

rebound response to initial HR 1799-related hypoglycemia with no toxicological relevance.

Blood urea nitrogen was significantly, dose-dependently lower in male rats of low and mid dose-groups and female rats of the high dose-group. Presumably, the alterations observed might reflect the anabolic state of the rats and are considered, therefore, to be due to the pharmacologic effect of the compound.

Serum creatinine concentrations were statistically significantly decreased in male rats of the mid dose-group. Since the difference was only marginal, it is considered incidental.

Serum sodium concentrations were statistically significantly lower in female rats of mid and high dose-groups. Since only minor alterations occurred, they are considered not relevant toxicologically. Serum chloride concentrations were statistically significantly lowered in female rats of the mid and high dose- groups. Since the differences were only marginal, they are considered incidental.

ATOX ID.2

PROVANTIS

RUN DATE: 19-SEP-2002

SUMMARY AND STATISTICAL EVALUATION

STUDY : 2001-0534 HUM 1964 - 12 month subcutaneous toxicity study in rats

FINAL VALUE

CLINICAL CHEMISTRY (MALE)

		GROUP 1 0 I.U./kg	GROUP 2 2.5 I.U./kg	GROUP 3 5 I.U./kg	GROUP 4 20 I.U./kg	GROUP 5 50 I.U./kg
Sodium (mmol/L)	MEAN	143	143	143	142	141
	S.D.	2	1	2	2	1
	N	25	26	28	21	10
Potassium (mmol/L)	MEAN	4.90	4.84	4.95	4.91	4.85
	S.D.	0.32	0.42	0.63	0.29	0.31
	N	25	26	28	21	10
Calcium (mmol/L)	MEAN	2.44	2.43	2.45	2.43	2.44
	S.D.	0.07	0.06	0.10	0.06	0.08
	N	25	26	28	21	10
Chloride (mmol/L)	MEAN	104	105	105	105	104
	S.D.	1	2	2	2	1
	N	25	26	28	21	10
Phosphorus (mmol/L)	MEAN	1.47	1.50	1.55	1.49	1.61
	S.D.	0.17	0.26	0.23	0.24	0.20
	N	25	26	28	21	10
Total Bilirubin (umol/L)	MEAN	8.3	8.2	8.5	7.6	8.8
	S.D.	1.7	1.5	1.8	1.1	1.6
	N	25	26	28	21	10
Glucose (mmol/L)	MEAN	11.15	12.28 +	13.76 +	14.96 +	20.39 +
	S.D.	2.76	3.06	2.65	2.67	3.96
	N	25	26	28	21	10
Creatinine (umol/L)	MEAN	41	41	41	41	38
	S.D.	3	3	3	4	3
	N	25	26	28	21	10

WILCOXON-TEST, TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES, IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL (P < 0.05).

+/- : SIGNIFICANTLY DIFFERENT FROM CONTROL
 N : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

APPEARS THIS WAY
ON ORIGINAL

ATOX 10.2

PROVANTIS

RUN DATE: 19-SEP-

SUMMARY AND STATISTICAL EVALUATION

STUDY : 2001-0534 - NMR 1964 - 12 month subcutaneous toxicity study in rats

FINAL VALUE

CLINICAL CHEMISTRY (FEMALE)

		GROUP 1 0 I.U./kg	GROUP 2 2.5 I.U./kg	GROUP 3 5 I.U./kg	GROUP 4 20 I.U./kg	GROUP 5 50 I.U./kg
Sodium (mmol/L)	MEAN	143	143	143	143	141
	S.D.	2	2	2	2	2
	N	29	28	28	23	14
Potassium (mmol/L)	MEAN	4.19	4.28	4.23	4.24	4.31
	S.D.	0.35	0.44	0.37	0.29	0.32
	N	29	28	28	23	14
Calcium (mmol/L)	MEAN	2.56	2.53	2.51	2.51	2.51
	S.D.	0.09	0.15	0.08	0.07	0.07
	N	29	28	28	23	14
Chloride (mmol/L)	MEAN	105	104	105	104	103
	S.D.	2	2	2	2	2
	N	29	28	28	23	14
Phosphorus (mmol/L)	MEAN	1.42	1.40	1.42	1.42	1.61
	S.D.	0.34	0.36	0.29	0.28	0.32
	N	29	28	28	23	14
Total Bilirubin (umol/L)	MEAN	8.8	7.8	7.3	8.3	8.9
	S.D.	3.2	1.4	1.3	1.5	1.9
	N	29	28	28	23	14
Glucose (mmol/L)	MEAN	10.70	10.29	11.31	12.50 +	13.04 +
	S.D.	2.59	2.29	2.33	2.58	3.11
	N	29	28	28	23	14
Creatinine (umol/L)	MEAN	42	44 +	45 +	45 +	44 +
	S.D.	3	3	4	3	3
	N	29	28	28	23	14

WILCOXON-TEST, TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES.
IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL (P < 0.05).

+/- : SIGNIFICANTLY DIFFERENT FROM CONTROL
N : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

Organ Weights:

HMR 1964: In relative terms, liver and spleen weights were significantly lowered in male rats of all dose-groups while brain weights were significantly lower in male and female rats of the high dose-group. Pituitary weights were significantly lower in male rats of high mid and high dose-groups and ovarian weights were significantly lower in female rats of the high dose-group vs. concurrent controls as shown in two tables below.

In view of the well-known fact that absolute brain weight represents a constant and robust parameter, lowered relative brain weights in high dose rats indicate that the alterations observed in other organs of male (liver, spleen, and pituitary) and female rats (ovaries) as well are due to the increased terminal body weights noted in the high dose-group. Since no correlating histopathologic lesions were seen, all organ weight alterations observed are considered not relevant toxicologically.

HR 1799: In relative terms, liver (mid dose males) and spleen weights (low and mid dose males) were significantly lower, and brain weights were significantly lower in female rats of mid and high dose- groups vs. controls. In contrast, thyroid weights were significantly increased in female rats of mid and high dose-groups.

Since no correlating histopathologic lesions were seen, all organ weight alterations are considered not relevant toxicologically. It should be mentioned that, because of only two male rats in the high dose-group surviving until termination of the study, statistical evaluations were not performed.

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ATOX 10.2

PROVANTIS

RUN DATE: 20-NOV-2002

SUMMARY AND STATISTICAL EVALUATION

STUDY : 2001-0534 - HUR 1964 - 12 month subcutaneous toxicity study in rats

FINAL VALUE

ORGAN WEIGHTS (MALE)

		GROUP 1	GROUP 6	GROUP 7	GROUP 8
		0	5	20	50
		I.U./kg	I.U./kg	I.U./kg	I.U./kg
BODY WEIGHT (g)	MEAN	651.8	688.9	611.4	741.5
	S.D.	100.0	74.1	117.2	72.8
	N	25	24	9	2
Heart Weight (g)	MEAN	1.541	1.604	1.462	1.533
	S.D.	0.208	0.177	0.284	0.163
	N	25	24	9	2
Liver Weight (g)	MEAN	19.748	19.705	16.512	22.190
	S.D.	3.620	2.880	3.349	5.148
	N	25	24	9	2
Kidneys Weight (g)	MEAN	3.348	3.388	3.301	3.846
	S.D.	0.640	0.466	0.649	0.931
	N	25	24	9	2
Spleen Weight (g)	MEAN	0.951	0.907	0.766	0.862
	S.D.	0.239	0.164	0.088	0.250
	N	25	24	9	2
Testes Weight (g)	MEAN	3.204	3.316	3.109	3.113
	S.D.	0.761	0.582	0.299	0.144
	N	25	24	9	2
Adrenals Weight (g)	MEAN	0.0478	0.0495	0.0414	0.0425
	S.D.	0.0103	0.0081	0.0093	0.0092
	N	25	24	9	2
Thyroid Weight (g)	MEAN	0.0347	0.0338	0.0286	0.0330
	S.D.	0.0142	0.0107	0.0101	0.0141
	N	24	24	9	2

NO STATISTICAL EVALUATION

APPEARS THIS WAY
ON ORIGINAL

ATOX 10.2

PROVANTIS

RUN DATE: 19-SEP-2002

SUMMARY AND STATISTICAL EVALUATION

STUDY : 2001-0534 - HMR 1964 - 12 month subcutaneous toxicity study in rats

FINAL VALUE

ORGAN WEIGHTS (FEMALE)

		GROUP 1 0 I.U./kg	GROUP 2 2.5 I.U./kg	GROUP 3 5 I.U./kg	GROUP 4 20 I.U./kg	GROUP 5 50 I.U./kg
BODY WEIGHT (g)	MEAN	384.6	359.4	372.6	395.6	424.4
	S.D.	46.9	46.9	36.9	49.7	47.0
	N	29	28	28	23	14
Heart Weight (g)	MEAN	1.020	1.048	1.081	1.073	1.189
	S.D.	0.111	0.129	0.077	0.113	0.095
	N	29	28	28	23	14
Liver Weight (g)	MEAN	11.349	10.944	11.292	11.745	12.724
	S.D.	1.442	1.726	1.420	1.525	1.216
	N	29	28	28	23	14
Kidneys Weight (g)	MEAN	1.998	2.026	2.035	2.045	2.235
	S.D.	0.247	0.265	0.189	0.193	0.264
	N	29	28	28	23	14
Spleen Weight (g)	MEAN	0.583	0.662	0.686	0.593	0.630
	S.D.	0.096	0.215	0.468	0.090	0.069
	N	29	28	28	23	14
Ovaries Weight (g)	MEAN	0.1181	0.1150	0.1165	0.1101	0.1126
	S.D.	0.0276	0.0297	0.0251	0.0276	0.0226
	N	29	28	28	23	14
Adrenals Weight (g)	MEAN	0.0633	0.0714	0.0724	0.0630	0.0824
	S.D.	0.0135	0.0183	0.0272	0.0117	1.9806
	N	29	28	28	22	13
Thyroid Weight (g)	MEAN	0.0233	0.0254	0.0240	0.0257	0.0309
	S.D.	0.0071	0.0073	0.0082	0.0090	0.0105
	N	29	28	28	23	14

NO STATISTICAL EVALUATION

Macroscopic observations:

No specific gross findings related to the administration site were noted in the various treatment groups of rats.

Microscopic observations:

No remarkable microscopic abnormalities were noted in the various treatment groups of rats except the mammary gland tumors in female rats.

An increased number of female rats with gross findings related to the mammary gland was noted in low, low mid, and high mid dose-groups HMR 1964 and mid and high dose-groups HR 1799 as shown in Table 6. The incidences of mammary tumors in HMR 1964- and HR 1799-treated groups were certainly high, compared to concurrent controls, which were similar to in-house historic controls (Table 7). However the incidences were not increased with the dose. In one female rat of the high mid dose-group (No. 215), two different types of mammary tumors occurred concurrently. Metastasis was not seen in any of the rats affected. In none of the rats, cause of death was attributed to the mammary tumor. The incidence of mammary tumors with the insulin analog HMR1964 did not significantly differ from the incidence observed with regular human insulin, HR1799.

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Table 6. Combined incidence of benign and malignant mammary tumors.

Group	HMR 1964					HR 1799		
	C0	D1	D2	D3	D4	P2	P3	P4
Number of rats	29	30	30	28	29	30	30	30
Mammary tumor bearers	-	6*	3	6*	3	3	6*	4

C0=Control; one-sided Exact Fisher Test: *) p<00.05; **) p<0.01.

Table 7. In-house historic data: combined incidence of benign and malignant mammary tumors.

Study Nos.	95.0017		94.0048		93.0622
Year	1995		1994		1993
Group	C1	C2	C1	C2	C0
Number of rats	50	50	30	30	30
Mammary tumor bearers	5	2	5	4	1

C1, C2=Independent control groups.

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Histopathology inventory

Study	F2001Tox0209		
Species	Rat		
Adrenals	X*		
Aorta	x		
Bone Marrow (sternum)	X		
Bone (femur)	X		
Brain	X*		
Cecum	X		
Cervix			
Colon	X		
Duodenum	x		
Epididymis	X		
Esophagus	X		
Eye	X		
Fallopian tube			
Gall bladder			
Gross lesions	X		
Harderian gland	X		
Heart	X*		
Ileum	X		
Injection site	X		
Jejunum	X		
Kidneys	X*		
Lachrymal gland			
Larynx	X		
Liver	X*		
Lungs	X		
Lymph nodes, cervical	X		
Lymph nodes mandibular	X		
Lymph nodes, mesenteric	X		
Mammary Gland	X		
Nasal cavity			
Optic nerves	X		
Ovaries	X*		
Pancreas	X		
Parathyroid	X*		
Peripheral nerve			
Pharynx			
Pituitary	X*		
Prostate	X*		
Rectum	X		
Salivary gland	X		
Sciatic nerve	X		
Seminal vesicles	X*		
Skeletal muscle	X		
Skin	x		
Spinal cord	X		
Spleen	X*		
Sternum	X		

Stomach	X			
Testes	X*			
Thymus	X			
Thyroid	X*			
Tongue	X			
Trachea	X			
Urinary bladder	X			
Uterus	X*			
Vagina	X			
Zymbal gland				

X, histopathology performed

*, organ weight obtained

Summary and Conclusions:

The twice daily administration of HMR 1964 at doses of 2.5, 5, 20, or 50 IU/kg or HR 1799 at doses of 5, 20, or 50 IU/kg to Sprague Dawley rats resulted in dose-dependent increased mortality in 20 and 50 IU/kg HMR 1964 and HR 1799 treated groups. In general, males and HR 1799 treated animals were more severely affected. Based on clinical observations and/or histopathological findings, cause of death was considered related to marked hypoglycemia. In 25 of the 159 rats dying early, clinical observations revealed signs typical for hypoglycemic episodes one or several occasions preceding death.

Several male animals of all study groups including the vehicle control showed an induration at the injection site in the dorsal neck region. Individual females of several groups also showed an induration at the injection site. In some of the animals showing induration at the injection site, additional observations like edema or wounds (oozing or scabby) were made. However, these symptoms were also seen in animals without indurations in the neck region. Incidence of palpable masses was minimally higher in HR 1799 mid and high dose females.

