Study title: HOE901- Subcutaneous Reproductive Toxicity Study in Himalayan Rabbits
Study No: and Report number/Doc. No.: 94.0564/ 95.0243 / 013939
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: Himalayan Rabbit/Chbb:HM(SPF)
Doses employed: 0.018, 0.036, 0.072 and 1 IU HOE36H
Route of Administration: Subcutaneously once per day from the 6th to 18th day of pregnancy.
Study Design: Potential embryotoxicity of HOE901 was tested in 20 mated female rabbits. Prior to the start of the study, the females were mated with fertile males in the morning during estrus. A total 5 groups: one control placebo group, three treated groups(low, mid and high doses) and one reference group(HOE36H) were used. Caesarian section and autopsy were performed on 29th day of pregnancy.

Number of animals/sex/dosing group: 20/group
Parameters and endpoints evaluated: Animals' behavior, physical conditions, body weight, food consumption and clinical chemistry were monitored including toxicokinetic studies. Uterus was open at caesarian section, and the live and dead fetuses; conceptuses undergoing resorption, placentas and corpora lutea in the ovaries were counted and examined macroscopically.

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. The findings obtained at autopsy and skeletal examination of the fetuses were evaluated separately for the fetuses and for the litters by the exact Fisher test at significance levels of 5% and 1%. Frequencies of findings obtained at autopsy and the skeletal examination of the fetus were compared with those of corresponding findings in previous control groups.

RESULTS:
Clinical signs: Most animals behaved as expected. Abortion was diagnosed from discolored urine on days 24 to 29 of pregnancy was noted in one dam from the high dose group. Two dams in this group had to be killed on days 11 and 12 of pregnancy, respectively. They exhibited a motoric attack within three hours of the 5th and 6th administration lasting for a few minutes. This violent activity appears to be due to hypoglycemic action of the test article.

Mortality: All animals survived until day 29 of pregnancy without remarkable findings. Two dams in the high dose group had to be killed on day 11 and 12 of pregnancy due to wild physical activities as described above. Both animals showed a weak and decreased activity on the day of killing after the violent somersaults with some bleeding from the nose and mouth.

Body weight: Body weight growth of the dams was not affected by the treatment of HOE901 or reference material. The rabbits with normal pregnancies in the groups treated with the test article showed normal body weight gains. In some animals from the high dose group periodic reduction of body weight was observed which was due to an increase in the intrauterine death rate of their conceptuses.
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 treated 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. In one dam of HOE36H group, food consumption was not recovered for the period from days 19-29 of pregnancy due to a computer operating error. There were several occasions that animals had scattered food so that accurate determination of food consumption was difficult at times.

Terminal and Necroscopic evaluations: In the three treated groups (low, mid and high doses), 19, 17, and 14 dams carried live fetuses to term. In the control group, 17 dams had live fetuses at caesarian section. One dam in the low dose group exhibited 4 corpora lutea of pregnancy in the ovaries and 3 empty implantation sites in the uterus. In the mid-dose group, clinically detectable abortion in one dam was confirmed at caesarian section. This dam had 9 corpora lutea in the ovaries and in the uterus 3 empty implantation sites beside 5 conceptuses undergoing resorption. In the high dose group, two dams aborted; one instance of abortion was diagnosed by presence of three corpora lutea in the ovaries, implantation sites being no longer detectable. In second case, 5 corpora lutea were present in the ovaries and two empty implantation sites and two conceptuses undergoing resorption were found in the uterus. In the control group, one dam delivered prematurely on day 29 of pregnancy, one dam aborted and one female was not pregnant.

The number of corpora lutea and implants in the dams of the various groups did not differ from those the control dams. The pre-implant embryonic death rate was rather high in one dam each in the three HOE901 treated groups and in four dams of the control group. The number of live fetuses in the low and mid-dose groups did not differ from that of the control. In the dams of the high dose group, the number of live fetuses was slightly reduced due to an increase in the number of conceptuses undergoing resorption.

Examination of the fetuses: The live fetuses delivered by caesarian section in the treated group showed normal state of physical development. Their mean body weights and lengths did not differ appreciably from the corresponding values in the control group. Only two live fetuses from the control group were fairly retarded in development and weighted 15.7 g and 17.2 g, respectively, compared to the approximate mean weight of 24 g. The sex ratio of the fetuses was relatively balanced in all groups except the high dose group which had more female than the males.

(EMBRYO-FETAL DEVELOPMENT) Dead embryofetal primordia were encountered in all groups. They died at a relatively early stage and had diameters up to a maximum of 19 mm in the treated groups, compared to 19 mm in control group. In the dams of the low and mid-dose groups, the number of conceptuses undergoing resorption was within the same range as that in the control group. In the dams of the high dose group, the

number of dead embryofetal primordia undergoing resorption was increased compared to that in the control.

Placenta: The placentas of the live fetuses in the treated groups and HOE36H treated group were unremarkable in the macroscopic examination. Their weights were not different from those of the control group, although there were relatively large degrees of variation. The survival rate of the fetuses in the three treated groups in the incubator 24 hr after delivery was 100% in each group as was that of the control group fetuses.

Terminal and Necroscopic evaluations: Morphological examination of the live fetuses revealed no malformations in the low dose group. In the mid-dose group, one fetus exhibited asymmetry of the visceral cranium, on both eyes open eyelids and displastic lenses. Another fetus from the same group had a bent forepaw. In the high dose group, one fetus showed an epical bone between the paired nasal and frontal bones.

Organ cross-section at autopsy revealed dilated ventricles of the brain in five fetuses *8.2%) from the high dose group. A hemorrhage in the thoracic cavity was seen in one fetus from the low dose and 4 (6.6%) from the high dose group. It appears that the increased incidences of the findings might be due to the hypoglycemic effect of the drug. The skeletons of the live fetuses in the treated group showed the same state of development as those of the control fetuses. Their state of ossification corresponded to the 29th/30th day of pregnancy.

Summary and Evaluation:

Subcutaneous administration of HOE901 at doses of 0.018 to 0.072 mg/kg during Day 6 to 18 of gestation induced hypoglycemia which was dose-dependent both, with respect to severity and duration. No clear maternal toxicity and/or toxic effect on the intra-uterine development of the conceptuses was detectable after the low dose group of HOE901 administration. The high dose induced hypoglycemia with an increase in abortion. The high dose also increased death with dead conceptuses with a decrease in live fetuses. The high dose did not increase malformations except ventricular dilatation of the brain, which might be due to an extension of HOE901 pharmacological action. The reference drug HOE36H led to hypoglycemia to the degree produced by the mid and high dose of HOE901. The reference compound also produced abortion and early intrauterine deaths as the cases with the high dose of HOE901. The top dose of HOE901 tested in this study was approximately 1.7 times of clinical dose, based on body surface comparison. But the top dose produced clearly drug-induced hypoglycemia in dams. In conclusion, there was no consistent and documentable embryotoxicity of HOE901 in Himalayan rabbits at doses up to 0.072 mg/kg/day.

Labeling Recommendations: Please see the Labeling Section on page 58.

GENETIC TOXICOLOGY:

I. Study Title: HOE901-Bacterial reverse mutation test
Study No/Document No/Report No: 96.0065/015407 /96.0154
Study Type: Mutagenicity test

Amendment #, Volume #58 and Page #218-248:
Conducting Laboratory: Hoechst AG, Pharma Development Corporate Toxicology, Frankfurt Germany
Date of Study Initiation/completion:2/13/1996/2/22/1996
GLP Compliance: yes
QA- Reports Yes (yes by ——————— No ( ):
Drug Lot Number: E003
Study Endpoint: In vitro mutagenicity

METHODOLOGY:
Strains/Species/Cell line: Salmonella typhimurium TA100, TA1535, TA1537 and TA98 and E. coli VP2uvrA
Dose Selection Criteria: Drug solubility limit
Basis of dose selection: Drug precipitation was noted at or above 2500 µg/plate.
Range finding studies: There was no dose range finding study.
Test Agent Stability: Visible precipitation occurred at or above 2500 µg/plate. The analysis was performed by ——————— on 1/8/1996.
Metabolic Activation System: Aroclor 1254 pretreated rat liver extract

CONTROLS:
Negative Controls:
a. untreated control
b. solvent controls

Positive Controls:
a. without S-9 fraction: Sodium azide(1µg/plate for TA100 and TA1535), 9-aminoacridine(50µg/plate for TA1537), 2-nitrofluorene(2.5µg/plate for TA98) and N-ethyl-N-nitro-N-nitrosoguanidine 2.5µg/plate for WpuvrA).
b. with metabolic activation: 2-aminoanthracene(0.5, 1, and 10 µg/plate for TA98 and 100, TA1535 and TA1537, and WP2uvrA, respectively.

Exposure Conditions: Minimal agar(1.5%), E medium with 2% glucose for 48 hours at 37°C
Incubation and sampling times: overnight
Doses used in definitive study: 4, 20, 100, 500, 2500, and 5000 µg/plate
STUDY DESIGN: Top agar was prepared for the Salmonella strains by mixing 100 ml agar(0.6%) with 10 ml of a 0.5 mM histidine-biotin solution. With E. coli histidine was replaced by tryptophan (0.5mM, 2.5 ml). 0.1 ml of culture medium, 0.1 ml test compound suspension and 0.5 ml of S9-mix were added to 2 ml of molten top agar at 45°C. After mixing, the liquid was poured into a petri dish with a 25 ml layer of 1.5% agar. Vogel-Bonner E medium with 2% glucose. Colonies were counted after 48 hour-incubation at 37°C.
ANALYSIS: Colonies of his^+ and trp^+ revertants were counted with —— counter for statistical evaluation.
No. slides/plates/replicates/animals analyzed: 3 plates per dose
Counting method: Bacterial colonies were counted microscopically.
Cytotoxic endpoints: The information was not provided by the sponsor.
Genetic toxicity endpoints/results: Not mutagenic in the absence or in the presence of the S-9 fraction.

