APPENDIX I

Protocol Report K 9001006

Title: An open single ascending dose pharmacokinetic study of enoxaparin after subcutaneous administration of 1.0 mg/Kg, 1.25 mg/Kg, 1.5 mg/Kg and 2.0 mg/Kg (additionally, two subjects were given a 2.5 mg/Kg dose) in 16 healthy volunteers (corresponding report [redacted] # 1753 May 3, 1994).

[redacted]

OBJECTIVE

To characterize the pharmacokinetics and tolerance of enoxaparin (200 mg/mL) after single subcutaneous injections of 1.0 mg/Kg, 1.5 mg/Kg, 2.0 mg/Kg and 2.5 mg/Kg in healthy male volunteers, in a dose ascending design.

METHODS:

Study Design:

The study was performed in 16 healthy male volunteers. It was an open, non randomized, single ascending dose study consisting in four successive single dose, subcutaneous injections of enoxaparin in ascending order. The design was altered from 2.5 mg/Kg to 1.25 mg/Kg in a protocol amendment.

Subjects:

Demographics:

	MEAN	%CV	RANGE
AGE (YEARS)	24.5	15	19-33
WEIGHT (KG)	71	12	57-90.6
HEIGHT (CM)	177.3	3	165-190

Treatment and Administration:

Each subject was given successively the following treatments as a single dose treatment:
Period 1 = 1.0 mg/Kg (n=16)
Period 2 = 1.5 mg/Kg (n=16)
Period 3 = 2.0 mg/Kg (n=16)
Due to protocol changes the following occurred in Period 4:
Period 4 = 1.25 mg/Kg (n=11). In addition, Subjects 1 and 4 given 2.5 mg/Kg dose, Subject 13 was given 1.1 mg/Kg and Subjects 5 and 7 dropped out.
The single dose injections were administered at 8 am after a 10 h overnight fast. The injection

site varied according to the study period, so that no single body site was repeated. A washout period of at least 15 days occurred between periods.

Formulation: UF-heparin Valori 5 dose was based on anti-Xa potency. This was referred to as Batch CB 4337 manufactured in the Dept. of Pharmaceutical Sciences, RPR, Antony, Cedex.100 mg/0.5 mL, actual potency 105.7 mg/0.5 mL or 10,570 IU anti-Xa/0.5 mL.

Blood Sampling:
Blood samples were taken at the following times: predose, 0.25h, 0.5h, 0.75h, 1.0h, 2.0h, 2.5 h, 3.0h, 3.5h, 4.0h, 4.5h, 5.0h, 6.0h, 7.0h, 8.0h, 10h, 12h, 16h, 24h, 30h and 36h.

Pharmacokinetic Analysis:
AUC, maximum plasma activity (Amax) and time of its appearance (Tmax), mean residence time (MRT), absorption half-life, distribution volumes (Vdβ and Vdss), Cls and t1/2β were determined for anti-Xa activity.

Analytical Method:
Amidolytic anti-Xa and anti-IIa assays were used on ex-vivo plasma samples. This method has been described in previous submissions. Quality control information for the current assays was provided with the individual study report and was satisfactory. Chronometric assays were also used: [redacted], aPTT, PT and thrombin clotting time (TCT).

Dates of analytical assays: August 7 - December 10, 1991 (for anti-Xa and anti-IIa activity) September 11 - February 4, 1992 (for [redacted] APTT, PT and TCT).