In hematology, a mild, but statistically significant prolongation of thromboplastin time was determined in some HMR1964 and HR1799 (2x5 and 2x20 IU/kg males only) treated groups. Since increases were only marginal and not always clearly dose-dependent, they are considered not relevant toxicologically.

Blood urea nitrogen was significantly decreased dose-dependently in HMR 1964 (high mid and high dose males, high dose females) and HR 1799 (low and mid dose males, high dose females) treated animals. Presumably, the alterations observed might reflect the anabolic state of the rats and are considered, therefore, to be due to the pharmacologic effect of the compound.

During necropsy, an increased number of female rats with gross findings related to the mammary gland was noted in low, low mid, and high mid dose- groups HMR 1964 and mid and high dose-groups HR 1799. Mammary tumors were found at low incidences in female rats of all HMR1964 and HR1799 dose groups, but not in controls. The lack of mammary tumors in controls is exceptional. The incidences of mammary tumors in treated groups were similar to the incidences of in-house historic control animals. In many cases, the incidences were not drug dose-dependent. More importantly, the incidences with HMR1964-treated rats were not different from those in rats treated with human insulin (HR1799).

Immunocytochemistry analysis revealed no significant differences between the HMR 1964 dose groups, the HR 1799 dose group and the control group with regard to proliferative activity in non-neoplastic mammary gland tissue. Therefore, it appears that a general mitogenic effect on mammary gland related to the compound HMR 1964 or HR 1799 is not likely.

3.4.4 Genetic toxicology:

The sponsor performed the following three genotoxicity studies with Apidra under acceptable experimental conditions. Bacterial reverse mutation test (Study#98.523), in vitro mammalian chromosome aberration test (Study#98.0524) and micronucleus study in SD rats (Study#2000.0959). These studies were reviewed in Original IND Review on 10/5/2001(see Appendix Page 88).

In the Ames test, insulin glulisine was not mutagenic at doses investigated (up to 5000 (g/plate) with any bacterial test systems. Furthermore, insulin glulisine did not induce chromosomal aberrations in vitro in mammalian cells (V 79 Chinese hamster cells) at the doses tested (up to 5000 µg/ml). There were also no chromosomal aberrations observed in vivo after administration of insulin glulisine to rats at all dose levels (up to 1000 IU/kg body weight), as indicated by examination of bone marrow cells for micronuclei.

3.4.5 Carcinogenicity:

A 12-month chronic toxicity study in rats was conducted with insulin glulisine in order to detect potential carcinogenicity. Human regular insulin (HR 1799) was included in the study design as a comparator. Mammary tumors occurred at similar incidences in all groups dosed with insulin glulisine (5, 10, 40 and 100 IU/kg/day) or with HR 1799 (40 and 100 IU/kg/day). Mammary tumors were absent in the control group (0 IU/kg). There was only a statistically significant increased tumor incidence in dose groups receiving 5 or 40 IU/kg insulin glulisine or 40 IU/kg HR 1799 (Fisher's-test, $p < 0.05$), but not in high dose groups receiving 100 IU/kg insulin glulisine or 100 IU/KG HR 1799. Furthermore, PETO analysis revealed no trend across all insulin glulisine test groups and indicated the lack of a positive dose dependency regarding mammary tumor incidences. Intercurrent deaths showed a compound-induced and dose dependent increase in mortality at dose levels of 40 IU/kg insulin glulisine or HR 1799. Generally, male rats were more severely

affected than females. At comparable dose levels, mortality was always higher in HR1799 treated groups.

In order to detect carcinogenic potential related to mitogenic activity of insulin glulisine (which was not visible in histopathology) proliferation was examined using Ki-67 immunohistochemistry. In the 6-month toxicity study, insulin glulisine did not exert an in vivo mitogenic effect on mammary glands of females at the highest dose of 80 IU/kg when compared to the untreated controls. The same was true for mammary glands from female rats in the 12-month toxicity study. This examination comprised all female rats from both insulin glulisine high dose groups (100 IU/kg and 40 IU/kg), from one HR 1799 high dose group (40 IU/kg) and from the control group. Both compounds did not induce a significant increase in proliferation in mammary glands in comparison to the untreated controls.

3.4.6 Reproductive toxicology:

Reproductive toxicology study was reviewed under IND#61,956 on 2/18/2002. The main findings are briefly described below. Reproductive and developmental toxicity studies were conducted with insulin glulisine and human regular insulin (HR 1799) as a reference compound in order to assess fertility, reproduction performance, embryo-fetal development and postnatal growth and development. In general, the effects of insulin glulisine were not different from those seen with HR 1799 and occurred only at maternal toxic dose levels. Insulin glulisine and HR 1799 were administered subcutaneously to female rats prior to mating, during mating, throughout pregnancy and during lactation, and to male rats prior to and during mating.

Insulin glulisine did not impair fertility and did not show any adverse embryo-fetal effects at all dose levels tested (up to 10 IU/kg). Insulin glulisine and HR 1799, each at doses up 8 IU/kg, did not induce adverse effects in rats on postnatal development, fertility or pregnancy of their progeny.

Insulin glulisine and HR 1799 were administered subcutaneously to female rabbits during embryogenesis and fetogenesis. Slightly increased post-implantation losses occurred at insulin glulisine dose levels of 1.5 and 0.5 IU/kg/day, whereas these were markedly increased with HR 1799 at the same dose levels. When pregnant rabbits were treated with insulin glulisine, two had abortions and two died at a dose level of 1.5 IU /kg body weight. At the same dose level, one rabbit had abortion and four were found dead after treatment with HR 1799. Furthermore, at dose levels of 1.5 and 0.5 IU /kg/day, a slightly increased incidence of skeletal defects after administration of both insulin glulisine and HR 1799 was observed. All adverse effects mentioned above are related to hypoglycemia due to an exaggerated pharmacodynamic action induced by the compound, insulin glulisine.

TITLE: Subcutaneous Toxicokinetic Studies in Pregnant Rats

Study no.: 2000.0967/Document#F2001Tox0051/Report#2000.1125

Volume # and page #: Module 4.2.3.5.2.3/0-92

Conducting laboratory and location: Aventis Pharma, D-65926 Frankfurt am Main

Date of study initiation: Jan. 24, 2001

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: Batch#1315 _____

Methods:

Nine to 11 week old female SD (Hsd) rats were used in this study. The virgin rats in the estrous phase were mated overnight. The day of detection of sperm in vaginal smears was defined as day 0 of gestation. Ten rats/group were given HMR1964 subcutaneously once daily at doses of 1, 3.15, or 10 IU/kg body weight from day 6-12 of pregnancy.

Observations:

The behavior and general health condition of the animals were observed twice daily (on weekends and public holidays once daily). Body weights were determined on days 0, 3, 6, 9, and 12 of pregnancy, and food consumption was recorded between days 0-3, 3-6, 6-9, and 9-12 of pregnancy. Blood samples were taken on day 12 of pregnancy at 0.25, 0.5, 1, 2, and 4 hours after administration of the test compound. On day 12 of pregnancy the animals were sacrificed and examined macroscopically with counts of corpora lutea and the conceptuses.

Results:

One rats of the 10 IU/kg group was found dead on day 9 of pregnancy. The cause of the death appeared to be related to the pharmacological action of the test compound because the animal had rolling convulsions, prone position, and stupor with irregular respiration.

Body weight and food consumption were not affected by the treatment. The animal of the HD group found dead exhibited a dark-brown discolored liver and there were no other visible changes in the remaining animals according to macroscopic examinations.

One animal each in the MD and HD groups did not become pregnant. Numbers of corpora lutea and implantation sites were not changed significantly by the treatment. The highest plasma drug concentrations in the LD, MD and HD groups were 11, 47, and 160 mg/ml, respectively, which were detected 15 minutes after the drug treatment. This is toxicokinetic study, but toxicokinetic findings in the pregnant rats are minimal except several parameters that were summarized in a table below.

Table 2: Pharmacokinetic parameters of HMR 1964 in plasma

Administration	Gender	Dose	t _{max}	c _{max}
HMR 1964		[IU/kg b. wt.]	[h]	[ng/ml]
7th administration	female	low	1	0.25
		medium	3.15	0.25
		high	10	0.25

TITLE: Subcutaneous Pre- and Postnatal Toxicity Study in RatsStudy no.: 2000.0942/Document#F2001Tox0295/Report#2000.1256Volume # and page #: Module 4.2.3.5.3.1/0-595Conducting laboratory and location: Aventis Pharma, D-65926 Frankfurt am MainDate of study initiation: Jan. 24, 2001GLP compliance: YesQA report: yes (x) no ()Drug, lot #, and % purity: Batch#1215 Verum**Methods:**Doses: 0 (control), 1, 3.15, and 8 IU/kg HMR1964; 1 and 8 IU/kg Human insulin (HR 1799) as a comparatorSpecies/strain: Female rat/ Hsd(SD), _____Number/sex/group or time point (main study): 23 mated female rats/groupRoute, formulation, volume, and infusion rate: Subcutaneous administrationSatellite groups used for toxicokinetics or recovery: N/AAge: 10-12 weeks oldWeight: Approx. 210 gUnique study design or methodology (if any): NA

Virgin female animals in the pre-estrous or estrous phase were mated overnight with fertile males and the day of sperm detection was defined as day 0 of gestation.

Indicated doses of HMR1964 or comparator were given as a solution from day 6 of pregnancy until day 21 post partum in a volume of 5 ml/kg.

Animals received the test compounds during embryogenesis and fetogenesis and during the lactation period. The dams were allowed to litter normally and rear their progeny to the stage of weaning. Growth, development and behavior of the progeny were assessed during lactation and after weaning. 23 males and 23 females were selected from as many litters as possible to produce the F₂-generation. These animals were subjected to a water

maze test, assessed for sexual maturity, mated and the dams allowed to litter. All animals were killed after parturition.

Observations:

Fo-generation:

Mortality and clinical: Daily once

Body weights were determined on Days 0, 3, 6, 9, 13, 16, and 20 of pregnancy and on Days 0, 4, 7, 10, 14, 17 and 21 post partum.

Food consumption was determined between Days 0-3, 3-6, 6-9, 9-13, 13-16, and 16-20 of pregnancy.

F1-Generation:

Examination during lactation: The number of stillborn and live young, the sex of the young and the presence of gross anomalies were determined. Live offspring was counted and weighed on Days 4, 7, 10, 14, 17, and 21.

Behavior and viability: Several times daily (on weekends and public holidays once daily)

Function tests: Between days 21 and 23 post partum function tests were performed in all animals. Function of the auditory and visual system was checked by pupil reactivity to light in partially dark-adapted animals.

Data processing and statistics:

All data except water maze test were recorded on-line and compiled by a data processing system. The statistical evaluation is based on the assumption of a monotone dose-response relationship. Except in the case of the highest dose, statistical comparisons of dose groups and the simultaneous control group at the 5% level were only carried out if significant effects were detectable in the higher dose group. In the univariate analysis, two-sided questions (food consumption and body weights) were generally tested as follows: a two-sided test with the high dose group was followed by one-sided tests for the low dose groups with the alternatives previously observed.

RESULTS

Fo-Generation

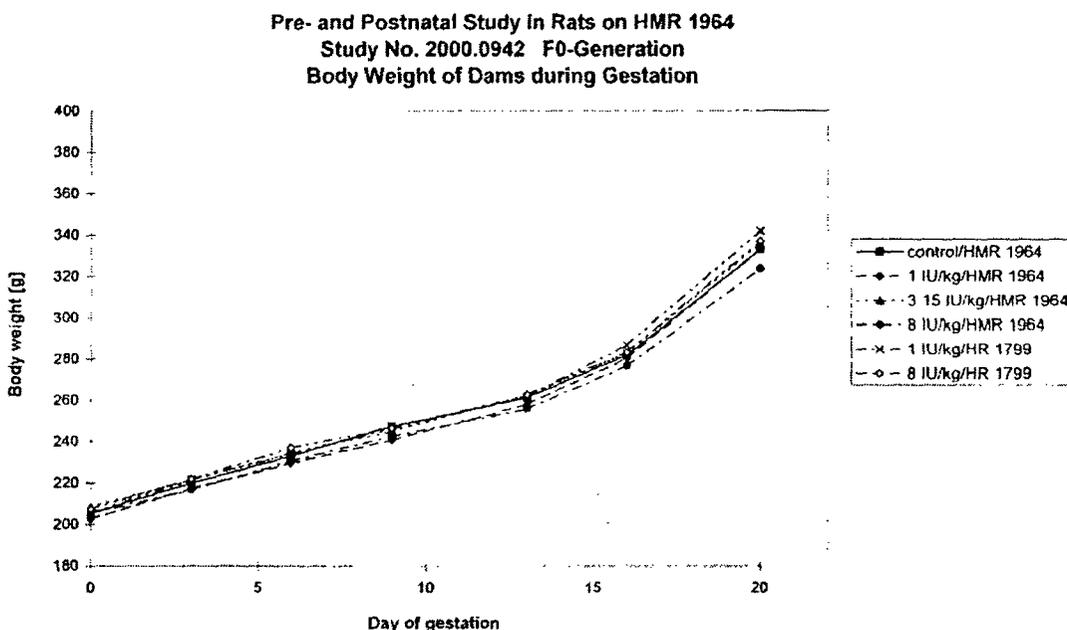
Mortality and clinical observations:

Four females from the 8 IU/kg HMR 1964 group were found dead on days 6, 22, 22, and 43 of the study (three deaths after delivery). Nine females from the 8 IU/kg HR 1799 group were found dead on days 6, 7, 18, 22, 22, 24, 24, 26, and 42 of the study (six deaths after delivery). Some of symptoms observed in these dose groups consisted of hypoactivity, bristling coat, prone position, saltatory and rolling convulsions, which appear to be related to the test-article-induced hypoglycemia.

Body weight and food consumption:

There were decreases in body weight gains on day 9 and 13 of pregnancy in the HD HMR 1964 group. Similar changes were observed in this group on day 0 and 4 during

lactation. Body weights were decreased to a significant degree in the animals treated with 1 or 3.15 IU HMR1964/kg on day 9 of pregnancy. However, the changes were minor without dose-dependency as shown in a figure below. Thus, the sponsor considered that the changes were not considered to be treatment-related.



