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Sponsor table 7.16, page 67

HIRUDIN: A SUBCUTANEOUS FERTILITY AND REPRODUCTIVE TOXICITY (SEGMENT I) STUDY IN RATS (MIN 931090)

Table 7.16: Summary of the reproductive parameters derived from full-term parental females (Mean ± Standard Deviation)

	Dose Level (mg/kg/day)			
Parameter	Control (0)	1	5	10
No. Pregnant Females	10	6	11	7
No. Implants	16.00±2.00	13.83±4.45	14.64±4.34	14.71±3.09
No. Viable newborn	14.40±2.63	13.17±4.67	13.36±4.01	13.57±3.10
No. Stillbirths	0.40±0.70	0.17±0.41	0.18±0.40	0.29±0.49
% Stillbirths	2.72±4.87	1.11±2.72	1.40±3.11	1.99±3.43
Postimplantation Loss	1.60±1.26	0.67±0.52	1.27±1.27	1.14±1.21
% Postimplantation Loss	10.32±8.05	6.04±6.11	7.95±7.97	7.81±7.89

Dams allowed to deliver: No significant differences in the gestation period between the groups were noted. The number of implantation sites, post-implantation loss, litter size, sex ratios was not affected by the treatment. Pups survival was significantly affected during days 0-4 (precull) of lactation in high dose group (control 96.25%, low dose = 100%, mid dose = 100% and high dose = 94%). However, post-culling survival of the pups and pups weights throughout lactation period were not affected by the treatment. Postnatal development and differentiation were comparable in all groups. There was no significant effect on fertility test and mating performance test of F_1 generation rats.

In conclusion, there were no adverse effects on the fertility and mating performance parameters of treated male and female rats at s.c. doses up to and including 10 mg/kg/day of CGP 39393.

2. **Segment II. Teratology in Rats** (Study # 896087)

Testing Laboratories: CIBA-GEIGY Ltd.,
Stein, Switzerland
Study Started: February 16, 1990

Study Completed: October 8, 1990

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Test Species: Albino rats (Tif: RAI f [spf], hybrids of RII/1 x RII/2; 2 month old females and of body weights rang: 190-210 g)

No. of Animals: 24 Pregnant rats/group

Drug Batch No.: 14/787/3

Methods: Pregnant rats were given subcutaneous Injections of CGP 39393 at 1, 5 and 10 mg/kg/day from day 6 through 15 of gestation. Control group animals received the vehicle (5% sterile aqueous mannitol) In similar manner. The volume of administration was fixed at 5 ml/kg. The selection of the doses were based on the preliminary toxicity study in which 3 Out of 8 pregnant rats (test # 896086) at the high dose level (15 mg/kg) died and had hemorrhages at the injection sites. Therefore in the main study, the highest dose selected to be 10 mg/kg/day. In the regular 3-month s.c. toxicity study the highest dose tested was also 10 mg/kg/day, and only toxicity observed was dysfunction of hemostasis mechanism. It should be noted that in the proposed clinical study the mode of administration of the drug is via i.v. bolus plus i.v. infusion. In the meeting on November 1, 1990, sponsor was asked to provide evidence of equivalence of two routes of administration in rats (s.c. versus i.v. bolus plus infusion) in the form of comparative kinetics. They have not done so. Pregnant dams were observed daily for mortality.and clinical signs. Body weights were recorded daily and food consumptions were recorded on days 6, 11, 16 and 21 of gestation. All dams were sacrificed on day 21 of gestation, and were examined for the number of corpora lutea, the number of implants, the number of dead or resorbed fetuses and number of live fetuses. The live fetuses were weighed and sexed. All fetuses were examined for gross anomalies. Approximately one half of the fetuses per litter eviscerated and examined for skeletal major/minor abnormalities, the remaining fetuses were examined for visceral abnormalities and variations.

Results: One dam (# 91) from high dose group was sacrificed on day 12 of gestation due to moribund condition. Local irritation and swelling at the injection sites were seen in treated dams. No significant effect on body weight gains and food consumptions were seen during the course of the study, except during 11-16 day of gestation high dose group dams consumed about 12% less than the control group dams. At necropsy dose related hemorrages were seen at injection sites and all animals in high dose group had hemorrhage at the injection sites. The number of corpora lutea, the number of implants, pre and postimplantation losses, number of live fetuses, sex ratio and mean body weight of live fetuses did not show any significant difference between the treated groups and the control group. External examination revealed cleft lip cleft palate in one fetus (# 59/02) of mid dose group and omphalocele/incomplete closer of abdominal wall were seen in one fetus (# 40/13) of low dose group, one fetus (# 67/04) of mid dose group and in two fetuses (# 73/13 and 95/05) from two litters of high dose group. The incidence rates of omphalocele/incomplete closer of abdominal wall in this study were 0%, 0.57% (4.16% of

litters), 0.60% (4.35% of litters) and 1.32% (9.5% of litters) in control, low dose, mid dose and high dose respectively. The historical incidence rate provided by the sponsor is 0.096% (5/5187 fetuses, 4/358 litters). Increased incidences of absent ossification were seen in metatarsal-1 [control = 10/154 (6.5%), low dose = 20/186 (10.8%), mid dose = 29/179 (16.2%) and high dose = 45/159 (28.3%)], proximal phalanx of posterior digit 2 [control - 48/154 (31%), low dose 72/186 (39%), mid dose 77/179 (43%) and high dose - 78/159 (49%)], digit 3 [control = 35/154 (23%), low dose = 43/186 (23%), mid dose = 54/179 (30%) and high dose = 69/159 (43%)], digit 4 [control = 34/154 (22%), low dose = 47/186 (25%), mid dose - 56/179 (31%) and high dose 72/159 (45%)], and digit 5 [control = 75/154 (49%), low dose = 98/186 (53%), mid dose = 111/179 (62%) and high dose = 114/159 (72%)]. Thus indicating delayed skeletal maturation at mid and high dose levels. Asymmetrically shaped sternebrae 1, 3, 4, 5 and 6; fused sternebrae 1 and 2; bipartite ossification of sternebrae 4 and 5; and poor absent ossification of metacarpal 5 and bipartite thoracic vertebral centers were seen in all groups, and these effects were not dose related. Thus in the present study the rate of incidence of omphalocele in treated rabbits increased up to 6.7 fold over the historical incidence rate, and this abnormality was dose related and in two litters of the high dose group. The data indicates that CGP 39393 is teratogenic in rats.

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Effect of CGP 39393 on Maternal and Fetal Parameters in Rats

Parameters Measured	Control	Low Dose	Mid Dose	High Dose
Total Mated	24	24	24	24
Died sacrificed	0	0	0	1
Pregnant	21	24	23	21
% Pregnant	87.5	100	95.8	87.5
# Surviving	100	100	100	100
# of Dam examined	21	24	23	21
# of Corpora lutea/dam	17.6	18.2	18.6	18.1
# of Implants/Dam	15.0	15.5	16.0	15.2
Preimplantation loss (%/dam)	14.5	14.7	13.5	15.8
Postimplantation loss (%/dam)	4.6	3.3	6.2	2.9
# Embryonic deaths/dam	0.7	0.5	1.0	0.4
# of Live Fetuses/dam	14.3	15.0	15.0	14.8
# of Live fetuses	300	360	346	310
Sex ratio (m/f)	1.09	1.04	1.04	0.96
Mean Fetal Weight (g)	5.3	5.3	5.2	5.2
Morphological Findings of Fetuses				
Gross Anomalies:				
Major Malformations:				
# of Fetuses Examined	300	360	346	310
# of Litters Examined	21	24	23	21
Generalized Edema	1	0	0	0

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% Fetuses affected	0.33	0	0	0
% Litters affected	4.76	0	0	0
Brachygnathia	1	0	0	0
% Fetuses affected	0.33	0	0	0
% Litters affected	4.76	0	0	0
Cleft lip/palate	0	0	1	0
% Fetuses affected	0	0	0.29	0
% Litters affected	0	0	4.35	0
Visceral:				
# of Fetuses Examined	146	174	167	151
# of Litters Examined	21	24	23	21
Major Anomalies:				
Omphalocele/ Incomplete Closer of Abdominal Wall	0	1	1	2
% Fetuses affected	0	0.57	0.60	1.32
% Litters affected	0	4.16	4.35	9.52

ADDENDUM TO THE STUDY:

In the Segment II Teratology study in rats, CGP 39393 treatment produced visceral malformations in developing fetus. In addition, the increased incidences of major skeletal fetal abnormalities of fused and asymmetric sternebrae, were also seen as shown in the table below:

Effect of CGP 39393 on Fetal Sternebrae Development Parameters

Parameters Measured	Control	Low Dose	Mid Dose	High Dose
Total Litters Studied	21	24	23	21
Poor Ossification (F/L):				
Sternebra 1	1/1	1/1	0	0
Sternebra 2	0	0	0	1/1
Sternebra 5	0	0	1/1	1/1
Sternebra 6	1/1	0	0	0
Fused (F/L):				
Sternebra 1	0	1/1	2/2	1/1
Asymmetric (F/L):				
Total	3/3	8/8	3/3	9/6
Sternebra 1	0	2/2	1/1	1/1
Sternebra 3	0	0	0	2/1
Sterner 4	1/1	0	0	3/2
Sterner 5	2/2	2/2	2/2	3/2
Sterner 6	0	4/4	0/0	0/8
Bipartite Stern brae (F/L):	0	0	1/1	0

In summary, CGP 39393 treatment in pregnant rats produced major fetal malformations of omphalocele, fused and asymmetric skeletal malformation of sternebrae. CGP 39393 is teratogenic in the study rats.

3. A Repeat Segment II. Teratology Study in Rats:
(Report # 92099/921030)

Testing Laboratories: Pharmaceuticals Division
CIBA-GEIGY Corp.,
Sumit, NJ.

Study Started: Not given

Study Completed: Not given

Report Date: December 22, 1992

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Test Species: Sprague Dawley (Crl:COBS (SD) BR)

No. of Animals: 26 Pregnant rats/group

Drug Batch No.: 810191

Methods: Previously sponsor submitted the result of s.c. Segment II teratology study in albino rats (Tif: RAIf [spf], hybrids of RII/1 x RII/2) in which s.c. doses of 1, 5 and 10 mg/kg/day were used. CGP 39393 produced cleft lip/cleft palate in one fetus of mid dose group and, omphalocele in one fetus of one litter of mid dose group and two fetuses from two litters of high dose group. The data also indicates delayed skeletal maturation at mid and high dose levels. Thus it was concluded that CGP 39393 is teratogenic in rats (see previous study at #2). To confirm this finding sponsor had repeated the present s.c. Segment II teratology study in Sprague Dawley rats. In repeat study, pregnant Sprague Dawley rats were given subcutaneous injections of CGP 39393 at 5, 10 and 15 mg/kg/day from day 6 through 15 of gestation. Control group animals received the vehicle (5% sterile aqueous mannitol) in similar manner. The volume of administration was fixed at 5 ml/kg. Pregnant dams were observed daily for mortality and clinical signs. Body weights and food consumption were recorded on days 0, 6, 8, 12, 16, and 20 of gestation. All dams were sacrificed on day 20 of gestation and examined for the number of corpora lutea, the number of implants, the number of dead or resorbed fetuses and number of live fetuses. The live fetuses were weighed and sexed. All fetuses were examined for gross anomalies. Approximately one third of the fetuses per litter eviscerated and examined for visceral

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abnormalities and variations, the remaining fetuses were examined for skeletal major/minor abnormalities.