Statistical methods: The information was not provided by the sponsor.

Criteria for Positive Results: 2-fold increase in the mean number of revertants per plate of at least one of the tester strain over the mean number of revertants per plate

RESULTS: The highest concentration was 50 mg/ml which provided a final concentration of 5000 µg/plate. The test article proved to be not toxic to the bacterial strains at the top dose. HOE901 did not cause a significant increase in the number of revertant colonies with any of the tester strains either in the absence or in the presence of S9-mix. There was no HOE901 dose-dependent effect.
Study Validity: The studies appear valid since the data were reproducible and all positive control produced significant increases in the number of revertant colonies.
Study Outcome: Negative

SUMMARY:
HOE901was not mutagenic in the bacterial strain tested with either with or without exogenous metabolic activation at the dose levels used.

II. Study Title: HOE901-In vitro mammalian chromosome aberration test in V79 Chinese hamster cells
Study No/Document No/Report No: 97.0135/016525 /97.0448
Study Type: Chromosome aberration test
Amendment #, Volume #59 and Page #80-115
Conducting Laboratory: Hoechst AG, Pharma Development Corporate Toxicology, Frankfurt Germany

Date of Study Initiation/completion:6/17/1997--2/13/1997
GLP Compliance: yes
QA- Reports Yes (yes by ———— No ( ):
Drug Lot Number: N001 and retention sample, L00173/006
Study Endpoint: Identification of chromosomal aberrations such gap, break, fragment, minute, deletion, and/ or exchanges

METHODOLOGY:
Strains/Species/Cell line: Mycoplasma-free V79 cell line in liquid nitrogen
Dose Selection Criteria: Cytotoxicity which was determined by photometric measurements of V79 cell cultures bred in microwell plates and stained with crystal violet
Basis of dose selection: The highest dose should reduce the survival rate to 20-50% and/or the mitotic index to approx. 50% compared with the corresponding solvent control.

Range finding studies: A preliminary toxicity test was performed for dose range finding study. Cytotoxic effects were determined by photometric measurement.
Test Agent Stability: Stability in the solvent was confirmed over 4 hours by HMR Germany, Pharma Qualtiy Control, on 8/1/1996.
Metabolic Activation System: Frozen S-9 fraction was used(Batch no. 8/26) from Aroclor 1254 pretreated rat liver extract.

CONTROLS:
Vehicle: Cell culture medium was minimal essential medium with Hanks-salts and 25 mM Hepes-buffer.
Negative Controls:
a. Untreated control
b. Solvent controls
Positive Controls:
a. Without S-9 fraction: Ethyl methane sulfonate(Batch 40606721) 500 µg/ml
b. With metabolic activation: cyclophosphamide(Batch 114555) 3.0 µg/ml

Exposure Conditions: Mycoplasma-free V79 cell lines of Chinese hamster lung fibroblasts were exposed to 4% CO_2 at 37°C in plastic flasks.
Incubation and sampling times: 18 hours after onset of treatment with HOE901 and 2 more hours after colcemide addition followed by washing and staining
Doses used in definitive study: 0, 1, 2.5, 5, 10, 25, 50, and 100 µg/plate with/out S9-mix
STUDY DESIGN: The highest dose should reduce the survival rate to 20-50% . The solvent control data are within the laboratory's normal control range for mutant frequency and the positive controls should cause a significant increase the frequency of aberrations.

ANALYSIS: The assay was considered valid if the solvent control data were within the laboratory's normal range for the spontaneous mutant frequency. And the positive controls had increased mutation frequency which was significant within normal range.
No slides/plates/replicates/animals analyzed: Only metaphases with 22+/- 1 chromosomes are included in the analysis.
Counting method: Chromosomal aberrations were counted and classified.
Cytotoxic endpoints: Statistically significant increase in the aberration rate(without gaps) with one or more of the concentrations tested as compared with the solvent controls, which was reproducible and concentration-related.
Genetic toxicity endpoints/results: Test article was not clastogenic at 100 µg/ml in the absence or in the presence of the S-9 fraction.
Statistical methods: Biometry of the results was performed with a one-sided Fisher-Exact test.

Criteria for Positive Results: Test article would be positive if 1)it induces a reproducible statistically significant increase in the aberration rate (without gaps) with one or more of the concentrations tested, and 2) there is a reproducible concentration-related increase in the aberration rate.
RESULTS: HOE901 was not mutagenic in this chromosome aberration test in vitro with cells of the V79 Chinese hamster cell line,
Study Validity: The test compound was not cytotoxic in the absence or in the presence of S-9 mix at the maximum solubility limit ———— . It appears that the chromosomal abberration test was performed in acceptable conditions. Statistical analysis procedure and criteria for valid assays were reasonable.
Study Outcome: HOE901 was not clastogenic in this chromosome aberration test in vitro with cells of the V79 Chinese hamster cell line,
SUMMARY: HOE901 was not clastogenic in this chromosome aberration test in vitro with cells of the V79 Chinese hamster cell line as summarized on the next page.

Main experiment Test group	Dose µg/ml	S9-mix	fixation interval (h)	Number of cells analysed 1	2	per cent aberrant cells incl. gaps mean 1+2	excl. gaps mean 1+2	exchanges mean 1+2
Solvent control d.-d. water	0.0	-	20	100	100	1.5	0.5	0.5
Hoe 901	25.0	-	20	100	100	2.0	1.0	0.5
Hoe 901	50.0	-	20	100	100	0.5	0.0	0.0
Hoe 901	100.0	-	20	100	100	1.5	1.0	0.0
Positive control EMS	500.0	-	20	25	25	48.0	48.0	22.0
Solvent control d.-d. water	0.0	+	20	100	100	1.5	1.0	0.5
Hoe 901	25.0	+	20	100	100	2.0	1.0	0.0
Hoe 901	50.0	+	20	100	100	1.0	0.5	0.0
Hoe 901	100.0	+	20	100	100	1.5	0.5	0.0
Positive control CPA	3.0	+	20	25	25	36.0	34.0	18.0
Solvent control d.-d. water	0.0	-	28	100	100	2.5	2.0	0.0
Hoe 901	100.0	-	28	100	100	4.0	2.0	0.0
Solvent control d.-d. water	0.0	+	28	100	100	3.5	2.5	0.0
Hoe 901	100.0	+	28	100	100	4.0	1.5	1.0

Repeat Test group	Dose µg/ml	S9-mix	fixation interval (h)	Number of cells analysed 1	2	per cent aberrant cells incl. gaps mean 1+2	excl. gaps mean 1+2	exchanges mean 1+2
Solvent control d.-d. water	0.0	-	20	100	100	2.0	0.5	0.0
Hoe 901	25.0	-	20	100	100	1.5	0.5	0.0
Hoe 901	50.0	-	20	100	100	2.5	1.5	0.0
Hoe 901	100.0	-	20	100	100	3.0	1.0	0.0
Positive control EMS	500.0	-	20	50	50	30.0	29.0	17.0
Solvent control d.-d. water	0.0	+	20	100	100	0.0	0.0	0.0
Hoe 901	25.0	+	20	100	100	1.0	0.5	0.0
Hoe 901	50.0	+	20	100	100	1.5	1.0	0.0
Hoe 901	100.0	+	20	100	100	1.5	1.0	0.5
Positive control CPA	3.0	+	20	50	50	22.0	22.0	11.0
Solvent control d.-d. water	0.0	-	28	100	100	2.5	1.5	0.0
Hoe 901	100.0	-	28	100	100	5.5	3.5	0.5
Solvent control d.-d. water	0.0	+	28	100	100	1.5	1.0	0.0
Hoe 901	100.0	+	28	100	100	3.5	2.0	1.0

III. Study Title: HOE901-Chromosome aberrations in vivo cytogenetic test in bone marrow cells of the Chinese hamster
Study No/Document No/Report No: 93.0347/012798 /93.0504
Study Type: Chromosome aberration test
Amendment #, Volume #59 and Page #152-175:
Conducting Laboratory: Hoechst AG, Pharma Development Corporate Toxicology, Frankfurt Germany
Date of Study Initiation/completion:6/1/1993/7/6/1993

GLP Compliance: yes
QA- Reports Yes (yes by [redacted] No ( ):
Drug Lot Number: Batch No.A002
Study Endpoint: Identification of aberrations such gap, break, fragment, minute, deletion, and/ or exchanges

METHODOLOGY:
Strains/Species/Cell line: Han:Chin/Five hamsters/sex/group
Dose Selection Criteria: Animal toxicity
Basis of dose selection: The highest sublethal dose of 750 IU/kg was selected for the main study, based on a preliminary range-finding study.

Range finding studies: In a preliminary study subcutaneous administration of 1000 IU HOE901 per kg had caused lethality in female hamsters.
Test Agent Stability: Stable until 10/1993 and guaranteed for 4 hours at room temperature as reported(8/25/1993).

CONTROLS:
Vehicle:85% glycerol [redacted] mg of m-cresol, and HCl(pH=4.0)
Negative Controls:a. untreated control and b. solvent controls
Positive Controls: Cyclophosphamide and Endoxan^{R} 50 mg/kg(subcutaneous)
Exposure Conditions: Please see Incubation below.
Incubation and sampling times: Bone marrow samples were taken out at 12, 24 or 48 hours after treatment.