RESULTS:
Pharmacokinetics:
Table 1- Anti-Xa concentrations (IU/mL) shown as mean ±SD:

DOSE mg/Kg	A_{24h}	A_{36h}	Amax	Normalized Amax (based on IU/mL) $X10^{-5}$
1.0	0.071 ±0.01 range =0.054-0.096	0.029 ±0.02 range =0.00-0.045	1.001 ±0.144 range =0.734-1.315	13.3±2.3
1.25	0.108 ±0.02 range =0.079-0.155	0.054 ±0.01 range =0.034-0.077	1.279 ±0.119 range =1.083-1.477	13.8±1.89
1.5	0.125 ±0.03 range =0.093-0.177	0.054 ±0.01 range =0.037-0.072	1.470 ±0.169 range =1.178-1.730	13.1±1.68
2.0	0.189 ±0.04 range =0.148-0.328	0.075 ±0.02 range =0.046-0.117	1.846 ±0.160 range =1.587-2.122	12.5±1.90

The Amax is linearly related to dose, however, as can be noted the line from the linear

regression analysis does not go through the origin.

Table 2: AUC for Anti-Xa activity (IU.h/ML) showing Mean ±SD

DOSE mg/Kg	$AUC_{24\ h}$ IU Anti-Xa.h/mL	AUC_{36h} IU Anti-Xa.h/mL	AUC_{inf} IU Anti-Xa.h/mL	Normalized AUCinf (based on anti-Xa dose)
1.0	10.13 ±0.89 range =8.56 -11.72	10.679 ±0.93 range =9.15-12.34	10.64 ±0.92 range =9.09-12.14	0.00142 ±0.00016
1.25	13.67 ±1.05 range =12.17-15.41	14.455 ±1.01 range =12.94-16.49	14.49 ±1.22 range =12.73-16.53	0.00156±0.00012
1.5	16.47 ±1.50 range =14.19-18.85	17.45 ±1.66 range =14.97-20.20	17.45 ±1.69 range =14.94-20.24	0.00158±0.00015
2.0	21.94 ±1.31 range =20.17-24.57	23.36 ±1.52 range =21.53-26.49	23.57 ±1.74 range =21.64-27.80	0.00156±0.00014

Table 3: MRT, Vdβ and Cls for Anti-Xa activity showing Mean ±SD

DOSE mg/Kg	MRT(h)	Vd_β L	Cls/F L/h
1.0	8.5 ±0.9 range =6.9 -10.4	5.12 ±0.81 range =3.76-6.54	0.72 ±0.09 range=0.59-0.84
1.25	9.2 ±0.8 range =7.8-10.5	4.87 ±0.41 range =4.3-5.81	0.65 ±0.06 range=0.54-0.73
1.5	9.3 ±0.7 range =8.3-10.9	5.09 ±0.59 range =3.91-6.17	0.64 ±0.06 range=0.59-0.81
2.0	9.9 ±1.0 range =8.9-13.1	5.38 ±0.88 range =4.18-8.03	0.64 ±0.07 range=0.55-0.77

Results for the [redacted] Mean±SD

DOSE mg/Kg	A(Δt) max (sec)
1	+ 63.05 ± 17.21
1.25	+ 77.35 ± 14.46
1.5	+ 85.06 ± 26.42
2.0	+ 105.6 ± 25.14

A(Δt) max was shown to be linearly related to dose [A(Δt) max(sec) = 39.51 + 0.004*Dose

$p<0.001$, $r=0.4806$.

Results for aPTT Mean±SD

DOSE mg/Kg	A max (s)	Tmax (h)	A(Δt) max (s)
1	41.04 ± 4.58	3.1 ±1.1	11.46 ± 2.2
1.25	43.39 ± 5.56	3.5 ± 1.3	13.35 ± 2.36
1.5	44.84 ± 6.55	3.5 ± 1.2	15.64 ± 3.39
2.0	51.41 ± 7.42	3.5 ± 0.9	21.86 ± 5.13

A(Δt) max was shown to be linearly related to dose [A(Δt) max(sec) = 0.765 +10.448*Dose $p<0.001$, $r=0.744$

Mean peak plasma concentration was around 3.5 hours for anti-Xa activity.

Compared to earlier studies, the anti-IIa activity could be more accurately measured due to the higher doses that were being used in this study. Mean peak plasma anti-IIa activity was recorded around 4.5 hours post-dose.