Results: Two dams (# 289 and 290) from high dose group died during study period and deaths were determined to be treatment related. Dose related hemorrhages and hematomas at the injection sites were seen in all treated dams. No significant effect on body weight gains and food consumption were seen during the course of the study. The number of corpora lutea, the number of implants, pre and postimplantation losses, number of live fetuses, sex ratio and mean body weight of live fetuses did not show any significant difference between the treated groups and the control group. In 15 mg/kg/day treatment group, 3 fetuses of 3 litters (1fetus/litter) had a single gross malformation of either edema, shortened hind limbs or umbilical hernia. No treatment related gross malformation was evident in any other group. Treatment related skeletal malformations were seen in 2/217 fetuses of high dose group, one fetus (from dam # 285) had shortened femur, fibula and tibia and another fetus had fused centrum. Increased incidences of absent ossification were seen in squamosals [control = 0/231 (0%), low dose = 1/237 (0.42%), mid dose = 0/228 (0%) and high dose = 3/217 (1.38%; 3/22 litters)], os pubis [control = 0/231 (0%), low dose = 0/237 (0%), mid dose = 0/228 (0%) and high dose = 3/217 (1.38%; 3/22 litters)], distal phalanges (forepaw) [control = 0/231 (0%), low dose = 0/237 (0%), mid dose = 0/228 (0%) and high dose = 3/217 (1.38%)], and metatarsus [control = 0/231 (0%), low dose = 0/237 (0%), mid dose = 0/228 (0%) and high dose = 4/217 (1.84%; 4/22 litters)]. Thus indicating delayed skeletal maturation at high dose level. In this repeat Segment II teratology study, the data indicate that CGP 39393 is teratogenic in rats and confirmed the previous findings.

4. **Segment II - Teratology in Rabbits:**
(Study #89-6074/Report #CBG513/901397)

Testing Laboratories: [redacted]

Study Started: April 3, 1990

Study Completed: February 14, 1991

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animal Species: Male (age and weight was not given) and female (16-24 weeks old, 3.4-4.0 kg) New Zealand White rabbits

No. of Animals: 16 Pregnant rabbits /group

Drug Batch No.: 14/019/1

Methods: Pregnant rabbits were given intravenous injections of CGP 39393 at 0-6, 2.0 and 6.0 mg/kg/day from day 6 through 18 of gestation. Control group animals received the vehicle (5% sterile aqueous mannitol) in similar manner. The volume of administration was fixed at 1 ml/kg. The selection of the doses were based on the preliminary toxicity study in which pregnant rabbits (n=6) were given 0.6, 3.0 and 15.0 mg/kg/day from day 6 through 18 of gestation. In this preliminary study there was a dose related increase in whole blood clotting time and this effect was most pronounced at 15 mg/kg/day dose level (clotting times in 3 out of 6 animals were greater than 90 min.) and no other toxicities were evident. Due to the potential difficulty of controlling bleeding at the injection site with 15 mg/kg/day, sponsor selected 6 mg/kg/day as the highest dose to be tested in the main study. Pregnant rabbits were observed daily during pregnancy, and their weights were recorded on 1, 6, 8, 10, 14, 19, 23 and 29 day of gestation. All surviving dams were sacrificed at day 29 of gestation, and were examined for the number of corpora lutea, the number of implants, number of early late resorptions, number of live/dead fetuses, identification of any malformed fetuses or uterine abnormalities. Live fetuses were weighted and sexed. All fetuses were eviscerated and examined for skeletal/visceral anomalies.

Results: Bruising (localized purple discoloration) at the injection sites were noted in the treated animals. There were no mortalities during study period. No significant effect on food consumptions and body weight gains were evident. There was one abortion at mid dose. The number of corpora lutea, the number of implants, number of live fetuses, sex ratio and mean body weight of live fetuses did not show any significant difference between the treated groups and the control group. Increased incidences of spina bifida were seen in the fetuses [control = 0/103 (0%), low dose = 1/107 (0.93%), mid dose = 11/143 (0.69%) and high dose = 6/135 (4.44%)]. At high dose spina bifida were seen in fetuses of 5 out of 15 litters. At high dose, all fetuses with spina bifida had flattened cranium and/or protruding occipital, 2 out of 6 had malrotated bind limb and 4 out of 6 showed reduced ossification of cranial bones. Sponsor submitted historical control data for the incidence of spina bifida in rabbit (strain not mentioned, laboratories(s) which conducted the study was not mentioned). In 1989 fourteen studies were conducted and 3 out of 1674 fetuses (206 litters) had spina bifida and in 1990 six studies were conducted and 1 out of 860 fetuses (100 litters) had spina bifida. Thus the rates of Incidence of spina bifida were 0.179% and 0.116% in 1989 and 1990 respectively (mean Incidence rate = 0.157%). In the

present study the rate of incidence of spina bifida in treated rabbits increased up to 28 fold over the historical incidence rate. There were some evidence of delayed ossification among the fetuses, however, sponsor did not provide the data in tabulated form. The data indicate that CGP 39393 is teratogenic in rabbits.

Effect of CGP 39393 on Maternal and Fetal Parameters in Rabbits

Parameters Measured	Control	Low Dose	Mid Dose	High Dose
Total Mated	16	16	16	16
Pregnant	13	15	15	15
% Pregnant	81.2	93.7	93.7	93.7
% Surviving	100	93.3	100	100
# of Dam examined	13	14	15	15
# of Corpora lutea/dam	11.8	11.7	12.3	11.7
# of Implants/Dam	9.4	8.8	11.5	11.0
# Embryonic deaths/dam	1.5	1.1	1.9	2.0
# of Live Fetuses/dam	7.9	7.6	9.5	9.0
# of live fetuses	103	107	143	135
Sex ratio (m/f)	1.18	1.21	0.81	1.02
Mean Fetal Weight (g)	44.2	45.9	42.3	41.9
Gross Anomalies:				
# of Fetuses Examined (excluding malformed fetuses)	101	103	138	122
# of Litters Examined	13	14	15	15
Irridial/retinal hemorrhage	0	1	0	2
% Fetuses affected	0	0.97	0	1.64
% Litters affected	0	7.14	0	13.33
Skeletal:				
Major. Anomalies:				
# Fetuses Examined	103	107	143	135
# Litters Examined	13	14	15	15
Sacral Spina bifida	0	1	1	6
% Fetuses affected	0	0.93	0.70	4.44
% Litters affected	0	7.14	6.66	33.33
Flat cranium/protruding Occipital	0	1	0	6
% Fetuses affected	0	0.93	0	0.44
% Litters affected	0	7.14	0	33.33
Malrotated hind limb	0	2	0	2
% Fetuses affected	0	1.87	0	1.48
% Litters affected	0	14.28	0	13.33
Minor Anomalies:				
# of Fetuses Examined (excluding malformed fetuses)	101	103	138	122
# of Litters Examined	13	14	15	15
Irregular cervical Ossification	3	2	1	6
% Fetuses affected	2.97	1.94	0.72	4.92
% Litters affected	15.38	14.28	0	20.00
Visceral:				
Major Anomalies:				
# of Fetuses Examined	103	107	143	135
# of Litters Examined	13	14	15	15
Single enlarg. Ventri.	0	0	0	1
% Fetuses affected	0	0	0	0.74
% Litters affected	0	0	0	6.66
Malformed systemic/ pulmonary arteries	1	0	0	2

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% Fetuses affected	0.97	0	1.40	1.48
% Litters affected	7.69	0	13.33	13.33
Malformed minor cervi./ Thoracic arteries	1	0	0	3
% Fetuses affected	0.97	0	0	2.2
% Litters affected	7.69	0	0	13.33
Minor Anomalies:				
# of Fetuses Examined (excluding malformed fetuses)	101	103	138	122
# of Litters Examined	13	14	15	15
Gall bladder reduced/ Bilobed/bifurcated	0	0	1	1
% Fetuses affected	0	0	0.72	0.82
Litters affected	0	0	6.66	6.66

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ADDENDUM TO THE STUDY:
The major malformation of hydrocephaly was observed in 0, 1, 0 and 1 fetuses of animals belonging to control, 0.6, 2.0 and 6.0 mg/kg/day treatment groups. Thus CGP 39393 treatment to pregnant animals produced major abnormalities spina bifida, flattened cranium and/or protruding occipital, malrotated hind limb and hydrocephaly.

5. Modified I.V. Segment II. Teratology Study in Rabbits. (Report # 94028)

Testing Laboratories: Pharmaceuticals Division
Ciba-Geigy Corp.
Summit, NJ

Study Started: August 18, 1993

Study Completed: June 27, 1994 (report date)

GLP Requirements: A statement of Compliance with the GLP regulations and quality assurance unit was included.

Tests Species: New Zealand White rabbits (27-27 weeks old; females body weight: 2.98-4.66 kg on day 0 of gestation).

Number of Animals: 40 pregnant rabbits/group

Drug Batch Number: Lot No. 810192

Methods: Earlier sponsor submitted the results of i.v. Segment II, teratology study in rabbits in which doses of 0.6, 2.0 and 6.0 mg/kg/day were used. Increased incidences of spina bifida were seen in fetuses [control = 1/103 (0%), low dose = 1/107 (0.93%), mid dose = 1/143 (0.59%) and high dose = 6/135 (4.44%)]. At high dose spina bifida were seen in fetuses of 5 out of 15 litters. At high dose, all fetuses with spina bifida had flattened cranium and/or protruding occipital, 2 out of 6 had malrotated hind limbs and 4 out of 6 showed reduced ossification of cranial bones. The

data indicated clearly that CGP 39393 is teratogenic in rabbits (for detail see my review dated 8/30/91). In the present study, sponsor repeated Segment II. teratology study in rabbits with some modifications. The design of standard Segment II. teratology study was modified to assess the time of gestation during which drug produced teratogenic effects. Only one i.v. dose level i.e. 6.0 mg/kg/day (1 ml/kg) was tested and there were 4 groups (n=40/group). Group 1 (control group) animals received vehicle (5% mannitol: 1 ml/kg) during day 7-19 of gestation, group 2 was treated during days 7-13 (early part of organogenesis) of gestation, group 3 was treated during days 14-19 (latter part of organogenesis) of gestation and group 4, was treated during-day 7-19 (overall organogenesis) of gestation. Pregnant rabbits were observed daily during pregnancy and their weights were recorded on days 0, 7, 10, 14, 20, 24 and 29 of gestation. Food intakes were recorded daily during 5-29 days of gestation. All surviving dams were sacrificed at day 29 of gestation and were examined for the number of corpora lutea, number of implants, number of early/late resorptions and number of live/dead fetuses. Live fetuses were weighed and sexed. All fetuses were eviscerated and examined for skeletal/visceral anomalies.