Doses used in definitive study: 0 and 750 IU/kg
STUDY DESIGN: The test substance with and without appropriate positive control agents was given to the animals in a dose of 750 i.u./kg bodyweight. Individual bone marrow preparation was fixed on slides and the set of chromosome was examined for chromosomal aberrations. The drug would be classified as clastogenic if it induced a significant increase in aberration rate.
ANALYSIS: Chromosomal aberrations were classified as gap, break, fragment, minute, deletion and exchanges, etc.
No. slides/plates/replicates/animals analyzed: 50 metaphases per animal were examined.
Counting method: Evaluation was determined by counting the aberration rate(excluding gaps).
Cytotoxic endpoints: Hamsters' lethality.
Genetic toxicity endpoints/results: The metaphases were examined for the following aberrations: gap, break, fragment, minute, deletion, exchanges including intrachanges, dicentrics, chromosome disintegration, ring formation and polyploidy.
Statistical methods: The sponsor did not provide this information.
Other: Biometry was not performed because the mean chromosome aberration rates in the substance groups were in the range of the negative control values.
Criteria for Positive Results: The test article was classified as clastogenic if it induced a significantly increased aberration rate(excluding gaps) at least one of the time points.

RESULTS: Animals from each group were killed 12, 24, or 48 hours after treatment of 750 IU/kg HOE901. Examinations for chromosomal aberrations in bone marrow cells

revealed no remarkable abnormalities. Five hamsters/sex were examined and 50 metaphases per animal were evaluated.

Study Validity: This study was conducted in acceptable conditions since the positive control produced various types of aberrations.

Study Outcome: The results lead to the conclusion that HOE901 is not clastogenic in the in vivo cytogenetic test in bone marrow cells of Chinese hamster as summarized below.

Table 1. Summary: Percentage of metaphases with aberrations per trial group

(10 animals per group; 50 metaphases per animal)

Trial group	Dose /kg bodyweight	Killing time hours after admin.	Metaphases with aberrations inclusive gaps		Metaphases with aberrations exclusive gaps		Metaphases with exchanges
			Mean	SD	Mean	SD	
negative control	0 i.u.	12	0.4	0.84	0.0	0.0	0.0
HOE 901	750 i.u.	12	1.6	2.64	0.0	0.0	0.0
negative control	0 i.u.	24	0.8	1.40	0.2	0.64	0.0
HOE 901	750 i.u.	24	1.4	1.64	0.0	0.0	0.0
Endoxan®	50 mg	24	14.0	3.52	12.8	3.42	5.2
negative control	0 i.u.	48	1.4	1.64	0.0	0.0	0.0
HOE 901	750 i.u.	48	1.0	1.42	0.0	0.0	0.0

IV. Study Title: HOE901-Detection of gene mutations in somatic cells in culture HGPRT-test with V79 Cells
Study No/Document No/Report No: 94.0274/013566 /94.0576
Study Type: Gene mutation test
Amendment #, Volume #59 and Page #116-143
Conducting Laboratory: Hoechst AG, Pharma Development Corporate Toxicology, Frankfurt Germany
Date of Study Initiation/completion:6/8/1994/7/27/1994
GLP Compliance: yes
QA- Reports Yes (yes by [redacted]; No ( ):
Drug Lot Number: Batch No. D001
Study Endpoint: Identification of mutant colonies, which do not synthesize HGPRT

METHODOLOGY:
Strains/Species/Cell line: Mycoplasma-free V79 cells
Dose Selection Criteria: The solubility of the test substance limits to [redacted] µg/ml.
Basis of dose selection: In the cytotoxicity experiment HOE901 was not toxic. Based on the finding, 100 µg/ml was determined in the absence/presence of S9-mix as the maximum dose level for mutagenicity tests.
Range finding studies: HOE901 was not toxic to the V79 cells in the absence/presence of S9-mix up to the limit of its solubility.
Test Agent Stability: The sponsor provided that the date of retesting was April, 1995.

CONTROLS:
a. Vehicle: Deionized water
b Negative Controls: Untreated control and Vehicle
c. Positive Controls:
1)Without S9-mix
EMS(Ethylmethanesulfonate) dissolved in the culture medium, 1 mg/ml=8 mM
2)With S9-mix
DMBA(9,10-dimethyl-1,2benzanthracene) dissolved in DMSO, 7.7 µg/ml= 30µM
Exposure Conditions: Under 4% CO_2 at 37°C
Incubation and sampling times: 4 hours and 9 days after subcultures were seeded.
Doses used in definitive study: 10, 25, 50, and 100 µg/ml

STUDY DESIGN: Two independent assays for mutation to 6-thoguanine resistance were performed in the absence of metabolic activation and three assays were performed in the presence of S9-mix. In the absence/presence of S9-mix, dose levels of 10, 25, 50, and 100 µg/ml were employed in all mutation assays.
ANALYSIS: Number of mutant colonies were counted in the absence/presence of S9-mix and mutation frequency (mutant colonies per 1 million cells) was calculated for statistical analysis.
No. slides/plates/replicates/animals analyzed: 6 wells per dose were examined.
Counting method: Mutant colonies of more than 50 cell were counted microscopically.
Cytotoxic endpoints: HOE901 was not cytotoxic at a dose of solubility limit.
Genetic toxicity endpoints/results: Please see the section of Results below.
Statistical methods: The biometry of the results was performed with the MANN-WHITNEY-U-TEST.

Criteria for Positive Results: The test article was classified as mutagenic if it induced a mutation frequency 3 times higher than the spontaneous mutant frequency reproducibly with one of the test substance concentrations.
RESULTS: No relevant reproducible enhancement of the mutant colonies or mutant frequency over the range of the solvent control was found with any of the concentrations used in the absence of S9-mix. In the presence of S9-mix, statistically significant increases were observed at the concentrations of 25 and 100 μg/ml in the first experiment. This effect was not dose-dependent and not reproduced in the second and third experiments.

Study Validity: This study was conducted in acceptable conditions since the positive control induced the number of colonies by 10 to 100 times of relevant controls.
Study Outcome: The results lead to the conclusion that HOE901 is not mutagenic in the HGPRT-test with cells of the V79 Chinese hamster cell line. The top dose for toxicity test was limited by poor solubility of the test article and two tests were negative while the first test was partially positive. It is reasonable to conclude that mutagenic potential of HOE901 is negative since Ames test also supports its view.

NDA Summary:

HOE901(Insulin Glargine Injection) is a human insulin analogue, which is 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. Preclinical studies indicate that HOE901 did not have remarkable cardiovascular, pulmonary, neurological, and renal effects in mice and rats at least 5 to 10 times of clinically relevant doses, although high doses produced numbers of systemic responses to drug-induced hypoglycemia, which was an extension of its pharmacodynamic action.

Toxicology studies in mice and rats revealed that HOE901 had no consistent drug-related adverse effects at doses which were 5 to 10 times higher than the clinical dose, except sedation, coma, and even deaths due to its hypoglycemic action. Data of blood glucose levels often were not in agreement with the hypoglycemia, which might be due to differences in blood sampling time and neutralizing antibody titers. Pharmacology study indicated that insulin glargine might be neutralized in subcutaneous tissue due to its acidity, leading to formation of microprecipitates. This might allow once-daily dosing to meet a patient's basal insulin needs.

The sponsor performed a combined fertility and prenatal and postnatal study, and an embryotoxicity study in Himalayan rabbits. Maternal toxicity due to HOE901-induced hypoglycemia was noted with some deaths, which was also reproduced by human insulin(HOE36H) due to hypoglycemia. Disregarding the pharmacodynamic drug effect (decrease in blood glucose), the NOAEL for the parental females was 0.108 mg/kg and for the parental males >0.360 mg/kg, corresponding to doses of approximately 3 and 10 IU per kg body weight, respectively. Embryotoxicity study showed that rabbits proved to be the most sensitive species. Due to dose-dependent hypoglycemia, maternal toxicity

(hypoglycemic shock, total litter loss) and embryofetal toxicity occurred in the middle- and high-dose groups.

HOE901 was not genotoxic in Ames test, V79 Chinese hamster ovary cells, gene mutations in somatic cells in culture HGPRT-test with V79 cells,
and bone marrow cytogenetic chromosomal aberration tests.

The two-year standard carcinogenicity studies were performed in mice and rats. Rats and mice were administered HOE901 orally at doses of 0.073, 0.182 and 0.455 mg/kg/day. Carcinogenic potential of HOE901 was negative in male mice since the submitted data were obtained from valid study and the results are justifiable. However, in female mice, there were insufficient number of animals at necropsy due to high mortality, although the deaths occurred on or around 1.6 years. The reviewer believes that the outcome of female mouse data was not conclusive due to high mortality in saline control group. This indicates that the study might not be valid for females.

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The 2-year carcinogenicity study with HOE901 in rats appeared to be valid based on the MTD in the range finding studies. HOE901 produced histiocytomas at injection sites in male rats but not females, which testified that the vehicle was probably not responsible for the tumors. However, it is true that the histiocytomas were found at the local injection sites of males in vehicle containing groups. 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. Thus, any morphological changes in injection sites should be closely monitored in patients receiving the drug.

Label Recommendation:

Carcinogenesis, mutagenesis, impairment of fertility

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Insulin glargine was not mutagenic in tests for detection of gene mutations in bacteria and mammalian cells (Ames- and HGPRT-test) and in tests for detection of chromosomal aberrations (cytogenetics in vitro in V79 cells and in vivo in Chinese hamsters).

The following two paragraphs were moved to Pregnany Category and modified (See below).

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Pregnancy

Teratogenic effects: pregnancy category —

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Subcutaneous reproduction and teratology studies have been performed with HOE901 and regular human insulin(HOE36H) in rats and Himalayan rabbits. The drug was given to female rats before mating, during mating, and throughout pregnancy at dose up to 0.36 mg/kg/day(approximately 7 times the recommended human subcutaneous starting dose of 0.008 mg/kg/day, based on ——— . In rabbits, doses of 0.072 mg/kg/day (approximately 2 times the recommended human subcutaneous starting dose, — was administered during organogenesis. The effects of HOE901 did not generally differ from those observed with regular human insulin in rat or rabbits.