Table 4- Anti-IIa concentrations (IU/mL) shown as mean ±SD:

DOSE mg/Kg	A_{10h}	A_{12h}	Amax
1.0	0.021 ±0.02 range =0.00-0.062		0.108 ±0.045 range =0.042-0.192
1.25	0.058 ±0.02 range =0.031-0.090	0.026±0.03 range =0.00-0.069	0.148 ±0.026 range =0.111-0.183
1.5	0.070 ±0.03 range =0.03-0.123	0.031 ±0.03 range =0.0-0.077	0.179 ±0.047 range =0.103-0.256
2.0	0.122 ±0.03 range =0.089-0.215	0.068 ±0.03 range =0.044-0.157	0.264 ±0.049 range =0.172-0.342

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Table 5: AUC, Cls/F for Anti-Xa activity (IU.h/ML) showing Mean ±SD Also the Ratio Anti Xa/IIa activity.

DOSE mg/Kg	AUC_{0-t}	Cls/F	Ratio AUC Anti-Xa/Anti-IIa
1.0	0.639 ±0.255 range =0.239-0.976	4.047 ±2.06 range =2.03-8.72	20.3 ±10.7
1.25	1.071 ±0.169 range =0.865-1.300	2.50 ±0.40 range =1.96-3.27	13.8±2.0
1.5	1.305 ±0.317 range =0.789-1.860	2.56 ±0.59 range =1.65-3.84	14.0±3.1
2.0	2.117 ±0.405 range =1.409-2.892	2.05 ±0.37 range =1.48-2.74	11.3±1.8

Note that:

It is important to note that the ratio of anti-Xa to anti-IIa activity in terms of AUC is 3 to 5.5 times higher that the potency ratio of 3.56 reported in the original submission on enoxaparin.

CONCLUSIONS:

1. Enoxaparin shows approximate dose proportionality for Anti-Xa activity based on clearance, normalized AUC and normalized Amax. Enoxaparin shows approximate dose proportionality for Anti-IIa activity based on clearance except at the 1.0 mg/Kg dose. This deviation at the lower dose may be related to the lower levels being measured rather than some other mechanism such as induction. The study was also a parallel design, so this needs to also be taken into account when making conclusions. The original submission for enoxaparin showed dose proportionality for anti-Xa activity from 20 to 80 mg of enoxaparin given subcutaneously.

2. It is important to note that the ratio of anti-Xa to anti-IIa activity in terms of AUC is 3 to 5.5 times higher than the potency ratio of 3.56 reported in the original submission on enoxaparin.

Protocol Report RP 54563Q-133

Title: A phase I, open, randomized, three period crossover study comparing the pharmacokinetic profile of two formulations of RP54563: single concentration ampoules (1 shot vs. 2 shots) versus double concentration ampoules, administered as 1.5 mg/Kg once a day subcutaneous treatment for 5 days to healthy males and females.

[redacted]

Study dates: August 03 to November 14, 1995

Objective: 1. Establish the pharmacokinetic profiles of two formulations of RP54563, administered as a once a day S.C. 1.5 mg/Kg dose for 5 days (100 mg/mL in 1 mL ampoules vs. 200 mg/mL in 1 mL ampoules).

2. To verify that the 200 mg/mL concentration does not affect the pharmacokinetic parameters of RP54563 (compared with 100 mg/mL concentration)

Assay dates: Sept. - Dec., 1995.
Assay site: RPR, Antony Cedex, France.

Batches: CB 06071 100 mg/mL 1 mL ampoules
CB06053 200 mg/mL 1 mL ampoules

Demographics:

All subjects were Caucasian

	MEAN ± SD	RANGE
AGE (YEARS) MALE FEMALE	25.8 ± 4.0 23.4 ± 2.6	21 - 32 18 - 28
WEIGHT (KG) MALE FEMALE	73.2 ± 7.67 57.7 ± 7.15	58 - 88 49 - 70
HEIGHT (CM) MALE FEMALE	180.1 ± 8.0 165.2 ± 6.0	168 - 191 152 - 175

METHODOLOGY:

Study design:

This was an open-label, randomized, three period, crossover study. 24 healthy *male* and *female* subjects completed the study.

