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Toxicokinetics: (See page 11 for therapeutic exposure ratio)

Toxicokinetics in 1-month dog study							
Administration	Gender	Dose	t _{max}	C _{max}	AUC _(0-24h)		
HMR1964		[IU/kg b. wt.]	[h]	[ng/ml]	[ng*h/ml]		
1st Administration	Male	control	0	5.0	—	31.4	
		low	1	0.8	—	48.1	
		medium	3	0.8	—	203.9	
		high	10	2.0	—	895.2	
	Female	control	0	5.0	—	19.2	
		low	1	0.7	—	44.5	
		medium	3	1.0	—	179.4	
		high	10	1.7	—	951.1	
	29th Administration	Male	control	0	1.5	—	18.6
			low	1	0.7	—	60.0
			medium	3	1.0	—	225.4
			high	10	1.5	—	914.8
Female		control	0	2.7	—	25.4	
		low	1	0.7	—	59.0	
		medium	3	1.5	—	302.5	
		high	10	1.2	—	1111.3	

* Samples were collected before administration and at 0.5, 1, 2, 3, 6 and 24 hours.

Conclusion: In 4-week subcutaneous toxicity study in dogs, one male in the high dose (10 IU/kg) and one female in the mid dose (3 IU/kg) were killed prematurely due to an extension of HMR 1964 hypoglycemic effect. The two doses are approximately 18 and 5 times therapeutic exposure, based on body surface comparison after a daily dose of 0.3 IU/kg. Besides the premature deaths, there were no treatment-related unexpected, detrimental effects on body weight, clinical chemistry, organ weight, and histopathology. NOAEL was 1 IU/kg/day, which therapeutic exposure ratio was 1.8, based on body surface comparison after a daily dose of 0.3 IU/kg/day.

Study Title: 6-Month Subcutaneous Toxicity Study in Dogs with 4-Week Recovery
Study/Report#: 2000.0325/2000.1131; **Document#** F2000TOX0570

Amended 4/03/2001; Vol #11; and pages#1-591

Conducting laboratory and location: Dept. Toxicology and Pathology

Hoechst Marion Roussel Deutschland GmbH, Frankfurt, Germany

Date of study initiation: June 14, 2000

GLP compliance: Yes

QA- Report Yes (x) No ()

METHODS:

Species/strain: Beagle dog/ _____

#/sex/group or time point: 4 dogs/sex/group

Age: 8 months

Weight: Male, 10.8 kg; Female, 9.3 kg

Dosage groups in administered units: 0, 0.5, 1, and 2 IU/kg

Route, form, volume, and infusion rate: Subcutaneous

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

ECG: Limb lead II of the ECG was recorded on Day 149 and Day 216 after the end of the recovery period.

Formulation/vehicle: Clear colorless solution/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

Histopathology: The following tissues were subjected to microscopic examinations.

Adrenals	Lymph nodes	Skeletal muscle (M. sartorius cran.)
Aorta	(mandibular, mesenteric, axillary)	Skin with mammary gland
Bone marrow	Jejunum	Spinal cord (cervical)
(middle sternal segment)	Kidneys	Spleen
Brain with Medulla oblongata	Liver	Stifle joint
Caecum	Lungs	Testes
Colon	Oesophagus	Thymus
Diaphragmatic	Ovaries	Thyroid with parathyroid
Duodenum	Pancreas	Tongue
Epididymides	Pituitary	Tonsils
Eyes (each with optic nerve)	Pyloric region	Trachea
Fundus (stomach)	Prostate	Urinary bladder
Gallbladder	Rectum	Uterus
Heart	Salivary glands	Vagina
Ileum	(parotid, mandibular, sublingual)	Injection sites
	Sciatic nerve	

RESULTS:

Mortality: Two dogs, one male and one female of the high dose group were sacrificed early. The cause of death was due to the extension of pharmacological action of the test article on study day 73 (male 8301) and 150 (female 8320). There were no other deaths.

Clinical signs: The two moribund dogs showed marked signs of hypoglycemia with tonic-clonic convulsions. HMR1964 did not produce any pharmacological or toxicological signs in the low and mid dose groups. All the other dogs of control, low, mid and high doses showed no clinical signs of hypoglycemia and remained in normal condition.

Reflex excitability and hearing tests revealed that there were no abnormal neurological incidents. There were no test article-induced changes in the electrocardiographic findings including QT-interval as shown below, although there was an increase in the parameter in safety study, which needs to be explained or documented in the Investigator's Brochure.

SUMMARY AND STATISTICAL EVALUATION

STUDY : 2000.0323 - HMR 1964 6 MONTHS + RECOVERY SUBCUTANEOUS

ELECTROCARDIOGRAM Q-T interval	SUMMARY AND STATISTICS			
	GROUP 1 0	GROUP 2 0.5 IU/kg b.wt.	GROUP 3 1.0 IU/kg b.wt.	GROUP 4 2.0 IU/kg b.wt.
FEMALES MEAN	0.18	0.20 M	0.20 M	0.22 M
S.D.				
N	1	1	1	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).
 COMMON CONTROL GROUP OR COMPLETELY POOLED ANALYSIS IF MORE THAN TWO DOSE*SEX GROUPS CONTAIN LESS THAN FOUR OBSERVATIONS.

*/- : SIGNIFICANTLY DIFFERENT FROM CONTROL (STATISTICAL ANALYSIS BASED ON CHANGES VERSUS PRELIMINARY VALUES).
 M : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

Ophthalmology: unremarkable.

Body weights: There was no remarkable drug-related change in body weight in the low and mid dose group, compared to the control. The female dogs of the high dose group (Group 4) showed significantly reduced body weight development from study day 98 onwards to 182 day as shown in two tables below. The tables also include data of male body weight at the same time period. The moribund sacrificed male of the high dose group had decreased body weight, which was not unexpected.

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SUMMARY AND STATISTICAL EVALUATION

STUDY : 2000.0325 - RDR 1964 6 MONTHS + RECOVERY SUBCUTANEOUS

BODY WEIGHT (kg)

SUMMARY AND STATISTICS

STUDY DAY		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0	0.5 IU/kg b.wt.	1.0 IU/kg b.wt.	2.0 IU/kg b.wt.
+0091	MALES MEAN	12.20	11.83	12.05	12.15
	S.D.	1.02	0.76	1.20	0.51
	N	4	4	4	4
+0098	FEMALES MEAN	11.08	11.08	10.20	10.98
	S.D.	0.82	0.36	0.82	0.74
	N	4	4	4	5
+0098	MALES MEAN	12.13	11.83	12.10	12.13
	S.D.	0.94	0.84	1.32	0.48
	N	4	4	4	4
+0105	FEMALES MEAN	11.15	11.23	10.30	10.96
	S.D.	0.82	0.26	0.80	0.63
	N	4	4	4	5
+0105	MALES MEAN	12.08	11.75	12.10	12.23
	S.D.	1.09	0.64	1.29	0.41
	N	4	4	4	4
+0111	FEMALES MEAN	11.20	11.35	10.28	10.74
	S.D.	0.94	0.31	0.78	0.79
	N	4	4	4	5
+0111	MALES MEAN	12.10	11.70	12.10	12.33
	S.D.	0.94	0.65	1.40	0.52
	N	4	4	4	4

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 (STATISTICAL ANALYSIS BASED ON CHANGES VERSUS PRELIMINARY VALUES).
 N : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

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BODY WEIGHT (kg)		SUMMARY AND STATISTICS			
		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0	0.5	1.0	2.0
			IU/kg b.wt.	IU/kg b.wt.	IU/kg b.wt.
STUDY DAY					
+0161	MALES MEAN	11.88	11.55	11.90	12.10
	S.D.	0.79	0.91	1.57	0.62
	N	4	4	4	4
+0168	FEMALES MEAN	11.50	11.30	10.45	10.70
	S.D.	0.65	0.41	0.75	0.83
	N	4	4	4	4
+0168	MALES MEAN	11.95	11.73	12.23	12.15
	S.D.	0.77	0.88	1.53	0.62
	N	4	4	4	4
+0175	FEMALES MEAN	11.48	11.18	10.63	10.73
	S.D.	0.62	0.38	0.71	0.85
	N	4	4	4	4
+0175	MALES MEAN	11.90	11.85	12.13	12.15
	S.D.	0.62	0.83	1.42	0.70
	N	4	4	4	4
+0182	FEMALES MEAN	11.35	11.18	10.45	10.63
	S.D.	0.66	0.51	0.75	0.82
	N	4	4	4	4
+0182	MALES MEAN	11.93	11.65	11.98	12.05
	S.D.	0.87	0.85	1.54	0.52
	N	4	4	4	4

Food consumption: No drug-related effect on food consumption was observed because dogs consumed usually their food completely.

Hematology:

Occasionally significant decreases were noted in the erythrocytes and hematocrit counts, but appeared to be not toxicologically relevant because the values were sporadic and were not associated with functional or morphologic changes. It appeared that other changes were not related to the test article.

Clinical chemistry:

There were no drug-treatment-related changes in dogs given HMR1964 at all doses except time-dependent decreases in serum glucose. Serum glucose was reduced in 0.5 – 24 hours after the HMR1964 administration. The peak effects were observed approximately 2, 3, and 6 hours after the administration of the low, mid, and high doses of HMR1964, respectively. Occasionally significant increases were noted in the parameter creatinine kinase, but appeared to be not toxicologically relevant because the values were sporadic and were not associated with functional or morphologic changes.

Urinalysis: There were no drug-treatment-related changes.

Organ Weights: HMR1964 did not have remarkable effects on major organ weights as shown below. Lung weight in male dogs in 2 IU/k/d group was reduced by 10% while the value was increased in the high dose group females without dose-dependency. There were sporadic changes in organ weight, which were largely due to the size of N, particularly after the recovery period. There were no clear abnormal organ weights that need to be documented.

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ATOX 10.2

ARTEMIS II

RUN DATE: 01-FEB-2001

SUMMARY AND STATISTICAL EVALUATION

STUDY : 2000.0325 - HMR 1964 6 MONTHS + RECOVERY SUBCUTANEOUS

FINAL VALUE

ORGAN WEIGHTS

SUMMARY AND STATISTICS

		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0	0.5	1.0	2.0
			IU/kg b.wt.	IU/kg b.wt.	IU/kg b.wt.
BODY WEIGHT (kg) NR	MALES MEAN	11.4	12.0	11.7	11.9
	S.D.	1.3	0.4	1.4	0.5
	N	3	3	3	3
	FEMALES MEAN	11.5	11.3	10.2	10.8
	S.D.	0.7	0.6	0.5	0.9
	N	3	3	3	3
Heart Weight (g)	MALES MEAN	100.067	98.333 M	102.700 M	97.567 M
	S.D.	9.253	4.561	9.074	6.250
	N	3	3	3	3
	FEMALES MEAN	93.067	101.667 M	80.133 M	99.133 M
	S.D.	13.115	8.361	11.293	14.069
	N	3	3	3	3
Lungs Weight (g)	MALES MEAN	101.100	101.333 M	96.167 M	91.567 M
	S.D.	5.415	4.760	14.523	7.569
	N	3	3	3	3
	FEMALES MEAN	85.767	86.833 M	81.367 M	95.400 M
	S.D.	5.994	5.160	6.158	5.474
	N	3	3	3	3
Liver Weight (g)	MALES MEAN	411.633	474.667 M	441.400 M	424.767 M
	S.D.	60.520	84.622	63.539	72.689
	N	3	3	3	3
	FEMALES MEAN	417.733	411.200 M	370.467 M	380.033 M
	S.D.	41.180	134.189	42.746	31.803
	N	3	3	3	3

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 NR : NO STATISTICAL EVALUATION I : CLASSIFICATION AS NORMAL/ABNORMAL NOT POSSIBLE

APPEARS THIS WAY
ON ORIGINAL

ATOX 10.2

APTOMIS II

RUN DATE: 01-FEB-001

SUMMARY AND STATISTICAL EVALUATION

STUDY : 1000.0325 - HDR 1964 6 MONTHS + RECOVERY SUBCUTANEOUS

FINAL VALUE

ORGAN WEIGHTS

SUMMARY AND STATISTICS

		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0	0.5	1.0	2.0
			IU/kg b.wt.	IU/kg b.wt.	IU/kg b.wt.
Kidneys Weight (g)	MALES MEAN	45.367	54.067 M	55.233 M	54.933 M
	S.D.	8.064	7.218	6.506	7.969
	N	3	3	3	3
FEMALES MEAN	42.867	44.600 M	45.267 M	44.833 M	
	S.D.	5.254	4.951	3.522	3.313
	N	3	3	3	3
Spleen Weight (g)	MALES MEAN	40.250	32.853 M	29.070 M	48.297 M
	S.D.	18.545	14.487	7.178	17.345
	N	3	3	3	3
FEMALES MEAN	27.187	25.270 M	40.720 M	24.533 M	
	S.D.	3.525	4.441	19.683	9.266
	N	3	3	3	3
Testes Weight (g)	MALES MEAN	19.587	22.900 M	16.657 M	16.447 M
	S.D.	2.818	1.480	1.872	5.119
	N	3	3	3	3
Ovaries Weight (g)	FEMALES MEAN	1.5667	1.1283 M	1.2587 M	1.2020 M
	S.D.	0.5844	0.1781	0.2293	0.3939
	N	3	3	3	3
Epididymis Weight (g)	MALES MEAN	4.400	4.647 M	4.697 M	3.483 M
	S.D.	0.793	1.071	0.266	0.676
	N	3	3	3	3
Uterus Weight (g)	FEMALES MEAN	12.170	5.273 M	5.260 M	5.340 M
	S.D.	10.275	2.067	0.936	0.649
	N	3	3	3	3

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 NE : NO STATISTICAL EVALUATION I : CLASSIFICATION AS NORMAL/ABNORMAL NOT POSSIBLE

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Gross and Histopathology:

Microscopic evaluation of dogs (male #8301) sacrificed moribund exhibited necroses of neurons in the hippocampus representing a histopathological evidence of hypoglycemia. At necropsy, the dog had abnormalities in heart, liver, kidneys, adrenal glands, digestive tract, urinary bladder and spleen suggesting a cardiovascular disorder. The hypoglycemia appeared to be induced the test article, although other dogs in the HD group did not display the same symptoms. For all gross observations at the injection site, corresponding histopathological findings were obtained. It may be concluded that HMR1964 did not produce pathological abnormalities except changes as extension of its hypoglycemic action. Histopathological findings of HMR1964 in injection sites of dog are summarized below, which had no significant abnormal effects of HMR1964 on skin injection sites as indicated below.