Results: There were no mortalities during the study period and no treatment related clinical signs were evident. There was one abortion in group 3 (does treated during days 14-19 of gestation), which was considered not to be treatment related. No significant effect on food consumptions and body weight gains were evident. The number of corpora lutea, the number of implants, number of early/late resorptions, percent of postimplantation loss, and number of live fetuses and sex ratio did not show any significant difference between the treated groups and control group, except in group 2 (does treated during day 7-13 of gestation), hirudin produced significant increases in the number of early resorptions (control = 0.24 $\pm$ 0.43, group 2 = 1.0 $\pm$ 1.83), post-implantation loss (control = 0.5 $\pm$ 0.86, group 2 = 1.11 $\pm$ 1.79) and percent of post-implantation loss (control = 5.22 $\pm$ 8.79, group 2 = 11.77 $\pm$ 18.66). These findings suggest embryotoxicity in group 2. Additionally, fetal weights were reduced by 5-6% compared to control values in group 3 (does treated during days 14-19 of gestation) and group 4 (does treated during days 7-19 of gestation). No significant effect on fetal weights was seen in group 2 (does treated during day 7-13 of gestation). Hence, the drug is fetotoxic when given during the latter part of gestation. A gross malformation i.e., gastroschisis was seen in 2/289 (0.69%) fetuses (2/35 litters 5.71%) in group 2. The incidences of gastroschisis were higher than the historical control values (0.02% by fetuses and 0.18% by litter). Additionally, malformation such as "eyes open" and hyperflexured forepaw were seen in 2 fetuses of two litters (does # 140 and 152) in group 4, no treatment related visceral malformations/variations were seen in any group.

Treatment related skeletal major malformations such as agenesis of centrum/vertebrae (control = 0/278, group 2 = 2/289, group 3 = 1/323 and group 4 = 2/289) and agenesis of ribs in group 2 (1/289) were seen. There were also some evidence of delayed ossification among the fetuses in treated groups. The data indicated that the drug is embryo toxic (increased early resorption & post-implantation loss), fetotoxic (decreased fetal weights) and teratogenic in rabbits.

Effects of CGP 39393 on Maternal and Fetal Parameters in Rabbits

	Dose Level (mg/kg/day) (dosing period/gestational days)			
	Control	Group 2	Group 3	Group 4
	(0)	(6)	(6)	(6)
Parameters Measured	(7-19)	(7-13)	(14-19)	(7-19)
No. of Females Inseminated	40	40	40	40
No. of Pregnant	34	35	37	34
% Pregnant	85.0	87.5	92.5	85.0
% Surviving	100	100	97.3	100
No. of Corpora Lutea/doe	12.79 ± 3.1	12.71 ± 2.1	12.64 ± 2.4	12.68 ± 2.5
No. of Implants/doe	8.68 ± 3.1	9.37 ± 2.3	9.61 ± 2.9	9.24 ± 2.6
No. of Early Resorptions/doe	0.24 ± 0.43	1.00 ± 1.83	0.33 ± 0.59	0.59 ± 0.92
No. of Late Resorptions/doe	0.26 ± 0.75	0.11 ± 0.32	0.31 ± 0.89	0.15 ± 0.50
No. of Live Fetuses/doe	8.18 ± 2.92	8.26 ± 2.73	8.97 ± 2.73	8.50 ± 2.79
% Post-implantation Loss	5.22 ± 8.79	11.77 ± 18.66	6.08 ± 10.07	8.33 ± 12.91
Sex Ratio (% males)	52.5	48.8	51.4	48.8
Mean Fetal Weight (g)				
Males	48.5 ± 1.0	47.6 ± 1.0	45.7 ± 1.0	45.7 ± 1.0
Females	46.6 ± 0.9	46.6 ± 0.9	44.5 ± 0.8	43.9 ± 0.9

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Morphological Findings of Fetuses				
	Dose Level (mg/kg/day) (dosing period/gestational days)			
	Control	Group 2	Group 3	Group 4
	(0)	(6)	(6)	(6)
Parameters Measured	(7-19)	(7-13)	(14-19)	(7-19)
No. of Fetuses Examined	278	289	323	289
No. of Litters Examined	34	35	36	34
Gross:				
Major Malformations:				
Eye Open	0	0	0	1
Filamentous Tail	0	1	0	0
Hyperflexure Forepaw	0	0	0	1
Short Tail	0	1	0	0
Gastroschisis	0	2 [2]	0	0
Minor Variations:				
Cyst in the Lumber Region	0	1	0	0
Visceral:				
Major Malformations:				
Ectopic Kidney	0	1	0	0
Minor Anomalies:				
Discolored Kidney	0	1	0	0
Skeletal:				
Major Malformations:				
Agenesis of Centrum/Vertebrae	0	2 [2]	1	2 [2]
Agenesis of Ribs	0	1	0	0
Minor Anomalies:				
Skull				
Hyoid - Not Completely Ossified	16 [10]	27 [13]	16 [10]	35 [14]
Sternebrae				
Not Ossified	19 [8]	18 [9]	49 [18]	40 [18]
Hindleg/Hindpaw				
Patellae - Not Ossified	7 [3]	11 [9]	28 [12]	36 [15]

( ) = Number in [ ] represents # of litters

6. Segment III. Perinatal and Postnatal Reproductive S.C. Toxicity Study in Rats: (Report No.93053)

Testing Laboratories: CIBA-GEIGY Corporation; Summit, NJ

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ON ORIGINAL

Study Started: December 30, 1992

Study Completed: December 6, 1993

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Pregnant Sprague Dawley rats; 8 weeks old weighing 224 to 296 g.

Drug Batch #: Lot No. 810192

Methods: Three groups of pregnant rats (21 rats/group) were subcutaneously injected hirudin (dissolved in a 5% mannitol solution, 1 ml total volume) at doses of 1, 5, or 10 mg/kg from gestation day 15 through lactation day 20. An additional group of 21 rats received equivalent volumes (1 ml) of the 5% mannitol vehicle and served as controls for the study. Observations of maternal mortality and clinical signs of toxicity were performed daily throughout the pre-dosing and dosing periods, with gross examinations of the injection site conducted during the dosing period and at necropsy. Maternal body weights were determined on gestational days 0, 6, 13, and 15, on day 20 during dosing and on lactation days 0, 7, 14, and 21. Following parturition, pups were counted, sexed and weighed and surviving pups were culled into standard litter sizes (N=8, 4 males and 4 females). Pups were also observed for the appearance of developmental markers (i.e. righting reflex [days 0-2], pinna detachment [days 3-11] ear canal opening [days 3-16] and eye opening [postpartum days 13-16]). Females sacrificed on postpartum day 21 and those which died prior to the scheduled sacrifice were examined grossly and the number of implantation sites were recorded for each of the animals. In addition, all pups including those culled on day 4 and those that died were grossly examination following death. No pathological examinations were performed on tissues in which gross observations were noted.

Results: Subcutaneous administration of hirudin (Rec-hirudin) produced a pooled blood at the injection sites at all doses tested (15 of 21 rats at the 1.0 mg/kg dose and in all rats tested (21 of 21) in each of the 5 and 10 mg/kg dose groups. Hirudin treatment also produced soft masses at the injection site in 4 of 21 and 7 of 21 rats belonging to 5 and 10 mg/kg/day treatment groups, respectively. Two of 21 rats of 10 mg/kg/day treatment group also exhibited treatment-related signs of blood and/or scabs at the injection sites, with one of the aforementioned animals also appearing generally pale. Three high dose females (Dam Nos. 4205, 4220, and 4219) were found dead during the dosing period on gestation days 19, 20, and 21, respectively. Death was attributable to excessive hemorrhages, as animals which died showed

one or more of the following: pooled blood at the injection site, blood filled uterine horns, dark lungs, pale organs and pale fetuses. No treatment-related effects on maternal food consumption were observed at any dose tested. Significant reductions in maternal body weights (-4.7%, day 14 only) and body weight gains (-66% days 7-14) were observed during the lactation period in the 5.0 mg/kg dose groups. However such effects were not observed at the 10 mg/kg dose. Therefore the biological significance of these body weight changes is unknown.

Hirudin had no effects on reproduction parameters (mean gestation durations, mean number of implantation sites, no. of live pups, no. or % of stillbirths, number or % of post-implantation loss) in the F_0 females. In addition, there were no treatment-related effects on pup sex ratio or pup survival. Pups of both sexes in the 5 mg/kg/day group exhibited slight reductions (-7 to -10%) in body weights compared to control animals, post culling, on days 7, 14, and 21. However, these differences may have resulted from normal biological variation, since the weights were reported to be in the normal range for the testing facility and no such differences were observed in the high dose groups. Clinical signs including: a cannibalized right arm in one 5 mg/kg/day pup and 2 litter mates at the 10 mg/kg/day treatment group which were darker in color/without milk, were considered incidental. Finally, Hirudin treatment had no effects on any postpartura developmental parameters.

In conclusion a subcutaneous dose of up to 10 mg/kg/day CGP 39393 in pregnant rats produced no effects on labor, delivery, food and body weight parameters or examined reproductive parameters of the F_0 generation or had no effect on lactation, postpartum survival and development (as measured by the appearance of various developmental parameters) of the F_1 generation.

MUTAGENICITY:

1. Salmonella/Mammalian-microsome Mutagenicity Test (Study # 906187)

Dates Studies Started and Completed: May 15, 1990 and May 30 1990

Strain Employed: Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537

Concentration Employed: 20-5000 mcg/0.1 ml in toxicity test, and 313-5000 mcg/0.1 ml in mutagenicity tests.

Solvent: [redacted]

Positive Control: Daunorubcin-HCI (10 mcg/0.1 ml), 4-nitroquinoline-n-oxide (0.25 mcg/0.1 ml), Sodium azide (5 mcg/0.1

Drug Batch No.: 810192

Methods: Cells with or without S9 activation mixture were incubated for 5 hours and 21 hours respectively, with the indicated concentrations of CGP 39393, along with the positive and negative controls. At the end of the experiment, the mutant frequency and the viability of the cells were determined. The test is considered positive **1)** if the mutant frequency is significantly greater than the control, shows a dose related response and is reproducible. **2)** The number of normalized mutant clones in the treated vs untreated cultures should differ by more than 20.

Results: CGP 39393 or its metabolites were not mutagenic at any of the concentration tested, ranging from 148 to 5000 µg/ml with or without metabolic activation (S9 fraction) in the forward mutation system. The positive controls showed a highly significant increase in mutant frequency.

3. Cytogenetic Test on Chinese Hamster Cells in Vitro (EC-Conform): (Study # 936152)

Testing Laboratories: Genetic Toxicity,
Ciba-Geigy Limited,
Basle, Switzerland

Study Started: July 12, 1993

Study Completed: March 18,1994

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Cells Employed: Chinese Hamster Ovarian Cells (cell line, CCL 61)

Concentration Employed: 39.06-5000 ug/ml were used for two initial studies and for the 3rd and 4th experiment in the confirmatory studies, whereas 312.5-5000 ug/ml concentrations were selected for the first and second confirmatory studies. There were a total of 6 studies, 2 original and 4 confirmatory.

Basis of Dose Selection: Dose levels are based on the preliminary studies of suppression of mitotic activity in cells by approximately 50-80% compared to controls. This was based on criteria for assay acceptance.

Solvent Control: [redacted]

Positive Controls: Mitomycin 0.2 ug/ml without metabolic activation. Cyclophosphamide 20.0 ug/ml with metabolic activation.

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Source of Metabolic Activation: Rat liver S9 fraction.

Drug Batch No.: 810192

Criteria of Genotoxic Effect: The percentage of metaphases containing specific chromosome aberrations in the exposed cells must be higher than 6.0 (based on historical negative control range). There should also be statistically significant differences from the respective negative control as well as a demonstration of a dose related response.