TRT A: a single 1.5 mg/Kg dose by sc injection of enoxaparin at a concentration of 100 mg/mL
TRT B: a single 1.5 mg/Kg dose by sc injection of enoxaparin at a concentration of 100 mg/mL, but injected as two concomitant injections of 0.75 mg/Kg in two different body sites
TRT C: a single 1.5 mg/Kg dose by sc injection of enoxaparin at a concentration of 200 mg/mL

All treatments were administered sc for 5 days in a randomized manner and each subject was crossed over to each treatment with a fourteen day wash-out between each treatment.

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Blood sampling:

Days 1, 3 and 4:	sampling was at 0.3 and 12 hours post dose
Day 5:	sampling was at pre-dose, 0.5, 1, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 10.0, 12.0, 16.0 hours post-dose.
Day 6:	Sampling was at 24 and 36 hours post Day 5 dose.

All samples were centrifuged at 1200g for 15 minutes at 4°C . Plasma samples were frozen at -80°C until analysis.

Biological Measurements:

Anti-Xa and anti-IIa activities, [redacted] and APTT were measured.

ANOVA (using proc mix procedure under SAS ™) and the two one-sided tests procedure was used in the statistical evaluation of bioequivalence. Data were analyzed by non-compartmental methods. The biological parameters for maximum activity level and area-under-the activity curve are Amax and AUC, respectively. This applies to both anti-Xa and anti-IIa activity. In addition to AUC, A(Δt) max ([redacted] clotting time prolongation) was used for [redacted]

Assay Methodology:

The amidolytic (chromogenic) assay methodology is the similar to that used in the original NDA 20-164 and is a validated method, report #1498. There is a full description of the assay in Dr. Hisham's July 1992 review. The assay at that time was found to be acceptable. The sponsors have provided assay validation information. Therefore, please refer to this information described in the review dated July 1992. The sponsor provided adequate QC data in Appendix A.5 (vol. 12) to this submission.

[redacted]
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RESULTS:

Arithmetic means ±SD for Amax, AUC_{0-24h}, $AUC_{0-\infty}$ on Day 5.

Anti-Xa Activity:

	Arithmetic		
	Mean ± SD	**CV%**	**Range**
Trt. A			
Amax IU/mL	1.37±0.23	16	0.94-1.95
AUC_{0-24h} h.IU/mL	14.25±2.93	21	8.79 -18.6
$AUC_{0-\infty}$ h.IU/mL	15.68±3.52	22	9.04-21.23
Trt. B			
Amax IU/mL	1.46±0.22	15	0.96-1.87
AUC_{0-24h} h.IU/mL	14.68±2.98	20	9.59-19.32
$AUC_{0-\infty}$ h.IU/mL	16.13±3.517	22	10.64-21.95
Trt. C			
Amax IU/mL	1.45±0.22	15	1.07-1.84
AUC_{0-24h} h.IU/mL	15.43±2.96	19	10.0-20.62
$AUC_{0-\infty}$ h.IU/mL	16.80±3.58	21	10.86-22.84

Anti-IIa Activity:

	Arithmetic		
	Mean ± SD	CV%	Range
Trt. A			
Amax IU/mL	0.23±0.05	20	0.15-0.31
$AUC_{0-8\,h}$ h.IU/mL	1.23±0.28	23	0.79-1.76
$AUC_{0-t\,h}$ h.IU/mL	1.54 ±0.61	40	0.85-3.03
Trt. B			
Amax IU/mL	0.24±0.05	19	0.14-032
$AUC_{0-8\,h}$ h.IU/mL	1.25±0.27	22	0.75-1.73
$AUC_{0-t\,h}$ h.IU/mL	1.52 ±0.56	37	0.75-3.14
Trt. C			
Amax IU/mL	0.26±0.05	19	0.17-0.35
$AUC_{0-8\,h}$ h.IU/mL	1.39±0.33	23	0.90-2.07
$AUC_{0-t\,h}$ h.IU/mL	1.77±0.67	38	1.03 -3.55