HMR1964 Effects on Skin Injection Sites in 6-Month Subcutaneous Toxicity Study in Dog@								
Sex	Male				Female			
Dose in IU/kg/d	0	0.5	1	2	0	0.5	1	2
Number of animals	3	3	3	4	3	3	2	4
Hemorrhage	3	3	3	3	3	3	2	4
Mixed cell infiltration								
Lymphoplasm. infiltr.*								
Foreign body granule								
Granulation tissue								
@Indicate the number of animals that were positive at the application sites. *Indicate infiltration.								

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Toxicokinetics: (See page 11 for therapeutic exposure ratio)

Toxicokinetics in 6-month dog study					
Administration	Gender	Dose [IU/kg body wt.]	t _{max} [h]	C _{max} [ng/ml]	AUC _(0-24h) [ng*h/ml]
HMR1964					
30th Administration	Male				
	control	0	3.8	—	30.0
	low	0.5	0.5	—	39.4
	medium	1	0.6	—	97.7
	high	2	0.8	—	173.6
	Female				
	control	0	1.8	—	29.5
	low	0.5	0.5	—	48.6
medium	1	0.5	—	67.5	
high	2	0.6	—	154.1	
176th/182st					
Administration	Male				
	control	0	2.8	—	34.9
	low	0.5	0.6	—	50.8
	medium	1	0.6	—	138.4
	high	2	0.9	—	170.8
	Female				
	control	0	3.3	—	43.1
	low	0.5	0.6	—	49.3
medium	1	0.5	—	57.6	
high	2	0.8	—	139.3	

Samples were collected at 0.5, 1, 2, 3, 6 and 24 hours after administration.

Conclusion: Two dogs from the high dose (2 IU/kg/day) group were sacrificed prematurely due to HMR1964-induced hypoglycemia. The low dose did not produce remarkable and test agent-related toxic effects on clinical signs, ECG, body weight, hematology, clinical chemistry, organ weight, and histopathology except dose-dependent reduction in blood glucose levels. Thus, NOAEL was 0.5 IU/kg/day, which gives AUC exposure ratio approximately 3-fold therapeutic exposure, based on daily clinical dose of 0.3 IU/kg. There were no test article-induced changes in the electrocardiographic findings including QT-interval as in the 4-week study in dogs, although there was an increase in the parameter in safety study.

VII. REPRODUCTIVE TOXICOLOGY:

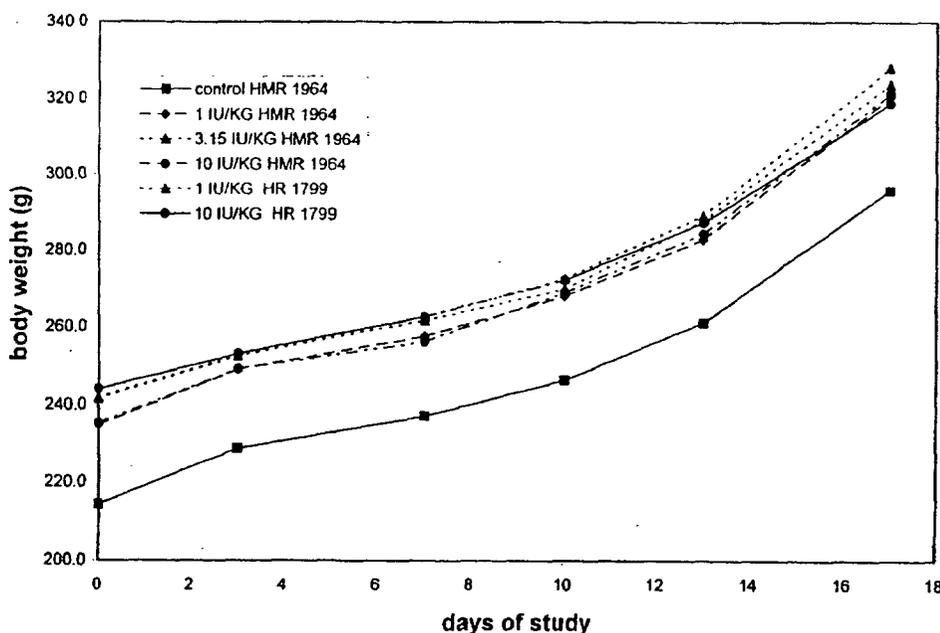
Study title: HMR 1964: Subcutaneous reproduction toxicity study in rats (Fertility Study)**Study/Report or Document#: 2000.00919/F2001TOX0147****Amendment #, Vol #13, and page#: 1-315****Site and testing facility: Aventis Pharma Deutschland GmbH, Drug Innovation and Approval, Drug Safety Evaluation, Mainzer Landstr. 500, Germany****Start of study: Dec. 12, 2000****GRP compliance: Yes****QA- Reports Yes (x) No ():****Lot/batch numbers: /1309 Verum; HR1799 (Batch H037)****Protocol reviewed by Division Yes () No (x):****METHODS:****Species/strain: Rat/ Hsd: (SD)****Number of animals/sex/dosing group: 23/sex/group****Age: 8-10 Week old****Weight: Male (305 gram), female (209 gram)****Doses employed: 0, 1, 3.15, and 10 IU/kg; 1 and 10 IU/kg of HR 1799 (Sponsor's human insulin product) were included in this study for comparison purpose).****Route of Administration: Subcutaneous****Study Design:** The rats received the test article at the indicated doses for 4 weeks (males) or 2 weeks (female) before mating and during the mating period. After the pre-mating period female animals were housed together with the assigned male for a maximum period of 4 weeks and caged individually after detection of sperm in vaginal smears, which was defined as day 0 of pregnancy. Behavior of the animals was observed several times daily, on weekends and holidays once daily.**Observations:** All animals were examined before the start of the study and were observed several times daily, on weekends once daily.**Body weight:** Body weights and food consumption were determined twice weekly in both sexes during the pre-mating dosing period and in females on days 0, 3, 6, 9, 13 and 17 of pregnancy.**Macroscopic examination:** All female rats were killed on day 17 of pregnancy to examine conceptuses, corpora lutea, and implantations and embryofetal primordia. Examination of sperm was performed in all males after mating. Sperm from the distal part of the cauda epididymis was examined for motility studies and other procedures were conventional.**Autopsy:** All animals were autopsied for visible changes. Testes, epididymides, ovaries and uteri were removed and preserved in 8% buffered formaldehyde solution for histopathological examination. But, no histopathological examination was performed because no test article related macroscopic changes were observed at necropsy.**Statistical evaluations:** Statistical evaluations were based on the t-tests and the test statistics of Wilks(Hartung and Elpelt, Multivariate Statistik, Oldenburg, Munchen, 1984).**RESULTS:**

Mortality: Two females from the 10 IU/kg HMR1964 group were found dead on day 7 and 21 of the study. Two males and two females from the 10 IU/kg HR 1799 group were found dead between days 7 and 28 of the study. There were no treatment-article related deaths in other groups.

Clinical signs: Rats in the control and low dose groups had no remarkable signs. Those animals that were found moribund had prone or lateral position, bristling coat and gasping before death.

Body weight: The mean absolute body weight of the males and females from the various groups was comparable with that of the respective control group during the pre-mating period. This was also true for the males as well as females during HMR1964 treatment. During pregnancy, body weight gains were increased significantly in all groups on days 0, 9, 13 and 17 and additionally on days 3 and 6 of pregnancy in the groups treated with HR 1799 as shown below. HR 1799 is one of the sponsor's insulin products.

Fertility study in rats with HMR 1964 and HR 1799
 Study No. 2000.0919
 Body weight (g) of females during gestation period



Food consumption:

The animals in the test groups consumed amounts of food comparable to those consumed by the control animals. During pregnancy, no significant drug effects on food consumption were observed.

Mating Results: There was a tendency for prolongation of the pre-coital period in the females treated with HMR1964, particularly in the group treated with HR1799 as shown below. Estrous cycle length and reproductive performance were not affected by the treatments. One female in low dose group became pregnant without sperm detection and pregnancy did not occur in 4 sperm-positive females from the control group.

Pre-coital period (Days) prolongation in drug-treated groups of female rats*						
Day@	0 - 3	4 - 7	8 - 12	13 - 16	17 - 27	Mean(Days)
Control	19*/83*	2/9	¼	0/0	¼	3.3
HMR 1 IU	10/45	7/32	1/5	0/0	4/18	6.5
HMR 3.15 IU	11/52	5/24	1/5	4/19	0/0	5.2
HMR 10 IU	11/52	5/24	1/5	4/19	0/0	5.2
HR 1 IU	9/41	3/14	5/23	3/14	2/9	7.1
HR 10 IU	6/29	5/24	5/24	3/14	2/10	8.0

@Indicates the pre-coital period in days after two sexes were paired in a cage. *The first number indicates the number of animal had mated during the indicated pre-coital period in days and the second number indicates the per cent of total animals. @Indicates observation duration in days.

Sperm Examinations: Epididymal sperm counts, motion, and motility were not affected by HMR1964 at the three doses tested. But, HMR1799 reduced sperm counts at high dose, 10 IU/kg, as shown below. Sperm motility was not affected by the treatment with HR1799.

Sperm counts and motility in Fertility Study in SD rats(Study#2000.0919)					
Drug	Dose	Counts/mg*	Motionless	Motile	No impairment
HMR1964	0	3394	21	37	42
	1	3390	22	36	43
	3.15	3312	24	38	38
	10	3485	23	37	40
HR1799	1	3347	21	38	42
	10	2917@	24	38	38

Values are expressed in % of total sperms except sperm counts, which were actual count per µg spermiate indicated by *. @ p<0.05.

Gestation and Uterine Findings:

Numbers of pregnancies as well as intrauterine development remained unaffected by the HMR1964 or HR1799. No differences were observed in the numbers of corpora lutea, pre-implantation losses, and conceptuses undergoing resorptions or dead embryos. Numbers of implantations and live conceptuses were not altered by the administration of the test compounds.

Necropsy Findings:

Autopsy findings revealed that there were no compound-related alterations of all animals except one female from the high dose group treated with 10 HMR1964 had an elevated liver lobe. One male from the high dose HR1799 had small testes, which were considered all incidental.

Conclusion: HMR1964 had no remarkable effects on body weight, food consumption, mating, and fertility of parent animals except a few mortality in HD groups due to hypoglycemia as a result of HMR1964 pharmacological action. No clear detrimental effects of HMR1964 on numbers of corpora lutea, pre-implantation losses, and conceptuses undergoing resorptions or dead embryos were observed in the low and mid dose groups. Maternal NOAEL = 3.15 IU for HMR1964/kg.

Study title: HMR1964-Subcutaneous Reproductive (embryo-fetal) toxicity study in rats

Study No: Report/Document#: 2000.0893/F2001TOX0033

Amendment #, Vol #14, page#1-325

Site and testing facility: Aventis Pharma Deutschland GmbH, Drug Innovation and Approval, Drug Safety Evaluation, Mainzer Landstr. 500, Germany

Start of study: Dec. 13, 2000

GRP compliance: Yes

QA- Reports Yes (x) No ():

Lot /batch numbers: /1215 Verum, H037 for HR 1799

Protocol reviewed by Division Yes () No (x):

METHODS:

Species/strain: Rat/ Hsd: (SD)

Numbers of Animals: 20/sex/group

Doses employed: 0, 1, 3.15, and 10 IU/kg; 1 and 10 IU/kg of HR 1799 were included in this study for comparison purpose.

Route of Administration: Subcutaneous

Study Design: The female rats received HMR1964 subcutaneously at doses of 0, 1, 3.15 or 10 IU/kg once daily from day 6-17 of pregnancy. The pregnancy day 0 was considered as the day after the detection of sperm in vaginal smears. HR 1799 was given similarly at doses of 1 or 10 IU/kg.

Behavior of the animals was observed several times daily, on weekends and holidays once daily. Body weights were determined twice weekly in females on days 0, 3, 6, 9, 13, 16, 18 and 20 of pregnancy and food consumption was recorded between days 0-3, 3-6, 6-9, 9-13, 13-16, 16-18, and 18-20 of pregnancy.

Macroscopic examination: All females were killed on day 20 of pregnancy and the fetuses removed by Caesarian section. Gravid uterus weight was recorded. The live and dead fetuses in the uterus as well as the conceptuses undergoing resorptions and corpora lutea were counted. Visceral and skeletal changes were subdivided into four categories; major defects, minor defects, variations, and retardations, based on the severity and/or the spontaneous incidence of the finding.

Statistical evaluations: Statistical evaluations were based on the t-tests and the test statistics of Wilks (Hartung and Elpelt, Multivariate Statistik, Oldenburg, Munchen, 1984).

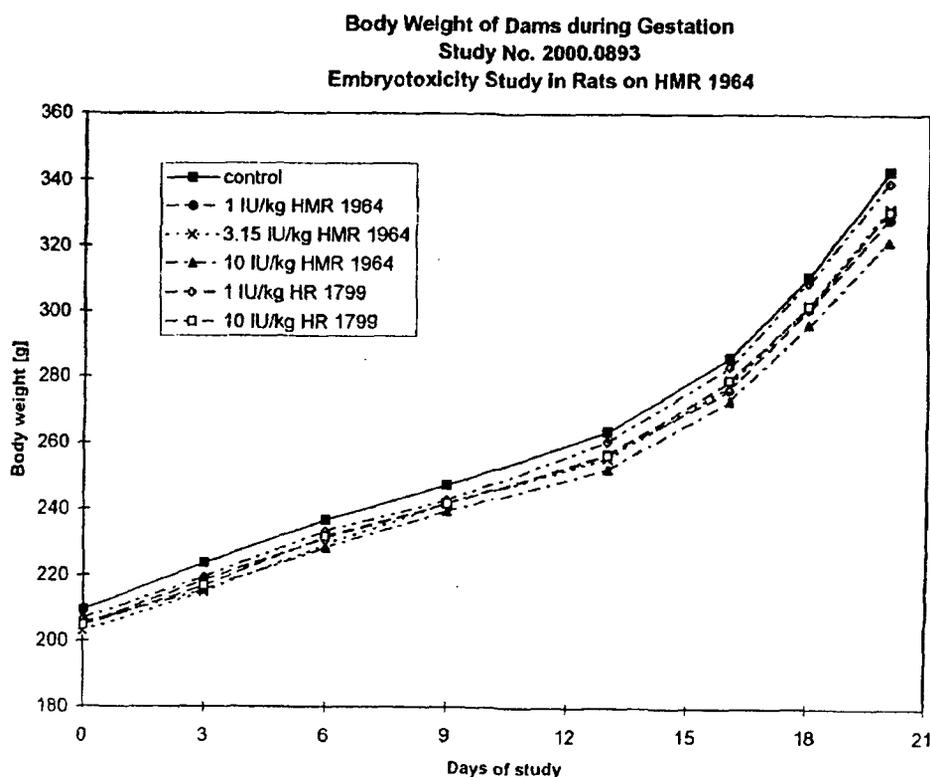
RESULTS:

Effects of Dam (F₀):

Mortality and Clinical Signs: Two animals of the HMR1964 high dose group died on day 6 of pregnancy after the first treatment. Four rats of HR 1799 high dose group were found dead between day 6 and 14 of pregnancy. Clinical signs of the animals were hypoactivity, prone position, lateral position, and rolling convulsions, which were likely related to the extension of pharmacological action of the drugs (hypoglycemia).