Methods: Chinese hamster cells were treated with various (39.06 - 5000 ug/ml) concentrations of CGP 39393 in the presence or absence of metabolic activator (S9 fraction). The cells were treated for different lengths of time. A sampling time of 18 hr was chosen to ensure analysis of first post treatment mitosis. An additional sampling time was selected, which was 24 hours after the first one, in case the cell cycle is delayed due to treatment with CGP 39393. In the presence of metabolic activation, treatment period was shortened by 3 hrs because prolonged exposure to S9 fraction would result in cytotoxic effect. Therefore, the treatment conditions are given in each experiment in the Table 1 and 2. At the end of the experiment, 200 metaphases were examined from two cultures in vehicle and the treated groups and at least 50 metaphases were examined in the positive controls. In addition, in these cell cultures, the DNA distribution was determined by flow cytometry. In this method, cultures were either treated with the test substance or with the vehicle, cells were then stained with DAPI (4', 6-diamino-2- phenylindole). Fluorescence of DAPI stain is measured with a ———— flow cyto-photometer. A disturbance of the cell cycle mediated by the test substance would indicate a substantial shift into DNA distribution pattern, which is compared with the pattern of the vehicle control.

Results: In the original study, 7.0, 6.0, and 4.5% of the cells metaphases with specific chromosomal aberrations were seen at 1250, 2500, and 5000 ug/ml concentration of the compound without metabolic activation. The negative and positive controls had 2% and 60% respectively, of metaphases with specific chromosomal aberrations in this study (Table 1). The mitotic index was determined in the original experiment 1 and 2, and in the confirmatory experiment 3 and 4 (Table 1 and 2). In the original study, with metabolic activation, no changes were recorded at any of the tested concentrations. Since the first experiment showed statistically significant increase in the chromosome aberrations at two dose levels, the study was repeated. In the repeat confirmatory study, the 1st original experiment could not be confirmed. In addition, no dose dependent increase in incidence of chromosome aberrations was observed. In the repeat confirmatory

experiment 2 with metabolic activation at 5000 ug/ml, a significant increase in chromosomal aberration was noted, but this was not considered genotoxic, because it was below the limit for a positive response (based on historical negative control data).

In the succeeding confirmatory experiment 3 and 4 (Table 1 and 2), these positive responses were not seen. However, in these studies (expt 3 and 4) positive controls were not included and should have been included to see that the experiment worked. The positive controls are very useful to demonstrate the adequacy of the experimental conditions to detect known mutagen. Since there was no dose related response in any of the studies (which is one of the criteria for a genotoxic effect), the sponsor suggests that the whole study is negative. Although, the DNA distribution determined by flow cytometry also showed no evidence of a cell cycle disturbance by CGP 39393, the chromosome aberration test is different. The fact, that 2 out of 4 experiments (which had both negative and positive controls) showed significant changes at certain doses, is of some concern. The dose response may not be easily demonstrable. Also, sponsor has selected an unusual way of setting the criteria for the negative controls. Their historical negative control data are derived from 1992 studies, which include all kinds of diluents including —— and ——— The present study only uses ——— ——— as a diluent. Therefore, historical negative controls should only be derived from buffer diluents. Using buffer as a diluent, the historical data for negative control range between 0-2.5, therefore, there may be actual statistical significance from the control. Because of these contradictory results, there is a need for the sponsor to repeat this test in human peripheral lymphocytes.

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Table 1

The effect of CGP 39393 on Chinese hamster ovary cells in vitro without metabolic activation.

Drug Conc µg/ml	Treatment 18 Hour				Treatment 42 Hour	
	Original Expt 1		Confirmatory Expt 1		Confirmatory Expt 3	
	% of cells with aberrations	Mitotic Index %	% of cells with aberrations	Mitotic Index %	% of cells with aberrations	Mitotic Index %
0	2.0	9.85	4.5	--	1.5	5.2
1250	7.0*	10.35	1.5	--	2.0	5.4
2500	6.0*	8.3	1.5	--	1.0	6.95
5000	4.5	10.00	3.0	--	0.5	5.3
Positive control	60.0***	--	78.0***	--	--	--

Table 2

Effect of CGP 39393 on Chinese hamster ovary cells in vitro with metabolic activation.

Drug conc µg/ml	Treatment 3 h/harvest time after treatment 15 h				Treatment 3 h/ harvest time after treatment 39 h	
	Original Expt 2		Confirmatory Expt 2		Confirmatory Expt 4	
	% of cells with aberration	Mitotic Index %	% of cells with aberrations	Mitotic Index %	% of cells with aberrations	Mitotic Index %
0	5.0	2.50	1.0	--	3.0	5.0
1250	5.0	2.80	3.0	--	3.0	6.85
2500	1.5	2.90	3.0	--	2.5	6.25
5000	5.5	2.50	5.0*	--	2.5	6.30
Positive control	40.0***	--	70.0***	--	--	--

*p 0.05 ≤ 0.01, ***p ≤ 0.001

ADDENDUM TO THE STUDY:

A significant increase in percent cells with aberrations was observed with metabolic activation ($p<0.05$) in 1 of out of 3 experiments as shown in above table. It increased the percent cells with aberrations without metabolic activation only at 1250 and 2500 ug/ml concentration and not on 5000 ug/ml concentration. This observation could not be confirmed in the repeat experiments in the presence and absence of metabolic activation. Thus CGP 39393 produced variable results in the test.

4. In Vivo Micronucleus Test in Rats
(Report - #916086)

Testing Laboratories: Genetic Toxicology
CIBA-GEIGY Ltd.
Basle Switzerland

Dates Studies Started and Completed: June 26, 1991 and November 1, 1991.

Test Species: Tif: RAIF (SPF) rats.

No. of Animals: 5 animals/sex/group.

Route of Administration: I.V.

Dose Levels: 125, 250 and 500 mg/kg (10 ml/kg).

Drug Batch No.: 810490

Basis of Dose Selection: Dose levels are based on the preliminary toxicity study.

Negative control: Saline (10 ml/kg)

Positive Control: Endoxane (20 mg/kg; i.p., 20 ml/kg)

Methods: Two experiments were conducted. In the first experiment, animals were given a single dose of vehicle or the test drug at 16, 24 or 48 hrs prior to sacrifice and preparation of the bone marrow (positive control animals were sacrificed at 24 hr after endoxane administration). In the second experiment, animals were given a single dose of vehicle, 125, 250 or 500 mg/kg of CGP 39393 24 hrs prior to sacrifice and preparation of the bone marrow (positive control animals were sacrificed at 24 hr after endoxane administration). On the ———stained slides, 1000 polychromatic erythrocytes per animal were examined for the presence of micronuclei.

Results: CGP 39393 did not induce an increase of micronucleated polychromatic erythrocytes in rats bone marrow. In contrast, the % of micronucleated polychromatic erythrocytes in endoxane treated group was markedly higher than the negative control. These findings suggest that CGP 39393 is not mutagenic in this test system. Dose levels of 250 and 500 mg/kg produced some deaths in this study.

SPECIAL TOXICOLOGY STUDIES:

1. Exploratory Immunotoxicological Study in Rabbits
(Study # 88-6128)

Study Started: September 12, 1988.

Study Completed: Not given.

Methods: This study was conducted to evaluate the immunogenic potential of the compound (formation of antibody, potential anaphylatic reaction), when given at a similar dose level as that intended for use in man (approx. 2 mg/kg/day). Five male and five female rabbits were given 2 mg/kg CGP 39393 as I.V. bolus injection daily for 10 consecutive days. Booster injections were given 4 and 8 weeks after the last primary injections. Prior to the first injection and one day before the booster injections a skin test was performed on each rabbit using histamine as a concurrent positive control and sterile 0.9% saline as a negative control. Blood samples were taken pretest, day 11 and 7 days after the first and second booster injections for the determination of specific antibodies, total protein and electrophoresis of proteins. Necropsy and histological examinations were not performed.

Results: No animal died during this study. There were no effects on body weight or food consumption. The skin tests were negative at all time points, and booster injections caused no clinical reactions. No specific antibodies were detected. Sponsor did not report the quantitative levels of immunoglobulins, total protein and electrophoresis results. Based on negative skin tests, lack of specific antibodies, and void of clinical signs of anaphylaxis or other allergic reaction, CGP 39393 is considered to have a very low potential for immunogenicity in the rabbit.

2. Exploratory Immunotoxicological Study in Dogs:
(Study # 88-6097)

Study Started and Completed: September 13, 1988 and December 1, 1988

Methods: This study was performed exactly the same way as the above mentioned study (# 88-6128), except in this study 6 beagle

dogs (3 males and 3 females) were given 2 mg/kg CGP 39393 as I.V. bolus injection daily for 10 consecutive days, followed by a booster injection on 4th and 8th weeks after the last primary injections. In addition, results of electrophoresis of proteins, levels of IgG, IgA & IgM were reported. Necropsy was performed 6 days after the second booster injection.

Results: No animal died during this study. There were no effects on body weight or food consumption, organ weight, gross and microscopic findings. Skin tests were negative at all time points, and booster injections caused no clinical reactions. Pretest and on day 11 no specific antibodies were found. However on day 46, a small quantity of specific antibodies were present in three of six dogs. After the second booster injection, on day 74, there were further increases in the level of specific antibody, but the titers remained at a low level. On day 46, a slight increase in IgG was seen in all dogs as compared to pretest, the levels of IgG decreased on day 74, but the levels were still somewhat higher than the pretest values in five of the six dogs. Although, there was no sign of anaphylaxis, and skin tests were negative, yet small levels of specific antibodies were detected in 50% of the animals, suggesting that CGP 39393 has low immunogenic potential in the dog.

3. Antigenicity Study of CGP 39393 in Guinea Pigs:
(Study # AG-1089)

Testing Laboratories: [redacted]

Study Started: August 9, 1990

Study Completed: December 12, 1990

Drug Batch No.: 810189

Animals: Male Hartley guinea pigs (310-477 g)

Active-Systemic Anaphylactic (ASA) Test:

Methods: Five to 10 guinea pigs per group were used in this study. One group of animals were sensitized by intravenous administration of 2 mg/0.5 ml/animal of CGP 39393 once a day, 3 times a week for a total of 10 times. Another group of guinea pigs was given 2 mg/0.5 ml/animal of CGP 39393 plus Freund's complete adjuvant (FCA) subcutaneously twice a week for two weeks (3 total doses). The positive control group (n=5) received 0.2 mg/0.5 ml/animal of ovalbumin (OVA) intravenously once a day, 3 times a week for a total of 10 times, another positive control group (n=5) received 0.2 mg/0.5 ml/animal of ovalbumin (OVA) plus FCA subcutaneously, twice weekly for two weeks (3 total doses). Systemic anaphylaxis

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was examined following i.v. injection of 3 mg/ml/animal of CGP 39393 at 14 days after the last sensitization dose (the positive control group animals received 3 mg/ml/animal of ovalbumin).

Results: All animals (5/5) sensitized with OVA died just after the 5th sensitization dose. No anaphylactic symptom was identified in any of the guinea pigs sensitized intravenously with CGP 39393. However 9/10 guinea pigs sensitized subcutaneously with CGP 39393 + FCA had anaphylactic reactions and 8 of these died. All positive control (OVA + FCA) group animals (5/5) had anaphylactic symptoms and 3 out of these 5 died.

Passive Cutaneous Anaphylactic (PCA) Test

Methods: The PCA reaction was examined with serum collected from CGP 39393 (i.v.), CGP 39393 + FCA (s.c.), and OVA + FCA (s.c.) treated animals at 14 days after the last sensitization dose (see above). Serial dilutions of the serum were injected intradermally on the back of guinea pigs (recipients, n = 5-10/group). Four hours later these animals were challenged with an intravenous injection (into vein of hind leg) of the respective antigen mixed with Evan's blue. After 30 min, animals were killed and examined for dyed extravasations in the back. Sites having dye spot of 5 mm in diameter is considered positive.