[redacted] **Clotting time prolongation:**

	Arithmetic		
	Mean ± SD	**CV%**	**Range**
Trt. A			
A(Δt)max s	104.5±16.6	16	82-139.1
AUC_{0-24h} h*s	1321±219	17	911-1734
$AUC_{0-\infty}$ h*s	1644±420	26	954-2545
Trt. B			
A(Δt)max s	108.4±15.8	15	85.1-145.3
AUC_{0-24h} h*s	1323±187	14	968-1588
$AUC_{0-\infty}$ h*s	1666±381	23	1025-2296
Trt. C			
A(Δt)max s	110.9±17.1	15	83-148.8
AUC_{0-24h} h*s	1401±227	16	1057-1871
$AUC_{0-\infty}$ h*s	1732±4041	23	1126-2595

Statistical results:

Note that single strength vs. double strength enoxaparin was shown to be bioequivalent in a single dose study, PK129, reviewed in April 1996 (NDA 20-164/SE1 (008)). The present study uses doses of enoxaparin (1.5 mg/Kg) likely to be used in the treatment of thrombosis or unstable angina.

ANOVA was using the PROC MIXED procedure in SAS™ including treatment, period, sequence, gender and subject(sequence) in the model.

Anti-Xa activity:

The Amin activities comparing Day 3 to Day 5 were not significantly different for the three treatment groups. Steady-state has probably been reached by Day 3. A gender effect was noticed in terms of the AUC being lower in females, but not on Amax nor AUC(0-24h), $p<0.05$. A statistically significant gender effect ($p<0.0001$) was noticed on parameters such as volume of distribution and terminal half-life, but not on total body clearance.

Anti Xa Activity - Mean Pharmacokinetic parameters (Day 5) for males vs. females ±SD

	Treatment A		Treatment B		Treatment C	
	Male	Female	Male	Female	Male	Female
Amax IU/mL	1.41±0.27	1.33±0.18	1.45±0.21	1.47±0.23	1.5±0.23	1.39±0.19
$A_{average}$ IU/mL	0.65±0.11	0.54±0.11	0.65±0.11	0.57±0.13	0.69±0.12	0.60±0.11
tmax h*	3	2.5	2.5	2.8	3.8	3.3
AUC_{0-24h}	15.55±2.67	12.69±2.67	15.57±3.14	13.79±3.14	16.54±2.84	14.31 ±2.74
AUC_{∞}	17.43 ±3.08	13.93±3.11	17.47±3.12	14.79±3.49	18.31±3.36	15.29±3.24
MRT	10.6±0.8	8.7±1.2	10.4±0.8	8.6±0.8	10.2±1.0	8.8±1.1
Vz/F	5.1±1.3	3.9±0.6	4.7±0.6	3.4±0.5	4.3±0.6	3.4±0.8
Vss/F	6.4±1.3	5.1±0.6	6.3±0.9	4.8±0.6	6.0±1.0	4.8±0.4
CL/F L/h mL/min	0.61±0.12 10.2±2.0	0.60±0.12 10.0±2.0	0.61±0.12 10.2±2.0	0.56±0.10 9.3±1.7	0.59±0.12 9.8±2.0	0.55±0.09 9.2±1.5
t1/2 h	5.8±1.0	4.6±0.8	5.5±0.7	4.2±0.6	5.2±1.1	4.3±0.8

*median

Anti-IIa activity:

Significant treatment differences were observed between A and C on Amax and AUC. Steady-state based on Amin was evident by Day 3. No significant gender effects were noticed on pharmacokinetic parameters except a borderline significance for AUC_{0-t} ($p=0.0324$).