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There are no well-controlled clinical studies of the use of insulin glargine in pregnant women. It is essential for patients with diabetes or a history of gestational diabetes to maintain good metabolic control before conception and throughout pregnancy. Insulin requirements may decrease during the first trimester, generally increase during the second and third trimesters, and rapidly decline after delivery. Careful monitoring of glucose control is essential in such patients. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing mothers

It is unknown whether insulin glargine is excreted in significant amounts in human milk. Many drugs, including human insulin, are excreted in human milk. For this reason, caution should be exercised when LANTUS is administered to a nursing woman. Lactating women may require adjustments in insulin dose and diet.

OVERALL SUMMARY AND EVALUATION:

Introduction:

Conventional long-acting insulin preparations are amorphous or crystalline insulin suspensions with protamine (NPH) and/or zinc as complexing agents. NPH insulin treatment often results in nocturnal hypoglycemic events due to unwanted plasma insulin peaks during the night. The absorption rate from the subcutaneous tissue and duration action are known to variable. Thus, it has been not easy to titrate accurately the blood glucose level for long-term care. A good regulation of glucose metabolism with insulins or antidiabetic agents is essential for the prevention of diabetic absorption rate from the subcutaneous tissue and duration action complications such as retinopathy, neuropathy, nephropathy, etc.).

HOE901(Insulin Glargine Injection) is a human insulin analogue, which is 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. In human, HOE901 was absorbed from the injection site significantly more slowly than NPH insulin when injected subcutaneously. Available data indicate that the in vitro and in vivo actions of HOE901 are similar to those of human insulin in lowering blood glucose, which might be suitable for once daily subcutaneous injection to provide a basal supply of insulin for patients with diabetes mellitus requiring insulin therapy. Pharmacology data support the idea that insulin glargine might be neutralized in subcutaneous tissue due to its acidity, which might allow once-daily dosing to meet a patient's basal insulin needs.

Safety Evaluation:

Data suggest that HOE 901 appeared to have little systemic effects except hypoglycemia as an extension of its pharmacodynamic action. HOE901 had no remarkable neurological, renal, cardiovascular effects in mice and rats at 10 IU/kg or in anesthetized rats and dogs at 3 IU/kg. Toxicology studies in mice and rats revealed that HOE901 had no consistent drug-related adverse effects at doses which were 5 to 10 times higher than the clinical dose, except sedation, coma, and even deaths due to its hypoglycemic action. Data of blood glucose levels often were not in agreement with the hypoglycemia, which might be due to differences in blood sampling time and neutralizing antibody titers.

The sponsor performed a combined fertility and prenatal and postnatal study, and an embryotoxicity study in Himalayan rabbits. Maternal toxicity due to HOE901-induced hypoglycemia was noted with some deaths, which was also reproduced by human insulin(HOE36H) due to hypoglycemia. Disregarding the pharmacodynamic drug effect (decrease in blood glucose), the NOAEL for the parental females was 0.108 mg/kg and for the parental males >0.360 mg/kg, corresponding to doses of approximately 3 and 10 IU per kg body weight, respectively. Embryotoxicity study showed that rabbits proved to be the most sensitive species. Due to dose-dependent hypoglycemia, maternal toxicity (hypoglycemic shock, total litter loss) and embryofetal toxicity occurred in the middle- and high-dose groups. HOE901 was not genotoxic in Ames test, V79 Chinese hamster

ovary cells, gene mutations in somatic cells in culture HGPRT-test with V79 cells, and bone marrow cytogenetic chromosomal aberration tests

The 2-year carcinogenicity studies 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 studies in female mice may not be valid because of high mortality of the animals. The findings should be stated in label appropriately. The 2-year carcinogenicity studies were carried out under acceptable conditions in rats. The malignant fibrous histiocytoma at the injection sites occurred in higher incidences in placebo control and HOE901 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 of malignant fibrous histiocytoma. The relevance of the animal finding to human is not known but examinations at the injection sites should be monitored in clinical use.

Clinical Relevance of Safety Issues:

In a standard 2-year carcinogenicity studies, malignant fibrous histiocytomas at the injection sites were observed in higher incidences in placebo control and HOE 901 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 the female rats, there was no significant increase in the malignant fibrous histiocytoma and human insulin (NPH) in a different vehicle (pH=7.0) did not produce the tumor. Therefore, it appears that vehicle but not the test article might promote the incidence of malignant fibrous histiocytoma. The relevance of the animal finding to human is not known, but examinations at the injection sites should be monitored in clinical studies.

Other Clinically Relevant Issues:

The acidity (pH=4) of HOE 901 vehicle might produce potentially discomfort at local injection sites.

Conclusions:

According to the sponsor's data presented in the NDA, HOE 901 had minimal toxicities in systemic pharmacology and toxicology in laboratory animals, although it exerted clearly the drug-dose dependent hypoglycemia, including coma and some deaths. Reproductive, genetic, and immunotoxicity studies were performed acceptably, which show that the potential toxic risk is rather small. The 2-year carcinogenicity studies in male mice and female rats are negative, although the results in female mice are not conclusive. In male rats, malignant fibrous histiocytoma at the injection sites occurred in higher incidences in placebo (vehicle) control and HOE 901 treated groups but not in saline control in male. The relevance of the animal finding to human is not known but examinations at the injection sites should be monitored in patients.

COMMUNICATION REVIEW: None

Labeling Review (NDA): Please see Label recommendation on page 58.
Investigator's Brochure/Informed consent review (IND): NA

RECOMMENDATIONS:

Internal comments: NA
External Recommendations (to sponsor):

[ ]

The submitted data might be justifiable to indicate that HOE901 was not carcinogenic in male mice. The reviewer believes that the outcome of female mouse data was not conclusive due to high mortality in all female groups including the saline control group. Some of the deaths occurred at or around 18 months from the onset of the study. But, the reviewer as well as reviewing statistician do not have the relevant and acceptable histopathological findings from the dead animals when they died.

Future development or NDA issues: None

DRAFT LETTER CONTENT FOR SPONSOR:

[ ]

Reviewer signature/team leader signature [Concurrence/Non-concurrence]

/S/

Herman M. Rhee, Ph. D.
Pharmacology Reviewer

/S/ V 2/3/00

Ronald W. Steigerwalt, Ph.D.
Pharmacology Team Leader

See TL memo to file. Comment on previous page will be discussed with sponsor during labeling negotiations. No need to send letter

cc: Original NDA
HFD-345
HFD510/Steigerwalt/H. Rhee/J. Rhee
Review Code: AE
Filename: ————

Draft date (# of drafts): 1/27/2000(4)
Memorandum of Non-concurrence (if appropriate, attached): None
Addendum to review (if necessary): None
Appendix/attachments:
1) Executive CAC Report
2) Final electronic copies of previous reviews(not signed)

APPEARS THIS WAY
ON ORIGINAL

Executive CAC
Date of Meeting: Dec. 21, 1999

Committee: Paul Andrews, Ph.D., HFD-150, Acting Chair
Joseph Contrera, Ph.D., HFD-900, Member
Robert Osterberg, Ph.D., HFD-520, Alternate Member
Ronald Steigerwalt, Ph.D., Team Leader
Herman Rhee, Ph.D., Presenting Reviewer

Author of Minutes: Herman Rhee, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

IND/NDA #: NDA 21081
Drug Name: Lantus (Insulin glargine injection)
Sponsor: Hoechst Marion Roussel, Inc.

Mouse Carcinogenicity Study or Mouse Dose Selection

In 3-month dose range finding studies with HOE 901 (Batch T3885), 10 NMRI mice/sex/group received 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 appeared to 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 12.5 I.U./kg was considered the MTD and an appropriate high dose for the 2 year bioassay. The top dose is approx. 8 times higher than the clinical dose (10 I.U.), based on body surface comparison. One I.U. of insulin is equivalent to approximately 0.04 mg.

In the 2 year carcinogenicity study in NMRI mice, hepatocellular adenomas (0,6,5,5,1) or carcinomas (6,7,3,3,1) occurred in male animals in all groups (saline, vehicle, LD, MD, HD). For adenomas, the Fisher exact test shows a positive p-value ($P<0.05$) for the vehicle control group and the low and mid-dose groups compared with the saline control. However, these incidences were within historical control ranges for CD-1 and $B6C3F_1$ mice, and there was no dose dependence for individual tumors or combined tumors. There were only 3 hepatic carcinomas in female mice without other remarkable tumors. The study appears to be valid for males, but the findings in females were inconclusive due to excessive mortality in all groups (only 3 females in the control saline group survived to scheduled necropsy).

Rat Carcinogenicity Study or Rat Dose Selection

In the 3 month 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 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 after the onset of drug administration, which forced the top dose to be reduced to 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 granulation 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 and an appropriate high dose for a life time study. Mid- and low doses were selected as 0.182 and 0.073 mg/kg, respectively including saline control and vehicle control groups.

In the 2 year carcinogenicity study, malignant fibrous histiocytomas at the injection sites were present in males of all groups except saline control and group 6 which had HOE36H, which is regular human insulin. This suggests that the vehicle might be responsible for the tumor incidence in males. As the dose of HOE901 increased, the tumor incidence was reduced. Although there was no statistically significant difference between vehicle (9) and test groups (13, 12, 6) in injection site histiocytoma incidence in males, vehicle and test groups were positive for injection site histiocytomas compared to the *saline* control group which had no tumors (p= 0.005, trend test). The excipients in the vehicles were:
a) Vehicle for test article contained- glycerol — mg, m-cresol 2.7 mg, and — NaOH/HCl (pH=4.0) to make 1 ml with distilled water,
b) Vehicle for human insulin (HOE36H) contained- glycerol — mg, m-cresol — mg, NaH_2PO_4 — mg, phenol — mg, and — NaOH (pH=7.3) to make 1 ml with distilled water.

Executive CAC Recommendations and Conclusions:

1. The Committee considered the two-year carcinogenicity study with HOE901 in rats to be valid based on the MTD in the 3 month and 1 year range finding studies. Although injection site histiocytomas were not seen in female rats, these tumors were found in all groups of male rats that received the HOE901 vehicle. The Committee recommended that the labeling report the histiocytomas found at the local injection site of males in the vehicle containing groups. Any morphological changes in injection sites should be closely monitored in patients receiving the drug.