Results: No PCA reaction was seen when sera obtained from the animals sensitized intravenously with CGP 39393 (PCA titer <2). However, drug specific antibody was detected in 9/10 animals when sera obtained from the animals sensitized with CGP 39393 + FCA were used. All positive control group animals had PCA reaction (mean PCA titer = 4000-32000). Thus CGP 39393 did elicit sensitization activity in passive cutaneous anaphylaxis test in guinea pigs.

Thus data suggest that CGP 39393 has antigenic potential in guinea pigs.

4. 5-Day Local Subcutaneous Irritation Study in Rabbits
(Test # 89-6059)

Study Started & Completed: April 10, 1989 and April 14, 1989.

Methods: This study was done to evaluate the local subacute subcutaneous irritation potential of CGP 39393 in rabbits, following once a day for 5 consecutive days. CGP 39393 was injected subcutaneously (40 mg/ml of 3% glucose, 1 ml/Kg and once daily) into the right shoulder of each of three female rabbits for 5 consecutive days. Tolerability was assessed based on gross observations during the study and microscopic examination of the injection site and underlying muscle tissue. In this study no

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separate control animals or vehicle were employed. Six hours after the last injection, the animals were necropsied.

Results: Grossly, slight hemorrhage was seen at the injection site in each rabbit. Microscopically the lesions at the injection sites were characterized by a slight to marked neutrophilic infiltration accompanied by a slight hemorrhage and minimal fibrosis. Two out of 3 rabbits showed a more pronounced inflammatory reaction, fibrin deposits and small amount of granulation tissue. A slight focal necrosis of the subcutaneous fatty tissue and of the cutaneous muscle was present in one of six animals. Due to the presence of hemorrhage and pronounced leukocytic infiltration, CGP 39393 should be evaluated in comparison to that of vehicle solution.

5. **Subcutaneous Irritation Studies in Rabbits**
(Test # 936221)

Testing Laboratories: Section of Toxicology/Pathology
Ciba-Geigy Limited
Basle, Switzerland

Study Started: October 7,1993

Study Completed: October 15,1993

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals Employed: Rabbits (chinchilla), 3-4 months old.

Methods: Two different formulations of CGP 39393, one in calcium chloride (2.66 mg) and the other in magnesium chloride (2.15 mg) were compared in two groups (3 each) of male rabbits. Both preparations contained 15 mg dose of the test article (batch # 224109A). These were administered by subcutaneous injection (0.5 ml) once daily for 5 consecutive days in the right shoulder, and only once, on the 5th day of the study, in the left shoulder. The control group (placebo) received the active ingredient (CGP 39393) without stabilizing agent i.e., magnesium or calcium chloride. Clinical signs were recorded daily. Body weights were recorded pretest and at the completion of the study. Local reactions were recorded pretest, as well as 3 and 6 hours post-test. Rabbits were killed by exsanguination under anesthesia, 6 hours after the last injection. Skin and subcutaneous tissues were examined, and both macro and microscopic (inflammation, fibrosis, hemorrhage, and calcification) changes were recorded.

Results: No clinical signs, local reactions (erythema, ischemia, edema or necrosis) or changes in body weight, were noted in any of

the rabbits. A single injection of either formulation had insignificant effects. However, both microscopic and macroscopic changes were observed after 5 days of injections. In the macroscopic studies, after repeated 5 days injection, the formulation in calcium chloride showed thickening of the subcutaneous tissue with adhesions. No changes were noted in the placebo group after 5 days of treatment. Microscopic changes in the group receiving calcium chloride formulation (after repeated dose), showed moderate inflammation in all 3 rabbits, 2 of the rabbits had moderate fibrosis and 1 had marked fibrosis. All 3 showed slight hemorrhage. One rabbit had slight calcification and the other 2 minimal calcification of the subcutaneous tissue. The placebo and magnesium chloride group showed minimal inflammation with fibrosis and hemorrhage. Thus, the two formulations of CGP 39393 are clearly different, when injected subcutaneously and are distinguishable by local changes, the calcium chloride formulation showed inflammation and was more irritative after repeated injection.

LABELING

The sponsor proposed labeling of CGP 39393 generally conforms to the format specified under CFR 21, subpart B, 201.50 to 201.57 dated April 1991 but based on the present review, the changes in the following sections/categories of the proposed label are recommended. These changes are described by first giving the text of the proposed version, followed by the reviewer's comments and the changed version of the label.

Sponsor proposed version:

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Reviewer's Evaluation: CGP 39393 was not mutagenic in the Ames test, the Chinese hamster lung cells (V79) forward mutation (HGPRT locus) test or rat micronucleus test. But in vitro Chinese hamster V79 chromosome aberration test, variable results of an increase in the number of aberrations in 1 out of 2 tests in the presence and none in the absence of metabolic activation were seen. Sponsor did not conduct any additional study but claimed that CGP 39393 was not mutagenic. CGP 39393 up to 10 mg/kg/day (59 mg/m^2/day) dose administered 64 days before mating and till sacrificed in male rats and, during mating and whole gestation period and lactation in females at the same dose, did not produce effect on fertility and reproductive parameters. The proposed label package insert of the substance under Carcinogenesis, mutagenesis and Impairment of

Fertility section of Animal Pharmacology and Toxicology should be appropriately changed.

Proposed Revised Text:

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2. Sponsor proposed version:

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Reviewer's Evaluation:

In first segment II Teratology study in pregnant rats, desirudin at a daily subcutaneous dose of 10 mg/kg (59 mg/mm^2/day or 5.4 times the human dose) or more during organogenesis period produced teratogenic malformations of cleft lip/cleft palate, fused/ bipartite sternebrae and omphalocele. In the repeat Segment II Teratology study in rats, fetal abnormalities of edema, shortened hind limb, and umbilical hernia were seen in animals included in 15 mg/kg/day (89.25 mg/m^2/day or 8 times proposed human dose) treatment group. A dose related and skeletal malformations of sternebrae were also seen. In two segment II Teratology studies in pregnant rabbits, intravenously administered CGP 39393 from the dose of 0.6 to 6 mg/kg/day (7.2 to 72 mg/m^2/day) produced gross fetal abnormalities of spina bifida, hydrocephaly, gastroschisis and skeletal abnormalities of agenesis of vertebrae centrum and

malrotated hind limbs. CGP 39393 was teratogenic in rats and rabbits and it should be given with caution in pregnant women. There are no adequate and well-controlled studies in pregnant women and it should be used during pregnancy only if potential benefit justifies its potential risk to the fetus.

In Segment III. Perinatal and postnatal reproductive toxicity study in pregnant rats, SC administered desirudin at 5 and 10 mg/kg/day (29.5 to 59 mg/m^2/day) produced maternal toxicity of retardation in the body weight, pooled bleeding (in all treatment groups dams) and soft masses at the injection sites in dams of 5 and 10 mg/kg/day treatment groups. The compound was maternal toxic in dams and deaths were due to bleeding (an overt pharmacologic effect). It did not show any effect on the reproductive parameters of labor, delivery and developmental parameters of F_1 fetuses.

Proposed revised Version:

[redacted]

SUMMARY AND EVALUATION:

CGP 39393 (rec-hirudin) a compound identical to naturally occurring peptide of leech hirudo medicinalis, exerted specific anti-thrombin activity (Ki = 231 fM) by binding with thrombin (1:1) and inhibiting thrombin induced platelet aggregation (EC_{50} = 3 nM). It showed linear and dose related anticoagulant effect by prolonging APTT and TT. Its antithrombotic activity was 4 to 5 times greater than unfractionated heparin (UH) in dog coronary artery thrombosis model. In dog venous shunt thrombosis model, a pretreatment of animals with 1.5 mg/kg, IV CGP 39393 prevented reocclusion after thrombolysis. It also prevented thrombosis in deep vein arterial injury model in pigs. It (1 mg/kg, i.v infusion) was 4 times more potent than heparin in angioplasty in rabbits. Based on these properties, CGP 39393 was considered useful in the deep vein thrombosis.

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Sponsor in the present NDA has requested to use CGP 39393 in the prevention of thromboembolic complications in patients undergoing elective hip replacement surgery at the subcutaneous dose of 0.3 mg/kg CGP 39393 twice a day for a period 9 to 12 days. In the cover letter, sponsor had stated that CGP 39393 would be administered in the prevention of deep vein thrombosis associated with hip and knee replacement surgery. This discrepancy in the use should be resolved before the approval of the application.

In support of this application, sponsor submitted the pre-clinical pharmacology in vitro and in vivo, absorption, distribution, metabolism and excretion (ADME) studies in vitro and in rats, dogs and rabbits, acute intravenous toxicity studies in rats, mice, dogs and monkeys; 1- and 3- month subcutaneous toxicity studies in rats and; 1- and 3- month intravenous toxicity studies in dogs; 14-day continuous intravenous infusion toxicity study in monkeys, subcutaneous Segment I. Reproductive toxicity study in rats, subcutaneous segment II. Teratology studies in pregnant rats and a repeat subcutaneous segment II. Teratology studies in pregnant rats, i.v. segment II. Teratology studies in pregnant rabbits and a repeat i.v. Segment II. Teratology studies in pregnant rabbits, subcutaneous perinatal and postnatal (segment III) toxicity study in rats, Ames test, gene mutation test in CHO V79 cells, in vitro chromosomal aberration assay in CHO cells and in vivo rat micronucleus test, special studies on exploratory local irritation study in rabbits, immunotoxicological studies in rabbits & dogs; 5-day subcutaneous irritation study in rabbits, and a study to evaluate its antigenicity in guinea pigs.

During the developmental stage, sponsor used methods I, II and III to improve upon the yield of the compound. Preclinical studies submitted under IND 34,046 were conducted with the compound prepared by method I and II. Only limited toxicity studies were done by using desirudin synthesized by method III. According to sponsor the purity of the compound was not affected by the change in the methods of production. The compound prepared by method III.

The pharmacokinetics studies with rec-hirdin were conducted in rats, dogs, rabbits, monkeys and baboons. An intravenous bolus injection of CGP 39393 had $t_{1/2\beta}$ value of 26.4 min in rats. Subcutaneous injection of CGP 39393 attained rat plasma peak concentration in 40 min. The bioavailability of subcuataneously administered CGP 39393, in comparison to intravenous route, was 83 and 86 to 90% in rat and man, respectively. CGP 3939 caused prolongation of APTT by 2.2 to 3 times in rats which was further increased by bilateral nephrectomy by >1.3 times. It was due to its impaired clearance. Intravenously or subcutaneously administered CGP 39393 was excreted in 4 hydrolyzed metabolites (not fully identified) in kidneys and only a small amount was

excreted in rat bile. In dogs, it was excreted rapidly and mostly detected in cortical portion of proximal or distal tubules. It was cleared completely from kidneys up to 6 hr of its administration. It was seen to pass placental barrier in pregnant rabbits. The bioavailability of subcutaneously administered CGP 39393 in comparison to intravenous route was 83 and 86 to 90% in rat and man, respectively.