Pregnancy and litter data:

Following animals did not become pregnant:

Control group: #64, #67, and #70

1 IU HMR 1964 / kg body weight: #84, #86, and #90

3.15 IU HMR 1964 / kg body weight: #103, #104, #105, and #115

8 IU HMR 1964/ kg body weight: #126, #128, #137, and #138

1 IU HR 1799 / kg body weight: #143, #148, #153, #154, #160, and #161

8 IU HR 1799 / kg body weight: #175 and #177

Animal #95 and #159 showed only implantation sites at necropsy. Following animals were killed after stillbirths (=day 0) or after loss of all pups during lactation:

Control group: #163 (day 4)

1 IU HMR 1964 / kg body weight: #78 (day 0), #83 (day 2)

3.15 IU HMR 1964 / kg body weight: #113 (day 0), #114 (day 1), #118 (day 4)

Litter parameters:

Numbers of implantations, live or dead pups, supernumerary implantation sites and birth index were comparable in all groups. Sex ratios of the pups were not altered by the administration of the test compounds as shown in Table 1.

Table 1: Reproduction parameters of F₀-animals

Parameter / Group	Control	HMR 1964 1 IU/kg	HMR 1964 3.15 IU/kg	HMR 1964 8 IU/kg	HR 1799 1 IU/kg	HR 1799 8 IU/kg
No. of dams littered	20	19	19	18	16	19
Stillbirths	0	1	1	0	0	0
Surviving / rearing dams	20	18	18	15	16	13
Pregnancy duration [days]	23.0	22.9	23.0	23.1	22.9	22.9
No. live pups per litter	11.0	11.8	11.5	11.2	12.1	12.1
No. live pups day 0	219	224	219	202	194	230
No. dead pups day 0	12	11	16	12	14	17
No. deaths during lactation	22	17	40	5	13	7
Implantation sites	235	250	254	221	218	255
- per litter	11.8	13.2	13.4	12.3	13.6	13.4
Supernumerary implantation sites	4	15	19	7	10	8
- per litter	0.20	0.79	1.00	0.39	0.63	0.42
- % of implantations	1.4	6.2	9.1	3.4	5.1	2.9
Sex ratio on day 0 [% males]	48.5	46.8	48.9	44.9	43.3	48.6
Unreared litters	1	1	2	0	0	0

Autopsy findings:

Macroscopic examination of the animals of the F₀-generation did not reveal clearly compound-related changes. The stomach and large intestine of one animal from the 8 IU HMR 1964 / kg group which died early was filled with gas. Another animal from this group killed at the end of the study showed a cyst on the left ovary. One animal from the 1 IU HR 1799/kg group did not litter and showed mass deposits in the vagina and the right uterus horn. Light yellow liquid in the uterus was observed in one animal from the 8 IU HR 1799 / kg group, which did not litter. Other findings were not considered to be compound-related due to the very low incidences and the lack of dose- dependency.

F₁-Generation:

Mortality and clinical observations:

Lactation: Clinical signs include hematoma, necrotic or missing tail tip, bite wound or swollen limb, which appeared not related to the treatment. Pup mortality [one animal of

the control group (#63), one animal of the 1 IU HMR 1964/kg group (#83) and two animals of the 3.15 IU/kg group (#114, #518)] might be related to lack of rearing their off-spring, which was not related to the doses of the article since this was not observed at the HD.

Post lactation: One male from the 1 IU HMR 1964/kg group was found dead one day after weaning and was replaced by another male from the same group. One male from the 8 IU HMR 1964/kg body weight group showed saltatory and rolling convulsions, gasping and decreased body temperature on day 12 after weaning and was found dead on the same day.

A kinked tail was observed in four males of the HD group from Day 25 - 29 after weaning and in two females from Day 1 or 49 onwards after weaning as shown below. It is remarkable that the abnormality was encountered only in the high dose group, not any other low dose groups. One male from the HD group (8 IU HR1799/kg) showed also necrotic tail tip from starting on day 42, and a missing tail tip from day 64. The treatment of the test article could lead to such effects in the offspring at this late stage of development is not known, although the frequency is small and was only observed at the HD.

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Clinical Observations - Symptoms By Period (with Animal Count)

Study : 2000.0942 F1 - Pre- and Postnatal Study in Rats on FMR 1964

		Day numbers relative to Start Date																								
Group		2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4
Sex	Clinical Sign	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6		
4m	ANIMALS ALIVE	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	
	ANIMALS NORMAL	22	21	19	19	19	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	
	Saltat. & rolling convulsions	
	Tail kinked	.	1	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4		
	Body temperature decreased	
	Gasping	
	Found dead	
	Killed: end of study	
4f	ANIMALS ALIVE	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	
	ANIMALS NORMAL	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	
	Tail kinked	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	Killed: end of study	

Body weights and food consumption of the pups:

Body weight gain and food consumption were comparable in all groups after weaning, although statistical evaluation revealed some differences. Increases in weight gain (D21) and food consumption (Day 42-45) in F1 male pups of HD females were observed. Decreased body weight (D42, 45, 59) and food consumption in female F1 pups of HD females were noted.

Since the changes occurred without any clear trend and were only slight, the sponsor decided that they are not considered to be treatment-related. Body weight and food consumption during gestation and body weight during lactation were remained unaffected by the administration of the test compound in the groups.

Physiological development:

Developmental landmarks such as unfolding of the ears, fur growth, incisor eruption and opening of the eyelids occurred at comparable times in all groups. Dates of vaginal opening, separation of the praeputium were also comparable in all groups.

Function tests:

All animals showed normal reaction to visual and auditory stimuli. Likewise, righting reflex was positive in all animals (individual data not shown, archived with the raw data). According to water maze test, learning, memory and learning a new situation were comparable in all groups. Disturbances in motor activity, coordination and equilibrium were not observed in F₁-animals.

Reproductive performance:

Two animals from the control group, one animal from the 1 IU HMR 1964/kg dose group and one animal from the 8 IU HR1799/kg dose group did not mate with their assigned partner within three weeks of housing together. In the 8 IU HR 1799 /kg group, the assigned male showed aplasia of the left testis and left epididymis at necropsy, which may be the cause for the negative mating result. All other females were mated and showed an intact estrous cycle. Mean pre-coital period was not altered by the treatment of the F₀ parent animals with the test compound.

Following animals did not become pregnant:

Control group: #217, and #223
1 IU HMR 1964/kg: #233
3.15 IU HMR 1964/kg: #249, #250, #256
8 IU HMR 1964/kg: #272, #275, #276, #280, #288, #292
1 IU HR 1799/kg: #295, #302, and #307
8 IU HR 1799/kg: #325

Gestation:

Behavior and general health condition of all animals remained normal throughout the gestation period. Body weight and food consumption were comparable in all groups. Statistical evaluation revealed decreased body weight gains in the 8 IU HMR 1964/kg group on days 9 and 13 of pregnancy and decreased body weight gains in the 1 IU and 8 IU HR1799/kg group on day 13 of pregnancy.

Food consumption was decreased to a statistically significant degree between days 0 and 6 of pregnancy in the animals dosed with 8 IU HR1799/kg body weight. However, all these changes were minor, and a clear dose-relationship was missing. Therefore, these effects are not considered to be toxicologically relevant. Mean duration of pregnancy was between 23.0 and 23.3 days in all animals, which appears not to be different in any groups significantly.

Parturition:

One female of the 1 IU HMR 1964/kg group (#224) and 3.15 IU HMR 1964/kg group (#267) did not litter and showed only empty implantation sites at necropsy. The treatment

of F₀-animals with HMR 1964 or HR 1799 did not affect the numbers of live young and stillborn of F₁-animals (see Table II below). Mean pup weights and sex ratios were comparable in all groups. Incidences of gross anomalies in pups from the treatment groups were also comparable.

Table II: Reproduction parameters of F₁ animals

Parameter / Group	Control	HMR 1964 1 IU/kg	HMR 1964 3.15 IU/kg	HMR 1964 8 IU/kg	HR 1799 1 IU/kg	HR 1799 8 IU/kg
No. of pregnant animals	19	20	19	17	20	21
Mean pre-coital period [d]	4.7	5.5	4.0	4.0	3.4	4.1
Duration of pregnancy [d]	23.3	23.1	23.3	23.0	23.3	23.2
No. live pups per litter	13.4	12.5	12.9	14.2	13.4	12.3
No. live pups	254	249	246	242	267	258
No. dead pups	6	11	13	0	23	9
Sex ratio [% males]	46.5	48.8	47.1	55.4	51.0	51.9

Autopsy findings:

One male animal of the 8 IU HR 1799/kg group showed aplasia of the left testis and left epididymis. The sponsor considers that the findings were not to be treatment-related since only one animal was affected.

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ARTEMIS II

Date: 20-Jun-2001 Page: 1

Intergroup Comparison of Gross Pathology Observations

2000.0942/F1^c. Pre- and Postnatal Study in Rats on HMR 1964

	MALES						FEMALES					
	HMR 1964				HR 1799		HMR 1964				HR 1799	
	0 IU/kg	1 IU/kg	3.15 IU/kg	8 IU/kg	1 IU/kg	8 IU/kg	0 IU/kg	1 IU/kg	3.15 IU/kg	8 IU/kg	1 IU/kg	8 IU/kg
Number of Animals on Study :	23	24	23	23	23	23	23	23	23	23	23	23
Number of Animals Completed:	(23)	(24)	(23)	(23)	(23)	(23)	(23)	(23)	(23)	(23)	(23)	(23)
EPIDIDYMIS;												
Aplasia	0	0	0	0	0	1	0	0	0	0	0	0
TESTES; LEFT;												
Aplasia	0	0	0	0	0	1	0	0	0	0	0	0

Summary and conclusion:

Groups of 23 mated female Sprague Dawley rats (mean weight: 206 g, 10-12 weeks of age, _____ received HMR 1964 (Batch 1215 Verum) at the dose levels 0, 1, 3.15, or 8 IU/kg body weight or HR 1799 (batch H037) at the dose levels of 1 or 8 IU/kg body weight subcutaneously once daily from day 6 of gestation until day 21 post partum. Behavior and state of health were observed daily in all groups. Body weights were determined during pregnancy and lactation, and food consumption was determined during pregnancy.

Four females from the 8 IU/kg HMR 1964 group were found dead on days 6, 22, 22 and 43 of the study. Nine females from the 8 IU/kg HR 1799 group were found dead on days 6, 7, 18, 22, 22, 24, 24, 26 and 42 of the study. Symptoms observed in these dose groups consisted of hypoactivity, bristling coat, prone position, saltatory and rolling convulsions, serous eye discharge and salivation, which might be secondary effects of the hypoglycemic effect of the test article.

Body weight gains were slightly and transiently decreased in the animals treated with 8 IU HMR 1964 per kg during pregnancy and lactation. There was a slight and transient decrease in food consumption in the groups treated with 8 IU HMR 1964 or 8 IU HR 1799 per kg body weight, which were not the test-article dose dependent.

Gestation length was not altered by the administration of the test compound. Number of implantations, live or dead fetuses, supernumerary implantation sites and birth index

were comparable in both groups. Sex ratio of the pups was not altered by the administration of the test compound.

Fertility of the progeny selected for mating remained unaffected by the treatment of the Fo-animals to the test compound. Likewise, pregnancy and parturition of the F₁-animals were not altered by the treatment of the parent animals with HMR 1964 or HR 1799. Some alterations were observed in F₁-animals. For example, a kinked tail was observed in four males of the HD group from Day 25 - 29 after weaning and in two females from Day 1 or 49 onwards after weaning.

In conclusion, administration of both HMR 1964 and HR 1799 caused clinical signs and mortality in the Fo-animals at the daily dose of 8 IU/kg body weight when administered during embryo- and fetogenesis and during lactation in Sprague-Dawley rats. There were no clear and consistent effects on birth parameters or lactation of the Fo-animals and on postnatal development, fertility or pregnancy of the F₁-animals. No adverse effects were observed after administration of HMR 1964 at the daily dose of 3.15 IU/kg body weight and after administration of HR 1799 at the daily dose of 1 IU/kg body weight. No Observed Adverse Effect Level (NOAEL) may be 3.15 IU HMR 1964/kg body weight and 1.0 IU HR 1799/kg body weight.

3.4.7 Local Tolerance Study:

TITLE: Single-Dose Local Tolerance Study in Rabbits after IV, S.C., IM and Paravenous Administration

Study no: 2000.0273 / Report#: F2000.0499/2000.0329/Document#: F2000Tox0499

Volume # and page #: Module 4 (Aventis 4.2.3.6.1) and page#0-23

Conducting laboratory and location: Aventis Pharma Deutschland GmbH, Drug Innovation and Approval, Lead Optimization, Frankfurt, Germany

Date of study initiation: April 27, 2000/Date of report: June 13, 2000

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: Batch#H601

Methods:

Animals: Two months old female rabbits, New Zealand White (———)/group

HMR 1964 (100 IU/ml) was injected as a single intravenous (0.5 ml), Para venous (0.1 ml), subcutaneous (0.1 ml) and intramuscular (0.5 ml) dose to groups of four female albino New Zealand White rabbits each. Isotonic saline solution (9 mg/ml) was administered as control.

Observations:

The animal's behavior was observed and the injection site was examined after 24 hours, and in two rabbits per group, also after 120 hours. Twenty-four and 120 hours after injection, two animals each were killed and the injected area was dissected and examined grossly and fixed in formalin (4 %) for histological assessment. To prevent potential hypoglycemic shock, the animals received a subcutaneous injection of 20 ml glucose solution (20%) below the nape skin immediately after administration of the test compound.

Results:

All animals tolerated the injections of HMR 1964 and the macro- and microscopic findings were as follows: There were no detrimental incidences after an intravenous administration of HMR1964, although there were slight hemorrhages in the area of the puncture sites. After paravenous injections of HMR1964 a moderate insudation and minimal infiltration were observed. Findings were qualitatively similar and included redness, insudation and infiltration after a subcutaneous or intramuscular administration.

