
IGF-1		0.13 ± 0.06		2308

Summary of Pharmacology:

The anticipated prolonged effect of HOE 901 was studied after subcutaneous injection at a dose of 0.3 IU/kg in fasted rabbits or dogs. A drug concentration of 100 IU/mL human insulin was used along with comparator(human NPH insulin). In rabbits, no significant difference between the HOE 901 preparations with a zinc concentration of — or — µg/mL was noted. The two HOE 901 preparations were also tested in dogs to evaluate the depot effect of the HOE 901 preparations. HOE 901 had a significant zinc-dependent delayed onset and prolonged duration of action. NPH insulin decreased blood glucose to its nadir at 3 hours after administration.

In a further study in dogs, the depot effect of drug and zinc concentration after subcutaneous injection was investigated at 6 drug concentrations in the range from 5 to 200 IU/mL (HOE 901 and human NPH insulin) and at 3 different zinc concentrations — 30, and — µg/mL). HOE 901 had a markedly extended depot effect compared with NPH insulin. The depot effect of HOE 901 (by onset and duration of hypoglycemia), was drug-concentration dependent and zinc-concentration dependent. Without zinc and at low drug substance concentration, the onset of effect of HOE 901 was similar to that of NPH insulin but the duration of hypoglycemia was about 2.5 hours longer. The extended depot effect of HOE 901 relative to human NPH insulin was obtained with similar variability of pharmacological profiles.

Studies in several in vitro cell lines such as human hepatoma HepG2 cell or human osteosarcoma cell lines indicated that HOE901 had 4 to 8 times higher affinity to IGF-1 receptors than human insulin products. HOE901 exhibited a 3 to 5 times higher mitogenic activity relative to human insulin in the thymidine incorporation assay in osteosarcoma cells. It is not clear that the mitogenic effect of the insulins in osteosarcoma cells is strictly mediated through the IGF-1 receptor. In a study with another insulin analogue (AspB10) an increase in induction of mammary tumor in a 1-year rat study was attributed to the dissociation rate from insulin receptor rather than

the relative increased affinity for the IGF-1 receptor. Moreover, the significance and relevancy of the in vitro findings to human clinical application is yet to explore.

SAFETY PHARMACOLOGY:

Neurological effects:

In mice, a sedation was observed after subcutaneous treatment with HOE 901 at a dose of 3, 10, 30 IU/kg, which was also noted with human NPH insulin at a dose of 10 IU/kg body weight. In all doses the animals showed a sedation starting at 20 to 25 minutes after dosing. The motility was reduced in all groups. All treated animals showed an increased food intake. One out of 6 animals died 2.5 hours after dosing in the groups receiving 30 IU/kg HOE 901 or 10 IU/kg human insulin. Potential influences of HOE 901 on ethanol- or pentobarbital-induced sleeping time were investigated in 2 mice after subcutaneous pretreatment with HOE 901 at a dose of 0.3, 1, or 3 IU/kg or human NPH insulin at a dose of 3 IU/kg body weight. HOE 901 did not potentiate the effect of barbiturate.

Cardiovascular effects:
Positive inotropic and chronotropic effect of insulin at supraphysiological doses is well known, which might be related to an increase in plasma norepinephrine levels and believed to be at least partly independent of hypoglycemia. In anesthetized rats, HOE 901 at a subcutaneous dose of 3, 10, 30 IU/kg body weight or human NPH insulin at a dose of 10 IU/kg body weight had no biologically significant influence on the blood pressure and heart rate, except minor decreases in the diastolic blood pressure at 30 and 60 minutes at all doses of HOE 901.

In anesthetized dogs, HOE 901 had no effects on cardiovascular parameters after i.v. injection at a dose of 0.3, 1, and 3 IU/kg body weight, which were not different from human NPH insulin tested at a dose of 3 IU/kg. HOE 901 caused a slight and short-lasting decrease in the systolic, diastolic, mean arterial and left ventricular blood pressures while dp/dt_{max}, heart rate, and cardiac output were increased over the test period.

Pulmonary effects:
Whole body plethysmometry study in anesthetized guinea pigs indicated that HOE 901 decreased pulmonary resistance by 30% from 20 to 60 minutes at doses of 0.3, 1, and 3 IU/kg body weight. The test article had no effects on other respiratory parameters in the study. The doses of 1 and 3 IU/kg body weight had no treatment-related consistent, remarkable adverse effects in respiratory system.

Renal effects: In rats, HOE 901 caused a minor increase in urine volume, sodium and chloride excretion, ratio Na^+/K^+ and urine osmolality at doses of 1.0, 3.0, or 10 IU/kg. These effects resolved by 6 hours after the drug treatment.

Conclusions: HOE901 did not produce clear treatment-related effects on neurological, cardiovascular, urinary, or respiratory system in mice, rats and dogs at doses at least 5 to 10 times of clinically relevant dose, based on body surface comparison. Data suggest

that HOE901 appears to have little systemic effects except hypoglycemia as an extension of its pharmacodynamic action.

Summary: -.

HOE 901 had no remarkable neurological, renal, and cardiovascular effects in mice and rats at 10 IU/kg or in anesthetized rats and dogs at 3 IU/kg. The doses are approximately 5 to 10 times higher than the proposed clinical dose, based on body surface comparison.

PHARMACOKINETICS/TOXICOKINETICS:

PK parameters:

Absorption and Bioavailability:

In human, HOE 901 was absorbed from the injection site significantly more slowly than NPH insulin when injected subcutaneously. There were no clinically relevant differences in absorption rates of radiolabeled HOE 901 when injected subcutaneously in the arm, leg or abdomen. Absorption rates of radiolabeled HOE 901 injected subcutaneously were similar in healthy subjects and subjects with type 2 diabetes.

Distribution:

Following s.c. injection of [^{125} I]HOE 901, high levels of radioactivity were detected at the site of injection at all time points up to 24 hours postdosing. The continuous supply of radioactivity as a result of this depot effect also explains the higher plasma concentrations observed after 24 hours as compared to those after intravenous dosing. Radioactivity levels were first detected in the plasma of animals at 1 hour, and even more markedly at 4 hours after s.c. dosing. Ubiquitous distribution with exclusion of the CNS was observed following both routes of i.v. and s.c. injections. The organs with the highest radioactivity levels were thyroid, stomach content, kidneys, skin, urinary bladder content and small intestine content. The extend of drug distribution after s.c. injections corresponds well to that observed after i.v. injection.

Metabolism:

HOE 901, the intermediate compound 21 A -Gly-30 B a-L-Arg-insulin, and the active metabolites 21 A -Gly-insulin (M1) and 21 A -Gly-des-30 B -Thr-insulin (M2) were recovered in human plasma samples taken during the first 24 hours after injection, but concentrations of each compound were near the lower limit of detection.

HOE 901 and a mixture of M1 and M2 were recovered from injection-site tissue in similar proportions, averaging about 50% each over the first 24 hours after injection, suggesting that HOE 901 is partially metabolized in the subcutaneous depot.

HOE 901 was degraded to a major extent by formation of M1 and M2 . The degradation of the molecule proceeds via cleavage of the arginines at the carboxy terminus of the B chain. The various studies on degradation indicated that metabolites M1 and M2 were formed presumably followed by endogenous catabolism. Investigations on the in vitro metabolism were performed using radiolabeled HOE 901. During incubation with rat adipocytes, the final degradation products formed consisted of ——— and a variety of peaks which are related to ——— intermediates. No interim degradation products like M1 or M2 have been found in the in vitro experiments.

Elimination:
Following i.v. injection to rats and dogs, HOE 901 is eliminated from the circulation with initial half-lives of 0.21 and 0.18 hours and terminal half-lives of 1.2 and 0.8 hours in rats and dogs, respectively. Following s.c. injection to rats and dogs, maximum concentrations are reached 2 or 4 hours postdosing, respectively. Elimination from plasma takes place with half-lives of 4.3 or 5.4 hours in rats and dogs, respectively. Limited information is available on the excretion pattern of HOE 901. In 2 studies after s.c. injection of HOE 901 to male rats and male dogs, urine was collected within the first day postdosing. In rats, no immunoreactivity was found in the eluates. In dogs, drug related immunoreactivity excreted in urine within 24 hours post dosing was estimated to be less than 1% of the dose administered. The remainder is probably either excreted as nonimmunoreactive compounds or recycled into the endogenous pool of amino acids.

Other studies:
No specific studies were conducted with HOE 901 to evaluate 1) repeated-dose absorption and pharmacokinetics because the toxicokinetics are considered conclusive, 2) protein binding because of a low order of magnitude for human insulin, 3) enzyme induction/inhibition (not relevant for human insulin), or 4) pharmacokinetic interactions because no such interactions known for human insulin.
Comments: There were no qualitative differences between species in the metabolism of HOE901, although there were some quantitative differences in PK data. In rats, HOE901 absorption was slower than that of human insulin after s.c. administration. The terminal half-life of HOE901 was 1.2 h in rats, while it was 0.8 h in human after an intravenous administration.

Summary: It appears that HOE901 was absorbed slowly both in rats and dogs after s.c. injection since the maximum concentrations were reached 2 to 4 hours postdosing. Plasma elimination half-lives were 4 to 5 hours in the two species. The highest level of drug was detected at the injection site up to 24 hours post-dosing, which demonstrated the slow distribution and absorption of the drug. Other tissues such as thyroid, stomach content, kidneys, skin, urinary bladder content and small intestine content also had a large amount of radioactive drug. There were two major metabolites: M1(21 A-Gly-30 B a-L-Arg -insulin), and M2 (21 A -Gly-des-30 B -Thr-insulin) in human as well as in animals.

TOXICOLOGY:

General Comments:
Acute toxicity of HOE 901 has been studied in mice and dogs using the subcutaneous route and in rats using both the subcutaneous and intravenous routes. Acute toxicity was comparable for mice and rats after subcutaneous and intravenous injections. The LD_{50} values were in the range of 1000 IU or higher in both species. Clinical signs in affected animals were attributed to excessive hypoglycemia and included abdominal position, irregular respiration and respiratory sounds, lacrimation, tremor and unsteady or ataxic gait.

Postmortem examinations of the animals, which died, and of those killed at the end of the observation period revealed no target organ toxicity in either species. The dog proved to be more sensitive than mice and rats. Dogs injected with a dose of 0.364 mg

HOE 901 per kg body weight or higher (corresponding to a dose of approximately 10 IU per kg body weight) died within 24 hours after administration. The postmortem examinations revealed changes known to occur after an excessive hypoglycemia.

Single-dose subcutaneous toxicity studies(mice:Document NO:013092; rat:Document No. 01668) were reviewed on original IND on 12/11/1995.

Study Title: HOE901-Single-dose subcutaneous toxicity study in mice
Study No: 95.0428/Doc. No. 015159/ Report#96.0024
Amendment #, Vol #, and page #: Vol.21 and p088-109
Conducting laboratory and location: Hoechst AG Pharma Development Corp., Frankfurt
Date of study initiation: Nov. 1, 1995
GLP compliance: Yes
QA- Report Yes (x) No ()

METHODS:
Dosing: 36.38 mg/kg
species/strain: Mouse/HsdWin:NMRI
#/sex/group or time point: 2 mice/sex/group
age: 5-6 week old
weight: 25-28 grams
satellite groups used for toxicokinetics or recovery: None
dosage groups in administered units: One
route, form, volume, and infusion rate: Subcutaneous, liquid, 0.25 ml /25 gram mice
Drug, lot#, radiolabel, and % purity: E003, as reported in QE0873/92-ho on 1/8/1995.
Formulation/vehicle: Glycerol(20 mg), m-Cresol (2.7 mg), —— HCl(pH=4.0), distilled water to make 1 ml)
OBSERVATIONS AND TIMES:
Clinical signs: were recorded during the first day continuously, from Day 2 on twice every day, on weekends and holidays only once.

RESULTS:
Clinical signs: Irregular respiration and respiratory sounds were noted.
Body weights: The parameter was decreased in a few of the surviving animals during the first week, but subsequently returned to normal.
Gross pathology: Macroscopic examination on dissection of the animals which died and of those killed at the end of the 2-week follow-up period revealed no morphological changes.
Conclusion: LD_{50} of HOE901 should be near 36.38 mg/kg in mice.