Acute toxicity studies were conducted at the single intravenous bolus doses of 332 and 1600 and 30 mg/kg in rats, mice and dogs, respectively. CGP 39393 did not produce any death among these animals. In monkey, a single intravenous dose of 100 or 300 mg/kg produced a dose related contusions on the hind limbs and pelvic areas and no deaths were reported. These studies did not provide any information on 'limiting toxicity'.

In 1-month subcutaneous toxicity study in rats, CGP 39393 was administered first at dose level of 60 mg/kg/day for 7 days. There were 9 deaths out of 30 animals treated with 60 mg/kg/day CGP 39393 during the first week of study due to dose related anemia and severe blood loss. The treatment was stopped and the study was restarted in two groups (15/sex/group) at the doses of 0 and 20 mg/kg/day for 28 days. Four out of 30 animals (2/sex) of 20 mg/kg/day treatment group died. Activated partial thromboplastin and thrombin time were erratic in rats. A significant increase in bilirubin and decrease in albumin were seen in animals of 60 mg/kg/day treatment group. Reddening and hemorrhage were seen in 20 and 60 mg/kg/day treatment group animals. A dose of 20 mg/kg/day was lethal and the 'highest tolerable dose' could not be identified. Based on the observed toxicity in animals included in 60 mg/kg/day treatment group, liver and kidneys could be the target organs of toxicity. However, the study was conducted at 1 dose level, thus was not a valid study.

In 3-month subcutaneous toxicity study (with 28 day-recovery period) in rats, 4 groups of rats (10/sex/group) were administered at doses of 0, 2.5, 5 and 10 mg/kg/day CGP 39393. The reversal of toxicity was examined in animals of control and high dose treatment groups. No clinical signs were observed in treated animals except dose related hematoma and hemorrhages at the injection sites. The intensity and incidences of hemorrhages were more in females than male rats. These hemorrhages were accompanied with granulation and fibrous tissue and inflammatory cells. After the recovery period minimal to slight fibrosis with few inflammatory cells, and microscopic hemorrhages were noted in the treated rats. No other treatment related histopathlogical findings were observed in the study. No specific target organ of toxicity was identified excepting dysfunction of hemostasis mechanism and 'no effect dose' was not established.

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In 7-day continuous intravenous toxicity study in male dogs, 3 groups of dogs (3/group) were administered intravenous infusion of 0, 1 or 4 mg/kg/hr CGP 39393 for 24 hr/day. The main findings at the end of 7 days of treatment were altered blood coagulation parameters, anemia, hemorrhage, and increased erythropoiesis. No specific target organ of toxicity was identified and 'no effect dose' was not established.

In 1-month subacute i.v. (bolus) dose toxicity study in dogs, slight hemorrhage and hematomas at the injection site were observed in animals belonging to 0 or 20 mg/kg/day (n=3/sex) treatment group. Severe increase of plasmatic coagulation parameters of partial thrombin time (PTT) and thrombin time (TT) were noted in all treated dogs 4 hrs post dosing. Blood chemistry parameters were not remarkable except on week 4, 1 male dog (#180) had elevated alanine aminotransferase (222%) and aspartate aminotrasferase (267%) and another female dog (#153) had elevated alanine aminotransferase (505%). Since the number of animals was small in control and treatment groups, no organ weight analysis was performed. Hemorrhage was seen at the site of injection in all treated animals and, subacute to chronic perivascular inflammation in the heart, liver, lungs and cecum/colon were noted in a male dog (#180).

In 3-month subchronic toxicity study, three groups of dogs were treated with the doses of 0, 10 and 25 mg/kg/day, i.v. CGP 39393. No clinical signs were observed except hemorrhages associated with the anticoagulant effect of the drug (exaggerated pharmacological effects). This incoagulability was protracted to at least 24 hr in some dogs independent of the dose. There were 4 deaths (2 in 10 mg/kg group, and 2 in 25 mg/kg group) during the experimental period due to preterminal severe hemorrhages in various organs/tissues including hemorrhage at the site of injection. Fibrin deposition, edema, inflammatory cell infiltration and granulation at the site of injection were much more severe in treated dogs than in control dogs. The vasculitis observed in 14/18 treated dogs was considered to be due to the immunogenic potential of the drug in dogs. Subsequently, sponsor reported that CGP 39393 formed antibody complex in plasma dogs and the antibodies were detected 4 hr post after 44^{th} dose of the compound. The concentration of the antibodies increased after 89^{th} dose and the $t_{1/2}$ of antibody complex of the compound was 21 hr, more than the $t_{1/2}$ of the native drug (3.8 to 4.1 hr) in the study. The increase of BUN, creatinine, bilirubin and cholesterol and proteinuria along with purulent myocarditis and fibrosis were noted in preterminally sacrificed dogs which suggested kidneys, liver and heart as the target organs of toxicity. The report did not identify the nature of the circulating antibodies in different organs, i.e., if it was in or not in tissues. CGP 39393 was immunogenic in the study dogs.

The 14-day continuous intravenous infusion toxicity study in monkeys was not a valid study as CGP 39393 treatment was stopped by day 2 among 4.0 mg/kg/day treatment group and by day 8 among 1 mg/kg/day treatment group because of treatment related deaths of animals among these treatment groups. No meaningful toxicity information could be obtained from the data of the study.

Segment I. Fertility and general reproductive performance study in Sprague Dawley rats was conducted at the subcutaneous doses of 0, 1, 5 and 10 mg/kg/day CGP 39393 in male (64 days before mating) and female (2 weeks before mating) to examine CGP 39393 treatment effects on F_0 (treated) and untreated F_1 rats. The treatment was continued during mating in males and, up to day 21 of lactation among females. No abnormal effects on the fertility parameters and reproductive performance of the treated male and female rats included in up to 10 mg/kg/day of CGP 39393 treatment groups were reported. No adverse effect in fertility and general reproductive performance of F_1 rats was noted. Subcutaneous dose of 10 mg/kg/day (59 mg/m^2/day) CGP 39393 was identified as a threshold dose in the study.

In segment II. Developmental and teratology study in pregnant rats, CGP 39393 was administered at subcutaneous dose of 0 (control), 1 (low), 5 (mid) or 10 (high) mg/kg/day (5.9 to 59 mg/m^2/day) from gestation day 6 through 15. Major developmental malformations of cleft lip in one fetus of mid dose treatment group and omphalocele in 1 and 2 fetuses of mid and high dose treatment groups, respectively were produced. The delayed skeletal maturation was seen among animals of mid and high dose treatment groups. Major skeletal malformations of fused and bipartite sternebrae were also seen in fetuses of animals of high dose treatment group. CGP 39393 was teratogenic in rats.

Sponsor repeated s.c. Segment II Developmental and teratology study in 4 groups of pregnant rats at the doses of 0, 5, 10 and 15 mg/kg/day CGP 39393 (29.5 to 88.5 mg/m^2/day) administered from gestation day 6 through 15 of gestation. Three fetuses of 3 litters of 15 mg/kg/day treatment group animals had gross malformation of edema, shortened hind limbs or umbilical hernia, respectively. Treatment related skeletal anomalies like shortened femur, fibula and tibia in 1 fetus and delayed skeletal maturation and fused centrums in another fetus of high dose treatment group animals were seen. The skeletal fetal abnormalities of sternebrae bipartite and irregular asymmetric sternebrae were also noted. Thus CGP 39393 produced teratological abnormalities in rat fetuses and confirmed the previous findings.
In segment II. Developmental and teratology study in rabbits, 4 groups (16/group) of pregnant animals were administered 0, 0.6, 2.0 or 6.0 mg/kg/day (from 7.2 to 72 mg/m^2/day) CGP 39393 intravenously from pregnancy day 6 to day 18. Malformations of hydrocephay in 1

fetus of 1 litter of each of 0.6 and 6 mg/kg/day treatment groups and, spina bifida in 1 and 6 fetuses of 1 and 5 litters of 2 and 5 mg/kg/day treatment groups, fore arm flexure/malrotated hind limb in 2 fetuses of each of 0.6 and 6 mg/kg/day treatment groups were seen. CGP 39393 produced teratological abnormalities in rabbits.

Segment II. Modified developmental and teratolcgy study was conducted in 4 groups (40/group) of pregnant rabbits to determine the critical/sensitive period for the developmental malformation in rabbits. Animals of group 1 and 4 were treated with intravenous doses at 0 and 6.0 mg/kg/day (72 mg/m^2/day) CGP 39393 from day 7 to day 19 of gestation. The animals of groups 2 and 3 were treated with intravenous dose of 6 mg/kg/day CGP 39393 from gestation day 7 to 13 (group 2) and, day 14 to 19 (group 3). Fetal toxicity of resorption and post implantation losses and, gross fetal malformation of gastroschisis were noted in 2 fetusus of 2 litters (group 2). Agenesis of ribs and centrum of vertebrae were seen in 1 fetus of an animal belonging to group 2. This study indicated that the period of group 2 rabbits (treated from day 7 to 13 during fetal development) was the most susceptible for producing teratological incidences.

In segment III. Perinatal & postnatal reproductive toxicity study in 4 groups of pregnant rats, subcutaneous doses of 1, 5, or 10 mg/kg/day CGP 39393 were administered from gestational day 15 through lactation day 20. Hirudin treatment did not produce any treatment related adverse effect on food consumption, body weight changes and maternal reproductive parameters of labor, delivery and lactation. In addition, hirudin had no effects on fetal postpartum survival and the developmental parameters.

The immunogenic potential of intravenously administered (bolus dose) CGP 39393 was evaluated in rabbits and dogs at the dose of 2 mg/kg/day for 10 days. In rabbits, the skin tests were negative and booster dose caused no reactions. In dogs, an increase of specific antibodies was seen from day 46 and a booster injection on day 74 increased specific antibodies (an increase in IgG antibodies) suggesting that CGP 39393 was immunogenic in the dog. Intradermal challenges in guinea pigs with CGP 39393 produced hypersensitivity and anaphylactic shock in guinea pigs sensitized by subcutaneous dose of CGP 39393.

In 5-day local subcutaneous irritation study in rabbits, a control group (vehicle treated) of animals was not used. Hemorrhage and pronounced leucocytic infiltration at the injection site were observed.

CGP 39393 was not mutagenic in Ames test, micronucleus test in rat (in vivo) and forward gene mutation test (HGPRT locus) in Chinese

hamster V79 cells but it showed variable results in vitro cytogenic test on CHO cells. It produced significant increase in the chromosome aberrations in two of the four experiments in the absence of metabolic activation. In the repeat confirmatory experiment conducted in the absence of control group, no increase in the chromosomal aberrations was seen. Thus CGP 39393 produced variable results in this test.

The proposed label of CGP 39393 is not acceptable in the present form and the sponsor should revise it as described under labelling section of the present review. CGP 39393 was seen to produce clastogenic effects in vitro chromosomal aberration test in CHO cells in the presence and absence of S-9 metabolic activation and, it caused developmental malformations in initial and repeat Segment II. Teratology studies in rats and rabbits.

CGP 39393 a thrombin inhibitor, is more potent and long acting anti-thrombin agent than unfractionated heparin and low molecular weight heparin, enoxaprin. In 3-month rat subcutaneous toxicity study, CGP39393 produced profuse bleeding, anemia, edema and inflammatory effects. No specific organs of toxicity were identified in rat study. In 3-month intravenous toxicity study in dogs, hemorrhage and inflammatory cell infiltration at the site of injection, increase in BUN, creatinine, bilirubin and cholesterol levels and proteinuria along with purulent myocarditis and fibrosis were noted in preterminally sacrificed dogs which suggested kidneys, liver and heart as the target organs of toxicity. CGP 39393 exerted immunogenic response and produced CGP 39393-antibody complex in dogs included in 3-month toxicity study. It was teratogenic in pregnant rat and produced major skeletal fetal abnormalities of omphalocele, edema, umbilical hernia and fused sternebrae. It was teratogenic in rabbits and malformation of hydrocephaly, gastroschisis, agenesis of ribs and fore arm flexure and malrotation of hind limb were seen. Sponsor submitted 1-month subacute toxicity study in a rodent species by the same route as intended to be used in human use.