2. The Committee recommended that the two-year carcinogenicity study with HOE901 in mice be considered adequate for males but inconclusive for females due to excessive mortality. This study and the findings should be reported in the label. The liver adenomas in males were not considered biologically significant because the incidence was within historical ranges and there was no dose dependency.

[ ]

/S/ 1/5/2000

Paul Andrews, Ph.D.
Acting Chair, Executive CAC

cc:\

/Division File, HFD 510
/Team leader, HFD-510
/Reviewer, HFD-510
/CSO/PM, HFD-510
/ASeifried, HFD-024

APPEARS THIS WAY
ON ORIGINAL

WITHHOLD 1 PAGE (S)

IND# ——

Dec. 11, 1995

Sponsor: Hoechst-Roussel Pharmaceuticals Inc., Sommerville, NJ
Tel: (908)231-2385; Fax: (908)231-3879 Dr. James DeMartino

Submission Date: Nov. 13, 1995
Assigned Date: Nov. 17, 1995

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
ORIGINAL REVIEW

1. Drug: HOE901 (rDNA Human Insulin Analogue)

TABLE OF CONTENTS

/S/

Herman M. Rhee, Ph.D.
Review Pharmacologist

/S/ 12/14

cc: Original IND/HFD IND
A. Jordan/H. Rhee

IND# [redacted]

Dec. 11, 1995

Sponsor: Hoechst-Roussel Pharmaceuticals Inc., Sommerville, NJ
Tel: (908)231-2385; Fax: (908)231-3879 Dr. James DeMartino

Submission Date: Nov. 13, 1995
Assigned Date: Nov. 17, 1995

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
ORIGINAL REVIEW

1. Drug: HOE901 (rDNA Human Insulin Analogue)

2. Structural Information: Human insulin was substituted by 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.

3. Pharmacological class: Human Insulin

4. Dosage form: [redacted] 5 ml vials that contain [redacted] The formulations have [redacted] additional components including [redacted] zinc [redacted]

5. Indication: Type I diabetes

6. Clinical: Study objective is to investigate the safety and tolerability of HOE901 in comparison to NPH in a total 240 Type I diabetes. Individually titrated dose of NPH-human insulin or HOE901 will be administered subcutaneously into the abdominal region once at bedtime.

I. PHARMACOLOGIC STUDIES

A. Effects of HOE901 on blood glucose level in rabbits

Seven fasted rabbits(unspecified strain) received HOE 901 at a dose of 0.2 IU/kg by ear vein injection. HOE901 had a faster and shorter blood glucose decreasing activity than regular insulin after an intravenous injection. But, HOE901 produced delayed onset and longer duration of blood glucose decrease after a subcutaneous administration.

B. Effects of HOE901 on blood glucose and plasma-insulin in fasted dogs

Twenty-four male Beagle dogs were fasted 18 hours prior to the study. Eight dogs/group were administered subcutaneously 0.3 IU/kg of regular insulin or one of two HOE901 preparations(HOE 901 with low and high zinc content as marked by HOE71 GT15 or HOE71 GT80). HOE901 had zinc-dependently delayed onset and longer duration of blood glucose decrease.

C. Study on insulin analogues: Characteristics of binding to the insulin receptor and activation of the receptor tyrosine kinase

The sponsor compared HOE 901 with regular human insulin and the insulin analog ASP(B10) insulin with respect to kinetics of receptor binding, activation of insulin receptor autophosphorylation and cellular substrates phosphorylation in fibroblast cells. The results showed that HOE901 behaved with receptor binding and promotion of mitogenesis like regular human insulin. But, ASP(B10) insulin was characterized by slower receptor dissociation rates and induced a prolonged phosphorylation state of the insulin receptor and receptor substrates.

D. Study on insulin analogues: Binding to the IGF-1 receptor and proliferative activity

Insulin and HOE901 were compared with their ability to bind IGF-1 receptors in human osteosarcoma cells(a subline of [illegible]) HOE901 exhibited a 5 to 8 times increased binding to the IGF-1 receptors, which correlates with a similar increase in mitogenic activity.

E. Insulin HOE 71 GT(15) and HOE 71 GT(80): Cardiovascular studies in anesthetized dogs.

Three female beagle dogs/group were administrated intravenously vehicle or vehicle containing HOE 71 GT(15) or HOE 71 GT(80) at a dose of 1 IU/kg. HOE 71 GT(15) caused a slight increase in systemic blood pressure and dP/dt, of which effect might be due to epinephrine release in response to the decrease in blood glucose.

F. Evaluation of the potential immunogenicity of the insulin analogue HOE 901 in rabbits and guinea pigs.

Three male and 3 female New Zealand White rabbits/group were immunized against HOE901 for a period of 26 weeks at weekly intervals with 40 µg per animal of either HOE901 0.54%, HOE 901 3.8%, human insulin, porcine insulin, or bovine insulin, including control vehicle. Three male and 3 female guinea

pigs/group were also immunized subcutaneously for a period of 25 weeks with 20 μg per animal of the same antigens as described above.

All animals except the animals in the control group developed antibodies. Bovine insulin injection produced a high titer of antibodies. Rabbit or porcine insulin produced a weak antibody titer. Similar results were obtained from the study of guinea pigs.

II. TOXICOLOGY

A. HOE 901: Single-dose subcutaneous toxicity study in mice (Doc#013092)

Two male and 2 female NMRI mice was administered subcutaneously a single dose of 1000 IU/kg of HOE901 and they were under observation for 3 weeks. The animals tolerated well without signs of toxicity. Macroscopic examination revealed no remarkable organ findings.

B. HOE901: Single-dose intravenous toxicity study in rats (Doc#011664)

[illegible] male and 2 female non-fasted Wistar rats, strain HOE:Wiskf were administered HOE901 intravenously at [illegible] or 1000 IU/kg. The rats tolerated the dose without signs of intoxication. Macroscopic examination on dissection of the animals killed at the end of the follow-up period revealed no remarkable organ damage.

C. HOE901: Single-dose subcutaneous toxicity in rats (Doc#011668)

Two male and 2 female non-fasted Wistar rats (Strain Hoe:WISKF) were administered HOE901 subcutaneously at a single dose of 1000 IU/kg. The HOE901 injection killed two animals (one each sex) within two days after administration. One of these animals showed the following symptoms: abdominal position, gasping respiration, lacrimation and periods of tremor. Macroscopic examination on dissection of the animals which died and of those killed at the end of the follow-up period revealed no morphological changes.

D HOE901: Subchronic (3-months) subcutaneous toxicity study in mice (Doc#013095)

1. Methods: Ten NMRI BR mice/sex/group were administered HOE901 (Batch T 3885) subcutaneously at doses of 0, 5, 10, or 20

IU/kg for 3 months.

2. Results:

a. Mortality: There were 7 deaths; 2 in low, one in medium, and 3 in high dose group, respectively. Since no animals died or had to be killed intercurrently in the control group these deaths could be compound-related, although the cause of death could not be determined histologically.

b. There were no remarkable changes in body weight gains or in food consumption.

c. There were no significant changes in hematology, clinical chemistry, or histological parameters.

3. Conclusion: Based on mortality data the sponsor concludeded that MTD would be 10 IU/kg.

E. HOE 901: Testing for subchronic (3 months) subcutaneous toxicity in male and female Wistar rats(Doc#013690)

1. Methods: Fifteen Wiskf Wistar rats/sex/group were administered HOE901(Batch #D001 and D001NF) subcutaneously at doses of 0, 0.145, 0.455, and 1.455 mg/kg for 90 days.

a. Mortality: Eleven males and 8 females from the high dose group died during the study. All deaths were due to drug-induced hypoglycemia. One rat in control group died of bacterial infection(Bacillus piloformis). No signs of compound-related neurological disturbances, opacity of the refracting media of the eyes, damage to the oral mucosa or impairment of dental growth were noted in the control or treated groups.

b. Body weight and food consumption were not affected significantly by the treatment.

c. Hematological examinations: There was no dose-dependent decrease in erythrocyte values in both sexes. Leucocyte counts were increased to a statistically significant degree in females of the low dose group, which also appeared to be not dose-related.

d. Clinical chemistry: Sodium levels were reduced in females of the high dose group. Reduced uric acid values was noted in males of the low dose group, which was not dose-dependent.

e. Organ weights: Increases in absolute and relative kidney weights occurred in females of the high dose group, which was not

detected by clinical chemistry or histopathology. Decreases in adrenal weights in females of the low and intermediate dose group were also noted, although there were no treatment-related histopathological changes detectable in the organs.

f. Microscopic and macroscopic findings:

No compound-related macroscopically visible changes were found at necropsy. In four animals of the high dose group, cortical infarction of the brain was evident, which was a morphological phenomenon of prolonged hypoglycemia. In the pancreas of the animals found dead, complete degranulation of the β cells in the islets of Langerhans could be seen as the pharmacological equivalent while in the rats which were sacrificed as scheduled degranulation state was only slight.

3. Conclusions:

Daily administration of HOE901 at the dose of 1.455 mg/kg for 3 months caused severe hypoglycemia, which resulted in death of 18 rats. There was no death at the dose level of 0.455 mg/kg/day. But, pharmacological effects could be demonstrated by histopathological examination. No observed effect level was 0.144 mg/kg/day.

F. Repeated-dose (12-month) subcutaneous toxicity study in rats (Doc#01[illegible]

. Methods:

Thirty SP rats/sex/group were administered HOE901 (Batch #T3885) subcutaneously at doses of 0 or 1.455 mg/kg/day for a year. But, the dose was reduced to 0.728 mg/kg after 12th injection.