Study Title: HOE901-Single-dose subcutaneous toxicity study in rat
Study No: 92.0383/Doc. No. 011667/ Report#92.0455
Amendment #, Vol #, and page #: Vol.21 and p130-140
Conducting laboratory and location: Hoechst AG Pharma Development Corp., Frankfurt
Date of study initiation: May 19, 1992
GLP compliance: Yes
QA- Report Yes (x) No ()

METHODS:
Dosing: 1000 I.U./kg
species/strain: Rat/HOE:WISKf(SPF71)
#sex/group or time point: 2 rats/sex/group
age: 5-6 week old
weight: 100-104 grams
satellite groups used for toxicokinetics or recovery: None
dosage groups in administered units: 1
route, form, volume, and infusion rate: Subcutaneous, liquid, 10 ml /kg

Drug, lot#, radiolabel, and % purity: E003, as reported in QE0873/92-ho on 1/8/1995.
Formulation/vehicle: Glycerol(20 mg), m-Cresol (2.7 mg), — HCl(pH=4.0), distilled water to make 1 ml)
OBSERVATIONS AND TIMES:
Clinical signs: were recorded during the first day continuously, from Day 2 on twice every day, on weekends and holidays only once.

RESULTS:
Clinical signs: All rats tolerated well without clear toxicities. There were no deaths.
Body weights: Body weight development was normal.
Gross pathology: There were no remarkable organ findings at the end of the follow-up period.
Conclusion: 1000 I.U.(approximately 40 mg)/kg was not lethal in rats under the experimental conditions.

Study Title: HOE901-Single-dose subcutaneous toxicity study in rats
Study No: 95.0427/Doc. No. 015157/ Report#95.0763
Amendment #, Vol #, and page #: Vol.21 and p142-164.
Conducting laboratory and location: Hoechst AG Pharma Development Corp., Frankfurt
Date of study initiation: Oct. 25, 1995
GLP compliance: Yes
QA- Report Yes (x) No ()

Dosing: 36.38 mg/kg
species/strain: Wistar rat/ HOE:Wiskkf(spf71)
#sex/group or time point: 2 rats/sex/group
age: 5-6 week old
weight: Approx. 100 grams
satellite groups used for toxicokinetics or recovery: None
dosage groups in administered units: One
route, form, volume, and infusion rate: Subcutaneous, liquid, 0.25 ml /25 gram mice
Drug, lot#, radiolabel, and % purity: E003, as reported in QE0873/92-ho on 1/8/1995.
Formulation/vehicle: Glycerol(20 mg), m-Cresol (2.7 mg), —— HCl(pH=4.0), distilled water to make 1 ml)

RESULTS: The maximum tolerable subcutaneous dose was at least 36.38 mg/kg in rats.
Clinical signs: Unsteady gait and there was no mortality.
Body weights: Product treatment had no effect.

Gross pathology: The animals killed at the end of the observation period showed no abnormal macroscopical changes.

Study Title: HOE901-Single-dose subcutaneous toxicity study in dog
Study No: 97011/Doc. No. 017464/ Report#96.0024
Amendment #, Vol #, and page #: Vol.21 and p196-255
Conducting laboratory and location: Hoechst Preclinical Development Laboratories, Japan
Date of study initiation: May 13, 1997
GLP compliance: Yes
QA- Report Yes (x) No ()

Dosing: 0.364 mg/kg(0.1 ml/kg) and 0.728 mg/kg (0.2 ml/kg)
species/strain: Beagle
#/sex/group or time point: 2 male dogs/group
age: 7-8 months old
weight: 11-11.7 kg
satellite groups used for toxicokinetics or recovery: None
dosage groups in administered units: 2
route, form, volume, and infusion rate: Subcutaneous, liquid, 0.25 ml /25 gram
Drug, lot#, radiolabel, and % purity: Batch 29 as specified in QE013/97-we
Formulation/vehicle: Glycerol(20 mg), m-Cresol (2.7 mg), — HCl(pH=4.0), distilled water to make 1 ml)

OBSERVATIONS AND TIMES:
Clinical signs: Observed at least once from pre-dose Day 7 to the day following admin.
Body weights: Determined on Pre-dose day 4 and on the day of admin.
Hematology: Determined in all animals twice before admin. and the day of admin.
Clinical chemistry: Blood was drawn from all animals twice (Pre-dose Day 7 and the days of admin).
Gross pathology: Necropsy was performed in all dead animals.

RESULTS:
Clinical signs: All dogs treated with the two doses were found dead the next morning after administration.
Body weights: This parameter was not determined.
Hematology: This parameter was not determined.
Clinical chemistry: This parameter was not determined except blood glucose, which was reduced to 50% of the pre-dose levels at 3 hours after the administration.
Gross pathology: In 2 dogs of the high dose and 1 dog of the low dose group, retention of the light yellowish to red hydrothorax and red spots scattered on the jejunum mucosa were observed. In one dog of the two groups, light reddish to red pericardial effusion was accumulated. It is not clear whether they were related to the test-article treatment.

Histopathology: All animals of the two groups had mild autolysis of the heart and the lung. One dog of the high dose group demonstrated mild, but localized cell infiltration of the liver. There were no control animal data since the sponsor did not use control or placebo group in this single-dose study.

Study Title: HOE901-One month subcutaneous toxicity study in rats
Study No: 93.0317/Doc. No. 012525/ Report#93.0968
Amendment #, Vol #, and page #: Vol.33 and p001-278
Conducting laboratory and location: Hoechst AG Pharma Development Corp., Frankfurt
Date of study initiation: April 27, 1993
GLP compliance: Yes
QA- Report Yes (x) No ()

species/strain: Rat/HOE:Wiskkf(Spf71)
#/sex/group or time point: 12 rats/sex/group
age: 8-9 weeks old
weight: Male(212 g) and female (207 g)
satellite groups used for toxicokinetics or recovery: None
dosage groups in administered units: Control(saline), HOE901(100 IU on Day 1 and 50 IU/kg afterward), HOE 31H(100 IU/kg) and HOE 36H(40 IU on day 1 and 20 IU/kg afterward)
route, form, volume, and infusion rate: Subcutaneous, liquid
Drug, lot#, radiolabel, and % purity: A002, as reported in QE0600/93-we.
Formulation/vehicle: Glycerol(— mg), m-Cresol (2.7 mg), —————— — and distilled water to make 1 ml)
OBSERVATIONS AND TIMES:
Clinical signs: Checked daily for mortality
Body weights: Checked weekly
Food consumption: Measured weekly
Clinical chemistry: Blood glucose was determined on days 22(females) and 23(males).
Gross pathology: All rats were checked at necropsy for potential abnormality.
Organ weights: Absolute and relative organ weights were determined.
Histopathology: 15 organs were examined.

RESULTS:
Clinical signs: One rat of the HOE901 and HOE 36H groups died after the first application. One male and one female of the HOE901 group were found dead on days 7 and 29, respectively. Behavior and general health condition of the other animals remained unaffected by the treatment.
Body weights: This parameter was not affected the treatment in any groups.
Food consumption: This parameter was not affected the treatment in any groups.

Effects of HOE901 on Blood Glucose Levels in 28-Days Studies in Rats*					
Group	Control	HOE901	I 924885	HOE 31H	HOE 36H
Dose	Saline	50 I.U./kg	100 I.U./kg	100 I.U./kg	40 I.U./kg
Male	5.97	2.74@	2.59@	2.37@	3.72@
Female	5.71	2.65@	2.48@	2.00@	2.31@

* The unit is in mM/L and @indicates P<0.05, compared to control.

Organ Weights: There were sporadic changes in the absolute and relative body weights. But it was not evident that the changes were the test article-related effects.
Gross pathology: Macroscopic changes were detected at necropsy, but they appeared not directly related to the treatment.

Histopathology: There was reduced beta-cell granulation in the islets of Langerhans in both sexes of the HOE901 groups and in males of the I 924885-treated group. There were also nonspecific treatment-related changes in the form of round-cell infiltration and tissue granulation was noted at injection sites in all groups. The changes are insignificant since the control group had the same changes.

Study Title: HOE901-3-Month subcutaneous toxicity study in rats
Study No: 94.0466/Doc. No. 013690/ Report#95.0186
Amendment #, Vol. #, and page #: Vol.34-36 and p004-917
Conducting laboratory and location: Hoechst AG Farm Development Corp., Frankfurt
Date of study initiation: Oct. 2, 1994
GLP compliance: Yes
QA- Report Yes (x) No ()

Dosing: 36.38 mg/kg
species/strain: Rat/Hoe:Wiskf(Spf71)
#/sex/group or time point: 10 rats/sex/group
age: 6 week old
weight: 25-28 grams
satellite groups used for toxicokinetics or recovery: 5 rats/sex/group for recovery study
dosage groups in administered units: Control(placebo), 0.1455, 0.4547, 1.455 mg/kg HOE901 and 0.4547 mg/kg of human insulin (HOE901 NF).
route, form, volume, and infusion rate: Subcutaneous, liquid
Drug, lot#, radiolabel, and % purity: D001/D001NF
Formulation/vehicle: Glycerol (— mg), m-Cresol (2.7 mg), and — mg — adjusted to pH 4 using — HCl and NaOH to make 1 ml.

OBSERVATIONS AND TIMES:
Clinical signs: General health of the animals was checked daily and neurological disturbances and eye opacity were examined weekly.
Body weights: This parameter was checked weekly throughout the study.
Food consumption: This parameter was checked weekly throughout the study.
Hematology: This parameter was checked at 6 weeks (interim value), the termination of the study (final value), and after the recovery period (recovery value). Blood was taken from a sublingual vein.
Clinical chemistry: This parameter was checked at the termination and after the recovery period.

Urinalysis: This parameter was checked at 5 weeks and 12 weeks. Due to animal deaths, scheduled urine analysis was not performed in females.
Gross pathology: After exsanguination, all rats were necropsied and checked for visible abnormalities.
Organ weights: The parameter was determined at necropsy and organ to body weight ratio was calculated.
Histopathology: 44 tissues were preserved in appropriate fixative for histopathological studies.
Other: Insulin antibodies were determined in blood samples that were taken at 1, 2, 4, 6, and 24 hours after 83rd/85th administration of test articles.

RESULTS:

Clinical signs: Eleven males and 8 females from the high dose group and 3 rats in HOE901 NF died during the study. No signs of compound-related neurological disturbances, or damage to the oral mucosa were noted in the control or treated groups.

Body weights: Body weight development was not remarkably affected by the administration of the test articles, although there were increases in the parameter in males of all groups on day 1, in males of the high dose group on day 16 and in males of the intermediate dose group on day 29.

Food consumption: The absolute and relative food consumption remained unaffected by the treatment throughout the study.

Ophthalmoscopy: There was no change in opacity of the refracting media of the eyes.

Electrocardiography: Not remarkable

Hematology: Interim findings: RBC counts were decreased (6%) to in males of the HOE901 NF group. In addition, there was a decrease in coagulation time (14%) in the animals of the high dose group and in the animals treated with HOE901 NF.

Terminal findings: Significant decreases in RBC counts and hematocrit values in both sexes of the HOE901 NF group were noted. Leukocyte counts were increased in females of the high dose group, of which finding may not be compound-related effect.

Recovery findings: There were no consistent changes in all parameters, which are related to treatment.

Clinical chemistry: Final finding: Sodium and chloride levels were decreased in females of the high dose group (<5%).

Recovery finding: There was decrease in uric acid in males of the low dose group, which appears to be dose-dependent, although it might not be statistically significant as shown below. However, the decrease may not be meaningful since the urine volume was increased due to free access to water.

Effects of HOE901 on Uric acid Levels in 3-month Toxicological Studies in Rats*					
Group	Placebo	Low Dose	Mid Dose	High Dose	HOE901NF
Dose	0 mg/kg	0.1455 mg/kg	0.4547 mg/kg	1.455 mg/kg	0.4547 mg/kg
Males	127	85	81	72	72
Females	87	128	86	81	84

*The unit was in µmol/L.

Urinalysis: Urine volume was increased in both sexes after approx. 5 weeks of the study as shown below.

Effects of HOE901 on Urine Volume in 3-month Toxicological Studies in Rats*					
Group	Placebo	Low Dose	Mid Dose	High Dose	HOE901NF
Dose	0 mg/kg	0.1455 mg/kg	0.4547 mg/kg	1.455 mg/kg	0.4547 mg/kg
Males	6.4	4.8	3.7	9.2	5.7
Females	5.0	4.5	4.9	7.8	5.7

*The unit is in mL.

