Appendix I

Formulation Summary:

Tables 1 and 2 compare the formulation of Org used in the phase I pharmacokinetic program with the proposed market formulation. The pivotal clinical trials #004-023 and #85148 have used formulation Lot # CP 087143 and CP 084126 respectively (telecon with Dr. Talarico, Medical Officer HFD-180, 10-5-95). All formulations used in pharmacokinetic program and in pivotal clinical studies are similar to the proposed marketed formulation with exception of Lot # CP081083 (used in two phase I pharmacokinetic studies).

Comment: The proposed marketed formulation can be considered bioequivalent to the formulations used in pivotal clinical trials.

Table 1

COMPARATIVE FORMULATION U.S. CLINICAL TRIAL BATCHES VS. MARKET FORMULATION

Drug Substance	Clinical Trials	Proposed Market Formulation
Raw Material Batch #	BP	N/A
Anti-Xa (Units/mg) Anti-Xa/Anti-IIa Ratio	16.1 54	- >22
Drug Product		
Formulation Lot #	CP087143	N/A
Study #	004-025	N/A
Org 10172 (Anti-Xa U/mL)	1250	1250
Sodium Sulfite (Anhydrous) Reagent Grade (mg/mL)	1.5	1.5
Hydrochloric Acid, NF and/or Sodium Hydroxide	to pH 7.0	to pH 7.0
Sodium Chloride[a]	+	+
Water for Injection, USP q.s. to	1.0 mL	1.0 mL
Nitrogen NF	q.s.	q.s.
Fill Volume	1.0 mL	0.6 mL

N/A - Not applicable

[a] – to isotonicity

Table 2

COMPARATIVE FORMULATION NON-U.S. CLINICAL TRIAL BATCHES VS. MARKET FORMULATION

DRUG SUBSTANCE	CLINICAL TRIALS				PROPOSED MARKET FORMULATION
Raw Material Batch # Anti-Xa (units/mg) Anti-Xa/Anti-IIa Ratio	K 8.0 80	BG 16.7 128	BJ 16.2 >162	BK 14.0 140	N/A - >22
DRUG PRODUCT					
Formulation Lot #	CP081083	CP084126/27	CP084132/33	CP086084	N/A
Study #	84063 85035	86026 87001 85023 86007 84063 85024 85011 85025 85014 85026 86025	85035	87001	N/A
Org 10172 (anti-Xa U/mL)	800	1250	1250	1250	1250
Sodium Sulfite (Anhydrous), Reagent Grade (mg/mL)	1.5	1.5	1.5	1.5	1.5
Hydrochloric Acid, NF and/or Sodium Hydroxide	to pH 7.0	to pH 7.0	to pH 7.0	to pH 7.0	to pH 7.0
Sodium Chloride	+	+	+	+	+
Water for Injection, USP q.s. to	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL
Nitrogen NF	q.s.	q.s.	q.s.	q.s.	q.s.
Fill Volume	2.0 mL	CP084126 = 0.6 mL CP084127 = 1.0 mL	CP084132 = 0.6 mL CP084133 = 1.0 mL	1.0 mL	0.6 mL

N/A = not applicable
* = to isotonicity

Four Period Crossover Dose Proportionality/Bioavailability Study for IV and SC Doses of Org in Healthy Male and Female Volunteers

Investigator and Site:

Study Dates: 10-9-92 to 11-3-92
Clinical Phase: I

Objectives: To define the pharmacokinetic profile of Org in healthy male and female volunteers after SC administration, to determine the dose proportionality of SC administered doses of Org over a range of 750-2250 anti-Xa units in healthy normal adult males and females, and to determine the absolute bioavailability of plasma anti-Xa and anti-IIa activity after a SC administered 750 anti-Xa unit dose of Org in healthy normal males and females.

Study Design: It was open label, 4 period, 3 way crossover, randomized dose proportionality study.
Treatments:
1. 750 anti Xa U IV
a. 750 anti Xa U SC
b. 1500 anti Xa U SC
c. 2250 anti Xa U SC

Subjects: A total of 24 subjects (12 male and 12 female) between the age of 19 to 50 years of age participated in this study. One male subject did not complete the study.

Formulation: Org supplied by Organon International in 1.0 ml glass ampules containing 1250 anti-Xa units (Lot #PD096).