Body weight: Body weight gain was decreased (approx. 10%) on days 3, 6,13, 16,18 and 20 in the group treated with 10 IU/kg HMR 1964. There were no significant differences between the control and other treated groups during gestation, indicating HMR1964 had no remarkable adverse effects on mean body weights. Please see the figure below for the details.

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Food consumption: The parameter was not affected by the treatment during gestation. There were sporadic changes in the parameter; for example, slight increase in food intake in the HR1799 10 IU/kg group during days 3-6 of administration (days 9-12 of gestation).
Other effects: No adverse effects were noted on the delivery, duration of gestation or gestation index in any of the treated groups. There were no abnormalities in nursing behavior.

Gross pathology: There were no abnormalities in any of the dams except one rat treated with HMR 1964 1 IU/kg had a light-brown coloration of the liver. The liver finding was thought to be incidental since there was no dose-dependency. A total twelve female rats did not become pregnant: 5 from the control, 2 and 3 from the LD and MD groups, respectively, and 2 from the HD group. There were also 3 females from the group dose with 1IU/kg HR1799. There was no increase in the incidence of early or late conceptuses undergoing resorptions.

Effects on fetuses (F₁):

Pre-implantation loss was increased in the 3.15 and 10 IU/k/d groups, of which increase appeared to be test article dose dependent. Post-implantation loss, early intrauterine

deaths and total intrauterine deaths were highest in the 1 IU/k/d group. However, that was not statistically significant and total live fetuses were not different from the control as shown in a table below. Sex ratio, fetuses' body weights, and placental weight were comparable in all groups including the 10 IU/k/d group.

One fetus from the high dose group had a shortened lower lip. One edematous fetus was observed in the group treated with 10 IU/kg HMR1964, which might be incidental because only one fetus was affected.

The incidence of wavy and thickened ribs was increased in the fetuses from the high dose group (9 of 123 fetus), which was not statistically significant (Please see the two tables below for these effects). In other cases, the findings were single incidences without dose-dependency. Thus, the compound-related effect is unlikely.

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IVENTIS PHARMA Deutschland GmbH

STUDY: EMBRYOTOXICITY STUDY
ANIMAL: Sprague Dawley Rat

PREPARATION: HMR 1964

STUDY NO. 2000.0893

SURVEY OF RESULTS DURING GESTATION AND AT CAESARIAN SECTION

		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0	1	3.15	10
		IU/KG	IU/KG	IU/KG	IU/KG
PRE-IMPLANTATION LOSS %	NE MEAN	4.06	4.60	9.44	12.54
POST-IMPLANTATION LOSS %	NE MEAN	3.92	19.32	8.03	8.96
- EARLY INTRAUTERINE DEATHS	TOTAL	11	45	17	17
	NE MEAN	0.58	2.50	1.00	0.89
	S.D.	0.51	4.58	1.41	0.94
% OF IMPLANTATIONS	MEAN	3.92	19.32	8.03	8.61
- DEAD FETUSES	NE TOTAL	0	0	0	1
	MEAN	0.00	0.00	0.00	0.05
	S.D.	0.00	0.00	0.00	0.23
% OF IMPLANTATIONS	MEAN	0.00	0.00	0.00	0.35
- TOTAL INTRAUTERINE DEATHS	TOTAL	11	45	17	18
	NE MEAN	0.58	2.50	1.00	0.95
	S.D.	0.51	4.58	1.41	1.03
- LIVE FETUSES	TOTAL	259	208	210	219
	MEAN	13.6	11.6	12.4	11.5
	S.D.	2.1	5.0	3.7	4.0

ANIMALS NOT SURVIVING TO DAY 20 AFTER MATING OR HAVING AN EARLY PM ON DAY 20 OR RESORPTIONS ONLY OR NONPREGNANT ANIMALS ARE EXCLUDED FROM THE MEANS.

* : SIGNIFICANTLY HIGHER THAN CONTROL - : SIGNIFICANTLY LESS THAN CONTROL
M STATISTICAL EVALUATION NOT POSSIBLE, GROUP CONTAINS LESS THAN 5 ANIMALS NE: NO STATISTICAL EVALUATION

APPEARS THIS WAY
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AVENTIS PHARMA Deutschland GmbH

STUDY: EMBRYOTOXICITY STUDY
ANIMAL: Sprague Dawley Rat

PREPARATION: EDR 1964

STUDY NO. 2000.0893

SURVEY OF RESULTS IN LIVE FETUSES AT CAESARIAN SECTION

		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0	1	3.15	10
		IO/KG	IO/KG	IO/KG	IO/KG
NUMBER OF FETUSES	TOTAL	259	208	210	219
	MEAN	13.6	11.6	12.4	11.5
	S.D.	2.1	5.0	3.7	4.0
% OF IMPLANTATIONS	MEAN	96.08	80.68	91.97	91.04
MALRE (%)	NE	57.5	50.5	49.0	47.9
BODY WEIGHT (G)	MEAN	3.5	3.7	3.7	3.5
	S.D.	0.2	0.4	0.3	0.2
CROWN/RUMP LENGTH (MM)	MEAN	36.5	38.1	37.5	36.6
	S.D.	1.0	1.8	1.2	0.9
PLACENTAL WEIGHT (G)	MEAN	0.50	0.58	0.48	0.48
	S.D.	0.05	0.22	0.04	0.06

*: SIGNIFICANTLY HIGHER THAN CONTROL - : SIGNIFICANTLY LESS THAN CONTROL
M: STATISTICAL EVALUATION NOT POSSIBLE, GROUP CONTAINS LESS THAN 5 ANIMALS NE: NO STATISTICAL EVALUATION

APPEARS THIS WAY
ON ORIGINAL

STUDY: EMBRYOTICITY
ANIMAL: SPRAGUE DAWLEY RAT

PREPARATION: HDR 1964

STUDY NO. 2000.0893

PAGE: 2

SUMMARY AND INTERGROUP COMPARISON OF MORPHOLOGICAL FINDINGS IN FORTUSSES

	CLASSIFICATION	GROUP 1		GROUP 2		GROUP 3		GROUP 4		GROUP 5		GROUP 6	
		NO	%										
<p>GROUP 1 GROUP 2 GROUP 3 GROUP 4 GROUP 5 GROUP 6</p> <p>0 1 3.15 10 1 10</p> <p>IU/KG IU/KG IU/KG IU/KG IU/KG IU/KG</p>													
<p>-----</p>													
<p>CAUDAL VENT. CENTRA</p>													
OSSEIFICATION OF LESS THAN 2	RETARDATION	29	21.3	21	18.9	16	14.8	17	15.0	9	8.1	23	18.7
		11	57.9	9	50.0	6	35.3	8	42.1	4	23.5	7	36.8
<p>-----</p>													
<p>STERNEBRA</p>													
FRAGMENTED, LONGITUDINALLY DISPLACED	MINOR DEFECT	1	0.7	1	0.9	0	0.0	2	1.8	1	0.9	0	0.0
		1	5.3	1	5.6	0	0.0	2	10.5	1	5.9	0	0.0
<p>-----</p>													
<p>NON- OR WEAKLY OSSIFIED</p>													
	RETARDATION	20	14.7	22	19.8	13	12.0	18	15.9	12	10.8	24	19.5
		13	68.4	10	55.6	8	47.1	12	63.2	9	52.9	9	47.4
<p>-----</p>													
<p>RIS</p>													
WAVY AND/OR THICKENED -	MINOR DEFECT	0	0.0	1	0.9	0	0.0	1	0.9	0	0.0	9	7.3
UNI- OR BILATERAL		0	0.0	1	5.6	0	0.0	1	5.3	0	0.0	3	15.8
<p>-----</p>													
EXTRA RIS - AT 1ST LUMBAR VERTEBRA	VARIATION	18	13.2	22	19.8	19	17.6	14	12.4	6	5.4	25	20.3
SHORT OR NORMALLY LONG -		9	47.4	11	61.1	10	58.8	8	42.1	5	29.4	12	63.2
UNI- OR BILATERAL													

JACKKNIFE-T-TEST (P < 0.05) BASED ON THE NUMBER OF FORTUSES
 *: SIGNIFICANTLY HIGHER THAN CONTROL M: EVALUATION NOT POSSIBLE (NE): NO STATISTICAL EVALUATION
 UPPER LINE: NUMBER OF AFFECTED FORTUSES LOWER LINE: NUMBER OF AFFECTED LITTERS

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STUDY: EDGRTOTOXICITY
ANIMAL: SPRAGUE DAWLEY RAT

PREPARATION: EHR 1966

STUDY NO. 2000.0893

PAGE: 3

SUMMARY AND INTERGROUP COMPARISON OF MORPHOLOGICAL FINDINGS IN FETUSES

CLASSIFICATION	GROUP 1		GROUP 2		GROUP 3		GROUP 4		GROUP 5		GROUP 6	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
SKELETAL DEFECTS												
PECTORAL GIRDLE												
SCAPULA - BENT COSTAL - RIGHT OR BILATERAL	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	2.4
	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	10.5
FORELIMB												
HUMERUS - SHORTENED - RIGHT,	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.8
RADIUS - BENT - RIGHT	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	5.3
FOREPAW												
METACARPAL 5 - NON-OSSIFIED - BILATERAL	13	9.6	11	9.9	4	3.7	8	7.1	1	0.9	12	9.8
	8	42.1	5	27.8	3	17.6	6	31.6	1	5.9	7	36.8
1ST TO 5TH TOE - PHALANX III - NON-OSSIFIED - BILATERAL	1	0.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	1	5.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
PELVIC GIRDLE												
PUBIS - NON-OSSIFIED - RIGHT OR BILATERAL	1	0.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	1	5.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

JACKKNIFE-T-TEST (P < 0.05) BASED ON THE NUMBER OF FETUSES
 * : SIGNIFICANTLY HIGHER THAN CONTROL M: EVALUATION NOT POSSIBLE (NE): NO STATISTICAL EVALUATION
 UPPER LINE: NUMBER OF AFFECTED FETUSES LOWER LINE: NUMBER OF AFFECTED LITTERS

Variations: In most cases the findings were single incidence without the drug dose-dependency in skeletal as well as visceral organs as shown below. There were no clear compound-related effects in retardations and no major defects from the dead fetus were observed (Please see table below).

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STUDY: EMBRYOTOXICITY
ANIMAL: SPRAGUE DAWLEY RAT

PREPARATION: FOS 1964

STUDY NO. 2000.0693

PAGE: 1

SUMMARY AND INTERGROUP COMPARISON OF MORPHOLOGICAL FINDINGS IN FOSTERES

CLASSIFICATION	GROUP 1		GROUP 2		GROUP 3		GROUP 4		GROUP 5		GROUP 6		TWO-SIDED TOLERANCE INTERVAL RANGE OF HISTORICAL CONTROLS (PER STUDY) GROUP SIZE 18
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	
EXTERNAL/VISCERAL DEFECTS OBTAINED AT MOST CROSS-SECTION													
LIVER													
LOBUS DEXTER ACCESSORIUS, LOBUS	1	0.0	1	1.0	1	1.0	2	1.9	0	0.0	0	0.0	0.0 - 2.7
SINISTER ACCESSORIUS, LOBUS SINISTER - HEMATOMA	1	5.3	1	6.7	1	5.3	2	11.1	0	0.0	0	0.0	
STOMACH													
DISPLACED DEXTRAD OR ENLARGED	0	0.0	0	0.0	0	0.0	1	0.9	0	0.0	1	0.9	0.0 - 0.0
	0	0.0	0	0.0	0	0.0	1	5.6	0	0.0	1	5.3	
BLOOD													
MINOR DEFECT	0	0.0	0	0.0	0	0.0	1	0.9	0	0.0	0	0.0	0.0 - 0.4
	0	0.0	0	0.0	0	0.0	1	5.6	0	0.0	0	0.0	
KIDNEY													
PELVIS - DISTENDED - RIGHT OR BILATERAL	1	0.9	0	0.0	1	1.0	0	0.0	3	2.9	2	1.7	0.0 - 5.0
	1	5.3	0	0.0	1	5.3	0	0.0	3	17.6	2	10.5	
URETER													
URETER - DISTENDED - LEFT OR BILATERAL	2	1.6	0	0.0	1	1.0	1	0.9	1	1.0	1	0.9	0.0 - 2.4
	2	10.5	0	0.0	1	5.3	1	5.6	1	5.9	1	5.3	

JACKKNIFE-T-TEST (P < 0.05) BASED ON THE NUMBER OF FOSTERES
 *: SIGNIFICANTLY HIGHER THAN CONTROL N: EVALUATION NOT POSSIBLE (NS): NO STATISTICAL EVALUATION
 UPPER LINE: NUMBER OF AFFECTED FOSTERES LOWER LINE: NUMBER OF AFFECTED LITTERS

APPEARS THIS WAY
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STUDY: EMBRYOTOXICITY PREPARATION: HMR 1964 STUDY NO. 2000.0893
 ANIMAL: SPRAGUE DAWLEY RAT
 PAGE: 1