RECOMMENDATIONS:

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1. From preclinical standpoint, the present application lacks a [redacted]

2. Sponsor should make the recommended changes in the proposed label (see above as under labeling section).

Yash M. Chopra, M.D., Ph.D. Date

COMMENTS:

Jasti Choudary, B.V.Sc, Ph.D. Date
Supervisory Pharmacologist, HFD-180

cc:
Original NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Chopra
HFD-345/Dr. Viswanathan

R/D Init.: J. Choudary (3/29/01)
YMC/4/11/2001/NDA 2121710 YC.doc
Deg: 4/17/01/5/3/01

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/s/

Yash Chopra
5/3/01 04:13:31 PM
PHARMACOLOGIST

Jasti Choudary
5/4/01 07:37:30 AM
PHARMACOLOGIST
1]ASupervisory Pharmacologist's memo. for labeling will follow. 2] 4-week monkey s.c. tox. study is needed to determine whether the toxicity profile in the 3-month dog i.v.tox study is only species specific or has relevance to human safety.

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Pharmacologist Review of NDA 21-271
(Amendment Dated July 17, 2001)

SPONSOR & ADDRESS: Aventis Pharmaceutical Inc., Bridgewater, NJ.

US REPRESENTATIVES: Quintiles, Kansas City MO.

REVIEWER: Yash M. Chopra, M.D., Ph.D., Pharmacologist

DATE OF SUBMISSION: July 17, 2001

HFD-180 RECEIPT DATE: July 18, 2001

DATE OF REVIEW: February 1, 2002

DRUG: [redacted]

SUBMISSION CONTENTS: Draft Protocol for 28-day toxicity study in Rhesus Monkeys.

Protocol for 28-Day S.C. Toxicity Study in Rhesus monkeys: The in-life study will be conducted at [redacted] a division of [redacted] and antibody measurements will be conducted at [redacted]

The study will be done by using 4 groups of 2 to 5 years old rhesus monkeys weighing about 2 to 5 kg at the dose schedule shown in the following table. Each of the animals will be treated subcutaneously with vehicle (3% mannitol) or desirudin solution (in 3% mannitol) in a volume of 0.167 ml/kg twice daily (10-12 hr apart).

Gr. #	# animals (M/F)	Total Daily Dose (mg/kg/day)	Compound Concentration (mg/ml)
1	4/4	0 (control)	0
2	4/4	0.5	1.5
3	4/4	2.5	7.5
4	4/4	10.0	30

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During the proposed study the EKG recordings and ophthalmoscopic examination in all animals of the study will be done prior to the treatment and in study week 4 (timed relative to Cmax). The blood samples for toxicokinetic parameters determination will be collected after 0 (prior to dosing), 0.5, 1, 2, 3 and 6 hr of the administration of the compound on study day 1 and 28. Blood samples for blood chemistry, hematology and coagulation parameters will be collected from all animals prior to treatment and on day 0, 14 and 28. The animals found dead during the study or sacrificed at the termination of the study will be necropsied. The observed organ pathology and histopathological changes will be assessed in animals of each of the study groups. Bone marrow smear (from 7th rib during sacrifice) of the animals will be prepared. The animals showing treatment related effects their slides would be examined microscopically. The antibody (anti-RPR 205511) determination will be made on the blood sample collected on prior to treatment and on study week 4. Sponsor has included the requested parameters in the proposed study; therefore the study is acceptable.

SUMMARY AND EVALUTION:

In response to Division's letters dated December 15, 2000 and May 14, 2001, sponsor has now submitted a plan for the conduct of 1-month subcutaneous toxicity study in rhesus monkeys. The study will be conducted in 4 groups at ——————— mg/kg/day, s.c. in 2 divided doses. The proposed study includes the estimation of toxicokinetics parameters and immunogenicity potential as requested by the Division. The study protocol is acceptable.

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RECOMMENDATIONS:

Sponsor should be informed that the submitted protocol is acceptable.

Yash, M. Chopra, M.D., Ph.D. Date
Pharmacologist,

COMMENTS:

J. B. Choudary, B.V.Sc., Ph.D. Date
Supervisory Pharmacologist, HFD-180

cc:
Original NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Chopra
HFD-180/Dr. Choudary

R/D Init.: J. Choudary 8/17/01
YC/deg: 09/22/01; 10/2/01/11/27/01; 2/1/02
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This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Yash Chopra
2/1/02 03:12:32 PM
PHARMACOLOGIST

Jasti Choudary
2/8/02 01:40:02 PM
PHARMACOLOGIST

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Pharmacologist Review of NDA 21-271
(Amendment Dated October 3, 2002)

SPONSOR & ADDRESS: Aventis Pharmaceutical Inc., Bridgewater, NJ.

US REPRESENTATIVES: Quintiles, Kansas City MO.

REVIEWER: Yash M. Chopra, M.D., Ph.D.,
Pharmacologist

DATE OF SUBMISSION: October 3, 2002

FDR/CEDR ECEIPT DATE: October 4, 2002

DATE OF REVIEW: February 28, 2003

DRUG: Iprivask^{R}/ [redacted]

SUBMISSION CONTENTS: 1. 28-Day toxicity and Toxicokinetics study in Rhesus Monkeys.

2. 42-Day immunization study in Rhesus monkeys.

1. **28-Day SC Toxicity and Toxicokinetic Study in Rhesus Monkeys:** (Study # DSE 2001-0102/1119-146-SBE)

Testing Laboratory: [redacted]

Study Started: July 31, 2001

Study Completed: April 24, 2002

Review Completion Date:

GLP Requirements: A statement of Compliance with GLP regulation was included.

Animals: Young 2.2 to 5.0 years old adult rhesus male and female monkeys with mean weight (males: 3.65 kg and females: 3.3 kg)

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Drug Batch #: Active substance batch # 796, 896, 197/297, 1297 and 199. Injection product Batch # 100, 200, 300, H8001, H9008, H1001.

Methods: Thirty two monkeys (16/sex) were randomized in 4 groups (4/sex/groeup) and given subcutaneous injection in the dorsal-scapular region at the doses of 0, 0.5, 2.5 and 10 mg/kg/day in 2 divided doses for 28 days (volume=0.167 ml/kg/dose, doses 12 hr apart). The dose selection of the study was based on the sponsor protocol submitted in amendment dated July 17, 2001. The high dose of 10 mg/kg/day of sponsor was acceptable. The control group monkeys were given 3% mannitol in 0.9% saline. All animals were observed twice daily 7 days before the first dose and each morning and evening for clinical signs and body weights were recorded twice weekly. Daily food consumption was assessed 7 days prior to treatment. Opthalmoscopic examinations and ophthalmic examinations were performed once pretest and once in week 4. Blood samples for serum chemistry, hematology and coagulation parameters assessment were collected prior to treatment (day 0) on prior to first dose on day 28 of study. Additionally, blood samples (2 ml) were collected before treatment and, at 0.5, 1, 2, 3 and 6 hr after the first dosing on day 1 and 28 for assessing the TK parameters at [redacted] The plasma concentrations were determined by an enzyme linked immunosorbent assay (ELISA)/ [redacted] with lower detection limit of [redacted] of the compound by using mouse monoclonal anti-hirudin antibodies, coated on to [redacted] An additional 2.0 ml blood sample from each animals of the each of the study groups was collected on day 1, 14 and 28 for determining the plasma antihirudin antibodies by ELISA method [redacted] If the sample value after subtracting the negative control value exceeds a cut off value of [redacted] it is considered positive. The absorbance value of negative controls (phosphate buffer saline/[redacted] was noted from [redacted] All animals sacrificed or killed during study and were subjected to complete necropsy. Organs like adrenals, kidneys, lungs, pituitary, thymus, brain and heart of each of the animals were weighed. These organs and spleen, seminal vesicles, testes with epididymides, thymus, thyroid + parathyroid and uterus, cervix, vagina, gross lesions, site of injection, gastrointestinal tract tissues, salivary glands, pancreas, spleen, gall bladder and cystic duct from all groups of animals were examined

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microscopically. Group mean±SD for body weight, clinical pathology parameters, organ weight changes will be collected and subjected to ANOVA and Dunnett's test.

Results:

a. **Observed Effects**: Slight to moderate bruises at right and left femoral areas from the blood withdrawn sites were seen in control and treated monkeys. Watery diarrhea in 2, 2, 3 and 1 males and, 2, 2, 3 and 0 females of 0, 2.5, 5.0 and 10.0 mg/kg/day treatment groups.

b. **Mortality**: One female belonging to 10 mg/kg/day treatment died of treatment related profuse bleeding.

c. Body Weight/Food Consumption/Water Consumption Changes: The body weights of the animals in the treated groups were comparable with the control groups animals during the study. The initial and final body weights of control male were 3.65 and 3.8 kg and these were 3.3 and 3.4 kg for the control females. The food consumption data of animals was not provided.

d. **Hematology/coagulation/Bone Marrow**: On day 28, one male (#R15997M) belonging to 0.5 mg/kg/day treatment group showed an increase of prothrombin time (PT) from 12.3 to 34.5 sec and, activated partial thromboplastin time (APTT) from 22.5 to 76.1 sec. The increase in APTT among females was from 26.0 to 40.0 sec in a female (#R16370F) belonging to 0.5 mg/kg/day treatment group, from 25.8 to 38.0 sec (in a female (R16372F) belonging to 2.5 mg/kg/day treatment and, from 27 to 43.0 sec in female (#R 16394F) belonging to 10 mg/kg/day treatment groups.

e. **Blood Chemistry/Urinalysis Changes**: On week 28, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of animal # R16394F of 10 mg/kg/day treatment group was increased by more than 2.4 and about 5 times than the pre treatment values showing a slight effects on the liver. There were no changes in the urinalysis of the animals.

f. **Toxicokinetic**: The blood samples collected during the study were sent to [redacted] for estimation of the toxicokinetic of RPR 205511. A treatment related peak plasma concentrations of RPR 205511 was seen in 45 to 1 hr of its administration

in study male and female animals as shown below in sponsor table (vol 5.5, pp 280):

A summary of the toxicokinetic parameters of RPR 205511 in plasma following daily subcutaneous dosing to Rhesus monkeys is shown below:

Dose (mg/kg)	Gender (N=4/group)[d]	AUC[a] ± SD (AUC/Dose)		C_{max}[b] ± SD (C_{max}/Dose)		T_{max}[c] (Range)	
		Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
0.5 mg/kg	M	49.4 ± 11.3 (98.8)	127 ± 57.7 (254)	19.2 ± 5.28 (38.4)	53.7 ± 28.4 (107)	1.5 (1-2)	1 (0.5-1)
	F	60.1 ± 4.52 (120)	65.9 ± 13.8 (132)	29.3 ± 15.3 (58.6)	30.0 ± 9.01 (60.0)	0.75 (0.5-2)	1 (0.5-1)
2.5 mg/kg	M	165 ± 90.1 (66.0)	478 ± 239 (191)	72.0 ± 34.9 (28.8)	165 ± 58.7 (66.0)	0.5 (0.5-1)	0.75 (0.5-2)
	F	356 ± 157 (142)	1030 ± 1010 (412)	150 ± 53.3 (60.0)	306 ± 316 (122)	0.75 (0.5-1)	0.75 (0.75-3)
10 mg/kg	M	1030 ± 40.4 (103)	1300 ± 247 (130)	369 ± 30.5 (36.9)	404 ± 71.3 (40.4)	1 (0.5-2)	1 (0.5-1)
	F[d]	1240 ± 84.1 (124)	1280 ± 203 (128)	432 ± 37.9 (43.2)	431 ± 45.6 (43.1)	1.5 (1-2)	1 (1-1)

[a] AUC is reported over the period 0-6 hr in units of nM*hr
[b] C_{max} is reported in units of nM
[c] T_{max} is reported as the median value in hr
[d] For 10 mg/kg, females on Day 28, there is no data for animal R16371-F (animal died)

On study day 28, the plasma concentration of RPR 205511 in males of 0.5, 2.5 and 10 mg/kg/day treatment groups were 2.8, 2.9 and 1.3 times the day 1 concentration. Among females, the concentrations were 1.1, 2.9 and 1.03 times the day 1 concentration in 0.5, 2.5 and 10 mg/kg/day treatment groups females. Thus an increase in the residence time/accumulation of the compound was noted on its prolonged use.

g. **Vital Signs/Physical Examination/Ophthalmic Examination:** The changes in the EKG tracings of animals on week 4 of the study were not significantly different than reported on week 1 (before the initiation of treatment). The compound did not show an adverse effect on the EKG.

h. **Organ Weight changes**: No treatment related effects were seen.

i. **Gross Pathology**: Pale discoloration of the carcass and/or multiple dark areas or foci in many different tissues and/or clot in the abdominal cavity and/or subcutaneous tissues were seen. Treatment related red discoloration at the site of injection in animals of treated groups was seen. The subcutaneous thickening at the site of injection was seen 1/sex animals belonging to 10 mg/kg/day treatment group.

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j. **Histopathological Changes**: Minimum to moderate grade treatment related bleeding at the site of injection in 1, 3, 4 and 4 males and, 3, 4, 4 and 4 females in 4/sex animals belonging to 0.5, 2.5 and 10 mg/kg/day treatment groups was seen. A large blood clot in the retroperitoneal area was seen in animal #R16371F (died on study day 28) of 10 mg/kg/day treatment group. The hemorrhage was present in subcutis of the skin and at the site for the withdrawal of blood. The leukocytic foci in heptocytes of 0, 3, 1 and 2 males and, 1, 3, 1 and 1 females belonging to 0, 0.5, 2.5 and 10 mg/kg/day treatment groups were seen. Neutrophilic intraglandular infiltrates in prostate were present in 0, 0, 1 and 1 males out of 4 males in each of 0, 0.5, 2.5 and 10 mg/kg/day treatment groups, respectively. Mononuclear cell infiltrates in the interstitium of the kidneys were reported in 1, 2, 3 and 3 females included in 0, 0.5, 2.5 and 10 mg/kg/day treatment groups, respectively. The bleeding was seen in cervix and vagina 1 out of 4 females of 10 mg/kg/day treatment group.

k. **Detection of Antibodies**: The anti-hirudin antibodies were estimated by ELISA technique validated at [redacted] In the present study, the negative control absorbance (phosphate buffer saline/[redacted] was from [redacted] and exceeds a negative control value of 0.392. The control values are supposed to be below 0.392 [redacted]. In some of the day 1 samples of male and female animals, the value exceeded the negative control value of 0.392. In the description of the method of estimation of antibodies in the submission, sponsor had done an additional subtraction of the negative control value of 0.392. By adopting the sponsor calculations, none of the samples would be positive. For the positive control sample of the study, the absorbance of the sample should be above 0.392. Two (#R16372F and #R16394F), 1 (#R15958F) and 1 (R#16371F) out of 4 females in 0.5, 2.5 and 10 mg/kg/day treatment groups showed greater than 0.392 absorbance (OD_{450}). One (#R16391M), 1 (#R14247M) and 1 (#R14221M) out of 4 males in each of 0.5, 2.5 and 10 mg/kg/day treatment groups also showed absorbance greater than 0.392 and were considered as positive for the antibodies. Thus the study animals developed antibodies to the compound during the test. The test for the detection of antibodies was positive.

In summary, r-hirudin up to the dose of 10 mg/kg/day produced minimum to moderate subcutaneous bleeding and inflammation at the site of injection and hemorrhage in the

liver. A dose of 10 mg/kg/day was lethal in the study and the target organs of toxicity were liver and site of injection. The animals treated with the compound induced antibodies.

SPECIAL STUDIES:

1. 42-Day Immunization Study of Intramuscularly Administered RPR 205511 in Rhesus Monkeys: (DSE 2001-0510/Sbi #1161-146)

Testing Laboratory: [redacted]

Study Started: July 3, 2001

Final Report Date: April 15, 2002

Review Completion Date:

GLP Requirements: A statement of Compliance with GLP regulation was included.

Animals: Young 2 to 6 years old adult rhesus male monkeys with mean weight of 2.5 to 2.7 kg

Drug Batch #: Active substance # 9200140087

Methods: The test was conducted in only 2 monkeys (1/sex), the male was treated at 0.5 mg/kg IM desirudin and female 2.0 mg/kg, IM desirudin on day 1 and 28 to observe immune response of the compound. The dose used in the study was low and the animals were treated for 2 days and the number of animals in study groups was inadequate. Nc useful data could be extracting from the study.

Results:

1. Observed Effects: Watery diarrhea was seen in male and female monkeys from PM of day -1 and throughout the study in male and female. None of the animals lost the body weight and the final body weights were 2.5 and 3.0 kg of male and female monkeys. The study was done in an insufficient number of animals (1/dose) with inadequate dose and the study data could not give any useful information.

In summary, the study animals were treated for 2 days and was done in an insufficient number of animals (1/dose) with inadequate dose. The study data could not be used to generate any information for inferring the possible immunogenic potential of the compound.

PROPOSED LABELING:

The proposed label of the sponsor conforms to CFR 21, Subpart B. But the following changes in the proposed existing label of the sponsor are suggested:

[

]

REVIEWERS COMMENTS:

The above paragraph should be replaced with the following paragraph as sponsor had included an additional information in the paragraph. The portion beginning from

[

]

should be deleted.

The recommended version "Under Section ***Animal Pharmacology and Toxicology: General Toxicity*** should be: Desiuridin produced bleeding, local inflammation and granulation at injection sites in rat and dog toxicity studies. In addition, it was immunogenic in dogs and formed antibody complexes resulting in prolonged half-life and accumulation

in dogs. Desirudin showed sensitisation potential in guinea pig immediate and delayed hypersensitivity models."

2. The subsection c) under ***Pregnancy***: *Teratogenic Effects*: Pregnancy Category C: reads as:

[ ]

should be replaced with text suggested previously by Dr. Jasti Choudary, Supervisory Pharmacologist in his memo dated May 4, 2001 to the NDA 21-271. The following is the text of suggested Preclinical portion of the label:

Pregnancy: *Teratogenic Effects*: Pregnancy Category C. Teratology studies have been performed in rats at subcutaneous dose range of 1 to 15 mg/kg/day (about 0.3 to 3 times the recommended human dose based on body surface area) and in rabbits at IV doses range of 0.6 to 6 mg/kg/day (about 0.3 to 3 times the recommended human dose based on body surface area) and have revealed desirudin to be teratogenic. Observed teratogenic findings were omphalocele, cleft palate, asymmetric and fused sterebrae, edema, shortened hind limb etc. in rats; and spina bifida, malrotated hind limb, hydrocephaly, gastroschisis etc in rabbits. There are no adequate and well-controlled studies in pregnant women. [redacted] should be used during pregnancy only if the potential benefits justify the potential risk to the fetus."

SUMMARY AND EVALUATION:

In the initial submission, the sponsor submitted a 28-day 1-month S.C. toxicity study in rats. Sponsor was asked in Agency letters dated September and December 11, 2000 to conduct a 28-day toxicity study in monkeys. In the present submission, sponsor submitted results of 28-day subcutaneous toxicity and toxicokinetic study in rhesus monkeys and 42-day immunization study of intramuscularly administered compound in rhesus monkeys.

The 28-day toxicity and toxicokinetic study in Rhesus monkeys was conducted in 4 groups (4/sex/group) at the subcutaneous doses of 0, 0.5, 2.5 and 10 mg/kg/day. A treatment related plasma concentrations were seen in animals and on day 28 the plasma concentrations were greater than on day 1. One of the animals in 10 mg/kg/day treatment group died on day 28 because of profuse bleeding. Treatment related inflammation and bleeding, leukocytic foci in hepatocytes, mononuclear cell infiltrates in the interstitium of kidneys in other animals of 10 mg/kg/day treatment group were seen. The identified target organs of toxicity were liver, site of injection and kidneys and 0.5 mg/kg/day was considered as no effect dose. The identification of the antibodies in the sample was positive if a sample value after subtracting a negative control value exceeds a cut-off value of 0.392. None of the samples from the study animal exceeded the value but in some of the animals the absorbance was greater than the negative control value and considered positive for the presence of antibodies. The antibodies were identified in 2, 1 and 1 out of 4 females and, 1, 1 and 1 out of 4 males in 0.5, 2.5 and 10 mg/kg/day treatment groups The test for the detection of antibodies was positive.

The 42-day immunization study was conducted at a low dose of 2 mg/kg/day and in the insufficient number of animals and by a route of administration other than the proposed clinical subcutaneous route of administration. This was not a valid study and not accepted.

The proposed label of the compound generally conforms to CFR 21, Subpart B. The proposed label of the sponsor under subsection Animal Pharmacology and Toxicology and Pregnancy should be modified as suggested under Labeling section of the present review.

RECOMMENDATIONS:

None.

Yash M. Chopra, M.D., Ph.D.,
Pharmacologist HFD-180

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Comments:

Jasti B. Choudary, B.V.Sc., Ph.D.
Supervisory Pharmacologist, HFD-180

cc:
Original IND
HFD-180
HFD-181/CSO
HFD-180/Dr. Chopra
HFD-180/Dr. Choudary

R/D Init.: J. Choudary 2/25/03

YMC NDA\21271B2c. 2/28/03

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PHARMACOLOGIST

Jasti Choudary
2/28/03 03:13:22 PM
PHARMACOLOGIST

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Addendum to February 8, 2002 Pharmacology Review of NDA 21-271
Amendment Dated July 17, 2001 (Desirudin Injection)

From: Yash M. Chopra,
Reviewer, HFD-180

Dated: February 28, 2003

In the above Pharmacology review, an error on page 2, second sentence under the section of 'Summary and Evaluation" was found. The correction to the error is made below by first giving the existing text of the review and then the corrected version.

Existing text: [redacted]

Corrected text: The study will be conducted in 4 groups of animals at the doses of 0, 0.5 2.5 and 10 mg/kg/day, s.c. in 2 divided doses.'

Yash M. Chopra)
Pharmacologist, HFD-180

cc:
Original NDA
HFD-180
HFD-181/CSC
HFD-180/Dr. Chopra
HFD-180/Dr. Choudary

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Jasti Choudary
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PHARMACOLOGIST

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