Summary and Conclusions:

HMR 1964 (Batch No. H 601), as a ready-to use aqueous formulation, with a compound concentration of 100 IU/ml, was injected as a single intravenous (0.5 ml), Para venous (0.1 ml), subcutaneous (0.1 ml) and intramuscular (0.5 ml) doses to groups of four female albino New Zealand White rabbits (NZW) BR). Isotonic saline solution (9 mg/ml) was administered as control.

The animals were able to tolerate HMR1964 even after an intravenous administration because they were given glucose. The macro- and microscopic findings were not remarkable. There were minor local reactions such as redness, insudation, and minimal granulocytic infiltrations after HMR administration via all routes.

3.4.8 Special Pharmacology Studies:**TITLE: Testing of recombinant Human Insulin (HMR1964) on Antigenic Determinants following Repeated Administration to Rabbits**

Study no: 189-04/ Report#: F2000.0326/Document#: 189-04

Volume # and page #: Module 4 (Aventis 4.2.3.7.1f2001tpx00118)/0-213

Conducting laboratory and location: Aventis Pharma, Deutschland GmbH, Marburg, Germany

Date of study initiation: Dec. 18, 2000/Date of report: Dec. 6, 2001

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: Batch#1309

Methods:

The purpose of this study was to test the potential immunogenicity of HMR1964. The sponsor used 18-week old female New Zealand White rabbits (2.4 ± 0.17 kg). Ten rabbits/group were assigned to one of the following four groups: 1) control, 2) bovine insulin, 3) HR 1799 human insulin, and 4) HMR 1964, respectively. Each animal treated with respective agents according to the following immunization protocol as shown below. Anti-insulin antibodies were determined by radioimmuno-precipitation (RIP), using radiolabeled HMR 1964, human and bovine insulin tracers.

Study day	Immunization (s.c.) – dose per animal
1	1 IU/animal (1:2 emulsified with Freund's complete adjuvant)
8	0.5 IU/animal (1:2 emulsified with Freund's complete adjuvant)
15	0.25 IU/animal (1:2 emulsified with Freund's complete adjuvant)
22	0.125 IU/animal (1:2 emulsified with Freund's complete adjuvant)
29	0.0625 IU/animal (1:2 emulsified with Freund's complete adjuvant)
36; 43; 50; 57; 64; 71; 78; 85; 92	0.2 IU/animal (without Freund's complete adjuvant) on each of the given study days

Observation:

Determination of body weight (individual data): Before first administration and on study days 8, 22, 36, 50, 64, 78, 92 and 99.

Collection of blood samples: On study days 1 (before first administration), 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 92 (immediately before dosing) and on study day 99. One sample from each animal was collected on each of the given days; serum was distributed between two vials and deep frozen at -20°C .

One set of serum samples was forwarded to the laboratories of _____
 _____ Anti-insulin antibodies in the study groups (HMR 1964, human insulin HR

1799, bovine insulin and HMR 1964-Placebo) were determined by a radioimmunoprecipitation (RIP) method.

Results:

Findings at administration site:

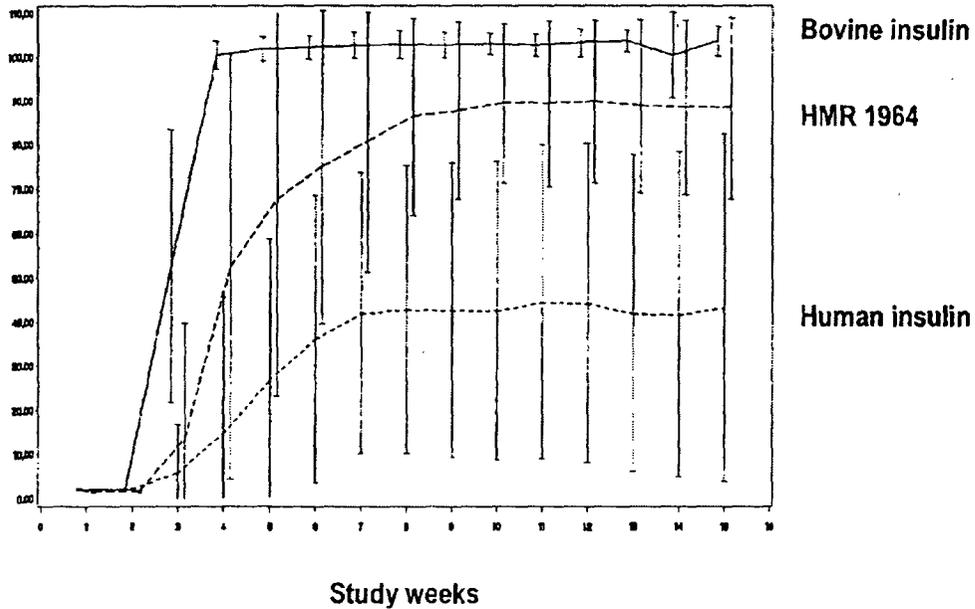
Compound administration triggered skin reactions at the injection site beginning three days after the first administration. Subcutaneous swellings were palpable in all rabbits. Until study day 6, nodular swellings and abscess formation were observed in all animals. Abscesses excreted yellowish turbid fluid and necrotized thereafter. Skin lesions were observed until the end of the study with a poor healing tendency. Keratoconjunctivitis was observed in one rabbit (animal no. 4009) on study day 6. This animal was treated with antibiotic eye ointment.

Mean binding in all dose groups were comparable with the placebo group at week 1 and 2, which indicates that all animals were negative for insulin antibodies before immunization. During immunization the number of responder animals increased until week 4, when all animals in each dose group were detected as responders as shown below. Human insulin binding shows an overall mean binding value of 31.54% with Cmax of 52.68% and median tmax at week 9. The high standard deviations in this group show individual animals' variability in antibody titers.

Mean binding for group (HMR 1964) were 66.78% with Cmax of 94.08% and median of tmax at week 7. The Wilcoxon rank sum test compares bindings of the treatment groups to the placebo. All treatment comparisons with placebo show significant differences for the target parameters' overall mean binding (%), last binding (%) and Cmax binding (%). Tmax values of the bovine insulin group show significant differences to placebo, whereas the tmax values of the human insulin group and the HMR 1964 group do not show significant differences to placebo.

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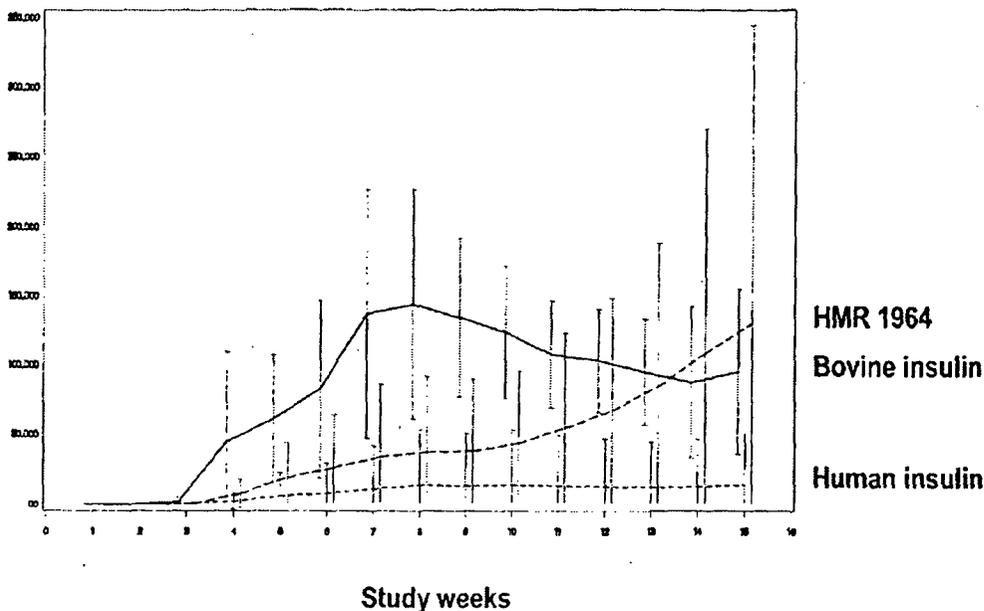
Binding (%)



Mean overall mean titer values were 8.42 (human insulin), 43.15 (HMR 1964) and 80.88 (Bovine Insulin) as shown below. Mean Cmax titer values were 14.50 (human insulin), 145.23 (HMR 1964) and 170.35 (Bovine Insulin). Cmax and SD for the HMR 1964 group is influenced by high titer values of the animals #4003, 4004 and 4010 (individual Cmax titer values of 676.7, 287.8 and 233.0). The Wilcoxon rank sum test shows significant differences between HMR 1964 vs. Bovine insulin for overall mean titer but not for last titer value or Cmax titer. HMR 1964 titers are different from the human insulin titers for overall mean titer, last titer value, and Cmax titer.

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titer



Conclusion:

Results of the HMR 1964 treatment group (group-no. 4) show mean bindings higher than after human insulin administration and lower than that of bovine insulin. Comparison of overall mean titer exemplify statistically significant lower titer in HMR 1964 versus bovine insulin ($p=0.0147$) and higher titer in HMR 1964 versus human insulin ($p=0.0089$). This suggests that the antigenicity of HMR 1964 must be significantly greater than human insulin, but somewhat less than bovine insulin.

TITLE: HMR1964 Determination of Insulin Antibodies in a Six Months Repeated Dose Toxicity Studies in Rats

Study no.: F2000.0326

Volume # and page #: Module 4 (Aventis 4.2.3.2.5 StudyF2001kin0121)

Conducting laboratory and location: Aventis Pharma, D-65926 Frankfurt am Main

Date of study initiation: February 21, 2001

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: Batch#1215

Methods:

Doses: 0, 5, 20, and 80 IU/kg bodyweight

Species/strain:

Number/sex/group or time point (main study): 10 rats/sex/group

Route, formulation, volume, and infusion rate: Subcutaneous administration(twice daily with 8 hours interval), formulation(final formulation), volume(one ml/kg)

Satellite groups used for toxicokinetics or recovery: None

Age: 6-7 weeks old

Weight: Male, 186 g; female, 147 g

Unique study design or methodology (if any): None

Observation and Results:

The presence of antibodies was estimated by determination of the binding of the corresponding ^{125}I -HMR 1964 tracer to the serum fraction and precipitation of the complex by polyethylene glycol. Controls and samples were measured in duplicate. 100 μl of control/sample was incubated with 200 μl of ^{125}I -Insulin tracer and 200 μl of assay buffer for 23 hours at room temperature. Separation of the antibody-bound and free radiolabeled ligand was done by centrifugation in 20% polyethylene glycol solution. The binding percentage of ^{125}I -HMR1964 was calculated as follows: Binding (%) = (Sample counts/total counts) x 100.

The individual and mean levels of HMR1964 tracer binding in the serum of the control and the dosed rats after the 24th and after the 181st administration are shown below. It appears that the HMR1964 tracer binding values in the control and treatment groups do not differ significantly because the values were in the range of non-specific binding, which was determined with the blank control.

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Administration	Gender	Dose	HMR 1964	
HMR 1964		[IU/kg b. wt.]	Tracerbinding [%B/T]	
24th Administration	male	control	0	5.08
		low	5	5.64
		medium	20	5.43
		high	80	5.42
	female	control	0	5.33
		low	5	5.39
		medium	20	5.81
		high	80	5.35
181st Administration	male	control	0	4.72
		low	5	4.66
		medium	20	4.24
		high	80	4.37
	female	control	0	4.99
		low	5	4.80
		medium	20	4.82
		high	80	5.16

Summary and conclusion:

The systemic exposure of HMR 1964 was examined in Sprague Dawley rats in a six months repeated dose toxicity study of HMR 1964. Determination of insulin antibodies was performed concomitant to toxicokinetics to support the interpretation of the results of the repeated dose toxicity study. Groups of 10 rats/gender/dose were administered HMR1964 single daily subcutaneous doses of 0, 5, 20 and 80 IU /kg body weight. The insulin antibody levels in serum were examined approx. 24 hours after the 24st and 181st administration. Insulin antibodies were determined by a semi- quantitative ¹²⁵I-HMR 1964-tracer binding assay. In conclusion, there was no increased binding of HMR1964 tracer in treated animals compared to the control after the 24th and 181st administration. Thus, it can safely say that antibody interferences in the toxicokinetic part of the study can be minimized.

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Insulin glulisine (HMR 1964, 3 β Lys-29 β Glu-human insulin) is an insulin analog obtained by structural modification of human insulin using recombinant DNA techniques and is designed to have similar biological activity as human insulin but a more rapid action when injected subcutaneously. The sponsor performed a series of pharmacology and toxicology studies on HMR1964 to support clinical studies. Pharmacology studies included in vitro and in vivo primary pharmacodynamic studies, safety pharmacology, drug interactions, and pharmacokinetic studies. To characterize the potential toxicity of the test article, single and repeated dose toxicology studies in mice, rats, rabbits and dogs were performed for various duration under GLP conditions according to ICH and EMEA guidelines. Required genotoxicity, reproductive and development toxicity studies, local tolerance and immunogenicity studies were also carried out acceptably.

In most preclinical studies HMR1964 behaved just like human insulin. However, the following several preclinical findings have to be documented.

A cardiovascular safety study was carried out by radiotelemetry method in instrumented free roaming Beagle dogs. The dogs received a single injection of HMR subcutaneously at doses of 0, 0.3 or 1 IU/kg. Various cardiovascular parameters were determined and QTc intervals were calculated. Statistically significantly prolonged QT intervals (Bazett's only) were observed in the treated groups associated with an increased heart rate. Uncorrected QT interval was shortened due to the drug-induced increase in heart rate and QTc corrected by Fridericia's formula was not significantly changed. Therefore, the prolongation of QTc corrected by Bazett's formula may be artifactual secondary to the increase in heart rate. The toxicological significance of these findings is unclear since QT prolongation was only observed in conjunction with increased heart rate and QT prolongation is known to occur secondary to insulin-induced hypoglycemia.