SUMMARY AND INTERGROUP COMPARISON OF MORPHOLOGICAL FINDINGS IN FORTUSES

CLASSIFICATION	GROUP 1		GROUP 2		GROUP 3		GROUP 4		GROUP 5		GROUP 6	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%

SKELETAL DEFECTS												

NUMBER OF FORTUSES EXAMINED	136		111		108		113		111		123	
NUMBER OF LITTERS EXAMINED	19		18		17		19		17		19	

SKULL												

INDIVIDUAL SKULL BONES - ELIGIBLE OSSIFICATION	5	3.7	3	2.7	1	0.9	1	0.9	0	0.0	1	0.8
	4	21.1	3	16.7	1	5.9	1	5.3	0	0.0	1	5.3

CERVICAL VERT. ARCH												

APLASIA - 4TH RIGHT	0	0.0	0	0.0	0	0.0	1	0.9	0	0.0	0	0.0
	0	0.0	0	0.0	0	0.0	1	5.3	0	0.0	0	0.0

THORACIC VERT. CENTRA												

FRAGMENTED 11TH	1	0.7	0	0.0	0	0.0	1	0.9	0	0.0	0	0.0
	1	5.3	0	0.0	0	0.0	1	5.3	0	0.0	0	0.0

LUMBAR VERTEBRA												

AMLAGE OF ONLY 3	0	0.0	0	0.0	0	0.0	1	0.9	0	0.0	0	0.0
	0	0.0	0	0.0	0	0.0	1	5.3	0	0.0	0	0.0

SACRAL VERT. ARCH/CENTRA												

NON-OSSIFIED	1	0.7	0	0.0	0	0.0	0	0.0	1	0.9	0	0.0
	1	5.3	0	0.0	0	0.0	0	0.0	1	5.9	0	0.0

JACKSONIVE-T-TEST (P < 0.05) BASED ON THE NUMBER OF FORTUSES
 *: SIGNIFICANTLY HIGHER THAN CONTROL N: EVALUATION NOT POSSIBLE (NR): NO STATISTICAL EVALUATION
 UPPER LINE: NUMBER OF AFFECTED FORTUSES LOWER LINE: NUMBER OF AFFECTED LITTERS

Summary and Conclusion:

Administration of both HMR1964 from Day 6-17 of gestation in SD rats caused clinical signs and mortality at the daily dose of 10 IU/kg. Slightly increased incidences of minor rib anomalies were observed in the high dose groups of both HMR1964 and HR1799. Neither maternal nor embryofetal toxicity were observed after administration of HMR 1964 at the daily dose of 1 or 3.15 IU/kg. Thus, NOAEL is 3.15 IU/kg HMR 1964 for maternal and developmental toxicity. The therapeutic exposure ratio was approximately 1.7 times, based on body surface comparison after a daily dose of 0.3 IU/kg.

Study title: HMR1964: Subcutaneous Embryo-fetal Toxicity Study in Rabbit

Study/Report#: Doc. No: 2000.0894/2000.1170/ F2001TOX0046

Amendment #, Vol #15, and page#1-281

Site and testing facility: Aventis Pharma Deutschland GmbH, Drug Innovation and Approval, Drug Safety Evaluation, Mainzer Landstr. 500, Germany

GRP compliance: Yes

QA- Reports Yes (x) No ():

Lot and batch numbers: 1215 Verum

Protocol reviewed by Division Yes () No (x):

Starting Date of Study: Dec. 11, 2000

METHODS:

Species/strain: Himalayan rabbit/Chbb.HM (SPF)

Age: 5 – 9 months old

Body weight: 2.5 kg

Number of animals/sex/dosing group: 20/group

Doses employed: 0(vehicle), 0.25, 0.5 and 1.5 IU/kg

Route of Administration: Subcutaneous

Study Design: Potential embryotoxicity of HMR1964 was tested in mated female rabbits. Prior to the start of the study, the females were mated with fertile males in the morning during estrus. Total 4 groups: one control placebo group and three treated groups (low, mid and high doses) were treated with the drug from the 6th to 18th day of pregnancy. Caesarian section and autopsy were performed on 29th day of pregnancy. In this study HR1799 and insulin were included for comparison purpose.

Observations: All animals were examined before the start of the study. General health of the rabbits was observed at least twice daily.

Body weight: The parameter was determined on days 0, 3, 6, 10, 13, 16, 19, 23, 26 and 29 of pregnancy.

Food consumption was recorded between days 0-3, 3-6, 6-10, 10-13, 13-16, 16-19, 19-23, 23-26 and 26-29 of pregnancy.

Other parameters: Toxicokinetic evaluation was also performed. The uterus was open at caesarian section, and the live and dead fetuses; conceptuses undergoing resorptions, placentas and corpora lutea in the ovaries were counted and examined macroscopically. Frequencies of findings obtained at autopsy and the skeletal examination of the fetus were compared with those of corresponding findings in previous control groups.

Statistical evaluations: Comparisons of dose groups and the control group at the 5% level were only carried out if significant effects were detectable in the higher dose group. Analysis of variance, t-test for body weight, and regression analysis were performed.

RESULTS:

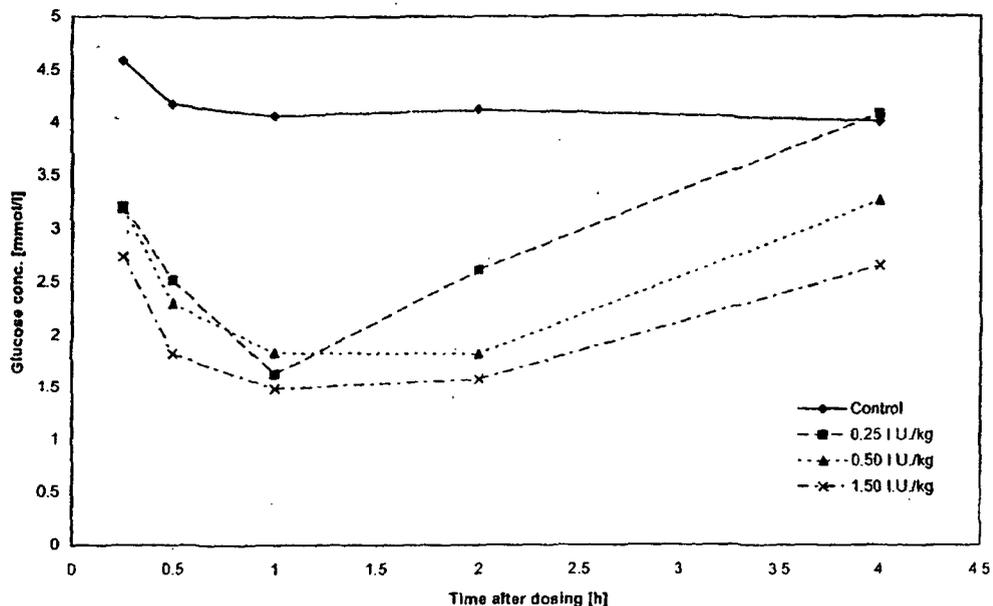
Mortality and clinical signs: Two animals of the high dose group died on day 8 and 12 of pregnancy. Two additional rabbits from the group were killed after abortion on days 23 and 27 of pregnancy. Symptoms observed in these dose groups consisted of hypoactivity, prone position, lateral position, salutatory and rolling convulsions, increased salivation, ataxia, panting, shallow respiration, faint heart beat, discolored urine and decreased hay consumption. No compound-related clinical signs were observed in the animals treated with 0.25 IU/kg or less HMR 1964.

Body weight and Food consumption: Body weight gain was comparable in all groups that treated with HMR1964. But, body weight gains was decreased in the animals treated with 1.5 IU/kg HR 1799 on study days 6, 26 and 29. Food consumption showed a slight increase in both groups treated with 1.5 IU/kg from day 6- 10 of the study. Food intake in the low and intermediate dose groups was comparable to controls throughout the study. Blood glucose: There was a dose-dependent decrease in blood glucose levels as shown below, which had returned to normal 4 to 8 hours after dosing. HR1799 had similar glucose lowering activity.

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Blood glucose levels in rabbits treated with HMR 1964
Study No.: 2000.0894



Terminal and Necropsy evaluations: There were slight decreases in live fetuses in high dose (107 vs 84), which was not dose-dependent. The decrease in live fetuses was related with an increase in post-implantation loss as shown below. The reduction in live fetuses and maternal deaths are likely related to hypoglycemia that was induced by the test article because convulsions were observed in the high dose groups. There were no appreciable intergroup differences in sex ratio (% male fetuses). There were sporadic necropsy findings that might not be considered to be the test-article related.

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RUN DATE: 9 Mar 2001

AVENTIS PHARMA Deutschland GmbH

STUDY: EMBRYOTOXICITY STUDY

ANIMAL: Himalayan Rabbit

PREPARATION: HMR 1964

STUDY NO. 2000.0894

SURVEY OF RESULTS DURING GESTATION AND AT CAESARIAN SECTION

		GROUP 1 0 I.U./kg	GROUP 5 0.25 I.U./kg	GROUP 6 1.5 I.U./kg
PRE-IMPLANTATION LOSS %	NE MEAN	6.20	12.12	7.39
POST-IMPLANTATION LOSS %	NE MEAN	15.63	13.43	38.17
- EARLY INTRAUTERINE DEATHS	TOTAL	16	17	41
	NE MEAN	0.89	0.89	2.93
	S.D.	1.32	1.10	2.34
% OF IMPLANTATIONS	MEAN	15.63	13.43	38.17 +
- DEAD FOETUSES	TOTAL	0	0	0
	NE MEAN	0.00	0.00	0.00
	S.D.	0.00	0.00	0.00
% OF IMPLANTATIONS	MEAN	0.00	0.00	0.00
- TOTAL INTRAUTERINE DEATHS	TOTAL	16	17	41
	NE MEAN	0.89	0.89	2.93
	S.D.	1.32	1.10	2.34
- LIVE FOETUSES	TOTAL	107	116	63
	NE MEAN	5.9	6.1	4.5
	S.D.	2.4	2.3	2.1

ANIMALS NOT SURVIVING TO DAY 29 AFTER MATING OR HAVING AN EARLY PM ON DAY 29 OR RESORPTIONS ONLY OR NONPREGNANT ANIMALS ARE EXCLUDED FROM THE MEANS.

+: SIGNIFICANTLY HIGHER THAN CONTROL - : SIGNIFICANTLY LESS THAN CONTROL
M: STATISTICAL EVALUATION NOT POSSIBLE, GROUP CONTAINS LESS THAN 5 ANIMALS NE: NO STATISTICAL EVALUATION

Examination of the fetuses: A total 6 females; 2 from the 0.25 IU/kg and 4 from the 0.5 IU/kg HMR1964 did not become pregnant. The live fetuses delivered by Caesarian section in the treated group showed normal physical development. Their mean body weights and lengths did not differ appreciably from the corresponding values in the control group.

External/Skeletal/Visceral Defects:

Blood in thoracic cavity and pericardium were slightly increased in 0.25 and 0.5 IU/k/d groups without dose-dependency as shown below. The toes of forepaw were shortened (7% litters and 1.2% fetuses examined, respectively) in the 1.5 IU/k/d group. Aplasia of thoracic vertebral arch was noted in the high dose group, of which incidences are 4.8% fetuses and 14.3% litters examined, respectively. The incidences of dysplasia of caudal vertebra and aplasia in lumbar vertebra were increased also in the high dose groups without a clear dose-dependency as shown below. Aplasia in ribs and nodular thickening of pectoral clavícula were observed in the 1.5 IU/k/d group. **The compound-related effect cannot be ruled out because these findings occurred clustered together in both high dose-groups.**

STUDY: EMBRYOTOXICITY
ANIMAL: HIMALAYAN RABBIT

PREPARATION: RMR 1964

STUDY NO. 2000-0884

PAGE: 1

SUMMARY AND INTERGROUP COMPARISON OF MORPHOLOGICAL FINDINGS IN FORTUSES

CLASSIFICATION	GROUP 1		GROUP 2		GROUP 3		GROUP 4		GROUP 5		GROUP 6		MINIMUM-MAXIMUM RANGE OF HISTORICAL CONTROLS (PER STUDY)	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%		
EXTERNAL/VISCERAL DEFECTS OBTAINED AT AUTOPSY														
=====														
NUMBER OF FORTUSES EXAMINED	107		106		83		84		118		63			
NUMBER OF LITTERS EXAMINED	18		17		14		14		19		16			
EXTERNAL														

FORTUS - RETAINED	RETARDATION	0	0.0	0	0.0	0	0.0	1	1.2	0	0.0	0	0.0	0.0 - 2.2
		0	0.0	0	0.0	0	0.0	1	7.1	0	0.0	0	0.0	0.0 - 11.0
BRAIN														

BRAIN - HYDROCEPHALUS EXTERNUM	MAJOR DEFECT	0	0.0	0	0.0	0	0.0	0	0.0	1	0.9	0	0.0	0.0 - 0.0
		0	0.0	0	0.0	0	0.0	0	0.0	1	5.3	0	0.0	0.0 - 0.0
THORACIC CAVITY														

CAVITY - BLOOD	MINOR DEFECT	0	0.0	1	0.9	1	1.2	0	0.0	1	0.9	0	0.0	0.0 - 1.8
		0	0.0	1	5.9	1	6.3	0	0.0	1	5.1	0	0.0	0.0 - 11.1
HEART														

APEX - DISPLACED - DEXTRAD	MINOR DEFECT	0	0.0	1	0.9	0	0.0	0	0.0	0	0.0	0	0.0	0.0 - 1.1
		0	0.0	1	3.9	0	0.0	0	0.0	0	0.0	0	0.0	0.0 - 5.0
PERICARDIUM - BLOOD														
	MINOR DEFECT	0	0.0	2	1.9	3	3.6	1	1.2	0	0.0	1	1.6	0.0 - 4.9
		0	0.0	2	11.9	3	18.9	1	7.1	0	0.0	1	7.1	0.0 - 24.3

2000-1170-0884

JACKSONVILLE-TEST (P < 0.05) BASED ON THE NUMBER OF FORTUSES
 * : SIGNIFICANTLY HIGHER THAN CONTROL N : EVALUATION NOT POSSIBLE (NE) : NO STATISTICAL EVALUATION
 UPPER LINE: NUMBER OF AFFECTED FORTUSES LOWER LINE: NUMBER OF AFFECTED LITTERS