Organ Weights: In general, there were no remarkable changes in the parameter throughout the study. Final finding: Increases in absolute and relative kidney weights occurred in females of the high dose group without clear signs of impaired organ

function. Recovery findings: relative adrenal weights were increased in female rats, which was not test article-dose dependent.
Gross pathology: No compound-related macroscopically visible changes were found at necropsy.

Histopathology: All rats found dead due to hypoglycemia except one death resulting from Bacillus piliformis infection. In four animals of the high dose group and one animal treated with HOE901 NF, cortical infarction of the brain might be a morphological sign of prolonged hypoglycemia. In the pancreas of the dead animals, degranulation of the β-cells in the islets of Langerhans was noted as the pharmacological action of the test article. There were no compound-related changes in rats of the low dose group. Lesions at the injection site consisted of cutaneous muscle necrosis, subcutaneous foreign body granuloma, and subcutaneous inflammatory cell reaction. These changes occurred in HOE901-treated animals as well as in control rats, which suggests that the lesions might not be compound-related.

Study Title: HOE901-6 Month toxicity subcutaneous toxicity study in rats
Study No: 95.0343/Doc. No. 014964/ Report#97.0434
Amendment #, Vol #, and page #: Vol.37-V39 and p003-863
Conducting laboratory and location: Hoechst AG Pharma Development Corp., Frankfurt
Date of study initiation: Aug. 30, 1995
GLP compliance: Yes
QA- Report Yes (x) No ()

Species/strain: Wistar rat/Hoe:Wiskf(SPF71)
#/sex/group or time point: 20 rats/sex/group
age: 6 weeks old
weight: 113 g(male) and 106 g(female)
satellite groups used for toxicokinetics or recovery: None
dosage groups in administered units: Placebo control, 0.073, 0.23 and 0.73 mg/kg
route, form, volume, and infusion rate: Subcutaneous, liquid
Drug, lot#, radiolabel, and % purity: E003, as reported in QE0112/96.
Formulation/vehicle: Glycerol(— mg), m-Cresol (2.7 mg), ——————, and — NaOH (pH=4.0) to make 1 ml with distilled water.

OBSERVATIONS AND TIMES:
Clinical signs: This parameter was checked daily.
Body weights: This parameter was checked weekly.
Food consumption: This parameter was checked weekly.
Ophthalmoscopy: This parameter was checked weekly.
Hematology: Blood samples were taken from a sublingual vein of non-fasted rats, which were collected at 1 and 3 months, at the end of treatment, and after 4 weeks recovery. Ten rats/sex/group were used.
Clinical chemistry: This parameter was checked at the end of treatment and after 4 weeks recovery by conventional methods. Ten rats/sex/group were used.
Urinalysis: The urine was collected overnight after withdrawal of food and water during the period. This parameter was checked at 1 and 3 months, and at the end of treatment. Ten rats/sex/group were used.

Gross pathology: Animals which died were dissected immediately as they were found.
Organ weights: The weights of 11 organs were determined at necropsy for absolute weights and relative organ weights were calculated based on 100 g body weight.
Histopathology: Organs or parts of thereof were submitted to microscopic examination in the case of the animals which died intercurrently.
Toxicokinetics: Blood was obtained from 2 rats/sex/group at 1, 2, 3, 4, or 7 hours after the 170th (males) or 169th (females) treatments. Blood samples from the rats that were sacrificed on and after 180th or 181st application were used as 24-hour values of toxicokinetic analysis.

RESULTS:
Clinical signs: Intercurrent deaths were 6 male and 3 female rats of the high dose group died between study days 149 and 182, which were considered to be caused by an exaggerated pharmacodynamic effect of the test product. In the rats, decreased spontaneous activity and / or prone position were noted approximately 4 weeks before deaths except several rats, which had no such symptoms. There were no other specific clinical signs considered to be treatment related.
Body weights: There was significant decrease in body weights for a few measurement points in the low dose group. The slight (under 5%) reduction appears incidental in males. However, in the females, there was a slight increase in the parameter, which appears to be test article dose dependent.
Food consumption: Mean absolute and relative food consumption was regular and not affected by the administration of the test article in both sexes.
Ophthalmoscopy: Weekly inspection of the eyes did not reveal any macroscopically visible compound-related changes.

Hematology: No compound-induced pathological changes were observed. The parameters were not affected by the test article treatment except several parameters as indicated below. In males, blood coagulation time and reticulocytes were reduced drug dose dependent manner. In female, hematocrit as well as RBC counts were decreased significantly in the high dose group.

Effects of HOE901 on Hematological Parameters in 6-month Toxicologic Studies in Rats				
Animal Group	Placebo	Low Dose	Mid Dose	High Dose
HOE 901 Dose	0 mg/kg	0.073 mg/kg	0.23 mg/kg	0.73 mg/kg
Male/Reticulocytes(Unit)	0.009	0.006*	0.005*	0.003*
Male/Coagulation Time(sec)	131	121*	116*	115*
Female/Hematocrit(Unit)	0.45	0.44	0.43	0.42*

*P<0.05.

Clinical chemistry: The parameters were not changed by compound treatment except a slight but dose depedent decrease of urea in males as shown below. The implication of treatment-related reduction of urea is not known. Serum glucose levels were not significantly different between the control and the treated groups when the parameters were analyzed after animal deaths. A statistically significant decrease of uric acid in mid dose males after the recovery period is considered to be incidental.

Effects of HOE901 on Clinical Chemistry in 6-month Toxicologic Studies in Rats				
Animal Group	Placebo	Low Dose	Mid Dose	High Dose
HOE 901 Dose	0 mg/kg	0.073 mg/kg	0.23 mg/kg	0.73 mg/kg
Male/Urea(mmol/L)	7.26	7.08	6.46*	6.23*

*P<0.05.

Urinalysis: No obvious treatment-related findings on the parameter were observed at the different examination time points.
Organ Weights: Organ weights were investigated at the necropsy as well as during recovery. Neither the absolute nor the relative organ weights was affected by the treatment in any study groups.
Gross pathology: No test article-related gross findings were seen in the intercurrently died or in other rats at terminal sacrifice.

Histopathology: Six of the 9 intercurrently died rats exhibited ganglion cell necrosis within the hippocampus and/or cerebrocortical laminated spongiform vacuolation with ganglion cell necrosis of varying degrees. All treated animals revealed dose dependent pancreatic β-cells degranulation which was reversed in most groups after the recovery period.
Toxicokinetics: A dose dependent increase of HOE901 serum levels was observed but slightly under proportionally in females, which was confirmed by AUC. Cmax after low dose was reached 1 hour after administration, while that of high dose group came 1-2 hours after treatment. Serum concentrations decreased to control levels at 2 hours, 3-4 hours and 7 hours after treatment in low, mid, and high dose groups, respectively.

Study Title: HOE901-12-Month subcutaneous toxicity study in rats (This study is a pilot study, which the sponsor used for dose range finding for carcinogenicity study).
Study No: 93.0622/Doc. No. 012937/ Report#95.0518
Amendment #, Vol #, and page #: Vol.40 and p001-350
Conducting laboratory and location: Hoechst AG Pharma Development Corp., Frankfurt
Date of study initiation: May 10, 1993
GLP compliance: Yes
QA- Report Yes (x) No ()

species/strain: Sprague-Dawley rats/CD(Sprague-Dawley)
#/sex/group or time point: 30 rats/sex/group
age: 4-5 weeks old
weight: Male(138 g), female(128 g)
satellite groups used for toxicokinetics or recovery: None
dosage groups in administered units: Control(placebo) and HOE901 1.455 mg/kg, which was reduced to 0.728 mg/kg at 112th injection.
route, form, volume, and infusion rate: Subcutaneous, liquid

Drug, lot#, radiolabel, and % purity: T3885, as reported on 7/71993 and repeated analysis on 1/5/1994.
Formulation/vehicle: HOE901 (1.455 mg), glycerol(– mg), m-Cresol (2.7 mg), —— HCl(pH=4.0), distilled water to make 1 ml
OBSERVATIONS AND TIMES:

Clinical signs: Checked twice a day for moribund and intercurrently dying animals.
Body weights: This parameter was checked weekly.
Ophthalmoscopy: This parameter was checked monthly.
Gross pathology: At necropsy, abnormality was checked by macroscopic examinations.
Organ weights: This parameter was checked at terminal. Organs and parts of organs were removed and preserved in fixing fluid for histopathological examinations.

RESULTS:
Clinical signs: Scabbed and open wounds on neck and forelimb were observed in control and treated groups in similar incidence. Mortality: The table below shows the statistics of animals died or had to be killed intercurrently.

Time Dependent Effects of HOE901 on Moratlity in One Year Toxicity Study in Rats*				
Study duration (Week)	Control(placebo)		HOE901 1.455 mg/kg	
	Male	Female	Male	Female
1 – 16	0	0	6	8
17 – 26@	0	0	1	0
27 – 40	0	0	4	2
41 – 53	4	0	9	4
Total Death	4	0	20	14
*Each group had 30 rats at the beginning and @Dose reduced to 0.728 mg/kg				

Body weights: There was statistically significant increase in body weight from the 2 weeks after the administration of HOE901. In males, the difference to the control animals was about 14% and in females about 18%, respectively.
Ophthalmoscopy: There were no remarkable changes in the eye examinations after the treatment.
Electrocardiography: Not reported.
Hematology: Not reported.
Clinical chemistry: Not reported.
Urinalysis: Not available

Organ Weights: Evaluation of the relative organ weights revealed no significant differences between control and HOE901 treated male rats. In females the relative weights of lungs, adrenals, brain and pituitary gland were lower than the corresponding control values. In females, weights of the heart and liver were increased by 13% and 24%, respectively, from the control.
Gross pathology: Palpation of skin for nodules is summarized in a table below.

HOE 901 Effects on Nodule Formation in Some Individual Rats with Histological Finding					
Rat #	Group	Sex	Kind of Nodules	First Noted (week)	Histology
47	Control	Female	Left Inguinal side	53	Carcinoma
89	HOE901	Male	Inguinal gland	44	Inflammation
106	HOE901	Female	Right side	38	Adenoma
117	HOE901	Female	Inguinal gland	48	Carcinoma

Histopathology: The fatalities in the dosed group were caused by compound-related hypoglycemia, which lead to cardiovascular failure. In the animals, β-cell degranulation

was noted in the Langerhans' islets of pancreases. Clear compound-related toxicity was not observed. Neoplastic and non-neoplastic findings were noted in both control and treated animals.

KEY STUDY FINDINGS:
In one year study in rats, a dose of HOE901 1.455 mg/kg killed 6 males and 8 females in the first 16 weeks. The dose was approximately 36 I.U., which was reduced to 0.728 mg/kg from the Week of 17. Therefore, the sponsor used HOE901 0.455 mg/kg as the top dose in 2–year carcinogenicity studies. The present study has limited value since no data or reports on hematology, clinical chemistry, toxicokinetics, or urinalysis were available.

Study Title: HOE901-Three month subcutaneous toxicity study in Beagle dogs
Study No: 94.0280/Doc. No. 0123559/ Report#94.0954
Amendment #, Vol #, and page #: Vol.52 and p001-376
Conducting laboratory and location: Hoechst AG Pharma Development Corp., Frankfurt
Date of study initiation: May 24, 1994
GLP compliance: Yes
QA- Report Yes (x) No ()

species/strain: Beagle dog, Hoe/Beak
#/sex/group or time point: 4 dogs/sex/group
age: 8 months old
weight: Male(11.4 kg) and female (10.2 kg)
satellite groups used for toxicokinetics or recovery: None
dosage groups in administered units: Control(saline), HOE901(0.036 mg/kg and 0.182 mg/kg)
route, form, volume, and infusion rate: Subcutaneous, liquid
Drug, lot#, radiolabel, and % purity: L00173/003
Formulation/vehicle: Glycerol(— mg), m-Cresol (2.7 mg), ———— HCl (pH=4), and distilled water to make 1 ml

OBSERVATIONS AND TIMES:
Clinical signs: Checked daily for mortality
Body weights: Checked weekly
Food consumption: Measured daily
Ophthalmoscopy: 8th and 14th weeks
EKG: 8th and 14th weeks
Hematology: 8th and 14th weeks
Clinical chemistry: Blood glucose was determined on days 22(females) and 23(males).
Urinalysis: 7th and 13th weeks
Gross pathology: All dogs were checked at necropsy for potential abnormality.
Organ weights: Absolute and relative organ weights were determined.
Histopathology: Organs or parts of them were examined.