Sprague-Dawley rats received HMR 1964 subcutaneously at doses of 0, 5, 10, 20, and 100 IU/kg/day for 12-month repeated dose study. An increased number of female rats with gross findings related to the mammary gland was noted in low, low mid, and high mid dose HMR 1964 groups and mid and high dose-groups HR1799. However the incidences were not increased with the dose. Metastasis was not seen in any of the rats affected. In none of the rats, cause of death was attributed to the mammary tumor. The incidence of mammary tumors with the insulin analog HMR1964 did not significantly differ from the incidence observed with regular human insulin, HR1799.

In pre- and post-natal toxicity study in female rats (HsD/SD), the rats received HMR 1964 subcutaneously at doses of 0 (control), 1, 3.15, and 8 IU/kg from day 6 of pregnancy until day 21 post partum. In F1 litters, a kinked tail was observed in four males of the HD group from Day 25 - 29 after weaning and in two females from Day 1 or 49 onwards after weaning. It is remarkable that the abnormality was encountered only in the high dose group, not any other low dose groups. These findings are not of concern because they occurred only at the highest dose which was associated with maternal toxicities such as deaths and hypoglycemia.

The detrimental actions of HMR1964 as indicated by the preclinical data presented in this NDA as well as in the original IND61, 956 appear to be similar to those with human insulin. Thus, glulisine appears to be reasonably safe and efficacious relative to other members of the short-acting insulin class, comprising regular insulin and rapid-acting analogs.

Unresolved toxicology issues (if any): None.

Pharmacology Recommendations:

The following preclinical findings should be included in labeling instructions.

a. Carcinogenicity, Mutagenicity, Impairment of Fertility

Standard 2-year carcinogenicity studies in animals have not been performed. In Sprague Dawley rats, a 12-month repeat dose toxicity study was conducted with insulin glulisine at subcutaneous doses of 2.5, 5, 20 or 50 IU/kg twice daily (dose resulting in an exposure 1, 2, 8, and 16 times the average human dose, based on body surface area comparison). There was a non-dose dependent higher incidence of mammary gland tumors in female rats administered insulin glulisine compared to untreated controls. The incidence of mammary tumors for insulin glulisine and regular human insulin was similar. The relevance of these findings to humans is not known.

Insulin glulisine was not mutagenic in the following tests: Ames test, in vitro mammalian chromosome aberration test in V79 cells and in vivo mammalian chromosome aberration test (erythrocyte micronucleus test). In fertility studies in male and female rats at subcutaneous doses up to 10 IU/kg once daily (dose resulting in an exposure 2 times the average human dose, based on body surface area comparison) had no clear adverse effects on male and female fertility, or general reproductive performance of animals were observed.

Pregnancy - Teratogenic Effects - Pregnancy Category C

Reproduction and teratology studies have been performed with insulin glulisine in rats and rabbits using regular human insulin as a comparator. The drug was given to female rats throughout pregnancy at subcutaneous doses up to 10 IU/kg once daily (dose resulting in an exposure 2 times the average human dose, based on body surface area comparison). Insulin glulisine did not have remarkable toxic effects on the embryo-fetal development in rats.

The drug was given to female rabbits throughout pregnancy at subcutaneous doses up to 1.5 IU/kg/day. Adverse effects on embryo-fetal development were only seen at maternal toxic dose levels inducing hypoglycemia. Increased incidence of post-implantation losses and skeletal defects were observed at a dose level of 1.5 IU/kg once

daily (dose resulting in an exposure 0.5 times the average human dose, based on body surface area comparison) that also caused mortality in dams. A slightly increased incidence of post-implantation losses was seen at the next lower dose level of 0.5 IU/ kg once daily (dose resulting in an exposure 0.2 times the average human dose, based on body surface area comparison), which was also associated with severe hypoglycemia but there were no defects at that dose. No effects were observed in rabbits at a dose of 0.25 IU/ kg once daily (dose resulting in an exposure 0.1 times the average human dose, based on body surface area comparison). The effects of insulin glulisine did not differ from those observed with subcutaneous regular human insulin at the same doses and were attributed to secondary effects of maternal hypoglycemia.

There are no well-controlled clinical studies of the use of insulin glulisine in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. 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.

Nursing Mothers: It is unknown whether insulin glulisine is excreted in human milk. Many drugs, including human insulin, are excreted in human milk. For this reason, caution should be exercised when APIDRA is administered to a nursing woman.

Pediatric Use: Safety and effectiveness of APIDRA in pediatric patients have not been established.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

3.7. APPENDIX/ATTACHMENTS

In this appendix, three documents are attached: Appendix I) original IND#61,956 dated Oct. 5, 2001, Appendix II) pharmacology review dated February 18, 2002 and Appendix III) Executive CAC minute dated March 20, 2001.

Appendix I**REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:**

KEY WORDS: Diabetes, obesity, insulin analogue

Reviewer Name: Herman M. Rhee, Ph.D., Pharmacologist

Division Name: Division of Metabolic and Endocrine Drug Products (DMEDP)
HFD#510

Review Completion Date: 10/5/2001; **Review Assigned Date:** 5/5/2001

Review number: 001

IND/NDA NUMBER: 61,956

Serial number/date/type of submission: 000/May 3, 2001/Commercial

Information to sponsor: Yes (x) No ()

Sponsor (or agent): Aventis Pharmaceuticals Inc., Bridgewater, NJ.

Manufacturer for drug substance: Aventis Pharmaceuticals Inc., Bridgewater, NJ.

DRUG: HMR1964 (rDNA human insulin analog)

Code Name: 199 15033

Generic Name: Not assigned

Trade Name: Not assigned

Table of Contents of IND 61,956 (HMR 1964)		
Description of Study	Study#	Page
Pharmacology, HMR1964 Binding Studies		4
Safety Pharmacology	99/11004/PH	5
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PK and TK studies		10
Table for Therapeutic Exposure Estimation		11
4-Week Subcutaneous Toxicity Study in Rats	99.0569	12
6-Month Subcutaneous Toxicity Study in Rats	2000.1110	17
1-Month Subcutaneous Toxicity Study in Dogs	1999.0699	23
6-Month Subcutaneous Toxicity Study in Dogs	2000.0325	27
Fertility Study in Rats	2000.00919	35
Embryo-Fetal Toxicity Study in Rats	2000.0893	39
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Bacterial reverse mutation test	98.523	56

In vitro mammalian chromosome aberration test	98.0524	59
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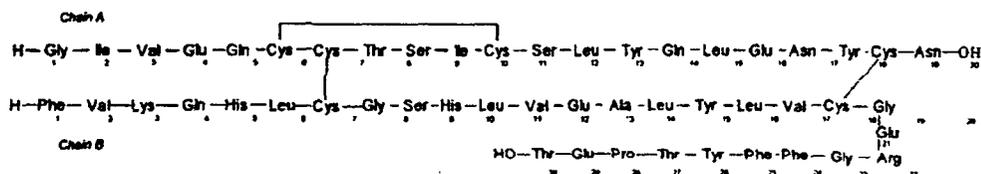
Chemical Name: 3^B-Lys-29^B-Glu human insulin/C₂₅₈H₃₈₄N₆₄O₇₈S₆

Excipients: Please see "Clinical Formulation" section.

CAS Registry Number: None

Molecular Formula/ Molecular Weight: 5823

Structure:



Relevant INDs/NDAs/DMFs:

NDA#20-563 (Humalog, LysPro), NDA#20-081 (Lantus), and NDA#20-986 (Novolog)

Drug Class: Fast-acting insulin analogue prepared by E. coli recombinant Indication: Diabetes

Clinical formulation:

HMR1964, which is equimolar to 100 IU/mL human insulin. The formulation contains 3.15 mg/mL m-cresol, 5 mg/mL NaCl, 6 mg/mL trometamol, 0.01 mg/ml polysorbate 20. HMR 1964 comes as a solution for injection in 10 mL vials

Route of administration: Intended routes of administration are s.c. and i.v. injection.

Proposed clinical protocol or Use:

Phase I - 0.3 IU/kg sc in 16 healthy volunteers for 7 days.

Phase III-330 patients for 6 months

Previous clinical experience:

Four phase 1 studies in healthy volunteers have been completed. A single dose of 0.1-0.3 IU/kg HMR1964 was administered s.c. or i.v. for pharmacokinetics, safety, tolerance, and PD studies in total 64 healthy male volunteers. Pharmacokinetic results of clinical study #1003 that was performed in Europe are summarized in a table below. $AUC_{0-clamp\ end} = 32,780 \mu IU \cdot min/ml = 546 \mu IU \cdot hr/ml$. The AUC value is equal to 19 ng.h/ml, dividing the AUC value by 28.6 $\mu IU/ng$.

Study 1003: Pharmacokinetic results

Variable	Geometric mean (N = 16)		
	HMR1964 (0.3 IU/kg) ^a	Regular human insulin	Insulin lispro
$AUC_{(0-1h)}$ [$\mu IU \cdot min \cdot mL^{-1}$]	6711.85	3516.46	7184.04
$AUC_{(0-1.5h)}$ [$\mu IU \cdot min \cdot mL^{-1}$]	11179.38	6197.85	11859.00
$AUC_{(0-2h)}$ [$\mu IU \cdot min \cdot mL^{-1}$]	15316.36	8862.56	15634.97
$AUC_{(0-clamp\ end)}$ [$\mu IU \cdot min \cdot mL^{-1}$]	32779.88	25210.74	26082.80
C_{max} [$\mu U/mL$]	162.68	99.19	179.92
t_{max} [min]	63 ^b	84 ^b	47 ^b
MRT [min]	139.59	171.31	112.69

^a The HMR 1964 formulation used in this study contained 7.5 $\mu g/mL$ Zn.

^b Median values.

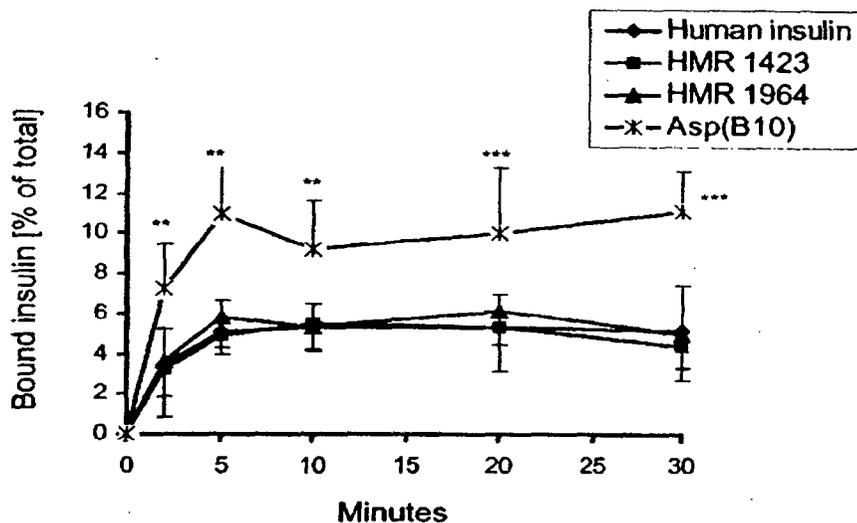
Disclaimer -- use of sponsor's material: use of sponsor's material: Relevant portions of the sponsor's IND submission may be used directly without any modification.

PHARMACOLOGY:

HMR1964 Binding Studies:

Insulin receptor association was analyzed utilizing ¹²⁵I-labelled insulin in rat fibroblast cells. The binding of HMR1964 to insulin receptor was comparable to that of human insulin, although the receptor affinity of Asp (B10) insulin was significantly elevated as shown in a fig. below.

To evaluate the binding to the IGF-1 receptor and mitogenic effect of HMR1964, studies were performed in rat cardiomyoblasts and human osteosarcoma cells. The IGF-1 receptor affinity of HMR 1964 was lower than that of human insulin.



Rat-1 fibroblasts over-expressing human insulin receptor isoform B were incubated with human insulin or the analogues. Ligand-association was determined for 0-30 min.

n = 6 ± SD.

** p<0.01, *** p<0.001.

HMR1964 Binding to IGF-1 Receptor:

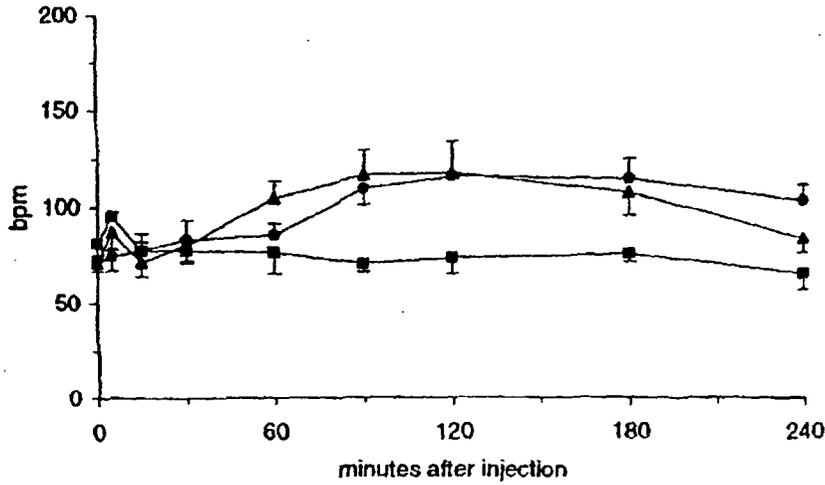
HMR binding to IGF-1 receptors was compared with insulin and insulin analogues, which is summarized in a table below.