2. Results:

a. Mortality: HOE901 killed 6 male and 8 female rats in 16 weeks after its treatment at a dose of 1.455 mg/kg, which forced to reduced to 0.728 mg/kg from Week 17. The total deaths by the end of the study was 20 in male and 14 female rats, respectively. There were only 4 deaths in the control male rats.

b. Clinical signs: No compound-induced behavioral and/or pathological changes were noted.

c. Body weight: HOE901 treated rats showed an increased body weight gain which was significant from study week 2 onwards. In males the difference to the control animals was about 14% and in females about 18%.

d. Organ weights: The relative organ weight was not different between control and HOE 901 treated male rats. In females, the relative weights of lungs, adrenals, brain and pituitary gland were significantly lower than the corresponding control values.

e. Palpation of skin for nodules is summarized below.

Animal No. sex/group	Nodule	First observation/week	Histology
47 female control	left inguinal side	53	mamma/carcinoma
69 male HOE901	inguinal region	24	skin/fibrosarcoma
89 male HOE901	inguinal gland	44	inflammation foreign-body
106 female HOE901	right side	38	mamma/adenoma
117 female HOE901	inguinal gland	48	mamma/carcinoma

f. Non-neoplastic findings: In the animals of the dosed group killed at the end of study, β-cell degranulation was found in the Langerhans' islets of pancreases. Toxic changes induced by the compound were not detected. Neoplastic changes observed in both the control and the treated group(tumors of the inguinal mammary gland, adenomas of the anterior pituitary and one instance each of hepatocellular adenoma and a fibrosarcoma in the subcutis). Other histological differences were not noted between control and medicated group.

g. Histological examinations: The fatalities in the dosed group were caused by compound-related hypoglycemia which lead to cardiovascular failure. Remarkable toxic changes induced by the compound were not detected. Neoplastic findings observed in both the control and treated group under item "e" above appear to be strainspecific.

3. Conclusion: The preliminary study may support the contention that HOE901 does not seem to have a carcinogenic potential in Sprague-Dawley rats. The daily dose of 1.455 mg/kg appears to be higher than the MTD.

G. Testing for toxicity by repeated(3-month) subcutaneous administration to Beagle dogs(Doc#013559)

1. Methods: Four Beagle(HOE:BEAK strain) dogs/sex/group were administered HOE901(batch# D001) subcutaneously at doses of 0.036, or 0.182 mg/kg for 90 days.

2. Results:

a. Mortality: All dogs survived until the scheduled end of the study.

b. Clinical signs: No compound-related impairment was observed. Testing for reflex excitability, postural reactions and hearing revealed no changes as compared with the initial status. No compound-related dental or ophthalmological changes were noted.

c. Food consumption: HOE 901 did not impair food consumption since there was no clear difference in the parameter between the control and treated groups.

d. Body weight: The individual development in the body weight curves of all dogs treated with HOE901 was essentially identical to that of the control curves.

e. Hematological findings: No remarkable changes in the measured parameters were noted after repeat treatment. An exception would be increased hemoglobin and hematocrit in high dose group males, although the values were within the normal physiological limits.

f. Clinical chemistry: In the high dose group animals, HOE901 produced hypoglycemia in 2-3 hours after its administration, which returned to the initial range approximately 20 hours after the administration. Alkaline phosphatase was increased in the females of the high dose group, which was reversible. None of the other parameters measured offered any indications of compound-related changes.

g. Urinalysis: No compound-related abnormalities could be detected.

h. Post mortem findings:

1) Macroscopic organ findings: No compound-related alterations were found at necropsy. Edema and bloody inhibition were common at drug injection sites, which was reversible.

2) Organ weight changes: There were no testing substance-induced abnormal findings.

3) Microscopic findings: There were no remarkable compound-

related abnormal alterations.

3. Conclusions:

HOE 901 administration did not induce substance-related abnormal effects in single daily doses of 0.036 and 0.182 mg/kg for 90 days. There were no clear sex-dependent differential actions of the testing substance.

III. REPRODUCTIVE STUDIES

A. Subcutaneous embryotoxicity study of HOE 901 and the reference drug HOE 36H in Wistar rats(Doc#013928)

1 Methods:

Twenty inseminated Wistar female rats/group were administered HOE901(Batch D001) subcutaneously at doses of 0, 0.072, 0.227, or 0.720 mg/kg/day from Day 7 to Day 18 of pregnancy. The last group of rats was given similarly 6.3 IU/kg of HOE36H(Batch L089) for comparison.

2. Results:

a. General findings: All the 20 mated females/group in [illegible] survived until scheduled caesarean section on Day 21. They did not show any impairment of behavioral or neurological signs during the course of the study.

b. Food consumption: HOE901 or HOE36H did not impair consumption of food in all groups. The reference drug HOE36H also had no effect on food consumption of the dams.

c. Body weight: Likewisely, dams' body weight was not affected by the administration of the test compound or the reference drug.

d. Blood glucose: In the high dose group, HOE901 reduced blood glucose within one hour of the administration, which returned to normal glucose level in 2 hours. The mid-dose of HOE901 had little or no effect on blood glucose levels.

e. Findings at caesarean section: At least 18 dams out of total 20 mated females were pregnant in each group. The number of corpora lutea and the number of implants in the dams of the HOE901 groups and the HOE 36H group did not differ from the number in the control animals. The number of live fetuses in the dams of treated groups did not deviate from that in the dams of the control group.

f. Findings of the fetuses: The fetuses delivered by caesarean

section in the drug treated groups showed normal state of physical development as in the control group. The mean body weights of the high dose group and the body lengths of the fetuses in all HOE group did not differ from the concurrent control values. The sex ratio in the fetuses was relatively balanced in all groups.

g. Dead embryofetal primordia: There was one dead fetus in a dam of the high dose group. In one litter of the HOE 36H group there was one markedly stunted dead fetus among three conceptuses undergoing resorption and ten live fetuses. The overall number of dead embryofetal primordia in the dams of the HOE 901 groups and the HOE 36H group did not differ from that in the concurrent control group.

h. The placentas of the fetuses in the drug treated groups were macroscopically unremarkable and their weights did not differ appreciably from those of the placentas of the control group.

i. Morphological examination: A fetus in the mid-dose group exhibited an encephalocele in the fronto-parietal region of the skull. Parts of the brain tissue lay between skull bones and the scalp. No malformations were observed in the 0.072 and 0.72 mg/kg groups.

j. Necropsy findings: In one fetus of the high dose group blood was found in the pericardium. In a [illegible] fetus of the HOE 36H [illegible] the inferior and medial lobe of the right lung were completely fused and in one control fetus the middle lobe of the right lung was bipartite. There was one instance of a hematoma in the liver of a fetus from the high dose group; one fetus each in the high dose group and in the control group displayed a unilateral distension of the renal pelvis.

k. Fetus skeletons: Skeletal ossification of fetuses in all groups was comparable. The percentage of fetuses showing slight or non-ossification of individual skull bones was between 18.0% and 23.3% in the dosed groups versus 19.4% in the control group. As to skeletal defects, one fetus in the high dose group and two fetuses in the HOE 36H group but also two fetuses in the control group exhibited dysplastic, longitudinally displaced or fragmented sternebrae. Waved and/or thickened ribs were observed in two or four fetuses per group in all 5 groups. Many fetuses from the drug treated groups had a short or normal length 14th rib on the first lumbar vertebra, uni- or bilateral. But, the number of fetuses affected in drug treated groups (24 - 42%) significantly exceeded the number of control fetuses with the same finding(10%).

l. Autopsy of the dams: The final autopsy revealed moderate uni- or bilateral renal pelvic dilatation in four dams of the low dose, in one dam each of the mid and high dose groups. In the

high dose group, renal pelvic dilatation was slight and unilateral.

3. Conclusions:

Subcutaneous administration of HOE901 produced a transient hypoglycemia for one to 3 hours of the administration, which did not produce apparent maternal or embryofetal toxicity. There was no compound-related malformations in any of the HOE 901 treated animals.

B. Subcutaneous embryotoxicity study of HOE901 and reference drug HOE36H in Himalayan rabbits(Doc# 013939)

1. Methods: Twenty mated female Himalayan rabbit/group were administered HOE901(Batch #D001) subcutaneously at doses of 0, 0.018, 0.036, 0.072 mg/kg/day, or 1 IU HOE 36H from Day 6 to Day 18 of pregnancy.

2. Results:

a. Clinical Signs: Two dams in the high dose group had to be killed on Day 11 and 12 of pregnancy. The animal killed on day 11 exhibited a motoric attack within 3 hours of the 5th and 6th administration lasting for a few minutes. The animal killed on day 12 of pregnancy showed similar motoric attack (banging against wall and/or somersaulting). One dam in the HOE36H group exhibited two short motoric attacks on Day 11 of pregnancy within 3-4 hours of the 6th administration. The animal died on Day 16 of pregnancy after several more motor attacks. All the other animals survived until day 29 of pregnancy without remarkable findings being observed, although there were frequent vaginal discharge of blood.

b. Food consumption: The amount of food consumed by the dams in drug treated group was comparable to that consumed by the control animals.

c. Body weight: Dams' body weights were not affected by the administration of HOE901 or the reference drug HOE36H. In some animals from the high dose group periodic reduction of body weight gain, which was due to an increase in the intrauterine death rate of their conceptuses.

d. Blood glucose: In the 0.018 mg/kg and in the 0.036 mg/kg HOE 901 groups, there was a maximum decrease in glucose levels of 31% and 56%, respectively, after 13th administration. In the high dose group, blood glucose reduced to 62% of the value preceding dosing and remained at a low level for more than 6 hours.

e. Examinations after caesarean section: In the 0.036 mg/kg HOE901 group, an abortion in one dam was confirmed at caesarean section. This dam had nine corpora lutea in the ovaries and in the uterus three empty implantation sites beside five conceptuses undergoing resorption. In the high dose group, two dams aborted. One instance of abortion was diagnosed by presence of 3 corpora lutea gravidities in the ovaries. In the case of the second abortion five corpora lutea were present in the ovaries and two empty implantation sites and two conceptuses undergoing resorption were found in the uterus.