RESULTS:
Clinical signs: All dogs survived until the scheduled end of the study. Test compound related impairments were not observed.

Body weights: This parameter was not affected by the treatment in all groups. The individual development in the body weight curves of all dogs treated with HOE901 was basically identical to that of the control curves.
Food consumption: This parameter was not affected by test article treatment in all groups. All dogs consumed their feed usually completely.
Ophthalmoscopy: No compound-related changes were seen.
Electrocardiography: There were no compound-induced changes in the P-R interval, QRS complex, QT interval, heart rate and functional picture.
Hematology: None of the measured parameters showed compound-related changes except a slight(3%) increase in hemoglobin and hematocrit in males of the high dose group.

Clinical chemistry: Serum glucose levels were reduced remarkably as expected in treated groups in 1,2 and 3 hours after administration. The drug effect was returned toward normal approximately 20 hours after the treatment. Alkaline phosphatase was increased(11%) in the males of the high dose group without dose dependency. There were sporadic increases or decreases of sodium, iron, chloride, bilirubin, creatinine, cholesterol, total lipid, and creatine kinase, which were still within the physiological limits.
Urinalysis: No compound-related changes were detected.
Organ Weights:There were sporadic changes in the absolute and relative body weights. But it was not evident that the changes were the test article-related effects.
Gross pathology: Macroscopic changes were detected at necropsy, but they appeared not directly related to the treatment.
Histopathology: There was reduced beta-cell granulation in the islets of Langerhans in both sexes of the HOE901 groups. There were also nonspecific treatment-related changes in the form of round-cell infiltration and granulation tissue were noted at injection sites in all groups. The changes are insignificant since the control group had the same changes.

Study Title: HOE901-Six month subcutaneous toxicity study in Beagle dogs
Study No:95.0342/Doc. No. 014959/ Report#93.0834
Amendment #, Vol #, and page #: Vol.53 and 54 and p001-430
Conducting laboratory and location: Hoechst AG Pharma Development Corp., Frankfurt
Date of study initiation: Oct. 23, 1995
GLP compliance: Yes
QA- Report Yes (x) No ()

species/strain: Beagle dog/hoe:BEAK
#/sex/group or time point: 4 dogs/sex/group
age: 15 months old
weight: Male(14.1 kg) and female (12.4 kg)
satellite groups used for toxicokinetics or recovery: None
dosage groups in administered units: Control(vehicle), HOE901(0.036 mg/kg, and 0.109 mg/kg)
route, form, volume, and infusion rate: Subcutaneous, liquid
Drug, lot#, radiolabel, and % purity: L00173/005/Batch E003, as reported in QE0112/96
Formulation/vehicle: Glycerol(— mg), m-Cresol (2.7 mg), NaOH(pH=4), and distilled water to make 1 ml

OBSERVATIONS AND TIMES:
Clinical signs: Checked daily for mortality, general health, and behavior
Body weights: Checked weekly
Food consumption: Measured daily
Ophthalmoscopy: Before the onset of study, 45th, 95th, 189th Days and at terminal
EKG: Before the onset of study, 45th, 94th, 189th Days and at terminal. HR, PR interval, QRS, QT interval, and functional picture were evaluated.
Hematology: Before the onset of study, 44th, 93th, 188th Days and at terminal
Clinical chemistry: Before the onset of study, 44th, 93th, 188th Days and at terminal
Urinalysis: Before the onset of study, 44th, 93th, 188th Days and at terminal using the urine collected overnight after withdrawal of food and water

Gross pathology: All dogs were checked at necropsy for potential abnormality after sacrifice using T61{200 mg N-[2-(m-metnoxy-phenyl-2-ethylbutyl-(1) γ-hydroxyl-butylamide; 50 mg 4.4'-methylene-bis-(cyclohexil-trimethyl-ammonium-iodide) and 5 mg 4'-butylaminobenzoyl-2-dimethylamino-ethanol-hydrochloride) for tissue fixation and staining.
Organ weights: Absolute and relative organ weights were determined at autopsy in all animals.
Histopathology: Organs or parts of organs were removed for microscopic examination at autopsy. Formaldehyde for all tissue and Schaffer's solution for bone and sternum; Bouin solution for pancreas.
Toxicokinetics: 2.5 ml Blood was obtained at Days 1, 8, 43, 97, 184 for drug analysis using RIA at

RESULTS:
Clinical signs: All dogs from the high dose group(0.109 mg/kg/day) had to killed prematurely on study days 13, 20, 47, 69, 74 and 79 because of poor clinical condition. The dogs of the control and low dose groups survived until the scheduled end of the study. The dogs in the high dose group showed severe signs of hypoglycemia with tonic-clonic convulsions including coma.
Body weights: This parameter was not affected by the treatment in all groups without exception throughout the study duration.
Food consumption: This parameter was not affected by the treatment in all groups, although the animals were offered 300 g food immediately after drug treatment to prevent potential hypoglycemia.
Ophthalmoscopy: This parameter was not affected by the treatment.
Electrocardiography: There were no compound-induced changes in the PR interval, QRS complex, QT interval, heart rate, and functional parameters.
Hematology: In all dogs of the low dose group, no changes were noted by the treatment. The high dose reduced hemoglobin(146 to 130 g/L) in males. Prothrombin time was increased from 6.64 sec to 10.64 sec in the high dose male. The parameter was slightly reduced (10.68 to 8.91 sec) in the high dose group females.

Clinical chemistry: There were sporadic changes in several parameters in the final analysis, which were not consistent from the values pretest and intercurrent assays. Thus the significance is negligible.
Urinalysis: No compound-related abnormalities were detected.

Organ Weights: There were sporadic changes in the absolute and relative body weights. But it was not evident that the changes were the test article-related effects.
Gross pathology: At the final autopsy one female dog of the low dose group had a bleeding in the pylorus area. Three prefinal killed dogs had focal red changes in the pyrolic area with an edema in the pancreas. There were no other remarkable gross abnormality.
Histopathology: Degranulation of the beta-cells in all treated groups was observed. However, grading of the degranulation was not performed because of individual fluctuation of the staining. In the treated two animals, there were necrotic changes in auricular cordis and bleeding in and around the wall. The changes are known as spontaneously developed alterations in dogs; possibly due to the poor clinical conditions. Similar changes were also noted in one control dog, which might be incidental.

Toxicokinetics:

Summary of Pharmacokinetic Parameters in 6-Month Dog Toxicology Study				
Study Day	Dose(mg/kg)	Tmax(Hr)	Cmax(mg/ml)	$AUC_{0\text{-}22h}$(ng/ml.hr)
8	Placebo	3.0	5.5	39.8
	0.036	3.3	8.1	64.7
	0.109	4.0	14.0	111.2
43	Placebo	3.3	6.0	43.9
	0.036	3.0	8.7	77.2
	0.109	3.7	12.4	132.4
97*	Placebo	2.8	8.4	49.6
	0.036	2.5	12.2	66.1
184*	Placebo	3.0	5.1	31.6
	0.036	3.0	5.0	44.8

*There were no data in the high dose groups due to premature animal sacrifice.

OVERALL TOXICOLOGY SUMMARY:
The sponsor provided toxicological data on HOE901 based on acute single or multidose studies in mice, rats, and dogs including standard carcinogenicity, reproductive and genetic toxicology studies. Single and multiple toxicity studies in mice, rats, and dogs using the subcutaneous route showed that the main effect was hypoglycemia as an extension of its pharmacodynamic effect at the high drug doses. Clinical signs in affected animals were attributed to excessive hypoglycemia and included abdominal position, irregular respiration and respiratory sounds, lacrimation, tremor and unsteady or ataxic gait. The dog proved to be more sensitive than mice and rats because dogs injected with a dose of 0.364 mg HOE 901 per kg body weight or higher (corresponding to a dose of approximately 10 IU per kg body weight) died within 24 hours after administration.

The postmortem examinations revealed changes known to occur after excessive hypoglycemia. Effects of HOE901 on cardiovascular system, hematology, and gross pathology indicate that there were no consistent drug-related toxicities in these animals. Animals which died and of those killed at the end of the observation period revealed no clear organ toxicity and histopathological changes except those attributable

to hypoglycemia. HOE901 toxicology was similar to that of human insulin, HOE36H, which was used as a comparator. Toxicological profile of HOE901 indicates that the potential adverse effects of this drug would be likely small when properly used, although potential inflammations at the injection site should be monitored as indicated in Labeling Section.

Addendum 1:Histopathology Inventory for IND #

Study	R#95.0186	R#97.0434	R#96.0834	R#95.0518
Species	Rat	Rat	Dog	Rat
Adrenals		X	X	x
Aorta			X	X
Bone Marrow smear			X	X
Bone (femur)			X	X
Brain	X	X	X	x
Cecum			X	X
Cervix				
Colon			X	X
Duodenum				X
Epididymis			X	X
Esophagus				X
Eye			X	X
Fallopian tube				
Gall bladder			X	
Gross lesions				X
Harderian gland				
Heart	X	X	X	x
Hyphophysis				
Ileum			X	X
Injection site			X	X
Jejunum			X	X
Kidneys	X	X	X	X
Lachrymal gland				
Larynx				
Liver	X	X	X	X
Lungs	X	X	X	X
Lymph nodes, cervical				X
Lymph nodes mandibular				
Lymph nodes, mesenteric				
Mammary Gland				
Nasal cavity				X
Optic nerves				X
Ovaries	X	X	X	X
Pancreas			X	X
Parathyroid				X
Peripheral nerve				X
Pharynx				
Pituitary	X	X	X	X

Prostate				X
Rectum			X	X
Salivary gland			X	X
Sciatic nerve				X
Seminal vesicles				X
Skeletal muscle				X
Skin				X
Spinal cord			X	X
Spleen	X	X	X	
Sternum				X
Stomach				X
Testes	X	X	X	X
Thymus			X	X
Thyroid	X	X	X	
Tongue			X	X
Trachea			X	X
Urinary bladder			X	X
Uterus			X	X
Vagina			X	X
Zymbal gland				

- organ weight obtained

CARCINOGENICITY

Study Title: 2-Year Carcinogenicity Study in Mice
Study Number/Doc#: 98.0211/014470
Volume Number/Page: 23-32/p003– p316
Test Facility: Hoechst Marion Roussel Deutschland GmbH, Frankfurt, Germany
Study Date(s): March 14, 1995-March 17, 1997

Date of Submission: 3/31/1998
GLP Compliance: Yes
QA Report- Yes (x) No ()
Study Type:
Species/strain: Mice/NMRI
Number of animals per group; age at start of study: 50/sex/group; 4-5 weeks old mice

Animal housing: Individually in transparent macrolon cage on soft wood granulate in an air-conditioned room
Drug Lot/Batch number(s): D001 and E003, 02D092, 01D098, and N110/01
Drug Purity / Stability / Homogeneity: The sponsor provided relevant data on Batch#D001 and E003 (QE0510/94-we/QE0112/96-we). However, there were no quality data provided on other batches as reported(Vol. 32, pages291-298).

DOSES: 0.073, 0.182, 0.455 mg/kg of test article (HOE901) in clinical formulation. The sponsor divided animals into 6 groups-1, 2, 3, 4, 5, and 6. Groups 1 and 2 were saline control and placebo. Groups 3, 4, 5 and 6 were 0.073, 0.182, 0.455 mg/kg of test article (HOE901), and HOE36H(0.45 mg/kg), respectively. HOE36H is regular human insulin. Control solution was 0.9% saline. Vehicle contains [redacted] mg of 85% glycerol, 2.7 mg m-cresol, and distilled water to make 1 ml with pH=4.0. In addition to that HOE36H had [redacted] mg phenol and [redacted] mg NaH_2PO_4 at pH=7.3

Basis of Dose Selection: In 3-month dose range finding studies with HOE 901 (Batch T3885), 10 NMRI mice/sex/group had 5, 10, or 20 I.U./kg. Two male mice of the low dose group and 3 females of the high dose group died intercurrently, which might be due to drug-induced hypoglycemia. There were no drug-induced consistent changes in body weight or food consumption in either sex. However, histological examination revealed a reduced granulation of β-cells in the Langerhans islets of pancreas in the intermediate and top dose groups. Based on these data, 10 I.U./kg was considered the MTD. The top dose is approx. 6 times of higher than the maximal therapeutic dose in human, based on body weight comparison. One I.U. is equivalent to approximately 0.04 mg insulin.