HMR1964 Affinity to IGF-1 Receptors in Osteosarcoma B10 Cells		
Test substance	IC ₅₀ Values (nM)	Relative affinity
IGF-1	0.043	11930
Human insulin	513	1.00
Asp(B10) insulin	145	3.54
HMR1964	3162	0.16

Safety Pharmacology (Document# 99/11004/PH):

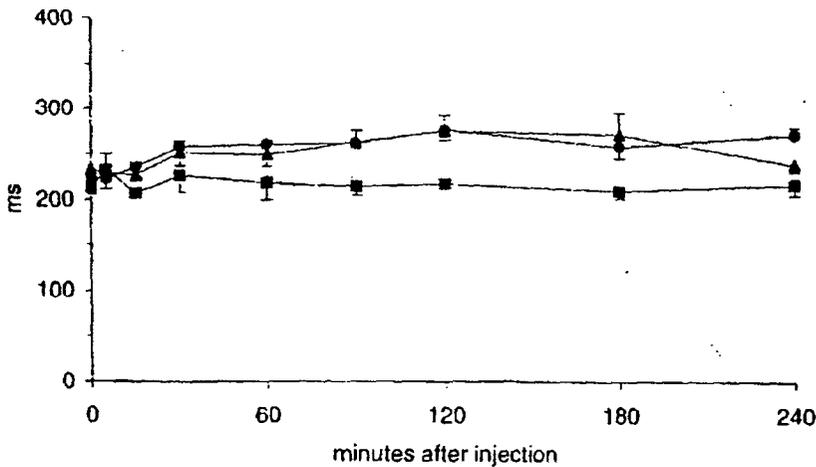
Three male or female beagle dogs per group were administered HMR1964 subcutaneously at doses of 0.3 or 1 IU/kg (Batch# 1081). Cardiovascular parameters were monitored telemetrically. There were dramatic increases in heart rate to 60 to 65% above initial values from 30 minutes after the treatment as shown below. An increase of corrected QT interval duration was noted after the treatment. Approximately 23% and 18% increases in QT intervals were observed in 1 IU/kg and 0.3 IU/kg groups, respectively. Breathing rate was also increased after the doses of 0.3 and 1 IU/kg. At maximum, the rate increased by 7 or 8 breaths/min from mean initial values of 23 or 24 breaths/min, an increase of approximately 30%. There were no remarkable drug effects on cardiovascular parameters such as blood pressure, PR interval, QRS complex duration or body temperature.

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Effect of a subcutaneous injection of HMR 1964 on heart rate in the conscious telemetered dog

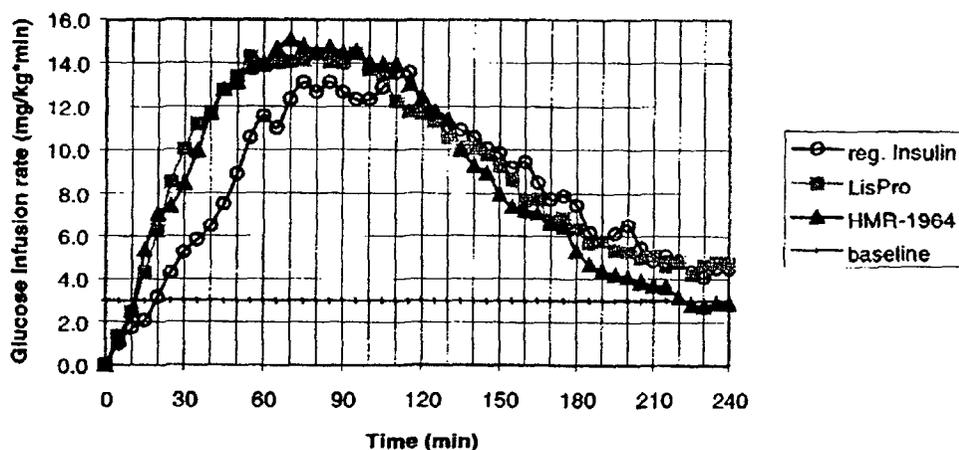
■ vehicle ▲ 0.3 I.U./kg ● 1 I.U./kg (3 dogs per group)



Effect of a subcutaneous injection of HMR 1964 on QT interval duration corrected by Bazett's formula in the conscious telemetered dog

■ vehicle ▲ 0.3 I.U./kg ● 1 I.U./kg (3 dogs per group)

Mechanism of Action: In dogs, the hypoglycemic activity of HMR1964 was faster than that of human insulin and comparable to LisPro as shown below. The mode of action of HMR1964 appeared similar to other insulin analogues. For example, according to the in vitro pharmacologic studies performed by the sponsor, HMR 1964 and human insulin have comparable insulin receptor association kinetics and maximal binding in transformed rat embryo fibroblast over expressing the insulin receptor. Steady state binding experiments in human insulin receptor preparations indicate that HMR 1964 has a slightly lower affinity to the insulin receptor than human insulin. Lipogenic activity and glucose transport in isolated rat adipocyte was slightly reduced compared to human insulin regarding the dose response.



Crossover glucose clamp results in dogs (n=18, 0.3 IU insulin/kg s.c.)

Immunotoxic Studies:

In preIND meeting on Nov. 7, 2000, the sponsor was requested to test the potential effect of HMR1964 on antibody formation. Groups of 4 dog/sex/dose were administered single daily s.c. doses of 0, 0.5, 1 and 21 IU/kg of the test article. The insulin antibody levels in serum were examined approximately 24 hours after the 30th and 176th administration. Insulin antibodies were determined by a semi-quantitative ¹²⁵I-HMR1964 tracer binding assay. The results were expressed as in % B/T {(sample counts/total accounts) x 100} as shown below. There was no increase in tracer binding in female groups at any doses of HMR1964. In male group, there was an increase in tracer binding in a few animals in the medium and high dose group after the 30th and 176th administration. The increase was observed in a few animals without dose dependency.

Insulin antibodies in 6-month dog study

Administration HMR1964	Gender	Dose [IU/kg body wt.]	Mean HMR1964 tracer binding [% B/T]
30th Administration	Male		
	control	0	4.07
	low	0.5	4.34
	medium	1.0	15.09
	high	2.0	6.80
	Female		
	control	0	4.07
	low	0.5	4.21
176th Administration	Male		
	control	0	4.25
	low	0.5	4.44
	medium	1.0	17.12
	high	2.0	5.84
	Female		
	control	0	4.00
	low	0.5	4.14
medium	1.0	4.47	
high	2.0	4.87	

Samples were collected approximately 24 hours after administration.

Ancillary Pharmacology Studies:

HMR1964 effects on DNA synthesis (thymidine incorporation) were comparable to human insulin as shown below. Stimulation of MAP kinase activation related to mitogenic effects was lower for HMR 1964 relative to human insulin. In rat fibroblast cells, HMR1964 and human insulin had the same mitogenic effect with a lower activity of HMR 1964 at 1 and 10 nM.

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Thymidine incorporation

Concentration [nM]	Human Insulin		HMR 1964		Asp(B10)		HMR 1423	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.00	1.0	0.43						
0.01	1.4	0.80	1.5	0.72	2.0	1.33	1.1	0.45
0.1	1.9	1.28	1.7	1.11	2.5	1.67	1.9	1.08
1.0	5.7	3.79	4.0	2.73	7.9	3.23	5.9	3.52
10	13.5	6.00	10.0	3.15	12.7	4.24	15.9	7.75

Summary of pharmacology:

Single dose s.c. administration of HMR 1964 to rats and dogs resulted in maximum concentrations at 0.167 and 1 hour post-dosing. HMR1964 was stable in vitro studies in human plasma, while it degraded rapidly in rat plasma. It appeared that HMR1964 has similar affinity to IGF-1 receptor with comparable mitogenic activity to human insulin.

In sponsor's preliminary study, the QT interval duration (corrected for heart rate variation by appropriate formulas) was increased dose dependently with an increase in heart rate in dogs. But, the test article had no effects on QT interval in 6-month dog study (#2000.0325). Such increases have been observed during hypoglycemia in man (Marques et al., Diabetic Medicine 14:648-54, 1997; Eckert and Agardh, Clin. Physiol. 6:570-5, 1998).

IGF-1 receptor affinity of HMR1964 has been compared with insulin in Hep G2 cells, and indicates that the receptor affinity was lower than that of human insulin. HMR1964 induces a higher IGF-1 receptor autophosphorylation compared to human insulin and DNA synthesis (thymidine incorporation) was comparable to that observed with human

insulin. Stimulation of MAP kinases activation by HMR1964 was less than human insulin.

In osteosarcoma cells, HMR1964 had reduced affinity to the IGF-1 receptor (20% relative to human insulin) and LisPro had a slight higher IGF-1 receptor affinity, compared to HMR1964. HMR1964 and insulin had a comparable degree of stimulation of thymidine incorporation in transformed rat embryo fibroblast cells. Thus, the carcinogenic potential of HMR1964 would not be expected to exceed that of human insulin.

PHARMACOKINETICS/TOXICOKINETICS:

PK parameters:

Absorption: Single intravenous and subcutaneous injections of HMR1964 in rats and dogs are summarized in a table below. The PK data after repeated-dose administration of HMR1964 will be discussed under toxicokinetic studies.

Variable	Rats		Dogs	
	0.5 mg/kg i.v.	2 mg/kg s.c.	0.05 mg/kg i.v.	0.05 mg/kg s.c.
C _{max} (ng/mL)				
t _{max} (h)	0.083	0.167	0.083	1
t _{1/2} (h)	0.025	0.35	0.92	1.11
AUC _∞ (ng*h/mL)	488.5	2025.6	130.2	55.1

Distribution: No studies have been performed and the sponsor has no plan to do tissue distribution of HMR1964.

Metabolism: Stability of ¹²⁵I-HMR1964 and _____ was studied in heparinized plasma at 37°C and subsequent processing and analysis by HPLC. Degradation or metabolic products were correlated to reference compounds available by comparison of retention times in HPLC. HMR1964 was stable during 8 hours incubation in human plasma, but highly unstable in rat plasma. One degradation product formed in both species was _____ by cleavage of the labeled 13^A-tyrosine. Another product was polar, which might be a _____. The sponsor decided not to further evaluate the potential metabolites, although they concluded that endogenous degradation of HMR1964 occurs by the same pathway as for human insulin metabolism.

Elimination: The sponsor did not perform specific studies on HMR1964 elimination.

Toxicokinetics:

Toxicokinetic data in 1-, 6-month rat and dog studies are summarized under individual studies. Based on AUC values available from the major toxicology studies, the

therapeutic exposure ratio were calculated as shown on page 11. The ratio was also calculated based on body surface comparison because the AUC ratios were quite variable due to limited sample sizes. Clinical $AUC_{0-6} = 19 \text{ ng.h/ml}$ after 0.3 IU/kg.

Table for Therapeutic Exposure Estimation

Therapeutic Exposure Ratios of HMR1964 in Major Toxicology Studies				
Study	Dose (IU/kg)	AUC(ng.h/ml)	AUC ratio*	BSA ratio@
1-Month Rat	50 ^{\$}	1880	99	27
	150	7106	374	82
	500	19088	1005	272
6-Month Rat	5 ^{\$}	165	9	3
	20	750	39	11
	80	4124	217	44
1-Month Dog	1	60	3	2
	3 ^{\$}	264	14	5
	10	2016	106	18
6-Month Dog	0.5 ^{\$}	50	3	0.9
	1	98	5	1.8
	2	155	8	3.6
Fertility Study in Rat	1			0.5
	3.15 ^{\$}			1.7
	10			5.5
Embryo-fetal Study in Rat	1			0.5
	3.15 ^{\$}			1.7
	10			5.5
Rabbit Repro	0.25 ^{\$}			0.27
	0.5			0.54
	1.5			1.64

*Based on clinical $AUC_{0-6} = 19 \text{ ng.h.ml}$ after 0.31 IU/kg. @Calculated based on body surface area comparison. \$ indicates NOAEL value.

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TOXICOLOGY:

Study Title: 4-Week Subcutaneous Toxicity Study in Rats

Study/Report: 99.0569

Document: F1999TOX0178

Amendment # 000: Vol #6, and page # 1-392

Conducting laboratory and location: Dept. Toxicology and Pathology, Hoechst Marion
Roussel Deutschland GmbH, Frankfurt, Germany

Date of study initiation: Sept. 21, 1999

GLP compliance: Yes

QA- Report Yes (x) No ()

METHODS:

Species/strain: Hsd: Sprague Dawley SD rats

#/sex/group or time point: 10 rats/sex/group for main study and 5 rats/sex/group for
kinetic study

Dosage groups in administered units: 0, 50, 150, and 500 IU/kg

Route, form, volume, and infusion rate: Subcutaneous

Drug, lot#, radiolabel, and % purity: Batch#1120, Inspected on 8/23/1999

Formulation/vehicle: m-Cresol(3.15 mg/ml), Trometamol(6.0 mg/ml), NaCl(5 mg/ml),
Tween 20(0.01 mg/ml), pH = 7.3 with NaOH and/or HCl

RESULTS:

Clinical signs: Nine males and three females from the high dose group died during the
study. There were two deaths in males from the intermediate dose group. It appears that
the deaths were due to drug-induced hypoglycemia secondary to fasting conditions for
urine sampling. Hypoactivity was noted in three males from the high dose group from
days 1-3 of the study.

Body weights: No marked change in the body weight gain was found in male, although
the parameter was increased in female rats.

Food consumption: Not remarkable.

Hematology: There were no treatment-related changes in 50 and 150 IU/kg male and female groups (Please see the table below). Adequate hematological evaluations were not possible in the 500 IU/kg groups in males because of limited number (N = 1) of surviving animal as shown below.

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ATOX 10.1

HMR Deutschland GmbH

RUN DATE: 08/11/99

SUMMARY AND STATISTICAL EVALUATION

STUDY : 99.0430 - 4 Weeks subcutaneous toxicity Study with HMR 1964 in rats

FINAL VALUE

HEMATOLOGY - RED CELL COUNT (MALE)

		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0	50	150	500
		IU/kg	IU/kg	IU/kg	IU/kg
Red blood cell count (10E12/L)	MEAN	7.52	7.44	7.52	7.31 M
	S.D.	0.30	0.24	0.21	
	N	10	9	8	1
Hemoglobin (g/L)	MEAN	149	147	152	152 M
	S.D.	6	4	4	
	N	10	9	8	1
Hematocrit (unity)	MEAN	0.46	0.45	0.46	0.47 M
	S.D.	0.02	0.01	0.01	
	N	10	9	8	1
MCV (10E-15 L)	MEAN	61	61	62	65 M
	S.D.	1	2	1	
	N	10	9	8	1
MCH (10E-12 g)	NE MEAN	20	20	20	21
	S.D.	0	0	1	
	N	10	9	8	1
MCHC (g/L)	NE MEAN	327	325	327	321
	S.D.	5	7	5	
	N	10	9	8	1
Reticulocytes (unity)	MEAN	0.011			0.008 M
	S.D.	0.005			
	N	10			1
Heinz Bodies (unity)	NE MEAN	0.000			0.000
	S.D.	0.000			
	N	10			1

WILCOXON-TEST, TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES, IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL (P < 0.05).