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STUDY: EMBRYOTOXICITY
ANIMAL: HINDALAM RABBIT

PREPARATION: HDR 1564

STUDY NO. 2000.0894

PAGE: 5

SUMMARY AND INTERGROUP COMPARISONS OF MORPHOLOGICAL FINDINGS IN FOSTUSES

CLASSIFICATION	GROUP 1		GROUP 2		GROUP 3		GROUP 4		GROUP 5		GROUP 6		MINIMUM-MAXIMUM RANGE OF HISTORICAL CONTROLS (PER STUDY)
	NO	%											
SKULL													

SPLINTING OF BONE ON PARIETAL BONE - UNI- OR BILATERAL	0	0.0	0	0.0	0	0.0	3	3.4	1	0.9	2	3.3	0.0 - 5.4
	0	0.0	0	0.0	0	0.0	1	7.1	1	5.3	2	14.3	0.0 - 21.4
EPICAL BONE BETWEEN BOTH PARTS OF NASAL AND FRONTAL BONE	5	4.7	4	3.8	4	4.8	1	1.2	6	5.2	5	7.9	0.0 - 6.4
	5	27.8	3	17.6	3	18.8	1	7.1	3	15.8	4	28.6	0.0 - 29.8
PERFORATION IN PARIETAL BONE - CIRCULAR OR OVAL-SHAPED - SMALL - UNI- OR BILATERAL	4	3.7	0	0.0	2	2.4	1	1.2	2	1.7	1	1.6	0.0 - 5.5
	4	22.2	0	0.0	2	12.5	1	7.1	2	10.5	1	7.1	0.0 - 24.7
FISSURE ON PARIETAL BONE - UNILATERAL	0	0.0	0	0.0	2	2.4	1	1.2	1	0.9	0	0.0	0.0 - 3.8
	0	0.0	0	0.0	2	12.5	1	7.1	1	5.3	0	0.0	0.0 - 21.4
VERTEBRAL COLUMN													

THORACIC VERTebra - SCOLIOSIS - DEXTRAD OR SINISTRAD	0	0.0	0	0.0	0	0.0	2	2.4	0	0.0	1	1.6	0.0 - 2.2
	0	0.0	0	0.0	0	0.0	2	14.3	0	0.0	1	7.1	0.0 - 7.7
LUMBAR VERTebra - SCOLIOSIS - SINISTRAD	0	0.0	0	0.0	0	0.0	1	1.2	0	0.0	0	0.0	0.0 - 0.0
	0	0.0	0	0.0	0	0.0	1	7.1	0	0.0	0	0.0	0.0 - 0.0
THORACIC VERT. ARCH													

APLASIA, REDUCED IN SIZE, DYSPLASIA, FUSED	0	0.0	0	0.0	0	0.0	4	4.8	0	0.0	3	4.8	0.0 - 2.2
	0	0.0	0	0.0	0	0.0	2	14.3	0	0.0	2	14.3	0.0 - 7.7

YACHTMANN-T TEST (P < 0.05) BASED ON THE NUMBER OF FOSTUSES
 * : SIGNIFICANTLY HIGHER THAN CONTROL #: EVALUATION NOT POSSIBLE (NR) : NO STATISTICAL EVALUATION
 UPPER LINE: NUMBER OF AFFECTED FOSTUSES LOWER LINE: NUMBER OF AFFECTED LITTERS

2000.1170.0368

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ON ORIGINAL

STUDY: EMBRYOTOXICITY PREPARATION: HMR 1964 STUDY NO. 2000.0894
ANIMAL: HIMALAYAN RABBIT PAGE: 7

SUMMARY AND INTERGROUP COMPARISON OF MORPHOLOGICAL FINDINGS IN FETUSES

CLASSIFICATION	GROUP 1		GROUP 2		GROUP 3		GROUP 4		GROUP 5		GROUP 6		MINIMUM-MAXIMUM RANGE OF HISTORICAL CONTROLS (PER STUDY)
	NO	%											
SKELTAL DEFECTS													
STEMMERA													
LONGITUDINALLY DISPLACED, DYSPLASIA, FRAGMENTED, FUSED	5	4.7	2	1.9	7	8.4	10	11.9	8	4.9	6	9.5	0.0 - 14.4
	3	16.7	2	11.9	3	10.8	6	43.9	5	26.3	6	43.9	0.0 - 55.0
NON- OR WEAKLY OSSIFIED	54	50.3	40	37.7	30	16.1	32	38.1	31	44.0	31	49.3	17.4 - 86.7
	14	77.8	15	80.2	12	15.0	10	71.4	15	78.9	12	85.7	46.7 - 100.0
RIB													
APLASIA, SHORTENED, FUSED - PROXIMAL, MEDIAL PART, COMPLETELY - UNI- OR BILATERAL	1	0.9	0	0.0	0	0.0	4	4.8	0	0.0	3	4.8	0.0 - 6.4
	1	5.4	0	0.0	0	0.0	2	14.3	0	0.0	2	14.3	0.0 - 15.4
EXTRA RIB - AT 7TH CERVICAL VERTEBRA - VARIATION SHORT OR NORMALLY LONG - UNI- OR BILATERAL	11	10.3	3	2.9	0	0.0	1	1.2	1	0.9	6	9.3	0.0 - 14.3
	7	38.9	2	11.8	0	0.0	1	7.1	1	5.3	3	21.4	0.0 - 50.0
EXTRA RIB - AT 13TH THORACIC VERTEBRA - VARIATION SHORT OR NORMALLY LONG - UNI- OR BILATERAL	2	1.9	0	0.0	0	0.0	1	1.2	2	1.7	3	4.8	0.0 - 11.6
	1	5.6	0	0.0	0	0.0	1	7.1	3	10.5	3	21.4	0.0 - 33.3
PECTORAL GIRDLE													
CLAVICULA - NODULAR THICKENED - MEDIAL PART - RIGHT OR BILATERAL	0	0.0	0	0.0	0	0.0	1	1.2	0	0.0	1	1.6	0.0 - 2.3
	0	0.0	0	0.0	0	0.0	1	7.1	0	0.0	1	7.1	0.0 - 13.3
FOREPAW													
5TH TOE - PHALANX II - NON-OSSIFIED - BILATERAL	1	0.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0.0 - 4.7
	1	5.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0.0 - 13.3

2000.1170.0570

JACKSON-K-T-TEST (P < 0.05) BASED ON THE NUMBER OF FETUSES
*: SIGNIFICANTLY HIGHER THAN CONTROL. #: EVALUATION NOT POSSIBLE (NR): NO STATISTICAL EVALUATION
UPPER LINE: NUMBER OF AFFECTED FETUSES LOWER LINE: NUMBER OF AFFECTED LITTERS

Summary and Conclusion:

Embryo-fetal toxicity of HMR1964 was examined in Himalayan rabbits at doses of 0.25, 0.5, and 1.5 IU/kg/day. The high dose killed 2 animals with signs of hypoglycemia. There were slight decreases in live fetuses in the group, which was related with an increase in post-implantation loss.

The incidence of dams with litter losses and resorptions was increased in the high dose group. Morphological examination of the fetuses revealed an increased incidence of fetuses showing anomalies in the region of vertebral column and ribs in the high dose

group. Neither maternal nor embryofetal toxicity was observed after administration of HMR1964 at dose of 0.25 IU/kg. The therapeutic exposure ratios were 0.3 and 0.5, respectively at 0.25 and 0.5 IU/kg, based on body surface comparison after a daily dose of 0.3 IU/kg. NOAEL < 0.25 IU/kg for both HMR1964.

PERINATAL AND POSTNATAL STUDIES of HMR1964 are ongoing in rats.

VIII. GENETIC TOXICOLOGY:

The sponsor performed three genetic toxicology studies: 1) Ames test, 2) In vitro mammalian chromosome aberration test in V79 Chinese hamster cells, and 3) In vivo mammalian erythrocyte micronucleus test in rats. The three tests were conducted by Aventis Pharma Deutschland GmbH, Drug Innovation and Approval, Drug Safety Evaluation under GLP condition at Mainzer Landstr. 500, Germany.

I. Study Title: HMR1964-Bacterial reverse mutation test

Study No/Document No/Report No: 98.523/017717/98.0905

Study Type: Mutagenicity test

Amendment #, Volume #15 and Page #289-320

Conducting Laboratory: Hoechst Marion Roussel Deutschland GmbH, Drug Innovation & Approval, Lead Optimization, Dept of Toxicology/Pathology

Date of Study Initiation/completion: 9/15/1998; 10/16/1998

GLP Compliance: yes

QA- Reports Yes (X), No ():

Drug Batch/Lot Number: :

Study Endpoint: In vitro mutagenicity

METHODOLOGY:

Strains/Species/Cell line: Salmonella typhimurium TA100, TA1535, TA1537 and TA98, and E. coli WP2uvrA pKM101

Dose Selection Criteria: A reduced rate of spontaneously occurring colonies and visible thinning of the bacterial lawn were used as toxicity indicators. Thinning of the bacterial lawn was evaluated microscopically as an index of drug-induced cytotoxicity.

Basis of dose selection: In this dose finding study, the drug-induced toxicity was noted at 5000 µg/plate. Thus, 50, 160, 500, 1600, and 5000 µg/plate were evaluated in the presence and absence of metabolic activation.

Range finding studies: Yes

Test Agent Stability: Stable in water, and stable against heat and light.

Metabolic Activation System: Arochlor 1254 (500 mg/kg body weight) pretreated rat liver extract for 5 days was used.

CONTROLS:

Negative Controls:

untreated control
solvent (water) control
vehicle controls (0 µg/plate)

Positive Controls:

Without S-9 fraction: Sodium azide (1 µg/plate for TA100 and TA1535), 9-aminoacridine (50 µg/plate for TA1537), 2-nitrofluorence (2.5 µg/plate for TA98) and N-ethyl-N-nitro-N-nitrosoguanidine (4.0 µg/plate for WpuvrA).

With metabolic activation: 2-aminoanthracene (0.5 to 30 µg/plate) for TA98, TA100, TA1535 and TA1537, and WP2uvrA, respectively.

Exposure Conditions: Pre-incubated with medium and S9mix for 8 hours at 37°C

Incubation and sampling times: 48 hours

Doses used in definitive study: 50, 160, 500, 1600, and 5000 µg/plate

STUDY DESIGN: The first mutation test was performed as the plate incorporation method in both the presence and absence of S9-mix using all bacterial tester strains at a wide range of concentrations of the test substance. Top agar was prepared for the Salmonella strains by mixing 100 ml agar (0.6%) with 10 ml of a 0.5 mM histidine-biotin solution. With E. coli histidine was replaced by tryptophan (0.5 mM, 2.5 ml). 0.1 ml of culture medium, 0.1 ml test compound suspension and 0.5 ml of S9-mix were added to 2 ml of molten top agar at 48°C. After mixing, the liquid was poured into a petri dish with a 25 ml layer of 1.5% agar, Vogel-Bonner E medium with 2% glucose. Colonies were counted after 48 hour-incubation at 37°C.

ANALYSIS: Colonies of his⁺ and trp⁺ revertants were counted with Artec counter for statistical evaluation.

No. slides/plates/replicates/animals analyzed: 3 plates per dose

Counting method: Bacterial colonies were counted microscopically.

Cytotoxic endpoints: Thinning of the bacterial lawn was evaluated microscopically as an index of drug-induced cytotoxicity.

Genetic toxicity endpoints/results: Not mutagenic in the absence or in the presence of the S-9 fraction.

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

Criteria for Positive Results: 2-fold increase in the mean number of revertants per plate of the appropriate vehicle control at complete bacterial background lawn.

RESULTS: Solvent, negative and positive control experiments with different bacteria strains demonstrated consistent findings in the presence and absence of S9mix. The indicated concentrations of the testing agent were incubated with various strains of bacteria in the presence and absence of S9mix. The duplicate experiments indicate that no significant increases in the number of revertant colonies was observed with any of the tester strains either in the absence or in the presence of S9-mix as shown below.

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TEST

STUDY ZR0562 TEST 01 SPONSOR DIVISION L

DATE TESTED 14/10/98
DATE COUNTED 16/10/98

COMPOUND L00526/001/001 NMR 1964

Batch: T 4132

COMMENTS: VOTOX 98.0523
ALL STERILITY CONTROL PLATES WERE STERILE

STRAIN	DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE		
						PLATE 1	PLATE 2	PLATE 3
TA 100 +S9	0.	175.7	11.0			163	183	181
	50.	166.0	12.5	0.9		165	179	154
	160.	175.3	15.8	1.0		189	179	158
	500.	185.7	9.3	1.1		196	178	183
	1600.	177.7	19.6	1.0		183	156	194
	5000.	175.0	7.5	1.0		167	176	182
TA 100 -S9	0.	183.0	16.5			173	174	202
	50.	181.3	10.4	1.0		178	173	193
	160.	173.7	6.7	0.9		166	177	178
	500.	166.0	13.1	0.9		172	151	175
	1600.	160.3	28.4	0.9		191	155	135
	5000.	157.3	7.8	0.9		155	151	166
TA 1535 +S9	0.	8.7	3.1			12	6	8
	50.	10.7	2.5	1.2		13	8	11
	160.	7.7	1.5	0.9		8	9	6
	500.	8.7	3.2	1.0		11	5	10
	1600.	8.0	1.0	0.9		7	9	8
	5000.	11.0	2.0	1.3		13	11	9
TA 1535 -S9	0.	10.0	2.6			8	9	13
	50.	8.3	5.0	0.8		13	9	3
	160.	9.7	2.5	1.0		12	10	7
	500.	8.3	2.1	0.8		6	10	9
	1600.	5.7	0.6	0.6		6	5	6
	5000.	7.7	3.1	0.8		7	5	11
TA 1537 +S9	0.	9.0	1.0			10	8	9
	50.	7.3	3.8	0.8		3	9	10
	160.	9.7	0.6	1.1		9	10	10
	500.	7.0	1.7	0.8		8	5	8
	1600.	5.0	0.0	0.6		5	5	5

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ON ORIGINAL

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ON ORIGINAL

TEST

STRAIN	DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/CONTROL	BACTERIAL LAWN	NO REVERTANTS/PLATE		
						PLATE 1	PLATE 2	PLATE 3
STUDY ZR0562	TEST 01	SPONSOR	DIVISION L					
						DATE TESTED 14/10/98		
						DATE COUNTED 16/10/98		
TA 1537	+S9 5000.	11.0	1.7	1.2		9	12	12
TA 1537	-S9 0.	8.7	1.5			10	9	7
	50.	10.7	2.1	1.2		10	9	13
	160.	8.3	3.1	1.0		5	11	9
	500.	6.7	2.5	0.8		4	7	9
	1600.	6.3	4.5	0.7		11	6	2
	5000.	6.3	4.9	0.7		12	3	4
TA 98	+S9 0.	25.0	3.6			21	26	28
	50.	26.7	2.5	1.1		27	24	29
	160.	22.7	1.5	0.9		24	23	21
	500.	23.7	5.5	0.9		20	21	30
	1600.	27.7	4.0	1.1		30	30	23
	5000.	25.3	3.1	1.0		26	22	28
TA 98	-S9 0.	19.7	3.1			17	19	23
	50.	21.7	2.1	1.1		21	20	24
	160.	20.3	2.1	1.0		18	22	21
	500.	28.0	3.5	1.4		30	27	30
	1600.	27.0	4.4	1.4		24	32	25
	5000.	22.7	1.2	1.2		22	24	22
MP2JvrA	+S9 0.	20.7	3.5			24	17	21
	50.	19.3	6.5	0.9		19	13	26
	160.	20.0	3.6	1.0		23	21	16
	500.	16.3	1.5	0.8		16	15	18
	1600.	21.3	1.5	1.0		23	21	20
	5000.	13.7	2.1	0.7		12	16	13
MP2JvrA	-S9 0.	18.3	4.5			14	18	23
	50.	20.7	2.1	1.1		20	19	23
	160.	22.7	3.1	1.2		22	26	20
	500.	17.3	2.5	0.9		15	20	17
	1600.	15.7	3.5	0.9		12	16	19
	5000.	17.7	2.1	1.0		17	16	20

Study Validity: The studies appear valid since the data were reproducible and the solvent control data are within the laboratory's normal control range for the spontaneous mutant frequency. In addition, all positive control induced increases in the mutation frequency, which were both statistically significant, and within the laboratory's normal range.