Relation to Clinical Use: This human insulin analog might have a long-acting property so that once daily subcutaneous injection provides a basal supply of insulin.
CAC Concurrence: No
Restriction Paradigm for Dietary Restriction Studies: NA
Route of Administration: Subcutaneous administration
Frequency of Drug Administration: Daily

Dual Controls Employed: Yes, saline and placebo controls
Interim Sacrifices: Yes
Satellite PK or Special Study Group(s): 15 male and 15 female mice served as satellite animals for toxicokinetic examinations.
Unscheduled Sacrifices or Deaths: Unscheduled animal death was processed for evaluation when the animals were moribund or dead.
Deviations from Original Study Protocol: Not noted.

STUDY RESULTS AND FREQUENCY OF MONITORING:

Clinical Observations: At the injection sites, there were scabs, swollen knotlike changes and open wounds with thrombosis. The per cent of such changes appeared to be higher in the placebo and treated groups, compared to the control as shown below. There were no clear treatment-related neurological changes in treated groups. HOE36H group is a reference insulin.

Effects of HOE901 on Incidences of Skin Abnormality at Injection Site*						
Sex	Control	Placebo	0.073	0.182	0.455	HOE36H
M	16	96	96	96	86	36
F	58	80	92	88	72	46

*Indicate % incidence in each group.

Mortality: Each group had 50 animals at Day O. The numbers of animals killed intercurrently and at terminal are shown in the first table below. The second table illustrates the numbers of mice surviving at indicated day. There were more deaths in the control group than the treated groups in females as shown in the tables and subsequent figure.

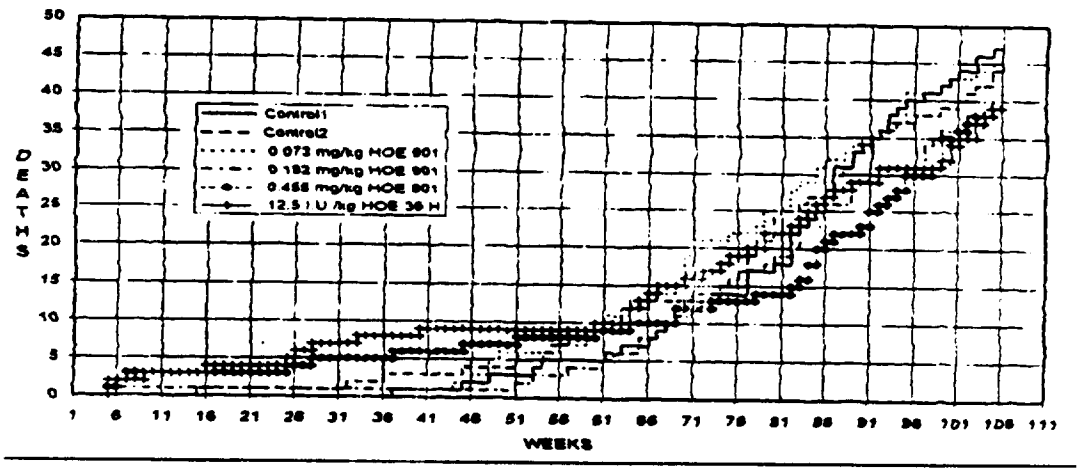
Effects of Placebo and HOE901 on Mice Mortality in 2-Year Carcinogenicity Study												
Sex	50 males/group at Day 0						50 females/group at Day 0					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Died	33	22	23	26	30	29	35	33	34	31	36	32
Intercurrent*	1	5	8	5	2	3	12	12	11	10	3	7
Terminal*	16	23	19	19	18	18	3	5	5	9	11	11
*indicate numbers of animals killed intercurrently or at terminal. Groups 1, 2, 3, 4, 5 & 6 had saline, placebo, 0.073, 0.182, 0.455 mg/kg of test article or 0.455 mg/kg HOE36H.												

Numbers of Mice Surviving on Indicated Day in 2-Year Carcinogenicity Study							
Sex	Day	Control	Placebo	0.073	0.182	0.455	HOE36H
M	0	50	65	65	65	65	65
M	401	48	48	47	45	44	48
M	548	42	42	43	38	38	39
M	597	35	38	40	35	36	35
M	646	30	32	34	31	29	30
M	695	23	30	28	24	22	22
M	730	16	23	21	20	19	19

F	0	50	65	65	65	65	65
F	408	45	42	42	46	42	41
F	506	37	36	29	37	38	33
F	604	21	21	18	24	28	23
F	653	12	14	12	20	23	19
F	702	6	11	7	14	15	16

Drug dose was in mg/kg and HOE36H dose was the same as the top dose of HOE901. The control animals had saline injections, and M and F indicate male and female, respectively.

CARCINOGENICITY STUDY OF HOE 901
Fig. 6: Mortality of female mice



Body Weight: The parameter was checked weekly for the first 2 weeks and biweekly for the remainder of study. HOE901 had little effect on male body weight, although HOE36H reduced the parameter by 15%, compared to saline control. In female, the low dose increased body weight by 35%, but the effects were not drug-dose dependent. The top dose increased the parameter in females by 12%, compared to saline control as shown below.

Effects of HOE901 on Mice Body Weight in 2-Year Carcinogenicity Study						
Sex	Control	Placebo	0.073	0.182	0.455	HOE36H
M	50/16*	65/23	65/21	65/20	65/19	65/19
M	18.0@	19.0	18.0	18.4	18.1	16.1
F	50/3*	65/6	65/6	65/1	65/12	65/12
F	12.7@	14.7	17.1	15.5	14.6	17.9
* indicate animal number at terminal and @ show weight in gram at terminal.						

Food Consumption: The parameter was checked weekly. Absolute food consumption in g/day was not different from the control in all treated group because the reduction was under 10% as shown below.

Effects of HOE901 on Food Consumption in 2-Year Carcinogenicity Study*						
Sex	Control	Placebo	0.073	0.182	0.455	HOE36H
M	6.3	6.5	6.4	6.4	6.4	6.3
F	6.2	6.1	6.2	5.8	5.6	5.7

*Represent mean value of food consumed in g/day.

Ophthalmoscopy: There were no remarkable ophthalmologic changes in treated animals in monthly examination.

Hematology: This parameter was checked at day 0 and final week (105 and 107 weeks). In general, the hematological parameters were not affected remarkably by the treatment. Statistically significant decrease in leukocyte in male mice from the high dose and HOE36H groups was noted, which might be incidental due to age-related variation. Evaluation of hematological parameters measured in animals killed intercurrently revealed pathological values in several of these mice without clear correlation with treatment.

Clinical Chemistry: There were no remarkable blood chemical changes in treated animals in monthly examination.

Organ Weights: The absolute organ weight was determined at necropsy and relative organ weights were calculated. Absolute and relative organ weights were similar in all study groups and there was no evidence that drug-induced changes in the parameter in both sexes.

Gross Pathology: Dead animals or killed intercurrently on the day after the last dose were subjected to macroscopic exam. Palpable masses were noted in all study groups. But, the incidence in the low dose male group was high as shown below and 9 mice were diagnosed histologically malignant fibrous histiocytoma in this group. Therefore, the incidences do not appear to be related to drug-dose.

Effects of HOE901 on Palpation for Nodules in 2-Year Carcinogenicity Studies in Mice												
Sex	50 Males per Group						50 Females per Group					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Mice#	4	5	12	4	3	3	5	5	4	2	3	5
Mass#	4	5	13	4	3	3	9	5	5	2	4	6

Mice# indicates the number of mice had the palpable masses and Mass# was the total number of the mass noted in all mice at the group. Group 1, 2, 3, 4, and 5 were saline control, placebo, 0.073 , 0.182, 0.455 mg/kg of test article (HOE901), respectively. Group 6 had HOE36H instead of HOE901. The acidity of HOE901 or excipients might not be responsible for this because control and placebo had similar counts while low dose only caused the highest incidence in males. There were 9 mice that had histiocytoma in the low dose(group 3) in male.

Histopathology: Standard microscopic examination was performed. Subcutaneous malignant fibrous histiocytoma at the injection sites occurred in male mice treated with HOE901 as indicated under Gross Pathology. There was no drug-dose dependency in the incidence of the malignancy. In males, control saline did not cause the incidence while the incidence was pronounced in low dose group only. It appeared that female mice had no such incidence.

Non-Tumor: At the injection sites, severe inflammatory reaction with fibrosis or sclerosis was noted as shown below. There was no clear sign or trend to distinguish the different groups which were treated with HOE901.

Incidences of Abnormality at Injection Sites in 2-Year Carcinogenicity Study in Mice						
Dosing Group	Control	Placebo	0.073	0.182	0.455	HOE36H
Male Incidence						
Epidermitis	4	7	4	12*	6	3
Inflammation S.C.	16	20	11	17	17	21
Fibrosis subcut.	42	35*	27*	43	45	42
Sclerosis subcut.	16	26*	25	31*	27*	14
Female Incidence						
Epidermitis	8	8	3	3	5	3
Inflammation S.C.	19	23	26	26	8*	27*
Fibrosis subcut.	46	38*	35*	45	38	33*
Sclerosis subcut.	15	23	16	30*	17	17

*indicates P<0.05, compared to saline control.

Tumor: In the liver, hepatocellular adenomas and carcinomas occurred in male animals in all groups, except for adenomas in the control group. For adenomas, the Fisher exact Test shows a positive p-value (P<0.05) for the placebo group, the low and mid-dose groups compared with the control as shown below. There was no drug dose-dependency in either type of tumors in male mice. There were only 3 hepatic carcinomas in female mice in placebo and the low dose groups without remarkable other tumors.

Incidences of Hepatic Adenoma & Carcinoma in 2-Year Carcinogenicity Study in Mice						
Male Group	Control	Placebo	0.073	0.182	0.455	HOE36H
Liver	50	50	50	50	50	50
Adenoma	-	6*	5*	5*	1	1
%	-	12	10	10	2	2
Carcinoma	6	7	3	3	1	3
Total	6	13	8	8	2	4
Animal#	6	13	8	8	2	4
%	12	26	16	16	4	8
*indicate P<0.05. Only 3 liver carcinomas were detected in female mice.						

Toxicokinetics: Blood samples were collected from a retrobulbar venous plexus at 1, 2, 3, 4, 7, or 24 hours after dosing in two/sex/group. One year and terminal determinations were performed. The results are summarized below.

Test Drug	Sex	Dose(mg/kg)	Tmax(hr)	Cmax(ng/ml)	AUC_{0-7h}(ng/mlxh)
HOE901	Male	0	2	15.9	62
		0.073	2*	13.9	53
		0.182	1	22.4	69
		0.455	1	133.0	204
HOE901	Female	0	-	2.2	14
		0.073	1	6.9	20
		0.182	1	28.6	45
		0.455	1	58.7	112

OVERALL INTERPRETATION For EVALUATION: There were no remarkable effects of HOE901 on body weight or food consumption in either sex throughout the study. One of the most important criteria for acceptable carcinogenic studies is sufficient number of animals at necropsy for statistical validation. In the present 2-year carcinogenic studies with male mice, there were at least 16 mice in saline control group at terminal. In all other groups there were at least 19 mice for histopathogical examination at necropsy. However, in female mice it was impossible to evaluate the data provided by the sponsor because of high mortality in saline and placebo control groups as described below(Please see Adequacy section).

The incidence of hepatic adenoma was rather high in placebo group. Low and mid dose-treated groups also had comparable numbers of hepatic adenoma, although there was only one such incidence in high dose group. The incidence of carcinoma was high in the saline control as well as placebo control groups, compared to the test article-treated groups. The difference was not statistically significant. According to the data provided by the sponsor, there were only 3 hepatic carcinomas detected in female mice(two in placebo and one in the low dose groups). But, the finding might not be meaningful because the data were derived from insufficient number of samples due to early animal deaths as stated above.

Adequacy of the carcinogenicity studies and appropriateness of the test model:
The main problem of the 2-year carcinogenicity study in mice was the fact that there was unacceptably high mortality in female saline control and placebo groups including the low dose group(Please see the mortality table). There were only 3 mice in control saline group at necropsy. Only 5 mice were available for the placebo and low dose groups, respectively, at the necropsy. The deaths were pronounced from the 13 months of the study. About a dozen mice died in the three groups in several months. In the mid and high dose groups there were only 9 and 11 mice available for macro- and microhistological examination at terminal. Thus, the data provided by the sponsor were not meaningful since no statistically valid analyses were possible in females. It is not clear the reason that caused the high mortality in the saline control and placebo groups. The vehicle contained — mg of 85% glycerol, 2.7 mg m-cresol, and distilled water to make 1 ml with pH=4.0.