+/- : SIGNIFICANTLY DIFFERENT FROM CONTROL
M : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

R

FINAL VALUE

RELATIVE ORGAN WEIGHTS MALES (g/kg bwmt)

		GROUP 1 0 IU/kg	GROUP 2 50 IU/kg	GROUP 3 150 IU/kg	GROUP 4 500 IU/kg
BODY WEIGHT (g) NE	MEAN	271.0	274.5	279.9	282.0
	S.D.	17.4	14.0	17.2	
	N	10	10	8	1
Heart Weight	MEAN	3.828	3.927	3.872	3.922 M
	S.D.	0.350	0.442	0.294	
	N	10	10	8	1
Lungs Weight	MEAN	4.948	4.615	5.490	4.993 M
	S.D.	0.625	0.725	0.940	
	N	10	10	8	1
Liver Weight	MEAN	38.017	35.431	34.277	34.539 M
	S.D.	1.308	1.675	1.725	
	N	10	10	8	1
Kidneys Weight	MEAN	7.143	6.822	6.672	6.926 M
	S.D.	0.577	0.349	0.333	
	N	10	10	8	1
Spleen Weight	MEAN	2.797	2.552	2.324	2.720 M
	S.D.	0.161	0.243	0.194	
	N	10	10	8	1
Testes Weight	MEAN	12.034	11.813	11.672	11.117 M
	S.D.	0.911	0.842	0.844	
	N	10	10	8	1
Adrenals Weight	MEAN	0.1357	0.1462	0.1383	0.1773 M
	S.D.	0.0200	0.0183	0.0154	
	N	10	10	8	1

WILCOXON-TEST. TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES, IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL (P < 0.05).

+/- : SIGNIFICANTLY DIFFERENT FROM CONTROL
M : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

Clinical chemistry: In males from the high dose group, no statistical evaluation was performed since only one animal survived until sacrifice. Decreased calcium and protein values with increased chloride levels in males from the low and intermediate dose group as shown below. The impact and significance are questionable since only one sex was affected.

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HMR Deutschland GmbH

RUN DATE: 27/10/99

SUMMARY AND STATISTICAL EVALUATION

STUDY : 99.0430 - 4 Weeks subcutaneous toxicity Study with HMR 1964 in rats

FINAL VALUE

CLINICAL CHEMISTRY (MALE)

		GROUP 1 0 IU/kg	GROUP 2 50 IU/kg	GROUP 3 150 IU/kg	GROUP 4 500 IU/kg
Sodium (mmol/l)	MEAN	139	139	139	143 M
	S.D.	2	1	1	
	N	10	10	8	1
Potassium (mmol/l)	MEAN	5.51	5.68	5.90	5.49 M
	S.D.	0.41	0.44	0.42	
	N	10	10	8	1
Calcium (mmol/l)	MEAN	2.49	2.43 -	2.39 -	2.39 M
	S.D.	0.06	0.06	0.06	
	N	10	10	8	1
Chloride (mmol/l)	MEAN	101	103 +	103 +	105 M
	S.D.	1	2	1	
	N	10	10	8	1
Phosphorus (mmol/l)	MEAN	3.06	3.06	3.08	3.17 M
	S.D.	0.14	0.17	0.14	
	N	10	10	8	1
Total Bilirubin (umol/l)	MEAN	4.1	3.8	3.7	4.0 M
	S.D.	0.8	0.8	0.9	
	N	10	10	8	1
Glucose (mmol/l)	MEAN	17.16	17.51	18.56	14.30 M
	S.D.	3.47	1.87	1.43	
	N	10	10	8	1
Uric Acid (umol/l)	MEAN	62	57	60	83 M
	S.D.	14	11	18	
	N	10	10	8	1

WILCOXON-TEST. TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES. IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL (P < 0.05).

+/- : SIGNIFICANTLY DIFFERENT FROM CONTROL
M : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

Urine analysis: Not remarkable.

Gross pathology:

Gross lesions observed in the animals found dead included empty stomach and thickened injection sites. The dead animal had congestion of organs that suggested cardio-respiratory failure, probably due to hypoglycemia. The injection sites showed foreign body granulomas, fibrosis, edema, fibrin deposits and myositis. The frequency and severity of the injection site problems were frequent in the control and the high dose group, indicating that the high volume of the vehicle used in these groups was not well tolerated.

Organ weights:

Spleen to body weight ratios were decreased to a statistically significant degree in males from the low and intermediate dose group and in females from all dose groups. These changes were not dose-dependent at least in females, and histopathological examinations did not reveal any compound-related changes in this organ. Liver weight was also reduced in the low and mid dose groups as shown below. The data were not adequate for the high dose in males due to limited survival and sample size as discussed previously.

STUDY : 99.0430 - 4 Weeks subcutaneous toxicity Study with HMR 1964 in rats

FINAL VALUE

RELATIVE ORGAN WEIGHTS MALES (g/kg bwwt)

		GROUP 1 0 IU/kg	GROUP 2 50 IU/kg	GROUP 3 150 IU/kg	GROUP 4 500 IU/kg
BODY WEIGHT (g) NE	MEAN	271.0	274.5	279.9	282.0
	S.D.	17.4	14.0	17.2	
	N	10	10	8	1
Heart Weight	MEAN	3.828	3.927	3.872	3.922 M
	S.D.	0.350	0.442	0.294	
	N	10	10	8	1
Lungs Weight	MEAN	4.948	4.615	5.490	4.993 M
	S.D.	0.625	0.725	0.940	
	N	10	10	8	1
Liver Weight	MEAN	38.017	35.431	34.277	34.539 M
	S.D.	1.308	1.675	1.725	
	N	10	10	8	1
Kidneys Weight	MEAN	7.143	6.822	6.672	6.926 M
	S.D.	0.577	0.349	0.333	
	N	10	10	8	1
Spleen Weight	MEAN	2.797	2.552	2.324	2.720 M
	S.D.	0.161	0.243	0.194	
	N	10	10	8	1
Testes Weight	MEAN	12.034	11.813	11.672	11.117 M
	S.D.	0.911	0.842	0.844	
	N	10	10	8	1
Adrenals Weight	MEAN	0.1357	0.1462	0.1383	0.1773 M
	S.D.	0.0200	0.0183	0.0154	
	N	10	10	8	1

WILCOXON-TEST. TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES, IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL (P < 0.05).

+/- : SIGNIFICANTLY DIFFERENT FROM CONTROL
M : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

**APPEARS THIS WAY
ON ORIGINAL**

ATOX 10.1

HMR Deutschland GmbH

RUN DATE: 21/10/99

SUMMARY AND STATISTICAL EVALUATION

STUDY : 99.0430 - 4 Weeks subcutaneous toxicity Study with HMR 1964 in rats

FINAL VALUE

RELATIVE ORGAN WEIGHTS FEMALES (g/kg bwwt)

		GROUP 1 0 IU/kg	GROUP 2 50 IU/kg	GROUP 3 150 IU/kg	GROUP 4 500 IU/kg
BODY WEIGHT (g) NE	MEAN	201.6	203.9	203.4	203.0
	S.D.	12.0	9.9	9.0	18.0
	N	10	10	10	7
Heart Weight	MEAN	4.097	4.094	4.049	4.059
	S.D.	0.334	0.369	0.489	0.250
	N	10	10	10	7
Lungs Weight	MEAN	5.700	5.717	5.304	5.342
	S.D.	0.763	0.812	0.386	0.522
	N	10	10	10	7
Liver Weight	MEAN	36.663	35.719	34.643	35.008
	S.D.	1.274	2.002	1.348	1.863
	N	10	10	10	7
Kidneys Weight	MEAN	6.472	6.501	6.307	6.339
	S.D.	0.512	0.583	0.460	0.231
	N	10	10	10	7
Spleen Weight	MEAN	3.213	2.773	2.897	2.765
	S.D.	0.434	0.367	0.372	0.240
	N	10	10	10	7
Ovaries Weight	MEAN	0.6204	0.6040	0.5973	0.5944
	S.D.	0.0762	0.0693	0.0753	0.0623
	N	10	10	10	7
Adrenals Weight	MEAN	0.2630	0.2504	0.2506	0.2614
	S.D.	0.0317	0.0481	0.0224	0.0305
	N	10	9	10	7

WILCOXON-TEST. TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES. IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL (P < 0.05).

/ : SIGNIFICANTLY DIFFERENT FROM CONTROL
 M : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

Toxicokinetics: (See page 11 for therapeutic exposure ratio)

Toxicokinetics in 1-month rat study

HMR1964 Administration	Gender	Dose [IU/kg]	t _{max} [h]	C _{max} [ng/mL]	AUC _{0-8h} [ng*h/mL]	AUC _{0-24h} [ng*h/mL]
1 st	Male					
	control	0	0.50	—	6.2	—
	low	50	0.25	—	1303.7	—
	medium	150	0.50	—	4557.4	—
	high	500	0.25	—	15696.9	—
	Female					
	control	0	0.50	—	7.8	—
	low	50	0.25	—	1876.1	—
	medium	150	0.50	—	5558.6	—
	high	500	0.50	—	14589.7	—
23 rd /28 th	Male					
	control	0	0.25	—	10.3	27.8
	low	50	0.25	—	1833.2	1861.0
	medium	150	0.50	—	7038.6	7111.9
	high	500	1.00	—	20903.0	21934.7
	Female					
	control	0	0.50	—	8.8	27.1
	low	50	0.50	—	1865.4	1899.1
	medium	150	1.00	—	7027.9	7105.8
	high	500	0.50	—	15872.9	16240.6

Samples were collected at 0.25, 0.5, 1, 3, and 6 hours after administration; 24-hour samples were taken following the 28th administration after exsanguination.

Hematology: A slight but significant reduction of red blood cells counts in high dose males was observed at the interim and final examination. A slight but significant increase in MCV was observed in all treated female and the high dose male groups as shown in two tables below.

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ON ORIGINAL

Toxicology Study Report - DSE 2000.1100
HMR 1964

ATOX 10.2

ARTENS II

SUMMARY AND STATISTICAL EVALUATION

STUDY : 2000.0326 - HMR 1964 - 6 month subcutaneous toxicity study in rats

INTERIM VALUE

HEMATOLOGY - RED CELL COUNT (MALE)

		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0	5	20	80
		I.U./kg	I.U./kg	I.U./kg	I.U./kg

Red blood cell count (10E12/l)	MEAN	8.50	8.37	8.55	8.18
	S.D.	0.33	0.17	0.36	0.29
	N	10	10	10	9
Hemoglobin (g/l)	MEAN	149	149	152	151
	S.D.	7	4	4	8
	N	10	10	10	9
Hematocrit (unity)	MEAN	0.47	0.47	0.48	0.48
	S.D.	0.02	0.01	0.01	0.03
	N	10	10	10	9
MCV (10E-15 l)	MEAN	55	57	56	59 +
	S.D.	2	1	1	2
	N	10	10	10	9
MCH (10E-12 g)	NE MEAN	18	18	18	19
	S.D.	1	0	1	1
	N	10	10	10	9
MCHC (g/l)	NE MEAN	320	316	320	311
	S.D.	5	3	3	6
	N	10	10	10	9
Reticulocytes (unity)	MEAN	0.029	0.031	0.028	0.030
	S.D.	0.002	0.004	0.002	0.002
	N	10	10	10	9
met-HB (%)	NE MEAN	2.07			1.66
	S.D.	1.64			1.16
	N	9			9

WILCOXON-TEST. TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES,
IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL (P < 0.05).

+/- : SIGNIFICANTLY DIFFERENT FROM CONTROL
M : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

Toxicology Study Report - DSE 2000.1100
HMR 1964

ATOX 10.2

ARTEMIS II

SUMMARY AND STATISTICAL EVALUATION

STUDY : 2000.0326 - HMR 1964 - 8 month subcutaneous toxicity study in rats

INTERIM VALUE

HEMATOLOGY - RED CELL COUNT (FEMALE)

		GROUP 1 0 I.U./kg	GROUP 2 5 I.U./kg	GROUP 3 20 I.U./kg	GROUP 4 80 I.U./kg
Red blood cell count (10E12/l)	MEAN	7.58	7.58	7.82	7.66
	S.D.	0.41	0.23	0.23	0.24
	N	10	10	10	9
Hemoglobin (g/l)	MEAN	141	143	147	146
	S.D.	5	4	5	5
	N	10	10	10	9
Hematocrit (unity)	MEAN	0.42	0.43	0.45 +	0.45 +
	S.D.	0.02	0.01	0.01	0.02
	N	10	10	10	9
MCV (10E-15 l)	MEAN	56	57 +	57 +	59 +
	S.D.	1	1	2	2
	N	10	10	10	9
MCH (10E-12 g)	NE MEAN	19	19	19	19
	S.D.	1	1	1	1
	N	10	10	10	9
MCHC (g/l)	NE MEAN	333	329	330	324
	S.D.	8	4	5	10
	N	10	10	10	9
Reticulocytes (unity)	MEAN	0.032	0.034	0.039	0.043 +
	S.D.	0.006	0.008	0.010	0.004
	N	10	10	10	9
ret-HB (%)	NE MEAN	1.61			1.24
	S.D.	1.02			0.50
	N	9			8

WILCOXON-TEST. TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES, IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL (P < 0.05).

+/- : SIGNIFICANTLY DIFFERENT FROM CONTROL
M : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

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Clinical Chemistry: In males in mid and high dose groups, total protein and globulin were decreased while the levels of creatinine and glucose were increased dose-dependently as shown below. The similar changes were observed in female rats. The above observations in both sexes were reversible after the recovery period.