Study Outcome: Negative

SUMMARY:

HMR1964 was not mutagenic in the bacterial strains tested either with or without exogenous metabolic activation at the dose levels up to the limit dose of 5000 µg/plate in duplicate assays.

II. Study Title: HMR1964-In vitro mammalian chromosome aberration test in V79 Chinese hamster lung (CHL) cell lines

Study No/Document No/Report No: 98.0524/017536/98.0908

Study Type: Chromosome aberration test

AMENDMENT #, VOLUME #15 AND PAGE #322-355

Conducting Laboratory: Hoechst Marion Roussel Deutschland GmbH, Drug Innovation & Approval, Lead Optimization, Dept of Toxicology/Pathology

Date of Study Initiation/completion: 9/8/1998-10/31/1998

GLP Compliance: yes

QA- Reports: (yes) or no ():

Drug Batch: T4132 and OP.3-13/98. Lot Number:

Study Endpoint: Identification of chromosomal aberrations such gap, break and fragment, deletion, and/ or exchanges.

METHODOLOGY:

Strains/Species/Cell line: Chinese hamster lung (CHL) cell lines

Dose Selection Criteria: According to ICH guidelines the top dose was set at 5000 µg/ml as a non-toxic, freely soluble test compounds.

Basis of dose selection: The top dose was 5000 µg/ml in the presence and absence of metabolic activation.

Range finding studies: HMR1964 doses tested were 500, 1000, 1500, 2000, 2500, 3000, 4000, and 5000 µg/ml in the range finding study. The highest dose level should reduce the survival rate to 20 – 50% and/or mitotic index to approximately 50% compared with the corresponding solvent control. Thus, the adequately spaced levels extending over at least one decadic logarithm were evaluated. In the event of clearly positive results at the 3 hours treatment time, it was not necessary to perform an evaluation of the 20 h treatment.

Test Agent Stability: Stable in cool place.

Metabolic Activation System: Arochlor 1254 (500 mg/kg body weight) pretreated rat liver extract for 5 days was used.

CONTROLS:

Vehicle: Cell culture medium was minimal essential medium with Hanks-salts and 25 mM HEPES-buffer.

Negative Controls:

Untreated control

Solvent controls

Positive Controls:

Without S-9 fraction: Ethyl methane sulfonate (Batch 40606721) 1500 µg/ml for 3 hour treatment, 400 µg/ml for 20 hour treatment.

With metabolic activation: Cyclophosphamide (Batch#603575B) 3 µg/ml

Exposure Conditions: Cultured hamster lung cells were seeded on-to slides (duplicate) then treated for either 3 hours (with and without S9mix) or for 20 hours (without S9mix). Colcemide was then added to arrest cell division. The chromosomes were stained and examined. The cells were exposed to at least three dose levels of HMR1964.

Incubation and sampling times: Mycoplasma-free V79 cells was thawed and kept at 37°C and 4% CO₂ in a flask. About 5 x 10⁵ cells were seeded into each flask in 30 ml of culture medium for 1-week culture.

Doses used in definitive study: 0, 500, 1600, 5000 µg/ml with/out S9-mix

STUDY DESIGN: The highest dose should reduce the survival rate to 20-50%, although HMR1964 did not exert any cytotoxicity at 5 mg/kg in vitro. The solvent control data are within the laboratory's normal control range for mutant frequency and the positive controls should cause a significant increase the frequency of aberrations.

No. slides/plates/replicates/animals analyzed: Only metaphases with 22+/- 1 chromosomes are included in the analysis.

Counting method: Chromosomal aberrations were counted and classified.

Cytotoxic endpoints: Statistically significant decrease in cell survival with highest dose > 50%.

Statistical methods: Biometry of the results was performed with a one-sided Fisher-Exact test.

Criteria for Positive Results: Test article would be positive if 1) it induces a reproducible statistically significant increase in the aberration rate (without gaps) with one or more of the concentrations tested, and 2) there is a reproducible concentration-related increase in the aberration rate (without gap). The test result would be negative if the incidence of aberration cell in each group was < 5%.

RESULTS: HMR1964 was not toxic for Chinese hamster cells at 5 mg/ml in vitro as shown in preliminary toxicity study. HMR1964 was not mutagenic in this chromosome aberration test in vitro with Chinese hamster lung cell line as demonstrated in 3 tables below.

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9.2 Preliminary experiment toxicity tables

21-629

Table 1a (3 hours treatment time without S9-mix):

	Dose µg/ml	S9- mix	Extinction in microwell plates mean less blank values	standard deviation	relative survival*
Solvent control	0	-	0,569	0,04	100,0
HMR 1964	500	-	0,620	0,04	109,1
	1000	-	0,583	0,02	102,5
	1500	-	0,588	0,04	103,3
	2000	-	0,587	0,02	103,2
	2500	-	0,619	0,03	106,8
	3000	-	0,588	0,04	103,3
	4000	-	0,575	0,02	101,1
	5000	-	0,616	0,02	108,3

Table 1b (3 hours treatment time with S9-mix):

	Dose µg/ml	S9- mix	Extinction in microwell plates mean less blank values	standard deviation	relative survival*
Solvent control	0	+	0,614	0,06	100,0
HMR 1964	500	+	0,570	0,07	92,7
	1000	+	0,599	0,10	97,4
	1500	+	0,637	0,16	103,6
	2000	+	0,531	0,03	86,3
	2500	+	0,583	0,03	94,9
	3000	+	0,595	0,07	96,8
	4000	+	0,571	0,02	93,0
	5000	+	0,601	0,04	97,8

Table 1c (20 hours treatment time without S9-mix):

	Dose µg/ml	S9- mix	Extinction in microwell plates mean less blank values	standard deviation	relative survival*
Solvent control	0	-	0,651	0,05	100,0
HMR 1964	500	-	0,558	0,03	85,4
	1000	-	0,572	0,06	87,9
	1500	-	0,601	0,07	92,4
	2000	-	0,557	0,06	85,5
	2500	-	0,619	0,06	95,1
	3000	-	0,609	0,13	93,6
	4000	-	0,683	0,07	101,9
	5000	-	0,647	0,06	99,5

Solvent Control = cell culture medium (MEM)

* relative survival (mean value / mean value corresponding control x 100)

9.3 Tables of the mitotic index and the number of polyploid cells

Table 2 (mitotic index):

First experiment		Dose µg/ml	S9- mix	treatment time (h)	mitotic index		mean	relative mitotic index percent*
Test group					1	2		
Solvent control Medium		0.0	-	3	9.1	7.5	8.3	100.0
HMR 1964		500.0	-	3	7.2	8.2	7.7	92.8
HMR 1964		1600.0	-	3	8.5	8.9	8.7	104.8
HMR 1964		5000.0	-	3	11.2	10.0	10.6	127.7
Positive control EMS		1500.0	-	3	6.0	8.0	7.0	84.3
Solvent control Medium		0.0	+	3	9.3	9.8	9.6	100.0
HMR 1964		500.0	+	3	5.8	6.6	6.2	64.6
HMR 1964		1600.0	+	3	7.3	6.9	7.1	74.0
HMR 1964		5000.0	+	3	6.4	7.5	7.0	72.9
Positive control CPA		3.0	+	3	10.0	12.5	11.3	117.7

Second experiment		Dose µg/ml	S9- mix	treatment time (h)	mitotic index		mean	relative mitotic index percent*
Test group					1	2		
Solvent control Medium		0.0	-	20	8.5	7.8	8.2	100.0
HMR 1964		500.0	-	20	6.4	5.8	6.1	74.4
HMR 1964		1600.0	-	20	6.1	6.7	6.4	78.0
HMR 1964		5000.0	-	20	6.3	6.7	6.5	79.3
Positive control EMS		400.0	-	20	5.8	5.5	5.7	69.5

* The mitotic index was determined in 1000 cells

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Table 3 (number of polyploid cells):

First experiment		Dose µg/ml	S9- mix	treatment time (h)	polyploid cells*		mean
Test group					1	2	
Solvent control Medium		0.0	-	3	2	3	2.5
HMR 1964		500.0	-	3	1	2	1.5
HMR 1964		1600.0	-	3	2	2	2.0
HMR 1964		5000.0	-	3	2	5	3.5
Positive control EMS		1500.0	-	3	1	2	1.5
Solvent control Medium		0.0	+	3	3	1	1.5
HMR 1964		500.0	+	3	2	1	1.5
HMR 1964		1600.0	+	3	1	3	2.0
HMR 1964		5000.0	+	3	1	5	3.0
Positive control CPA		3.0	+	3	4	5	4.5

Second experiment		Dose µg/ml	S9- mix	treatment time (h)	polyploid cells*		mean
Test group					1	2	
Solvent control Medium		0.0	-	20	1	0	0.5
HMR 1964		500.0	-	20	1	0	0.5
HMR 1964		1600.0	-	20	0	1	0.5
HMR 1964		5000.0	-	20	1	3	2.0
Positive control EMS		400.0	-	20	0	0	0.0

* The number of polyploid cells was determined in 1000 cells

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9.4 Summary tables

Table 4:

First experiment Test group	Dose µg/ml	S9- mix	treatment time (h)	Number of cells analysed		percent aberrant cells		
				1	2	Incl. gaps mean 1+2	excl. gaps mean 1+2	exchanges mean 1+2
Solvent control Medium	0.0	-	3	100	100	0.5	0.0	0.0
HMR 1964	500.0	-	3	100	100	1.0	0.0	0.0
HMR 1964	1600.0	-	3	100	100	0.0	0.0	0.0
HMR 1964	5000.0	-	3	100	100	1.5	1.0	0.0
Positive control EMS	1500.0	-	3	50	50	18.0	15.0	10.0
Solvent control Medium	0.0	+	3	100	100	1.5	0.0	0.0
HMR 1964	500.0	+	3	100	100	0.5	0.0	0.0
HMR 1964	1600.0	+	3	100	100	0.5	0.5	0.5
HMR 1964	5000.0	+	3	100	100	0.0	0.0	0.0
Positive control CPA	3.0	+	3	50	50	18.0	18.0	12.0

Second experiment Test group	Dose µg/ml	S9- mix	treatment time (h)	Number of cells analysed		percent aberrant cells		
				1	2	Incl. gaps mean 1+2	excl. gaps mean 1+2	exchanges mean 1+2
Solvent control Medium	0.0	-	20	100	100	0.5	0.5	0.0
HMR 1964	500.0	-	20	100	100	0.0	0.0	0.0
HMR 1964	1600.0	-	20	100	100	1.0	0.5	0.0
HMR 1964	5000.0	-	20	100	100	0.5	0.0	0.0
Positive control EMS	400.0	-	20	50	50	16.0	16.0	9.0

Study Validity: It appears that the chromosomal aberration test was performed under acceptable conditions. Solvent control data were within the laboratory's normal control range for the spontaneous mutant frequency. Positive controls responded appropriately within the laboratory's historical values.

Study Outcome: HMR1964 was not clastogenic in this chromosome aberration test in vitro with Chinese hamster lung cell line.

SUMMARY: The sponsor performed standard chromosome aberration test with doses of 500, 1600, and 5000 µg/ml of HMR1964. The procedures of experiments, criteria for positive results, and analysis methods were acceptable. HMR1964 was not mutagenic in this in vitro Chinese hamster lung cell line.

III. Study Title: HMR1964-Micronucleus Study in Sprague Dawley Rats.

Study No.: DSE2000.0959/Document No:F2001TOXX0065/Report No:

Study Type: Clastogenicity test

Amendment #, Volume # and Page #360-384

Conducting Laboratory: Hoechst Marion Roussel Deutschland GmbH, Drug Innovation & Approval, Lead Optimization, Dept of Toxicology/Pathology

Date of Study Initiation/completion: 2/22/1991-/3/20/1991

GLP Compliance: yes

QA- Reports Yes

Drug Lot Number: Batch No. 1215 _____

METHODOLOGY:

Strains/Species/Cell line: Hsd:Sprague Dawley 5 rats/sex

Dose Selection Criteria: HMR1964, 1000 IU/kg, was the maximum dose because of a limitation to the highest tolerable subcutaneous application volume as demonstrated in preliminary dose range finding study.

Basis of dose selection: 1000 mg/kg was selected as the highest level in dose range finding study. Four levels were established by dilution of the highest concentration to 300, 100, and 0 IU/kg, including the control and positive groups.