Evaluation of Tumor Findings:
In male mice, HOE901 increased incidences of hepatic adenoma in placebo, low and mid dose-treated groups. The significance of the increase is questionable because the

placebo treatment increased the incidence and the top dose did not cause the tumor. This might indicate that the excipients might have to with the incidence, although the findings of the top dose group does not support the view. The findings might not be significant because the historical incidence of hepatocellular adenoma is about 18.6% in male CD-1 mice in 2-year studies so the present findings are within the range of historical controls. The incidence of hepatic carcinoma was rather high in placebo control group. Saline control group also had comparable numbers of hepatic carcinoma, of which incidence is also in the range of historical values. All test article-treated groups including HOE36H treated group had much lower incidence compared to the two control groups. However, the interpretation of data and subsequent evaluation should be accepted with reservation due to high animals' mortality, particularly in females.

SUMMARY CONCLUSIONS AND RECOMMENDATIONS

The results of 2-year carcinogenicity study in mice indicate that the data from the male might be valid, but not the data from the females. In male mice, the incidence of hepatic adenoma was statistically significant in placebo, low, and mid dose-treated groups. But, the biological significance is questionable due to lack of dose dependency and positive findings in placebo group. The significance of carcinoma is also likely to be negligible because both saline and placebo controls had a similar incidence compared to the test article-treated groups. The three hepatic carcinomas detected in female mice are not interpretable because the data were derived from insufficient number of samples due to early animal deaths. It appears that the 2-year carcinogenicity studies in mice may be valid in male. But, the studies in female mice may not be valid because of high mortality of the animals. The findings should be stated in label appropriately.

Acceptability of Study(s) or Overall Testing Approach: Male studies only
Major Tumor Findings: Negative
Non-neoplastic Findings: Not significant
Biological Significance: None
Potential Clinical Implications of Findings: None

Recommendations for Further Analysis:

[ ]

The submitted data may be justifiable to indicate that HOE901 was not carcinogenic in male mice. The reviewer believes that the outcome of female mice cell data was not conclusive due to high mortality in saline control group.

Study Title: Carcinogenicity Study in Rats
Study Number/Report#/Document#: 95.0017/ 98.0212/014319
Volume Numbers: 41/P004-51/P332
Test Facility: Hoechst Marion Roussel deutschland GmbH, Frankfurt, Germany
Study Date(s): 2/09/1995-2/21/1997

Date of Submission: 3/31/1998
GLP Compliance: Yes
QA Report- Yes (x) No ()
Study Type: Regular

Species/strain: Rat/CD(Sprague-Dawley)

Number of animals per group; age at start of study: 50/sex/group; 4-5 weeks old
Animal housing: Individually in transparent macrolon cages
Drug Lot/Batch number(s): D001, 02D92, 01D098, N110/10, and E003
Drug Purity / Stability / Homogeneity: The sponsor provided relevant data on Batches #D001 and E003 (QE0510/94-we/QE0112/96-we). However, there were no data provided on other batches such as N110/10 and 02D092.

DOSES: Control, Placebo, HOE901(0, 0.073, 0.182, and 0.455 mg/kg)

Basis of Dose Selection: In the 3 months study HOE901 (Batch:D001), 15 Wistar rats(strain:Hoe:WISKf/SPF71)/sex/group received HOE901 subcutaneously at doses of 0.1455, 0.455, and 1.455 mg/kg. The drug treated groups were generally comparable to control and/or placebo groups. The top dose produced intercurrent death of 11 males and 8 females. Following histopathological examinations these deaths were considered to be due to severe hypoglycemia. In addition, extensive degranulation of β-cells in the islets of pancreas of the dead animals was supporting evidence of the hypoglycemic action of the test article. Urine volume was increased in both sexes of the top dose group. In the 12-month pilot study HOE901(Batch: T3885), 30 Sprague Dawley rats(Strain:CD)/sex/group received HOE901 subcutaneously at doses of 0.723 and 1.455 mg/kg. Six male and female rats in the top dose group died within the time frame of 111 consecutive days, which forced to a reduction of the top dose as 0.723 mg/kg beginning on study day 112 for the rest of testing period. There was an additional death of 14 male and 8 female rats intercurrently at the dose group of 0.723 mg/kg by the end of the study. The cause of deaths was considered to be due to hypoglycemia which lead to cardiovascular failure. In this group of rats, β cell degranulation was also confirmed as an expression of the pharmacological action of the test article. Scabbed and open wounds on neck, forelimb and flank were observed in control and drug treated groups. Based on these data 0.455 mg/kg(approx. 12.5 I.U./kg) was considered the MTD for a life time study. Mid- and low doses were selected as 0.182 and 0.073 mg/kg, respectively including saline control and placebo groups.

Relation to Clinical Use: This human insulin analog might have a long-acting property so that once daily subcutaneous injection provides a basal supply of insulin.
CAC Concurrence: No
Restriction Paradigm for Dietary Restriction Studies: NA
Route of Administration: Subcutaneously
Frequency of Drug Administration: Once daily
Dual Controls Employed: Yes, saline and placebo(glycerol, m-cresol, pH 4.0)
Interim Sacrifices: Yes(Please see mortality table below)
Satellite PK or Special Study Group(s): Yes, 3 rats/sex/group
Unscheduled Sacrifices or Deaths: Unscheduled animal death was processed for drug evaluation when the animals were moribund or dead.

Deviations from Original Study Protocol: NA

STUDY RESULTS AND FREQUENCY OF MONITORING:

Clinical Observations: In all animals including the control group, scab induration and swollen deposit were noted at the injection site. The number of affected animals with those findings was increased in male placebo and HOE901 treated groups, which was not clear in female groups. The increased incidence in male groups was not dose dependent. Thus, it appears that the incidence was not due to compound-induced pathological consequence, although the placebo increased the incidence in both sexes.

Mortality: Unscheduled animal death was checked twice daily except weekends and holidays when the animals were checked once weekly. There was all increase in mortality in placebo(group 2), HOE 901 and HOE36H treated male groups. In females, mortality increased significantly in the HOE901 and HOE36H groups as shown below. Treatment-duration dependent mortality was also documented, which shows that the majority of preliminary deaths occurred during the 2nd year of study duration except the top dose male group.

Effects of HOE901 on Mortality in 2-Year Carcinogenicity Study in Rats												
Sex	50 males for each 6 group						50 females for each 6 group					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Died	15	24*	36*	33*	47*	44*	11	12	17	18	35*	28*
Inter@	12	15	8	11	2	1	18	19	15	16	8	13
Term+	23	11	6	6	1	5	21	19	18	16	7	9

*indicate P<0.05. Inter@ and Term+ indicate killed intercurrently and at study end, respectively.

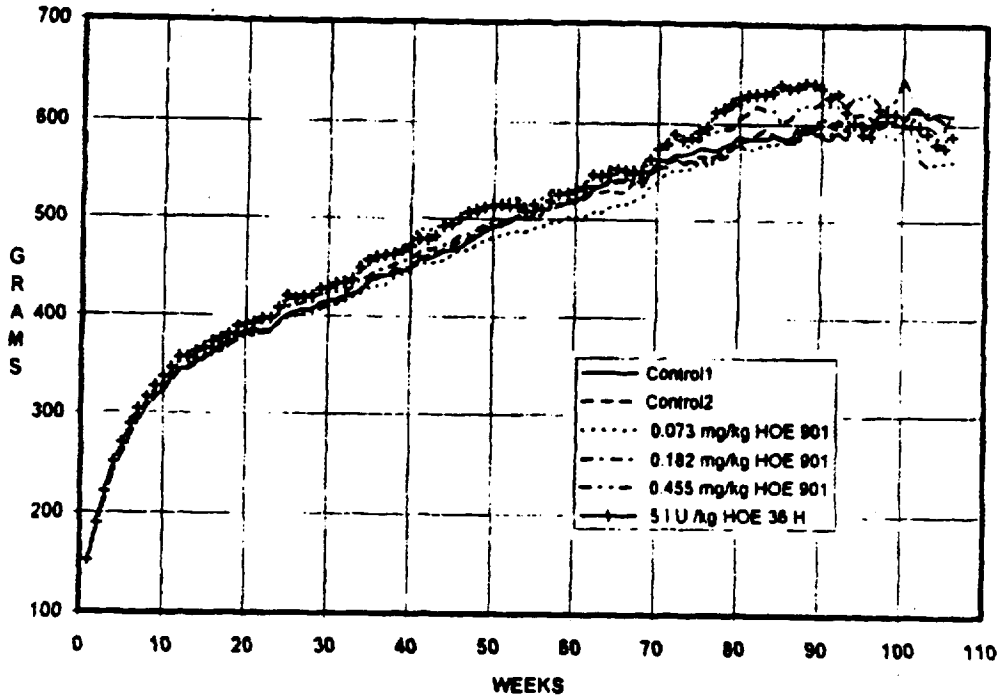
Time-Dependent Mortality in 2-Year Carcinogenicity Study in Rats												
Sex	50 males for each 6 group						50 females for each 6 group					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Wk 1-26	1	1	1	1	7	-	-	-	-	1	2	-
Wk 27-52	-	5	4	1	14	2	1	2	2	-	6	3
Wk53-78	12	9	14	15	11	12	9	7	10	9	11	11
Wk79-107	14	24	24	26	17	31	19	22	20	24	24	27
Total*	27	39	44	44	49	45	29	31	32	34	43	41
%@	54	78	88	88	98	90	58	62	64	68	86	62

*indicate cumulative number of animal deaths up to week 107. @ indicates percentage of 50 animals that were started at the beginning of the study.

Body Weight: The parameter was checked twice weekly during the first 3 months of study and once weekly for the remainder of study. Body weight development was regular and comparable in all groups in both sexes. Males of the low and mid dose HOE901 groups tended to slightly higher mean body weight values during a period from week 20 to week 80, which might be related to treatment of the test article. Single statistically significant difference such as Days 5, groups 4 and 5 females, Day 281 group females was considered incidental(please see the figure below). Furthermore, the statistical analysis in later part of the study may not be meaningful because of markedly reduced animal numbers in many groups(Please see the mortality data).

CARCINOGENICITY STUDY OF HOE 901

Fig. 2: Body weight development of female rats

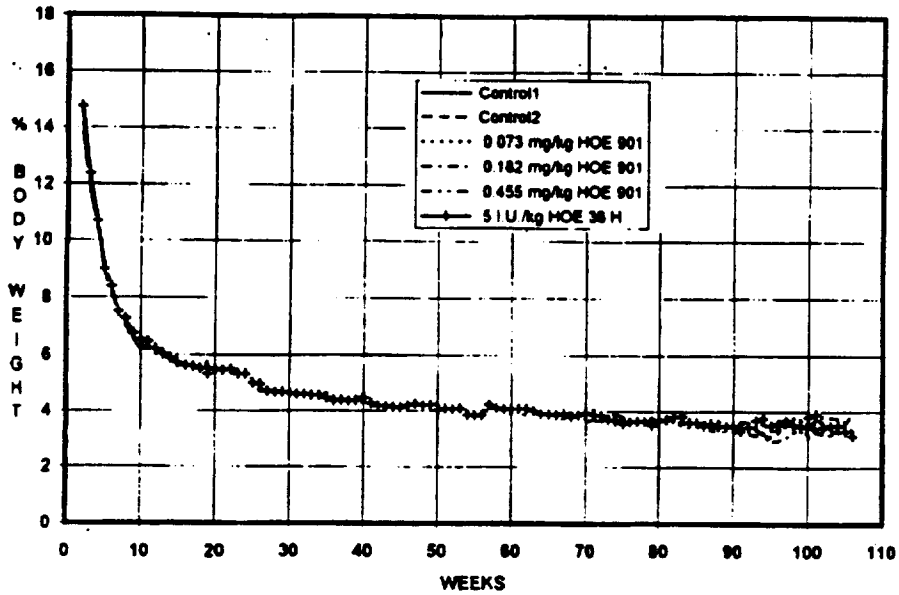


Food Consumption: Food consumption was calculated based on weekly examination. Overall absolute and relative food consumption were regular and not affected remarkably by the administration of HOE901 and HOE36H in both sexes as shown in table and figure below. There was a slight increase(8%) in relative food consumption in the high dose HOE901 males as in the table.