ATOX 10.2

ARTENIS II

RUN DATE: 07-MAR-2001

SUMMARY AND STATISTICAL EVALUATION

STUDY : 2000.0326 - NMR 1964 - 6 month subcutaneous toxicity study in rats

INTERIM VALUE

CLINICAL CHEMISTRY (MALE)

		GROUP 1 0 I.U./kg	GROUP 2 5 I.U./kg	GROUP 3 20 I.U./kg	GROUP 4 80 I.U./kg
Sodium (mmol/l)	MEAN	150	151	149	150
	S.D.	1	1	1	2
	N	10	10	10	8
Potassium (mmol/l)	MEAN	6.21	6.14	6.11	6.35
	S.D.	0.13	0.30	0.15	0.48
	N	10	10	10	8
Calcium (mmol/l)	MEAN	2.60	2.63	2.60	2.63
	S.D.	0.03	0.05	0.05	0.07
	N	10	10	10	8
Chloride (mmol/l)	MEAN	103	104	101	102
	S.D.	2	2	2	2
	N	10	10	10	8
Phosphorus (mmol/l)	MEAN	2.22	2.19	2.16	2.26
	S.D.	0.15	0.14	0.16	0.29
	N	10	10	10	8
Total Bilirubin (umol/l)	MEAN	4.3	3.5	4.5	4.2
	S.D.	0.7	0.5	0.5	0.6
	N	10	10	10	8
Glucose (mmol/l)	MEAN	6.14	6.33	6.79 +	7.76 +
	S.D.	0.39	0.45	0.47	0.78
	N	10	10	10	8
Creatinine (umol/l)	MEAN	51	53	52	58 +
	S.D.	5	3	4	6
	N	10	10	10	8

WILCOXON-TEST. TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES, IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL (P < 0.05).

+/- : SIGNIFICANTLY DIFFERENT FROM CONTROL
 M : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

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ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

ATOX 10.2

ARTEMIS II

RUN DATE: 07-MAR-2001

SUMMARY AND STATISTICAL EVALUATION

STUDY : 2000.0326 - HMR 1964 - 6 month subcutaneous toxicity study in rats

INTERIM VALUE

CLINICAL CHEMISTRY (FEMALE)

		GROUP 1 0 I.U./kg	GROUP 2 5 I.U./kg	GROUP 3 20 I.U./kg	GROUP 4 80 I.U./kg
Sodium (mmol/l)	MEAN	148	149	148	148
	S.D.	1	1	1	1
	N	10	10	10	10
Potassium (mmol/l)	MEAN	6.09	6.14	6.34	6.14
	S.D.	0.39	0.54	0.41	0.39
	N	10	10	10	10
Calcium (mmol/l)	MEAN	2.82	2.83	2.58	2.57
	S.D.	0.03	0.05	0.06	0.03
	N	10	10	10	10
Chloride (mmol/l)	MEAN	101	102	102	101
	S.D.	2	2	2	2
	N	10	10	10	10
Phosphorus (mmol/l)	MEAN	2.04	1.95	2.05	2.02
	S.D.	0.18	0.10	0.12	0.15
	N	10	10	10	10
Total Billirubin (umol/l)	MEAN	4.7	4.6	5.2 +	5.5 +
	S.D.	0.5	0.8	0.6	0.7
	N	10	10	10	10
Glucose (mmol/l)	MEAN	6.39	6.44	6.82	7.11 +
	S.D.	0.42	0.48	0.59	0.67
	N	10	10	10	10
Creatinine (umol/l)	MEAN	53	56 +	60 +	59 +
	S.D.	3	4	4	4
	N	10	10	10	10

WILCOXON-TEST. TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES. IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL (P < 0.05).

+/- : SIGNIFICANTLY DIFFERENT FROM CONTROL
M : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

Urinalysis: Not remarkable

Organ weight: Statistically significant decreases in absolute and relative liver weights were observed in mid and high dose males, which was persisted after the recovery period in high dose males. Relative spleen weights were also reduced in high dose males after the recovery period, of which significance is questionable. Relative liver weight in females was not reduced even after the 4-week recovery period, which was different from the case of males as illustrated below.

	Final Values				After 4-Week Recovery			
	0	5	20	80	0	5	20	80
Dose*	0	5	20	80	0	5	20	80
Male#	10	10	8	4	10	10	9	5
B. Wt.	460	504	482	475	501	473	457	491
Heart	1.50	1.41	1.33	1.50	1.37	1.40	1.44	1.41
Liver	16.23	14.24	14.14	14.84	14.04	15.39	13.18	12.85
Adrenal	0.046	0.045	0.043	0.041	0.045	0.046	0.043	0.047
Spleen	0.859	0.901	0.837	0.837	0.892	0.823	0.825	0.789
Female#	9	10	10	9	10	10	9	9
B. Wt.	259	288	280	270	295	292	286	280
Heart	0.85	0.98	0.86	0.95	0.97	0.91	0.93	0.95
Liver	7.49	8.33	7.84	7.60	8.43	7.98	8.58	8.33
Adrenal	0.048	0.051	0.049	0.055	0.051	0.051	0.053	0.056
Thyroid	0.019	0.019	0.018	0.019	0.018	0.020	0.017	0.020

@Values are in gram and * unit is in IU/kg. Male# and Female# stand for the numbers of males and females, respectively.

Gross pathology: An empty stomach was found in 6 of the premature decedent rats, which might be a contributing factor for the deaths in the high dose groups. The other macroscopic findings observed did not correlate with drug dosing.

Microscopic observations:

The intercurrent death of one female in the control group (#146) had a spontaneous malignant fibrous histiocytomas as cause of death. The other dead animals did not have specific signs including microscopic findings that might suggest cause of mortality. Thus, hypoglycemia induced by the test article might be presumed responsible for the deaths.

Toxicokinetic Studies: (See page 11 for therapeutic exposure ratio)

Toxicokinetics in 6-month rat study						
Administration	Gender	Dose	t _{max}	C _{max}	AUC _(0-6h)	AUC _(0-24h)
HMR1964		[IU/kg body wt.]	[h]	[ng/ml]	[ng*h/mL]	[ng*h/ml]
22nd Administration	Male					
	control	0	0.50	—	10.8	-
	low	5	0.25	—	143.7	-
	medium	20	0.50	—	622.0	-
	high	80	0.50	—	3349.8	-
	Female					
	control	0	0.50	—	10.7	-
	low	5	0.25	—	106.8	-
	medium	20	0.25	—	625.8	-
	high	80	0.25	—	3210.2	-
176th/182st Administration	Male					
	control	0	6.0	—	10.1	34.3
	low	5	0.25	—	140.6	169.8
	medium	20	0.25	—	750.8	779.5
	high	80	0.5	—	4589.7	4717.9
	Female					
	control	0	1.0	—	12.4	35.3
	low	5	0.25	—	138.3	160.4
	medium	20	0.25	—	692.4	720.5
	high	80	0.25	—	3487.0	3530.1

Samples were collected at 0.25, 0.5, 1, 3, and 6 hours after administration; 24-hour samples were taken following the 181st administration after exsanguination.

Conclusions: After 6 months of treatment, a marginal but significant prolongation of PT was observed in the 20 and 80 IU/kg/day groups. There was slight but significant reductions of red blood cells counts in 80 IU/kg males. The absolute and relative liver weights were decreased in males of the 20 and 80 IU/kg/day groups. During necropsy, the stomach was found to be empty in two of the rats that died prematurely. The deaths appeared to be related to anticipated pharmacological action of insulin. NOAEL was 5 IU/kg/day in both sexes and AUC_{0-24h} values were 165 ng.h/ml as a mean of both sexes. AUC exposure ratio was 9, based on a clinical dose of 0.3 IU/kg/d.

Cardiac Effects of HMR1964 in 4-Week Subcutaneous Toxicity Study and after 4-Week Recovery Period in Male Dog								
Determination@	Final Values at Week 4				Values after Recovery			
Dose in IU/kg/d	0	1	3	10	0	1	3	10
Numbers of dog	3	3	3	2	1	1	1	0
Heart rate in bpm	83	97	83	65	80	150	90	
P-R interval*	0.09	0.09	0.10	0.10	0.11	0.11	0.09	
Q-T interval*	0.21	0.20	0.20	0.21	0.21	0.18	0.21	
QRS*	0.04	0.04	0.04	0.04	0.04	0.03	0.04	
@Indicate the time when the determination was made. *Unit is in second.								

Ophthalmology: Unremarkable.

Hematology:

An increase in MCV was noted in the final value, but might not be of toxicological relevance as it was sporadic. None of the incidental findings were related to the treated article.

Clinical chemistry:

There were no drug-treatment related changes in dogs given HMR1964 at any dose except time-dependent decreases in serum glucose.

Urinalysis: There were no drug-treatment related changes.

Organ Weights:

The heart weights in males were increased while the parameter was reduced in female dogs as shown below. Female lung weights were also reduced while liver weights were increased in the 10 IU/kg/day groups in both sexes. Similar increases in prostate weight were observed in males as brain weights in the 10 IU/kg/day group were increased in males.

Thyroid weights were increased in the low and mid dose groups in both sexes, but there were no differences between the control and in the high dose groups. The fluctuations in organ weight values were largely due to small sample size, which were even greater after 4-week recovery period. This again confirmed the inadequacy of the 4-week subcutaneous toxicity study in dogs.

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AVENTIS Pharme

RUN DATE: 13/01/00

SUMMARY AND STATISTICAL EVALUATION

STUDY : 99.0429 - HNR 1964 1 MONTH + RECOVERY SUBCUTANEOUS

FINAL VALUE		SUMMARY AND STATISTICS			
ORGAN WEIGHTS		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0	1	3	10
		IU/kg b.wt.	IU/kg b.wt.	IU/kg b.wt.	IU/kg b.wt.
BODY WEIGHT (kg) ME	MALES MEAN	10.4	11.9	11.6	11.6
	S.D.	0.4	0.9	1.6	0.3
	N	2	2	2	2
	FEMALES MEAN	11.3	10.1	10.7	10.2
	S.D.	0.1	1.3	0.6	0.8
	N	2	2	2	2
Heart Weight (g)	MALES MEAN	86.350	96.400 M	92.400 M	96.000 M
	S.D.	3.889	20.789	11.314	8.910
	N	2	2	2	2
	FEMALES MEAN	107.750	94.550 M	83.450 M	78.850 M
	S.D.	3.182	18.880	8.132	0.919
	N	2	2	2	2
Lungs Weight (g)	MALES MEAN	92.000	93.100 M	89.750 M	89.650 M
	S.D.	6.081	17.536	3.606	10.677
	N	2	2	2	2
	FEMALES MEAN	91.950	81.650 M	81.300 M	76.300 M
	S.D.	3.323	13.789	11.455	2.970
	N	2	2	2	2
Liver Weight (g)	MALES MEAN	388.400	432.900 M	405.600 M	433.950 M
	S.D.	16.688	26.446	40.588	20.153
	N	2	2	2	2
	FEMALES MEAN	386.150	314.350 M	358.600 M	426.600 M
	S.D.	12.092	2.758	13.718	61.235
	N	2	2	2	2

T-TEST, TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES, IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL (P < 0.05).

+/- : SIGNIFICANTLY DIFFERENT FROM CONTROL
 N/A : GROUP (>= 4 ANIMALS) CLASSIFIED AS NORMAL/ABNORMAL n/a : GROUP (< 4 ANIMALS) CLASSIFIED AS NORMAL/ABNORMAL
 M : EVALUATION NOT POSSIBLE ME : NO STATISTICAL EVALUATION I : CLASSIFICATION AS NORMAL/ABNORMAL NOT POSSIBLE

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ON ORIGINAL

ATOX 10.1

AVENTIS Pharme

RUN DATE: 13/01/00

SUMMARY AND STATISTICAL EVALUATION

STUDY : 99.0429 - HMR 1964 1 MONTH + RECOVERY SUBCUTANEOUS

FINAL VALUE		SUMMARY AND STATISTICS			
ORGAN WEIGHTS		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0	1	3	10
		IU/kg b.wt.	IU/kg b.wt.	IU/kg b.wt.	IU/kg b.wt.
Prostate Weight (g)	MALES MEAN	2.860	3.125 M	4.720 M	4.225 M
	S.D.	0.453	0.332	2.319	3.967
	N	2	2	2	2
Adrenals Weight (g)	MALES MEAN	0.9195	1.0615 M	1.0355 M	1.0290 M
	S.D.	0.0177	0.1704	0.0544	0.1174
	N	2	2	2	2
	FEMALES MEAN	1.3530	0.9445 M	1.1145 M	1.1570 M
	S.D.	0.1711	0.1450	0.0912	0.0354
	N	2	2	2	2
Thyroid Weight (g)	MALES MEAN	0.6385	0.9140 M	0.9110 M	0.6160 M
	S.D.	0.2256	0.2206	0.0834	0.0537
	N	2	2	2	2
	FEMALES MEAN	0.7895	0.8090 M	0.8895 M	0.7570 M
	S.D.	0.0389	0.1796	0.0686	0.1697
	N	2	2	2	2
Brain Weight (g)	MALES MEAN	76.550	75.350 M	77.450 M	82.950 M
	S.D.	9.263	2.051	6.152	0.919
	N	2	2	2	2
	FEMALES MEAN	76.350	75.600 M	73.100 M	74.700 M
	S.D.	1.909	1.273	0.141	10.748
	N	2	2	2	2
Pituitary Weight (g)	MALES MEAN	0.0635	0.0735 M	0.0650 M	0.0765 M
	S.D.	0.0049	0.0205	0.0113	0.0021
	N	2	2	2	2

T-TEST. TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES, IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL (P < 0.05).

+/- : SIGNIFICANTLY DIFFERENT FROM CONTROL
M/A : GROUP (>= 4 ANIMALS) CLASSIFIED AS NORMAL/ABNORMAL n/a : GROUP (< 4 ANIMALS) CLASSIFIED AS NORMAL/ABNORMAL
M : EVALUATION NOT POSSIBLE RE : NO STATISTICAL EVALUATION I : CLASSIFICATION AS NORMAL/ABNORMAL NOT POSSIBLE

Gross and Histopathology:

Microscopic evaluation of dogs sacrificed moribund or at study termination failed to detect any histopathological correlates for the reported hypoglycemia. In the male high dose animal a slightly increased grade of hemosiderin deposition in the spleen was observed compared to the other dosed and control dogs. For all gross observations at the injection site, corresponding histopathological incidences and findings in the treated groups were comparable to the control.