Range finding studies: No mortality was observed at 1000 IU/kg, although it was not possible to increase the subcutaneous dose due to limited solubility of HMR1964 in buffer medium. Full study was performed using 100, 300, and 1000 IU/kg levels.

Test Agent Stability: Stable under cool and light-resistant condition

CONTROLS:

Vehicle: Solvent Buffer

Negative Controls: a). Untreated control; and b). Solvent controls

Positive Controls: Endoxan (batch#98630A), 40 mg/kg, was given orally once, although HMR1964 was given subcutaneously twice by an interval of 24 hours.

Exposure Conditions: Please see "Incubation" below.

Incubation and sampling times: Bone marrow samples (femora) were collected at 24 or 48 hours after treatment.

Doses used in definitive study: 0, 100, 300, and 1000 mg/kg

STUDY DESIGN: The test substance and positive control were given to the animals. Individual bone marrow preparation was fixed on slides and the specimen was observed microscopically. Micronucleated polychromatic erythrocytes were counted.

ANALYSIS: 2000 polychromatic erythrocytes were counted for each animal. The number of cells with micronuclei (not the number of individual micronuclei) was recorded. The ratio of polychromatic erythrocytes to 200 normochromatic erythrocytes was determined. Incidences of micronuclei in the test compound group were compared with positive and negative control groups.

No. animals analyzed: 5 rats/sex/group

Counting method: Microscopically with 1000 x magnification.

Cytotoxic endpoints: There was no toxicity with HMR1964 as an insulin analogue.

Genetic toxicity endpoints/results: Micronuclei formation.

Statistical methods: Significance in the incidence was analyzed by Wilcoxon test based on binomial distribution with significance levels of 5%.

Criteria for Positive Results: The test article was classified as mutagenic if it induced significant dose-related increases in micronucleated polychromatic erythrocytes, compared with negative control groups.

RESULTS: Subcutaneous administration of 1000 IU/kg HMR 1964 resulted in the death of 3 males. These animals were replaced. No signs of toxicity were observed and no macroscopic findings were noted upon dissection. The mean incidence of micronuclei in the 0, 100, and 1000 IU/kg and in the positive control groups were 0.11, 0.17, 0.11, 0.21, and 1.14 in males, respectively, as shown below. The values in females were also comparable.

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10.1 SUMMARY TABLES AND STATISTICS

Test compound: HMR 1964

Table 2: Summary Tables and Statistics

Sex	Dose mg/kg b.w.	killing time	Number of animals	Poly / animal counted	Poly/Ery Mean	Poly/Ery SD Mean	Poly with MN Mean	Poly with MN [%] Mean	Poly with MN SD Mean
male	0 - Control	24 h	5	2000	0,49	0,05	2,2	0,11	0,10
male	100	24 h	5	2000	0,55	0,03	3,4	0,17	0,08
male	300	24 h	5	2000	0,50	0,02	2,2	0,11	0,05
male	1000	24 h	5	2000	0,49	0,08	4,2	0,21	0,04
male	40 - Endoxan	24 h	5	2000	0,44	0,07	22,8	1,14	0,30
female	0 - Control	24 h	5	2000	0,49	0,04	3,2	0,16	0,04
female	100	24 h	5	2000	0,51	0,02	3,4	0,17	0,08
female	300	24 h	5	2000	0,53	0,04	3,4	0,17	0,04
female	1000	24 h	5	2000	0,47	0,04	3,4	0,17	0,06
female	40 - Endoxan	24 h	5	2000	0,47	0,05	27,0	1,35	0,29

Sex	Dose mg/kg b.w.	killing time	Number of animals	Poly / animal counted	Poly/Ery Mean	Poly/Ery SD Mean	Poly with MN Mean	Poly with MN [%] Mean	Poly with MN SD Mean	Mut. I.
pooled	0 - Control	24 h	10	2000	0,49	0,04	2,70	0,1	0,07	1,0
pooled	100	24 h	10	2000	0,53	0,03	3,40	0,2	0,07	1,3
pooled	300	24 h	10	2000	0,51	0,04	2,80	0,1	0,06	1,0
pooled	1000	24 h	10	2000	0,48	0,06	3,80	0,2	0,05	1,4
pooled	40 - Endoxan	24 h	10	2000	0,45	0,06	24,9*	1,2	0,30	9,2

Mut. I. = Mutagenic index
 Control = Vehicle (vehikel)
 * = significantly different from control (p < 0,05)

A cross comparison of individual data and pooled data may show discrepancies since the values are rounded.

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Study Validity: This study was conducted in acceptable conditions since the positive control group produced an expected increase in polychromatic erythrocytes while the negative control had no effects.

Study Outcome: The results lead to the conclusion that HMR1964 was not mutagenic in the in vivo micronucleus test in rats.

Summary of Genotoxicity Study:

The sponsor performed three genetic toxicology studies: 1) Ames test, 2) In vitro mammalian chromosome aberration test in V79 Chinese hamster cells, and 3) In vivo mammalian erythrocyte micronucleus test in rats

The three studies that were documented under Report numbers, 017717, 017536, and F2001TOX0065, respectively, were conducted under acceptable conditions with valid batches of HMR1964 as summarized in a table below. The three tests indicate that the test article was not mutagenic.

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Species (strain) Number, Age, Weight Report number	Test compound (batch number) Dose/Route Formulation/ Vehicle	Study design (Laboratory) GLP status	Results
Genetic toxicity			
Salmonella typhimurium (TA 100, TA 1535, TA 1537, TA 98) <i>Escherichia coli</i> WP2uvrA Ref. Doc. No. 017717 (5)	HMR1964 (batch mixture Kromesalil C18 Op-3-13/98) 0, 50, 180, 500, 1800-5000 µg/plate deionised water	Objective: Evaluation of gene mutations in bacteria. Design: Ames test, two independent mutagenicity studies were conducted, each in the absence and in the presence of a metabolising system derived from rat liver homogenata. (Aventis Pharma Deutschland GmbH, Drug Innovation & Approval, Lead Optimization, Department of Toxicology/Pathology) GLP study	HMR1964 is not mutagenic in these bacterial test systems, either with or without exogenous metabolic activation, at the dose levels investigated.
V 79 Chinese hamster cells Ref. Doc. No. 017536 (11)	HMR1964 (batch T4132, preliminary experiment; Op3-13/98, main experiment) 3 h: 500-1600-5000 µg/mL (±S9-mix) 20 h: 500-1600-5000 µg/mL (without S9-mix) cell culture medium (MEM)	Objective: Induction of chromosomal aberrations <i>in vitro</i> Design: <i>In vitro</i> mammalian chromosome aberration test; two experiments with duplicate cultures were used for each concentration (Aventis Pharma Deutschland GmbH, Drug Innovation & Approval, Lead Optimization, Department of Toxicology/Pathology) GLP study	HMR1964 is not mutagenic in the chromosome aberration assay.
Rat (Hsd: Sprague Dawley) 5M/5F per group 6 weeks M: mean 194.8 g F: mean 150.8 g Ref. Doc. No. F2001TOX0065 (43)	HMR1964 (batch 1215 Verum) 0, 100, 300, 1000 IU/kg body wt. Placebo (batch 1310) Positive control Endoxan® (40 mg/kg body wt., once orally)	Objective: Induction of micronuclei in bone marrow cells <i>in vivo</i> , following two s.c. injections. Design: <i>In vivo</i> mammalian chromosome aberration test. Study evaluation included clinical observation, macroscopic examination, and analysis of the bone marrow. (Aventis Pharma Deutschland GmbH, Drug Innovation & Approval, Lead Optimization, Department of Toxicology/Pathology) GLP study	No signs of toxicity were observed and the dissection revealed no test substance-related macroscopic findings. HMR1964 is negative in the micronucleus test.

Note: F: female; M: male; wt.: weight; MEM: minimal essential medium; GLP: good laboratory practice

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Summary and Conclusion:

HMR1964 simulates insulin secretion as a result of its interaction with the insulin receptor as an insulin analogue. The effect is characterized by a rapid onset of activity and rapid reversal upon removal of the drug, which were comparable to LisPro. In dogs, the hypoglycemic activity of HMR1964 was faster than that of human insulin. The mode of action of HMR1964 appeared similar to other insulin analogues such as Lantus or LisPro. In vitro pharmacologic studies demonstrated that HMR 1964 and human insulin have comparable insulin receptor association kinetics and maximal binding in transformed rat embryo fibroblasts overexpressing 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 adipocytes was slightly reduced compared to human insulin regarding the dose response.

HMR1964 treatment was without effects on the central nervous system, pulmonary, and renal systems. HMR1964 increased HR and QT interval in female dogs in safety study (Document#99/11004/PH) at a dose of 1 IU/kg. The findings were not confirmed in 1- and 6-month subsequent toxicological studies in dogs with doses up to 10 IU/kg/day, which is approximately 18-fold therapeutic exposure, based on body surface area comparison. The 4-week subcutaneous toxicological study in rats used 500 IU/kg/day as a top dose which killed 9 male rats out of 10 animals because of animal fasting. Similar findings were observed in 6-month toxicity study in rats. Pharmacology and toxicology profile of HR1799, the sponsor's insulin was similar to that of HMR1964 at the same doses.

Fertility and pre-natal studies with HMR1964 were performed in SD rats. The HMR1964 top dose was 10 IU/kg, which was approximately 5 times clinical exposure based on body surface area comparison. There were no remarkable drug-related effects on parental body weight, food consumption, duration of gestation, and gross pathology. HMR1964 at doses of 1, 3.15, and 10 IU/kg in SD rats during Day 6 to 17 of gestation did not produce clear the test article-related severe teratogenic toxicity. Slightly increased incidences of minor rib anomalies were observed in the high dose group in rats. No clear maternal toxicity and/or toxic effect on the intra-uterine development of the conceptuses was detectable after the low and intermediate dose groups of HMR1964(0.5 and 1.7 times human exposure based on body surface area).

HMR1964 at doses of 0.25, 0.5, and 1.5 IU in Himalayan rabbits during Day 6 to 18 of gestation killed 2 animals of the high dose group with signs of hypoglycemia. There were slight decreases in live fetuses in the high dose, which was related with an increase in

post-implantation loss. Aplasia of thoracic and lumbar vertebral arch was observed in the high dose group fetuses. Nodular thickening of pectoral clavicular girdle was also observed in the group. Thus, the compound-related effect cannot be ruled out because these findings occurred clustered together in high dose groups. No clear maternal toxicity and/or toxic effect on the intra-uterine development of the conceptuses was detectable after the low and intermediate dose groups of HMR1964(0.3 and 0.6 times human exposure based upon body surface area).

HMR1964 was tested for its potential genotoxicity according to the standard ICH battery tests. The sponsor performed Ames test, in vitro chromosome aberration study using Chinese hamster lung cells and in vivo mammalian erythrocyte micronucleus test in Sprague Dawley rats. The three studies were performed under acceptable GLP conditions with valid evaluation criteria, of which results lead to the conclusion that HMR1964 did not display mutagenic potential.

RECOMMENDATIONS (External to Sponsor):

- 1) In the cardiovascular safety study (Document# 99/11004/PH), HMR1964 increased the QT-interval and heart rate in dogs. However, the sponsor's 1- and 6-month toxicology studies in dogs did not confirm the finding (Document#F1999TOX0166 and F2000TOX0570). Please explain the discrepancy.

- 2) The sponsor suggested that necrosis of neurons in a restricted area of the hippocampus were considered as histopathological evidence of HMR1964-induced hypoglycemia. Which area of the hippocampus was affected first and why is the area vulnerable, compared to other parts of the nervous systems such as the hypothalamus or peripheral nerves?

Herman Rhee, Ph.D.
Review Pharmacologist

Jeri El Hage, Ph.D.
Team Leader

cc: IND61,956
HFD-510/El Hage/H.Rhee/Rheej

Appendix II. Pharmacology Review dated 2/18/2002

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: 2/18/2002; Review Assigned Date: 1/23/2002

Review number: 003

IND/NDA NUMBER: 61,956

Serial number/date/type of submission: #044 on 1/23/2002/Commercial

Information to sponsor: Yes (x) No ()

Sponsor (or agent): Aventis Pharmaceuticals Inc., Bridgewater, NJ, 1

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

DRUG: HMR1964 (rDNA human insulin analog)

Code Name: 199 15033

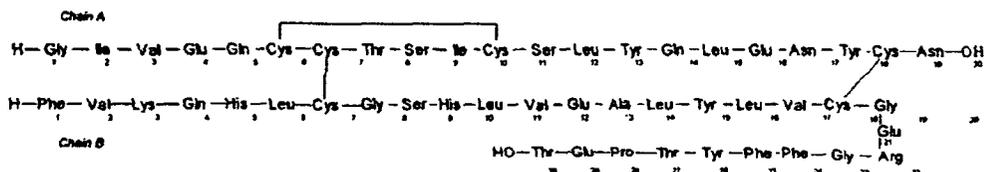
Chemical Name: 3^B-Lys-29^B-Glu human insulin/C₂₅₈H₃₈₄N₆₄O₇₈S₆

Excipients: Please see "Clinical Formulation" section.

CAS Registry Number: None

Molecular Formula/ Molecular Weight: 5823

Structure:



Relevant INDs/NDAs/DMFs: IND# _____ NDA#20-563 (Humalog, LysPro), NDA#20-081 (Lantus), and NDA#20-986 (Novolog)

Drug Class: Fast-acting insulin analogue prepared in E. coli by recombinant technology.

Indication: Diabetes

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

Background:

The study is in Phase II at this time. In Phase I studies, HMR1964 (1 - 0.3 IU/kg sc) was used in 16 healthy volunteers for 7 days. Over 300 subjects will be used in Phase III studies for 6 months. In the submission, the sponsor wishes to document HMR1964 effects on reproductive system in rats. In addition, the sponsor included regular insulin, HR1799 for comparison purposes.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:**Study title: Subcutaneous pre- and postnatal toxicity study in rats**

Key study findings: HMR 1964 and human insulin HR1799 were tested for their potential effects on reproductive system in rats. Four rats from HD dose (8 IU/kg) group died (18%) of presumable hypoglycemia as a result of insulin action. There were no remarkable reproductive toxicological changes in F₀- or F₁-generation. Other observations and findings have no impact on clinical drug development. NOAEL= 1 IU/kg for both HMR 1964 and HR 1799.