In the HOE 36H group there were two intercurrent deaths, one abortion and one instance of intrauterine deaths only. The number of live fetuses in the low and mid dose groups did not differ from that of the control group. In the dams of the high dose group, the number of live fetuses was somewhat reduced.

f. Examination of the fetuses: The mean body weights and lengths of live fetuses delivered in the treated groups did not differ appreciably from the corresponding values in the control group. The sex ratio of the fetuses was balanced in the low and mid dose groups. In the high dose group the female fetuses were more than the males.

g. Dead embryofetal primordia: In the dams of the low and mid dose group, the number of conceptuses undergoing resorption was within the same range as that in the control group. In the high dose group the number of dead embryofetal primordia undergoing resorption was slightly elevated compared to that in the control group.

h. Placentas: The weights of placentas in the treated groups did not differ from those of the control fetuses.

i. Survival rate of the fetuses in all groups including the control group was 100%.

j. Morphological examination of live fetuses: Essentially there was no malformation in the control and low dose group fetuses. In the mid dose group, one fetus exhibited asymmetry of the visceral cranium and the lens of the left eye was reduced in size. In another fetus from the same group one forepaw was bent lateral in the carpal joint. In the high dose group, one fetus showed an epactal bone between the paired nasal and frontal bones.

k. Examinations at autopsy and organ cross-section:

Five fetuses from the high dose group had slightly dilated ventricles of the brain(see Tab. 1). In one fetus from the low dose group and in four fetuses from the high dose group, a hemorrhage in the thoracic cavity was seen(Tab. 1). This sort of findings was not unique to the treated group since blood was seen

in the orbital cavity in three control fetuses.

1. Skeletons of the live fetuses: A relatively large number of fetuses in the dose groups and in the control group exhibited sternebrae which were either poorly ossified or were not yet ossified. In one control fetus the metacarpal bone of both paws was not yet ossified. In a single fetus from the low dose group, the left 12th rib was shortened. Two fetuses in the mid dose group and two control fetuses exhibited a short additional rib at the 7th cervical vertebra left or bilaterally.

m. Autopsy of the dams: The final autopsy revealed no remarkable organ abnormalities in any of the dams from the treated groups. This holds true for the two dams in the high dose group, which were killed in extremis on pregnancy Day 11 and 12, respectively. In the dam from the HOE 36H group which was found dead on Day 16 of pregnancy, no organ changes were observed either. One dam in the latter group exhibited a peribulbar inflammation and exophthalmos of the right eye, which had developed due to an infection after the animal had been bled from the orbital sinus.

3. Conclusion:

In general, HOE 901 treatment did not cause impairment of behavior or unusual condition without remarkable changes in food consumption. Five fetuses exclusively from the high dose group, [illegible] dilated brain ventricles. The sponsor attributed the increased incidence of these findings in the high dose group to hypoglycemic actions of the test compound. Although hypoglycemia is known to produce different types of malformations, the causal relationship between the blood levels of glucose and dilation of brain ventricle is not known.

IV. MUTAGENICITY STUDY

A. HGPRT-Test with V79 Cells: Detection of gene mutations in somatic cells in culture(Doc# 013566)

1. Methods:

Male Sprague Dawley rats received a single intraperitoneal injection of Aroclor 1254(500 mg/kg) 5 days before sacrifice. From the pooled livers of the treated animals S9 fraction was prepared. Approximately 10^6 mycoplasma-free V79 cells/experimental point were treated with the test substance or control solvent in the presence or absence of S9 fractions for 4 hours. The doses of HOE901(Batch D001) were 10, 25, 50, and 100 µg/ml. Twenty four hours after treatment, the cells were fixed

and stained with crystal violet and cell survival was determined by measurement of the crystal violet extinction. Positive control agents were ethylmethanesulfonate(1 mg/ml) without metabolic activation and 9,10-dimethyl-1,2-benzanthracene(7.7µg/ml) with S9 fraction.

2. Results:

HOE901 was not cytotoxic to the V79 cells in the absence or in the presence of metabolic activation. No relevant reproducible enhancement of the mutant colonies or mutant frequency over the range of the solvent control was found with any of the concentrations used in the absence of metabolic activation by S9-mix. In the presence of S9-mix significant increases were observed at the concentrations of 25 and 100 µg/ml in the first experiment. But, this effect was not dose dependent and not reproduced in the second and third experiment.

3. Conclusion: The test compound appeared to be not mutagenic in the HGPRT-test with cells of the V79 Chinese hamster cell line.

B. Chromosome Aberrations in Vivo Cytogenetic Test in Bone Marrow Cells of the Chinese Hamster(Doc#012798)

1. Thirty-five Chinese hamsters(Strain Han:Chin)/sex/group were administered subcutaneously HOE901 (Batch # A002) at a dose of 750 IU/kg. [illegible] control agent was endoxan [illegible], which was given subcutaneously. [illegible] animals were killed at 12, 24, or 48 hours after the drug treatments and the bone marrow was collected. The bone marrow suspension was fixed in 1.5 ml of fixative (methanol and glacialacetic acid mixture). A few drops of the suspension were stained for the examination of chromosomal aberrations.

2. Results:

All animals survived after administration of 750 IU of HOE 901/kg without noticeable toxicity. After the drug administration no indication of cytotoxicity as reduction of the mitotic index and no increase in the aberration rate exclusive and inclusive gaps was noted in the 12, 24 and 48 hour groups as compared with the negative control. No increase in chromosome aberrations after administration of the test substance in all dose groups were observed.

3. Conclusion:

HOE901 was not mutagenic in the in vivo cytogenetic test using bone marrow cells of Chinese hamster.

V. HOE901 Absorption, Distribution, Metabolism and Excretion

A. Study Title: HOE901-^{125}I Radioluminographic Distribution in Male Rats after a Single Intravenous or Subcutaneous Dose(Doc#013571)

1. Methods: Four Wistar(Hoe:Wiskf/SPF71) male rats/group were administered HOE901 subcutaneously or intravenously at a dose of 1.5 mg/kg, which was labelled with radioactive iodine(9.8 MBq/ml). After 5 min, 1, 4, and 24 hours after either s.c. or i.v. injection of the drug, the animals were rapidly frozen for the preparation of longitudinal sections. [redacted] was obtained from [redacted] which were exposed to the sectioned tissue slices for 15 minutes or 24 hours.

2. Results:

a. Distribution in organs at different time after iv injection

At 5 min after iv administration of HOE901, the radioactivity was distributed in the whole organism except the CNS and posterior segments of intestine. The highest radioactivity was noted in the kidney at that time. Other tissues such as liver, lungs, blood, salivary glands, adrenals, spleen and regions of stomach wall also showed high radioactivity. By 1 hour after iv injection, the [redacted] levels had [redacted]ributed so that the highest radioactivity was noted in the thyroid, followed by renal cortex, contents of urinary bladder, skin, stomach. By 4 hour after iv injection, the highest radioactivity was seen in the thyroid. One day after iv dosing, high intensity of radioactivity was noted around the thyroid.

b. Distribution in organs after subcutaneous injection

Radicactivity was restricted to the injection site at 5 min after subcutaneous administration(Fig. 1), which was distributed to entire body in 1 hour after sc administration. The kidney, thyroid, renal cortex, bladder, stomach, liver and pancreas had relatively high concentrations. One day after sc dosing, the highest radioactivity concentrations were found at the injection site and in the thyroid(Fig. 1).

3. Conclusion: The sc injection of HOE901 compared with iv dosing produced lower, but longer-lasting tissue radioactivity. The maximum concentrations in the different organs and tissues were lower than after iv injection. The excretion after both iv and sc administration occurred by the same days, but it was slower after sc injection.

VI. Summary and Conclusion

HOE901 is a recombinant DNA human insulin analogue, which contains Zinc [redacted] Subcutaneous treatment with HOE901 resulted in a delayed lowering of blood glucose in animal models, which was comparable to zinc containing human insulin. HOE901 was shown to be well tolerated and it had a similar immunogenic potential to human insulins. HOE901 did not produce remarkable reproductive and mutagenic toxicity. However, it appears that HOE901 does not offer any significant therapeutic improvement over existing antidiabetic agents.

VII. Attachment: Table 1 and figure 1.

VIII. Recommendation(Letter to the sponsor)

According to autopsy finding of HOE901 embryotoxicity study in Himalayan rabbits(Doc #013939), 5 fetuses of the high dose (0.072 mg/kg) group had dilation of brain ventricle. You attributed the symptom to hypoglycemia-induced by HOE901. Is there direct evidence that a similar degree of ventricular dilation could be reproduced by hypoglycemia-induced by other means such as insulin treatment?