Effects of HOE901 on Food Consumption in 2-Year Carcinogenicity Study in Rats							
Food Con.	Sex	Control	Placebo	0.073	0.182	0.455	HOE36H
Absolute Consumption*	Males	33.6	33.2	34.9	33.9	33.8	33.5
	Females	24.6	24.2	24.0	24.5	24.7	25.4
Relative Consumption@	Males	4.8	4.9	4.9	4.8	5.3	4.9
	Females	5.7	5.6	5.6	5.6	5.7	5.7

*Represents mean food consumption in g/day and @ expressed in g/100 g/day.

CARCINOGENICITY STUDY OF HOE 901
Fig. 3: Food consumption of male rats



Ophthalmoscopy: There were no remarkable findings that were documented.

Hematology: Blood samples were taken prior sacrifice at the time of final dissection (week 106-107). For the animals in a moribund status, blood samples were obtained before the event as far as possible. In general, there was no change in the hematological parameters investigated in all groups of both sexes. An exception would be the fact that the increased mean hemoglobin value in group 5 females, which was statistically significant (Placebo value 127 vs 143 g/L). An evaluation of parameters measured in animals killed intercurrently revealed pathological values in some of those rats. However, no clear correlation with the treatment could be established due to sporadic findings.

Clinical Chemistry: There were no remarkable findings that were documented.

Organ Weights: 13 organs were examined at necropsy. In general, the parameter was not affected by the treatment. There was slight, but statistically significant decrease in mean relative spleen weight in HOE36H treated females, which appears to be no toxicological implication. However, it should be considered that the number of animals examined at final dissection was reduced markedly due to intercurrent death in some groups.

Gross Pathology: Palpable masses were observed in all groups including the control and placebo groups. The incidence frequency in all groups in both sexes was similar except the low dose group of males as shown in a table below. In this group, 15 animals had malignant fibrous histiocytoma at the injection site.

Effects of HOE901 on Formation of Papable Masses in 2-Year Carcinogenicity Studies												
Rat Sex	50 males for each 6 group						50 females for each 6 group					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Σanimals	10	14	20	14	8	8	32	26	28	28	22	30
Σmasses	10	14	25	16	8	8	56	42	44	43	30	52

Histopathology: 47 organs or parts thereof were examined microscopically for histological evaluation. Increased incidence rates of malignant fibrous histocytomas at the injection site were present in males of the placebo group and all HOE 901 treated groups. In females, placebo, low and mid dose groups were affected.

Non-Tumor: Utilizing blood samples obtained from 10 rat/sex/group on 3, 6, 12 and 24 months after the onset of study, antibodies titer was determined. In the control HOE901 tracer binding was comparable to the baseline values(3.3%). In the low dose group a slight increase in binding values (10-20%) occurred. In the medium and high dose groups, a further dose-dependent increase in binding was observed. These results demonstrate a clear correlation between dose and increased tracer binding values indicating the formation of insulin antibodies for HOE901 administration. Chronic tissue inflammation and subcutis sclerosis were also observed in placebo as well as treated groups in male as well as in females, although the incidences were high in males. Granuloma and muscle degeneration were noted in the mid dose group of males, but the incidences were not drug-dose dependent because the high dose group had no such occurrence.

Incidence of Non-Tumors in 2-Year Carcinogenicity Studies in Rats												
Rat Sex	Male						Female					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Total Rat#	50	50	50	50	50	50	50	50	50	50	50	50
Rats Examined	49	50	50	49	48	49	49	50	49	50	50	49
Inflammation	2	12*	9*	19*	12*	23*	6	6	7	9	8	8
Granuloma	2	1	5	11*	2	6	-	3	2	4	1	2
Granulation	-	-	2	4	1	1	-	3	5*	1	-	1
Sclerosis	-	25*	25*	27*	17*	25*	-	10*	17*	16*	14*	10*
Subc. Edema	-	4	-	5*	-	3	1	7*	6	1	5	6
Hemorrhage	-	5*	1	4	5*	6*	-	5*	7*	2*	6*	6*
Degenerated@	-	2	-	6*	-	1	-	1	3	-	-	1
Siderophages	-	1	-	2	1	1	-	1	1	1	-	1

@indicate degeneration in skeletal muscles. *indicate $P<0.05$, compared to saline control.

Tumor: There were no clear tumors in control groups of either sex except histiocytoma as indicated below. The malignant fibrous tumors at the injection sites were present in males of all groups except saline control and group 6 which had HOE306H. This suggests that the vehicle(Please see the components in Method Section) might be

responsible for the incidence. As the dose of HOE901 increased, the incidence was reduced as the mortality increased in male, although the causal relationship is not known. The excipient components in HOE901 and HOE36H were slightly different as specified under "Methods".

Incidence of Malignant Fibrous Histiocytoma in 2-Year Carcinogenicity Studies in Rats												
Sex	Male						Female					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Total Rat#	50	50	50	50	50	50	50	50	50	50	50	50
Rats Examined	49	50	50	49	48	49	49	50	49	50	50	49
Histiocytoma	-	9*	13*	12*	6*	-	-	1	2	1	-	-

*indicate P<0.05, compared to saline control.

Toxicokinetics: Blood samples were taken from the retrobulbar venous plexus from non-starved rats according to the following schedules. From 3 rats/sex/group, blood was collected at 1, 2, 3, 4, 7, and 24 hours after drug administration on day 28, 189. The peak drug levels in serum were reached within a few hours after subcutaneous injection. Increased times to peak concentrations were noted in male with increasing dose as shown below(See table). Cmax as well as AUC were increased according to the size of doses. The two parameters were generally increased as a function of exposure duration, which might be related to the development of antibodies if it was not accumulated in the system. Gender dependent differences were not significant, although the AUC values were a little low in the female groups.

Parameters *		Tmax(hour)			Cmax(ng/ml)			$AUC_{0\text{-}7h}$ (ng.h.ml)		
Sex	Dose@	28	190	370	28	190	370	28	190	370
Male	0	24	1	1	8.6	10.1	15.3	47	47	72
	0.072	1	1	1	28.0	23.4	34.8	64	71	106
	0.182	1	1	1	47.4	36.1	88.7	80	112	224
	0.455	1	1	1	32.2	76.9	159.0	101	251	539
Female	0	2	1	4	4.1	5.6	8.3	26	34	48
	0.072	1	1	1	5.56	19.4	28.3	28	49	60
	0.182	1	1	1	32.8	52.4	84.8	56	96	135
	0.455	1	1	1	54.4	124.0	169.7	98	196	344

*Indicated parameters were determined at 28, 190 and 370 days after the onset of study. @ dose was in mg/kg.

OVERALL INTERPRETATION AND EVALUATION

In general, the 2-year carcinogenicity study in rat was conducted in an acceptable manner. There were test article dose-dependent deaths in both male and female rats. Apparently the mortality in both sexes appeared to be due to the extension of pharmacodynamic effects of test article such as hypoglycemia and coma. Body weight development was regular and comparable in all groups in both sexes, although there were sporadic changes. The test article dose-dependent elevation of blood level, Cmax, and AUC were also well documented. It appears that the male rats had significantly high incidence of histiocytoma, compared to saline control. However, the incidence was not test article dose-dependent since there were 13, 12, and 6 incidences in low, mid, and high dose groups. Furthermore, the level of significance was not significant when one compares with the placebo control group because it had 9 such incidences.

Adequacy of the carcinogenicity studies and appropriateness of the test model:

The sponsor performed 3 months study for dose range finding purpose in Wistar HOE rats, while they used Sprague-Dawley CD rats in 2-year carcinogenicity studies. In dose range finding studies, the top dose, 1.455 mg/kg , killed 11 males and 8 females out of 15 wistar rats due to drug-induced hypoglycemia. Thus, they selected the top dose as 0.455 mg/kg with 0.185 and 0.073 mg/kg as mid and low doses. Despite of the difference in strain, there were some indications of pharmacological actions such as granulations of pancreatic β cells. Thus, the dose selection appears to be acceptable. However, there were only 11 male rats available in the placebo group for necropsy, although there were 23 rats available in saline control group. Thus, it is not clear the high mortality in the male of the treated group was purely due to pharmacological action of drug.

Evaluation of Tumor Findings:

There were no clear tumors in any groups of either sex except histiocytoma. The malignant fibrous histiocytomas at the injection sites were present in males of all groups except saline control and group 6 which had HOE306H. The level of significance, compared to saline control, was positive. However, there was no test article dose-dependency because the incidence was reduced as the dose increased. Saline control and HOE36H treated groups had no indication at all, which suggests the components of the placebo might play a role. In female, there was no indication of any tumor except the histiocytoma, of which incidence appears no great concern.

SUMMARY CONCLUSIONS AND RECOMMENDATIONS

The 2-year carcinogenicity study was carried out under acceptable conditions in rats. The malignant fibrous histiocytoma at the injection sites occurred in higher incidences in placebo control and HOE treated groups but not in saline control in male. The acidity(pH=4) of vehicle alone is probably not responsible for it since there was no such incidence in female rats. In male rats, vehicle but not the test article might promote the incidence of malignant fibrous histiochytoma. The relevance of the animal finding to human is not known but examinations at the injection sites should be monitored in clinical use.

Acceptability of Study(s) or Overall Testing Approach:
The 2-year carcinogenicity study in female rats was conducted in reasonably acceptable set up, although the sponsor used different strain of rats from the dose range finding study. The basis of dose selection appears tc be acceptable at least in female rats, based on mortality findings in the carcinogenicity study. The high mortality of females in the high dose group and HOE36H group might be due to the extention of drug action as discussed previously. In males, there was high mortality in all groups except the saline control group. The high mortality in placebo group in male rats (39 deaths out of total 50) in 2-year carcinogenicity studies casts some doubt on the validity of the present studies.

Major Tumor Findings: Malignant fibrous histiocytomas at the injection site occurred in higher incidence in all males except the saline and HOE36H groups. All the positive groups had common vehicle since the placebo as well as HOE901 were in the vehicle. The pH of HOE36H was 7.3 while the pH of the placebo solution was 4.0. The sponsor indicated that the acidity (pH=4.0) of the HOE901 vehicle was considered to be causative for the tumor formation. However, similar malignant fibrous histiocytomas at the injection site were not significant in the placebo or in any HOE901 treated groups in female.

Non-neoplastic Findings: In males, inflammation and sclerosis were noted in all groups except saline control and HOE36H groups. Granuloma, subcutaneous edema, and skeletal muscle degeneration were only observed in the mid dose group without a dose-dependency. In female, hemorrhage was noted in all group except the saline control group without drug-dose dependency. Sclerosis was also observed in females including the placebo group, which might be due to HOE 901 common excipients.

Biological Significance:
There were no tumors indicated in the 2-year carcinogenicity studies in rats except malignant fibrous histiocytoma, particularly in males. In human, the maligant fibrous histiocytoma accounts for 20 to 30% of soft tissue sarcomas. They are multilobulated, and infiltrative, but deceptively circumscribed. There are several variants, for which 5-year survival is about 50%(Schneider et.al., Histol. Histopathol. 14:845,1999). The relevancy of the animal finding to human is not known but examinations at the injection sites should be monitored in clinical use.

Potential Clinical Implications of Findings: The pH of HOE901 was acidic, while HOE36H had pH 7.3. The low pH had no detrimental effects in mice. The therapeutic use of HOE901 in the acidic vehicle may not represent a cancer hazard for humans based on human experience with acidic insulin formulations over decades. However, the malignant fibrous histiocytoma that observed in male rats require to be documented clearly in label.

IMMUNOTOXICOLOGY:

Study title: **Immunogenicity studies in rabbits and guinea pigs**
Study No: and number: Ref.#42; Doc.#014616, Vol#18, Page#192-203
Site and testing facility: Hoechst AG, Frankfurt, Germany
GRP compliance: No
QA- Report Yes ( ) No (x):
Lot and batch numbers: Not specified.