Study no.: 2000.0942

Volume #1 and 2, and page #: 1-595

Conducting laboratory and location: Aventis Pharma Deutschland GmbH

Date of study initiation: 1/16/01

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, Batch#: 1215 Veri

Formulation/vehicle: Placebo solution for HMR1964, batch#1310.

Methods:

Species/strain: Female Rat/Hsd: Sprague Dawley

Doses employed: Control, HMR 1, HMR 3.15, HMR 8 IU/kg; HR1799 1 and 8 IU/kg

Route of administration: The rats received the test compounds of HMR 1964 at doses of 0, 1, 3.15 or 8 IU/kg body weight subcutaneously once daily from day 6 of pregnancy until day 21 post partum.

Study design: Pregnant animals received the test compounds during embryogenesis and fetogenesis, and during the lactation period. Twenty-three males and females were selected from F₁ generation to produce the F₂ generation. Virgin females in the pre-estrous or estrous phase were mated overnight with fertile males and were caged individually after detection of sperm in vaginal smears. The day of sperm detection was defined as day 0 of gestation.

Number/sex/group: 22/group (6 groups)

Parameters and endpoints evaluated: Please see individual study.

Observations and measurements:F₀-Generation:

All animals were examined before the start of the study and the animals' general health conditions were observed several times daily (on weekends and holidays once daily). 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:

Examinations during lactation: After delivery the number of stillborn and live young, the sex of the young and the presence of gross anomalies were determined in all litters. Live offspring were counted and weighed after birth and on days 4, 7, 10, 14, 17, and 21 thereafter. Animals were weighed twice weekly and food consumption was measured at these time points. Behavior and viability were observed several times daily (on weekends and holidays once daily).

Dates of vaginal opening (separation of the praeputium in males) were checked and mating experiments were performed at 12-week post partum. Pregnancy was confirmed at necropsy by the detection of implantation sites or normally developed corpora lutea. After delivery, the number of stillborn and live young, the weight and sex of the live young and the presence of gross anomalies were determined.

Macroscopic Examination:

F₀-animals were killed after weaning of the pregnancy by CO₂-inhalation. Animals of the F₁-generation not used for behavioral and mating experiments were killed by CO₂-inhalation after termination of the reflex tests. All animals were autopsied and checked for macroscopically visible changes. Uteri of all females of the F₀-generation were preserved in formaldehyde solution.

Data Processing and Statistics:

All data except water maze test were recorded on-line and compiled by a data processing system. T-tests and the test statistics of Wilks (Hartung and Elpelt, 1984), and Wilcoxon test were calculated. The number of implants and live pups, the birth index and the percentage of dead pups, supernumerary implantation sites, viability on day 4 post partum, weaning and capacity to survive on day 21 post partum were analyzed on one-side Wilcoxon tests.

Results:1) F₀-Generation:

Mortality: Four females from the 8 IU/kg HMR 1964 group were found dead on days 6, 22, 22 and 43 days of the study (3 deaths after delivery). Nine females from the 8 IU/kg

HR1799 group were found dead on days 6, 7, 18, 22, 24, 24, 26 and 42 of the study (6 deaths after birth). Symptoms of these animals consisted of hypoactivity, bristling coat, and salivation and rolling convulsions, which appeared to be related to hypoglycemic action of insulin.

Clinical signs: There were no remarkable clinical signs of intoxication in other groups except one animal from the 3.15 IU HMR 1964/kg group (#106) that showed prone position on day 22.

Body weight: There were decreases in body weight on days 9 and 13 in HD group, which was not drug-dose dependent, as shown below. During lactation, body weight gains were transiently reduced on day 4.

Day	0 IU/kg	1 IU/kg	3.15 IU/kg	8 IU/kg
0	205	203	209	205
3	222	217	221	217
6	233	230	234	231
9	247	241*	245*	243*
13	261	258	263	256*
16	282	280	283	277
20	333	333	336	324

@ Number of dams was at least 18 rats in all groups. *Indicate P< 0.05.

Food consumption: Food consumption was slightly, but significantly decreased between days 9-13 of pregnancy in the 8 IU/kg HMR 1964 group and between days 13-16 in the groups treated with 8 IU/kg HMR 1964 and HR 1799, respectively (Data not shown).

Toxicokinetics: No data were presented.

Pregnancy and Litter Data:

The following two tables show the numbers of non-pregnant dams and litter data. Implantation sites in excess of the normal number appeared to increase in HMR1964 treated groups, although the increases were not dose dependent as the case of the percentage of implantations. There were no such observations in HR1799.

Group	Numbers of Non-pregnant rats	Implantation Sites only	Stillbirths or All Loss
Control	3(#64, #67, #70)		1(#63 on day 4)
1 IU HMR 1964	3(#84, #86, #90)	#95	2(#78, #83)
3.15 IU/kg 1964	4(#103-5, #115)		3(#113-4, #118)
8 IU/kg 1964	4(#126, 128, 137-8)		
1 IU HR1799/kg	6(#143, 148, 153-4,	#159	

	160-1)		
8 IU/kg HR1799	2(#175, #177)		

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

Litter parameters calculated from dams which littered

@HR 1799 is a regular insulin that was used for comparison purpose.

Autopsy Findings: The stomach and large intestine of one animal from HD HMR 1964 group which died early was filled with gas as shown. Another animal from the group killed at the end of the study showed a cyst on the left ovary. One animal from the 1 IU HR 1799 group did not litter and showed mass deposits in the vagina and the right uterus horn. In HD HR 1799 group, one rat had yellow liquid in the uterus. It is difficult to associate the findings with the treatment due to low incidences and the lack of dose-dependency.

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ARTEMIS II

Intergroup Comparison of Gross Pathology Observations

2000.0942/P - Pre- and Postnatal Study in Rats on HMR 1964

	FEMALES				
	HMR 1964				
	0 IU/kg	1 IU/kg	3.15 IU/kg	8 IU/kg	1 IU/kg
Number of Animals on Study :	23	23	23	23	23
Number of Animals Completed:	(23)	(23)	(23)	(23)	(23)
STOMACH;					
Filled with gas	0	0	0	1	0
OVARY (1E6); LEFT;					
Cyst(s); clear	0	0	0	1	0
VAGINA;					
Filled with mass	0	0	0	0	1
UTERUS;					
Filled with fluid; light yellow	0	0	0	0	0
LARGE INTESTINE;					
Filled with gas	0	0	0	1	0

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F₁-Generation:

Mortality and Clinical Observations: Clinical signs during lactation consisted of insufficiently suckled or non-suckled pups, hematoma, and necrotic or missing tail tip or swollen limb. One male from LD HMR 1964 group was found dead on day after weaning and was replaced by another male from the same group. One male from HD HMR 1964 group showed hypothermia and convulsions on day 12 weaning and was found dead on the same day. Kinked tails were observed in 4 males of HD HMR 1964 group from day 25-29 after weaning and in 2 females from day 1 or 49 onwards after weaning, which was not observed in low dose groups.

Body Weights and Food Consumption: There were slight increased weight gains in males in HD HMR 1964 group on day 21 and decreased weights in HD females on days 42, 45 and 59. Body weights of the pups were comparable at birth and during lactation. Increased food consumption in HD males between days 42-45 and decreased food consumption in HD HR 1799 females between days 31-35. These changes were slight and not dose-dependent since there were no clear trends.

Reproductive Performance:

Table below shows the pregnancy data during 3 weeks of housing of males and females together in different groups. Regular insulin (HR1799) had no effect on pregnancy, but HMR 1964 increased slightly non-pregnancy rate in HD group.

Pregnancy Data in 21 Day after Drug Treatment in F ₁ Generation				
Drug	Dose(IU/kg)	N*	Pregnant	No Pregnancy
HMR1964	0	23	19	4
	1	23	21	2
	3.15	23	20	3
	8	23	17	6
HR 1799	1	23	20	3
	8	23	21	2

* Indicate number of female rats in each group.

Body weight and food consumption were comparable in all groups, although there were minor exception. The body weight gains were reduced in HMR HD group on days 9 and 13 of pregnancy and in HR1799 HD group on day 13 of pregnancy. Mean duration of pregnancy was between 23 and 23.3 days as shown below. Two females of HMR 1964 IU group (#224) and 3.15 IU group (#267) did not litter and showed only empty implantation sites at necropsy. Either HMR 1964 or HR 1799 had no significant effect on numbers of live young and stillborn of F₁-generation as shown below.

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

(Summary tables and Statistics page 133-136, Individual data page 520-549)

Terminal evaluations:

One male animal of HD HR 1799 group showed aplasia of the left testis and left epididymis as shown below. The findings appear to be not related to the treatment due to low incidence and lack of drug-dose dependency.

Intergroup Comparison of Gross Pathology Observations							
Sex	Pathological Findings in Rats during Pre- and Postnatal Study	HMR 1964 Dose (IU/kg)				HR 1799 Dose (IU/kg)	
		0	1	3.15	8	1	8
Male	Epididymis Aplasia	0	0	0	1	0	0
	Testes(left) Aplasia	0	0	0	1	0	0
Female	Abnormal Organs or	0	0	0	0	0	0

Visible Lesions	0	0	0	0	0	0
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Reproductive and developmental toxicology summary and conclusions:

Administration of both HMR1964 and HR 1799 during embryo- and fetogenesis and during lactation produced clinical signs and mortality in the F₀-generation at the daily dose of 8 IU/kg body weight in Sprague-Dawley rats. The deaths in the HD group appeared to be related to treatment-induced hypoglycemia. There were no clear treatment-induced abnormalities in birth parameters or lactation of the evidence on F₀-generation and on postnatal development, fertility or pregnancy of the F₁-generation. No documentable adverse effects were observed after administration of HMR1964 at a dose of 1 IU/kg or less. NOAEL = 1 IU HMR 1964 and 1.0 IU HM 1799 per kg body weight.

Labeling recommendations: None

RECOMMENDATIONS (External to Sponsor):

The sponsor provided pregnancy data after 3 weeks of housing of males and females in different groups as shown below. Regular insulin (HR1799) had no effect on pregnancy, but HMR 1964 increased slightly non-pregnancy rate in HD group. Why do the numbers of non-pregnant rats differ from those in individual data in Vol. 2, pages 496-501?

Pregnancy Data in 21 Day after Drug Treatment in F ₁ Generation				
Drug	Dose(IU/kg)	N*	Pregnant	No Pregnancy
HMR1964	0	23	19	4
	1	23	21	2
	3.15	23	20	3
	8	23	17	6
HR 1799	1	23	20	3
	8	23	21	2

* Indicate number of female rats in each group.

Herman Rhee, Ph.D.
Review Pharmacologist

Jeri El Hage, Ph.D.
Team Leader

cc: IND61,956
HFD-510/El Hage/H.Rhee/Rheej
Appendix III. Executive CAC minute

Executive CAC
March 20, 2001

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Frank Sistare, Ph.D., HFD-910, Alternate Member
Jim Farrelly, Ph.D., HFD-530, Alternate Member
Jeri ElHage, Team Leader and Presenting Reviewer

Author of Draft: Jeri ElHage

The following information reflects a brief summary of the Committee discussion and its recommendations. Only brief narrative summaries of the one and six-month rat studies were provided, therefore, full data were not available for review.

IND # 61,956

Drug : HMR 1964

Sponsor: Aventis Pharmaceuticals

Contact: Faraneh Attarchi, Ph.D. Phone: 816-767-6483; Fax : 816-767-7385

Background: Insulin and its short-acting analogs have been demonstrated to produce mammary tumors in rats. Some insulin analogs are more potent mammary tumor inducers than native insulin. The relative potency of the insulin analogs to produce tumors can not be predicted by receptor binding activity or short-term in vitro assays in mammary tumor cell lines. These observations have lead to a divisional requirement for a one-year chronic toxicity study in rats for the insulin analogs with the assessment of tumor endpoints.

Rat Chronic Toxicity Protocol: Aventis proposed use of subcutaneous doses of 2.5, 5 and 10 IU HMR 1964/kg/day in Sprague Dawley rats (30/sex/dose). The selection of the mid and high doses was based upon mortality presumed to result from drug-induced hypoglycemia. The 6-month rat study demonstrated the absence of mortality with 5 IU/kg/day, infrequent deaths(4/40) at 20 IU/kg/day, and deaths in 13/40 rats dosed with 80 IU/kg/day. In addition to the mortality endpoint, Aventis suggested that the doses were adequate since Cmax with the proposed doses were approximately 7.5, 15 and 30 times the Cmax with the human dose.

Executive CAC Recommendations and Conclusions:

1. The Division and ECAC do not concur with the dose selections proposed by Aventis for the following reasons:

a. The study to be conducted is a one-year chronic toxicity study and dose selections should be made based upon the dose selection criteria for toxicity studies, namely, high doses should produce frank toxicity. In addition, the doses proposed are 10 to 20-fold lower than those studied with other insulin analogs, and would not permit a valid comparison between products.

b. Use of Cmax criteria for dose selection is not appropriate since the rat study was conducted with once per day dosing and humans are dosed three times per day. For this product, AUC data provide more accurate systemic exposure comparisons across species. Systemic exposures (AUC) with the doses proposed for use in the rat study all produce daily exposures less than therapeutic.

2. The Division recommends that Aventis conduct the study evaluating doses of 5, 20, and 50 IU/kg, twice per day. The insulin comparator arm should evaluate a comparable high dose of 50 IU/kg, bid. Twice per day dosing is recommended to permit evaluation of maximal doses, while attempting to minimize deaths secondary to hypoglycemia.
3. In addition, the sponsor should avoid fasting of the animals. Use of satellite animals or a temporary one day suspension of dosing is suggested if experimental animals must be fasted for blood sampling. Many of the hypoglycemia-related deaths observed with HMR 1964 and other insulin products appear to be associated with planned or inadvertent fasting.
4. The Executive Carcinogenicity Assessment Committee (ECAC) commented that this one-year study is not a carcinogenicity study (inadequate duration, sample size, etc) and, therefore, does not fall under the Special Protocols Guidance or require eCAC review. However, the ECAC did review and concur with the Division's revised dose selections, as described in item 2 above.

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

Cleared for Faxing:

Jeri ElHage, Ph.D.
Pharmacology Supervisor
Metabolic and Endocrine Drug Products

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/s/

Herman Rhee
2/24/04 01:19:08 PM
PHARMACOLOGIST

Jeri El Hage
2/25/04 11:55:49 AM
PHARMACOLOGIST