/S/

Herman M. Rhee, Ph.D.
Review Pharmacologist

cc: Original IND/HFD IND
A. Jordan/H. Rhee

Table 1

HOECHST AKTIENGESELLSCHAFT SECTION: REPROTO

T E R A T O L O G Y - INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

STUDY NO: RK0695 TITLE: EMBRYOTOXICITY IN RABBITS O HOE 901

FISHER'S EXACT:- GROUP 1 COMPARED WITH ALL OTHER GROUPS (* P < 0.05 ** P < 0.01, ONE SIDED)

	CLASS	GROUP 1 0 MG/KG		GROUP 2 0.018 MG/KG		GROUP 3 0.036 MG/KG		GROUP 4 0.2 MG/		GROUP 5 (HOE 36 N) 1 IU/KG		MIN - MAX RANGE OF FINDINGS IN FOETUSES PER GROUP (%)
		INCIDENCE BY FOETUS/LITTER										
		NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	
EXTERNAL/VISCERAL DEFECTS OBTAINED AT AUTOPSY AND ORGAN CROSS-SECTION												
NUMBER OF FOETUSES EXAMINED		94		119		115+		61		86		
NUMBER OF LITTERS EXAMINED		17		19		17		14		16		
EXTERNAL												
RETARDED FOETUS	RET	2	(2.1)	0		0		0		0		0
		2	(11.8)	0		0		0		0		
HEAD/EYE												
FACIAL PART OF THE SKULL - ASYMMETRICAL	MAJ	0		0		1	(0.9)	0		0		0
		0		0		1	(5.9)	0		0		
LENS DYSPLASTIC - BILATERAL,												0
LENS REDUCED IN SIZE - LEFT,												0
OPEN EYELID - BILATERAL												—
EYE												
BLOOD IN ORBITAL CAVITY - LEFT OR BILATERAL	MIN	3	(3.2)	0		0		0		0		—
		1	(5.9)	0		0		0		0		
BRAIN												
ALL VENTRICLES SLIGHTLY DILATED	MIN	0		0		0		5**	(8.[illegible]	0		—
		0		0		0		2	(14.[illegible]	0		
THORACIC CAVITY												
BLOOD IN THORACIC CAVITY	MIN	0		1	(0.8)	0		4*	(6.[illegible]	0		—
		0		1	(5.3)	0		2	(14	0		

+ * OF THESE 1 FOETUS - FINDINGS NOT AVAILABLE - LOSS OF DATA DUE TO OPERATING EI - EXCLUDED FROM FISHER'S EXACT TEST

WITHHOLD 1 PAGE (S)

NDA 21-081

February 4, 2000

DRUG: LANTUS™; HOE 901; insulin glargine injection
INDICATION: Control of hyperglycemia

TEAM LEADER MEMO TO FILE REGARDING PRECLINICAL PHARMACOLOGY/TOXICOLOGY ISSUES FOR NDA 21-081 (LANTUS™; HOE 901; INSULIN GLARGINE INJECTION)

The following statements are based upon Dr. Rhee's pharmacology review of NDA 21-081 and from records of IND ———

LANTUS™ is a recombinant human insulin with the modification of the natural human insulin molecule in which 2 arginines are substituted to positions 31 and 32 of the β-chain of human insulin and replacing the asparagine at position 21 of the α-chain with glycine. This is designed as a long acting insulin presumably by the slow release of microprecipitated product from the injection site. All appropriate studies were adequately performed to permit a safety evaluation of this product suitable for approval.

In general, the preclinical studies performed with this agent indicated that the toxicological findings with LANTUS™ are similar to human insulin and in most cases, are likely due to the expected hypoglycemia at high dose levels.

Carcinogenicity: The carcinogenicity assessment of compounds such as modified insulins is problematic. In general, the standard 2-year bioassay approach is not appropriate for biotechnology products. However, in some cases, particularly where mitogenic or potential carcinogenic effects may be suspected, some kind of approach is necessary to provide information regarding carcinogenic potential. General approaches are outlined in the ICH S6 document for biotechnology products. In this case, the standard 2-year bioassays were performed in both rats and mice.

In male rats, there were findings of injection site histiocytomas in groups treated with vehicle or vehicle plus LANTUS™. This did not occur in female rats, the saline control or an insulin comparator group in which the vehicle was similar, but a different pH. This was not dose related, but appeared to be related to the presence of vehicle. This cannot be clearly attributed to the vehicle alone, however, since the finding was not evident in females. The significance of this finding to humans in not known, but the executive CAC expressed concern when the results were presented to the committee and recommended that the findings be described in the label (see copy of eCAC minutes).

In mice, there were no tumor findings in males or females related to treatment. However, due to excessive mortality in the female arm of the study (apparently not related to drug toxicity since there was high mortality in the control group), the data could not be interpreted. Since standard 2 year studies in two species are not generally recommended for insulin analogs, this does not represent a deficiency in the safety evaluation package, but the e-CAC again recommended that the label reflect that the female arm of the study was inconclusive.

Genotoxicity: LANTUS™ was not genotoxic in the Ames bacterial mutagenesis assay, a mammalian cell mutagenesis assay (V79 HGPRT assay), a mammalian cell clastogenicity assay (V79 chromosomal aberration assay) or an *in vivo* micronucleus test in mice.

Mitogenicity and relative IGF-I receptor binding: The mitogenicity and relative IGF-I receptor binding were slightly higher for LANTUS™ compared to human insulin.

Reproductive toxicity: The high doses used in the reproductive toxicology study induced clinical signs consistent with significant hypoglycemia, and thus were performed with adequate exposure levels. In the NDA review, there is not an extensive discussion of animal findings. However, during the IND review (see IND reviews in the appendix to Dr. Rhee's review) there was considerable concern raised regarding findings of ventricular dilatation in 5 fetuses in 2 litters of rabbits at a dose approximately twice human exposure based on surface area (mg/m^2) comparisons. These appear to be similar (but not identical) to findings reported for human insulin when tested in animal models. The clinical significance would appear to be the same as for insulin. However, according to 21 CFR 201.57(f)(6)(c) this is an adverse effect in the fetus and thus justifies a category C for the pregnancy category. Following is an excerpt from an e-mail (scanned from a printed copy, the electronic version is not available) that I sent to Dr. Herman on October 1, 1998 regarding my view of the findings in rabbits. My opinion on this topic has not changed since then:

> I looked over the files for HOE 901 regarding the reproductive findings. The dilatation of the ventricles occurred in 5 fetuses of 2 litters in the rabbit study. A total of 14 litters were examined at this dose. This did not occur at lower doses in the rabbit study or in the rat study. There were also some delays in ossification and an occasional malformation particularly a low incidence of findings in the eyes (single occurrence of each malformation) that are not clearly treatment related. , but this does not seem like a serious problem to me. While the delays in ossification occurred with Lis-Pro, there is no mention of the ventricular finding. This was a different strain of rabbit and since the incidence was so low with HOE, it is not surprising that this may not have been picked up. They cited some insulin studies, which have some similar findings with ossification and the occasional eye defect, but they did not mention the ventricular effects. I presume that they did not occur with insulin. One author apparently did report dilation of brain ventricles in fetuses from rats treated with insulin (the study was done in 1962, so I don't know how reliable it is). There was apparently no dilation of ventricles found in studies with other hypoglycemic agents (which were not specified). Overall, this sounds like something that should be included to in the label unless they provide enough evidence to attribute this to hypoglycemia.
>
> [ ]
>
> According to the humalog label, carcinogenicity studies were not performed. I found the pregnancy labeling interesting and think we should model the HOE after the humalog label. However, the findings in the repro studies would probably categorize HOE as a category C. The humalog label is reproduced below.

Pregnancy--Teratogenic Effects--Pregnancy Category B—Reproduction studies have been performed in pregnant rats and rabbits at parenteral doses up to 4 and 0.3 times, respectively, the average human dose (40 units/day based on body surface area. The results have revealed no evidence of impaired fertility or harm to the fetus due to Humalog. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Although there are no clinical studies of the use of Humalog in pregnancy, published studies with human insulins suggest that optimizing overall glycemic control, including postprandial control, before conception and during pregnancy improves fetal outcome. Although the fetal complications of maternal hyperglycemia have been well documented, fetal toxicity also has been reported with maternal hypoglycemia.
Insulin requirements usually fall during the first trimester and increase during the second and third trimesters. Careful monitoring of the patient is required throughout pregnancy. During the perinatal period, careful monitoring of infants born to mothers with diabetes is warranted.

Based on the above discussion, the team leader proposes modifications to the labeling proposed in Dr. Rhee's review as follows:

Carcinogenesis, mutagenesis, impairment of fertility

[ ]

[ ]

Insulin glargine was not mutagenic in tests for detection of gene mutations in bacteria and mammalian cells (Ames- and HGPRT-test) and in tests for detection of chromosomal aberrations (cytogenetics in vitro in V79 cells and in vivo in Chinese hamsters).

[ ]

[ ]

; In a combined fertility and prenatal and postnatal study in male and female rats at subcutaneous doses up to 0.36 mg/kg/day (approximately 7 times the recommended human subcutaneous starting dose,

[ ]

Pregnancy

Teratogenic effects: pregnancy category

[ ]

Subcutaneous reproduction and teratology studies have been performed with Lantus and regular human insulin(HOE36H) in rats and Himalayan rabbits. The drug was given to female rats before mating, during mating, and throughout pregnancy at doses up to 0.36 mg/kg/day(approximately 7 times the recommended human subcutaneous starting dose of 0.008 mg/kg/day, based on mg/m^2). In rabbits, doses of 0.072 mg/kg/day (approximately 2 times the recommended human subcutaneous starting dose, based on mg/m^2) were administered during organogenesis. The effects of Lantus did not generally differ from those observed with regular human insulin in rat or rabbits. However, in rabbits, five fetuses from two litters of the high dose group exhibited dilatation of the cerebral ventricles. Fertility and early embryonic development appeared normal.

↳ does not need to be underlined in label

CONCLUSION

The pharmacology team leader recommends that this application should be approved (AP) from a pharm/tox standpoint pending appropriate modifications to the label (including the histiocytoma findings in the rat study and a category C listing).

/S/ 2/4/00

Ronald W. Steigerwalt, Ph.D.
Pharmacology Team Leader, DMEDP

cc: NDA Arch
HFD510
HFD510/Steigerwalt/HRhee /Koller/JRhee
Review Code: AP (pending labeling revisions)
Filename: _____

APPEARS THIS WAY
ON ORIGINAL

MEMORANDUM
March 13, 2000

DRUG: LANTUS®; HOE 901; Insulin glargine injection

I have review the NDA action package and the proposed product label and have found both to be acceptable from a pharmacology/toxicology perspective. Minor labeling changes were made; no other actions indicated.

/S/

Kenneth L. Hastings, D.V.P.H.
Acting Associate Director, ODE II
Pharmacology/Toxicology

APPEARS THIS WAY
ON ORIGINAL