METHODS:
Species/strain: Rabbits/White New Zealand and guinea pigs/DHP
Doses employed: 40µg antigen/animal with complete Freund's adjuvant(1:1). The dose of guinea was 20 µg under similar conditions to rabbits.
Route of Administration: Subcut. Injection at the neck and shoulder regions
Rationale: Insulins are known to induce antibodies in patients. The development of high antibody titers in rabbits might be indicative for the human response, whereas guinea pigs are known to produce antibodies even to human insulin. Also, neutralizing antibodies in toxicology studies has an important impact on study interpretation.
Number of animals/sex/dosing group: 3/sex/6 groups for both rabbits and guinea pigs
Endpoints: Determination of antibody titer after test compound or human insulin.
Observations: In rabbits, HOE 901 induced a significantly lower antibody response compared to human, porcine and bovine insulin. In guinea pigs, all tested insulins induced high antibody levels. However, when a decline of antibody titers was induced by a change of the immunization procedure, HOE 901 again reached the lowest antibody titer.

Timing: Immunization weekly over a 26-week period with the control or with the antigen. From week 13 onward, the immunization was performed every 2 weeks in both rabbits and guinea pigs.

Overall Summary(**Results**): All rabbits were negative for analogue/insulin antibodies before immunization. The data show that the fewest number of rabbits had an antibody response when administered HOE 901 versus the other insulins. After 26 weeks, only 1 out of 12 animals in the 2 HOE 901 groups raised an antibody response. In contrast, 4 or 5 of the 6 rabbits in the human, porcine or bovine groups had an antibody response at the 26-week time point. The level of antibody production was very low in both HOE 901 groups throughout the study. In contrast, bovine insulin had the strongest antibody response. In fact, human, porcine, and bovine insulin had a statistically higher percentage of antibody binding in rabbits than either HOE 901 preparations from day 100 onward. All guinea pigs were negative for analogue or insulin antibodies prior to immunization. After immunization all animals of each group developed high antibody levels. When the immunization protocol was changed from weekly injections with adjuvant to bi-weekly injections without adjuvant, antibody titers began to decline. At the end of the 25-week immunization period, HOE 901 [redacted] had the lowest antibody titer, followed by human insulin, bovine insulin, HOE 901 [redacted] and porcine insulin with the highest titer.

Conclusions: The results from these investigations in rabbits and guinea pigs indicated that HOE 901 had a similar immunogenic potential as regular human, porcine, or bovine insulin.

REPRODUCTIVE TOXICOLOGY:

Study title: HOE901- Subcutaneous reproduction toxicity study in rats
Study No: and Report number/Doc. No.: 94.0608/ 96.0021 / 013942
Site and testing facility: Hoechst Pharma Development, Frankfurt, Germany
GRP compliance: Yes
QA- Reports Yes (x) No ( ):
Lot and batch numbers: Batch D001(Report#QE0510/94 on 4/15/1994)
Protocol reviewed by Division Yes ( ) No (x):

METHODS:
Species/strain: Wistar rat/Hoe:WISKf(SPF71)
Doses employed: 0.036, 0.108 and 0.360 mg/kg
Route of Administration: subcutaneously once per day
Study Design: Combined fertility and pre- and postnatal study design. All the treated males and females received the test compound, the control solution or the reference drug subcutaneously once per day. The males were continuously treated from day 28 prior to mating until impregnation of either their treated or untreated allocated female or until the end of further mating attempts with two untreated females. The treated females received the test substances or the control solution during a 14-day pre-mating treatment period, during the mating period and throughout pregnancy and the 21-day lactation period.
Number of animals/sex/dosing group:25/sex/group
Parameters and endpoints evaluated: Mating, sperm examination, examination at birth and during lactation, function tests, FI examination after weaning including behavior, sexual maturation, mating and fertility test, and autopsy of the parent and pups
Statistical evaluations: Statistical evaluations were based on the assumption of a monotone dose-response relationship. Comparisons of dose groups and the control group at the 5% level were only carried out if significant effects were detectable in the higher dose group.

RESULTS:
Clinical signs: In the low and mid-dose groups, there were no abnormal clinical signs. In the high dose group, hypoglycemic signs such as convulsions and coma were manifested.
Mortality: The males of the P generation did not show any impairment of behavior or general condition attributable to the various dosages of the test compound. There was also no impairment of behavior or general condition in the treated females of the 0.036 and 0.108 mg/kg groups. In the 0.36 mg/kg group, 5 dams dropped out prematurely and 3 dams died between days 22 to 23 after mating.
Body weight: This parameter of the males and treated females from the various groups was comparable with that of the respective control group during the pre-mating period. This holds also true for the males of HOE36H group as reference. There were also no compound-dependent effects on weight development in the lactating females of the 4

groups and the reference drug group, which is consistent with comparable food consumption data(Please see Section below).
Food consumption: The parameter of the males and treated females from the various groups was not impaired during the pre-mating treatment period. The animals in the test groups consumed amounts of food comparable to those consumed by the control animals. During pregnancy, the various doses were not affected remarkably on the parameter.

(- FERTILITY IN MALES): Most of the males in the various groups inseminated both their treated and untreated female partners. Two males each in the high dose and HOE 236H groups and one control male inseminated only the untreated females. One male in the high dose group failed to inseminated either of its allocated partners. Thus, male mating index was 100% in all groups except the high dose group which was 96%. Examination of sperm in the cauda epididymis of part of the animals showed that the ratio of spermatozoa exhibiting local movement was similar in the all groups including the control.

(- FERTILITY AND EARLY EMBRYONIC DEVELOPMENT IN FEMALES)
The estrus cycle of treated female rats was normal since most rats had estrus in 4 days. One female in the low dose group had estrus on day 5 after mating. A few animals had prolonged metestrus/diestrus two or three times during the mating period. The majority of the treated females in the three groups became pregnant after the first two or three mating attempts, as did the control dams. One female each in the high dose, HOE36H, and control did not become pregnant. The number of days that required by the females until pregnancy materialized was comparable in HOE901 treated or untreated control animals as shown below.

Days	Control		HOE 901 (mg/kg) 0.036		0.108		0.360		HOE 36 H (IU/kg) 3	
	treated	un-treated	treated	un-treated	treated	un-treated	treated	un-treated	treated	un-treated
0 - 4	19	22*	18	13	17	19*	19	18	18*	16
5 - 9	1	1	3	4	3	1	4	2	3	3
10 - 14	2	1	-	4	2	3	1	1	1	1
15 - 19	-	1	4	-	-	1	1	1	-	4
20 - 24	1	-	-	-	-	-	-	2	-	1
25 - 29	1	-	-	2	1	-	-	-	1	-
30 - 34	-	-	-	-	-	-	-	1	-	-
35 - 39	1	-	-	1	1	1	-	-	1	-
40 - 44	-	-	-	-	-	-	-	-	1	-
45 - 49	-	-	-	-	1	-	-		-	-
n	25	25	25	24	25	25	25	25	25	25

* One of these dams was impregnated within the first 5 days of the mating period without spermatozoa being found in the vaginal smear.

Pregnancies: All impregnated females in the mid- and high dose groups had normal pregnancies and normal delivery. From the 24 pregnant females in the HOE36H group, one dam died overnight from day 22 to 23 of pregnancy. Of the dams, which littered normally, all gave birth between the 22nd and 24th day of pregnancy. This is typical for the rat strain used. The mean duration of pregnancies in the HOE901 treated groups was 22.5 –22.6 days, while the duration was 22.5 days each in the HOE36H and the control group. The duration of pregnancies in the individual treated dams is summarized on the next page.

Days	Control	HOE 901 0.036 mg/kg	HOE 901 0.108 mg/kg	HOE 901 0.360 mg/kg	HOE 36 H 3 IU
22	4	2	3	1	4
22 - 23*	17	22	17	19	16
23	1	1	1	1	1
23 - 24*	2	-	3	-	1
n	24	25	24	21	22
Mean	22.5	22.5	22.6	22.6	22.5
Pregnancies not included in the means	-	-	-	$1_{(1)}$ $1_{(2)}$ $1_{(3)}$ $1_{(4)}$	$1_{(1)}$ $1_{(4)}$

* Birth overnight
(1) Pregnancy without spermatozoa being found
(2) Killed during delivery on pregnancy day 24
(3) Died during delivery on pregnancy day 23
(4) Died overnight on pregnancy day 22/23, shortly before delivery

In-life observations:
The treated dams from all groups gave birth to live pups. The number of implants, of live, and dead pups did not differ from that of the control dams. The pups were normally developed and their weights were comparable with those of the control pups. There was no increase in the frequency of dead pups at birth in either the test compound groups, the reference drug group or the control group. Post-delivery examinations of the litters from the untreated females gave results, which all were within the normal range of the rat strain used.

Of the dams, which gave birth to live pups, two in the high-dose group failed to rear their litters. One dam in this group died on the first day of lactation period shortly after birth and the second on day 19 of lactation period. The behavior and general health condition of all the other dams which reared their pups up to the time of weaning remained undisturbed during lactation period.

Terminal and Necroscopic evaluations:
One rat in the low dose group showed aplasia of the right testis. One third of normal size in one male from that group was also noted. One male from the high dose group had small whitish granular spot in the renal pelvis of the right kidney. Individual males from the various group exhibited slightly or moderately enlarged renal pelvis, uni- or bilaterally. Uterine and vaginal findings in the P generation females were not remarkable. There were three rats from each low, HOE36H and control group, which had moderately dilated renal pelvis. Dissection of the pups at weaning revealed a uni- or bilateral moderate or marked renal pelvis dilatation in single animals from all treated groups.

(EMBRYO-FETAL DEVELOPMENT)

In-life observations: The general behavior of the pups in the treated group during the three-week lactation period was comparable with that of the control pups. In all the treated groups but also in the control group, several pups were suckled insufficiently. Several litters from the treated group showed impaired coat growth during the second or third week of lactation period, which was seen in the control group. Body weight gain of the pups in the treated groups throughout the lactation period was comparable with that of the controls. Physiological development of the pups such as pinna separation, start of coat growth, incisor eruption, and eyelid opening revealed no abnormalities in the groups treated with test article.

Terminal and Necroscopic evaluations: There were individual cases of renal pelvis dilatation. The dilatation observed in one male each from the low and HOE36H groups, which was bilateral and moderate in the right kidney. One female from the HOE36H group was killed due to exophthalmos and enlargement of the left eyeball exhibited sclera hemorrhages and a hematoma in the lower part of this eyeball.

Dams: The body weight development of the dams from all HOE901 treated groups, HOE36H group, and the control group was not statistically different throughout the pregnancy and on the day of birth.
Offspring: Separate clinical examinations of the offspring selected for rearing started at weaning on day 22 after birth. Most of all clinical signs were not unexpected although there were some differences such as prolonged delivery in the mid dose group. The male and female offspring showed normal development of external genital organs. In all the males of the various experimental groups including the control, preputial separation occurred on days 28-40 after birth with a maximum on days 35-37 in treated groups and on day 35 on the control group. Vaginal opening was seen in all the females of the groups on days 26-39, which was not different from the treated and control groups.

Fertility of F_1 generation females: The estrus cycle of the females was intact. All the females were estrous within four days from the start of taking vaginal smears. And almost all the females in the various groups became pregnant after the first or second mating attempt. Individual animals in the various groups were impregnated after the 3rd to 6th mating attempt. The number of days from the start of mating until the detection of sperm in the vaginal smear was not significantly different between the treated and the control groups. Likewise, the number of matings required by the females until pregnancy materialized was not statistically different from one group to another.

Fertility of F_1 generation males: Almost all males in the various experimental groups inseminated and impregnated their allocated female partner. One male in the 0.36 mg/kg group and one control group male, which inseminated but did not impregnate their allocated partner, impregnated untreated females not belonging to the study. Mating index and the fertility index were 100% in all groups including the top dose group.

PRENATAL AND POSTNATAL DEVELOPMENT, INCLUDING MATERNAL FUNCTION

In-life observations: All impregnated females in the treated groups gave birth to live pups. HOE901 did not affect the duration of pregnancy. All dams gave birth between the 22nd and 24th day of pregnancy.

Offspring: Separate clinical examinations of the F_1 generation animals revealed that there were no gross abnormalities.

Terminal and Necroscopic Evaluations:
Dams: There were no remarkable abnormalities.
Offspring: There were no consistent treatment-related findings in offspring.

Summary and Evaluation: Combined fertility and pre- and postnatal study with HOE901 was performed in Wistar rats. The HOE901 top dose was 0.36 mg/kg, which was approximately 7 times of clinical dose, based on body surface comparison, which did not produce remarkable drug-related effects on parental fertility, F1 sexual maturation, mating, and autopsy findings.