Specimens: Blood samples were withdrawn at the time of administration 0 hr and at .08, .25, .5, .75, 1, 1.5, 2, 4, 8, 12, 24, 36, 48 and 72 hr. There was a seven day washout between periods.

Assay:

Results: Tables 1 to 4 summarizes individual and mean pharmacokinetic parameters at different dose levels. Figures 1 and 2 illustrate the dose proportionality. There was trend of lower Cmax and AUClast in males than in females (Figures 3 and 4). The absolute bioavailability of subcutaneous dose was nearly 100%. Plasma anti IIa levels were below quantifiable levels.

Conclusion:
For the subcutaneous route of administration, the doses from 750 to 2250 anti Xa units were found to be dose proportional.

Sheet5 Chart 3

Fig 1

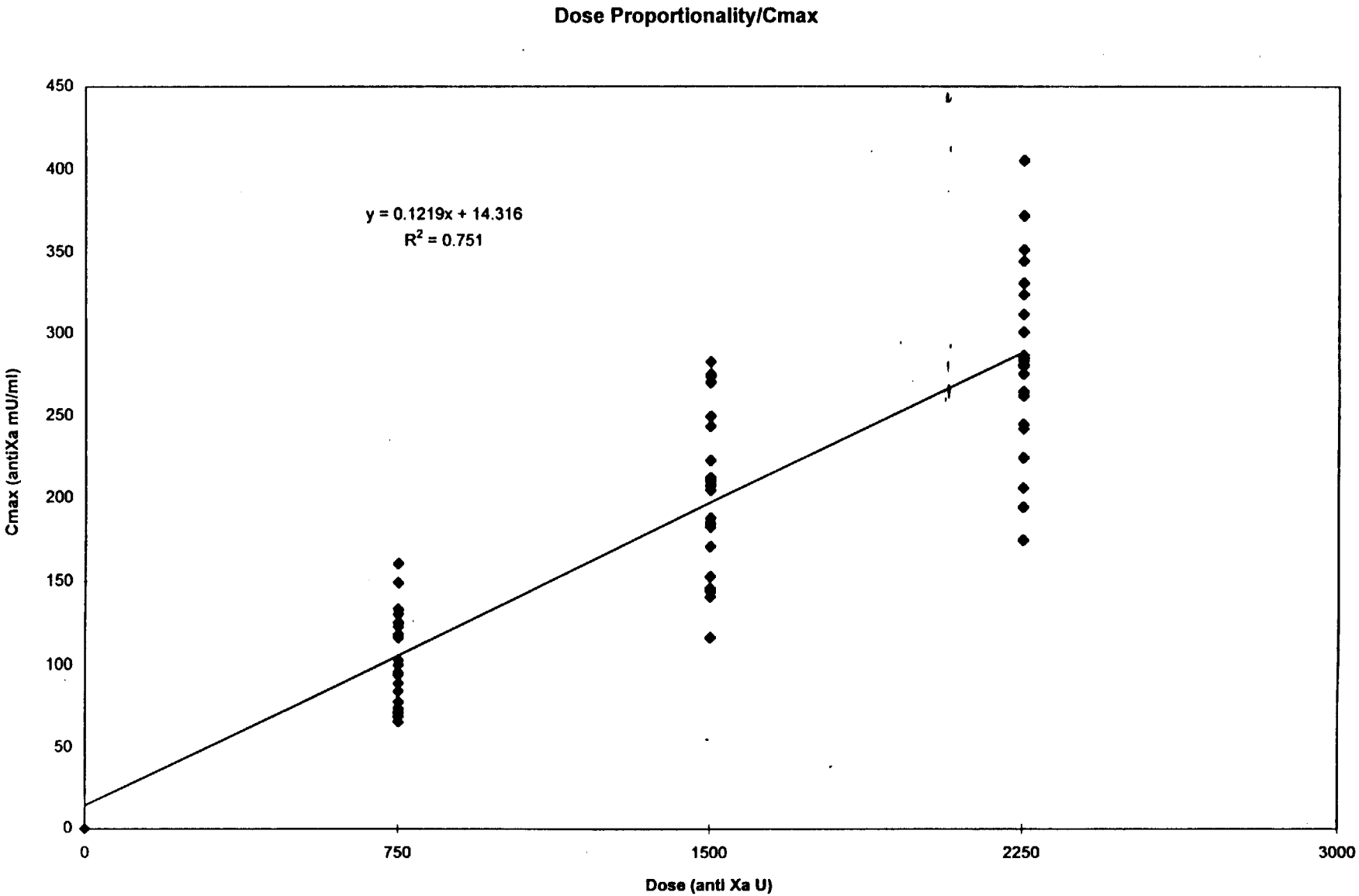
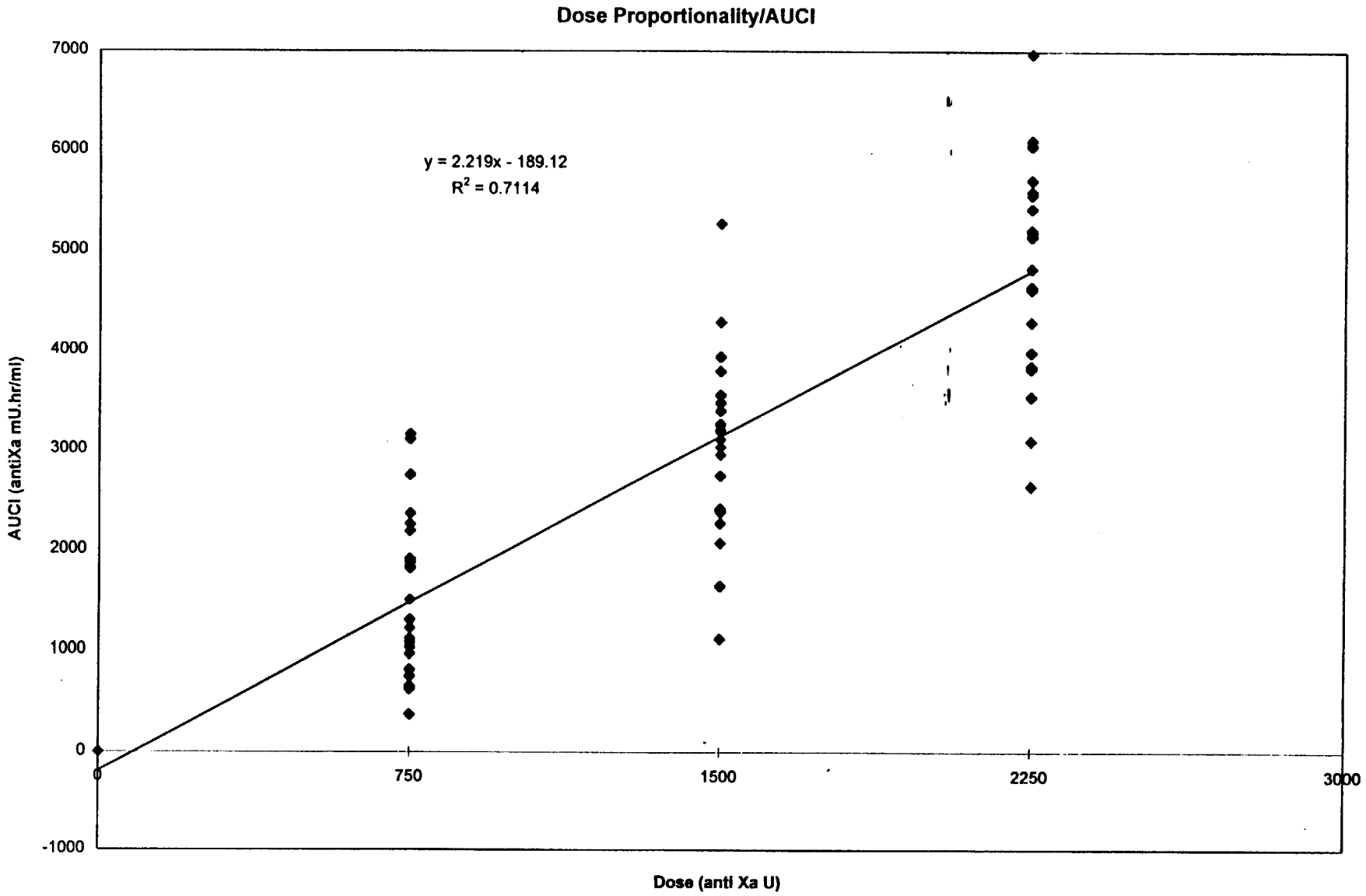


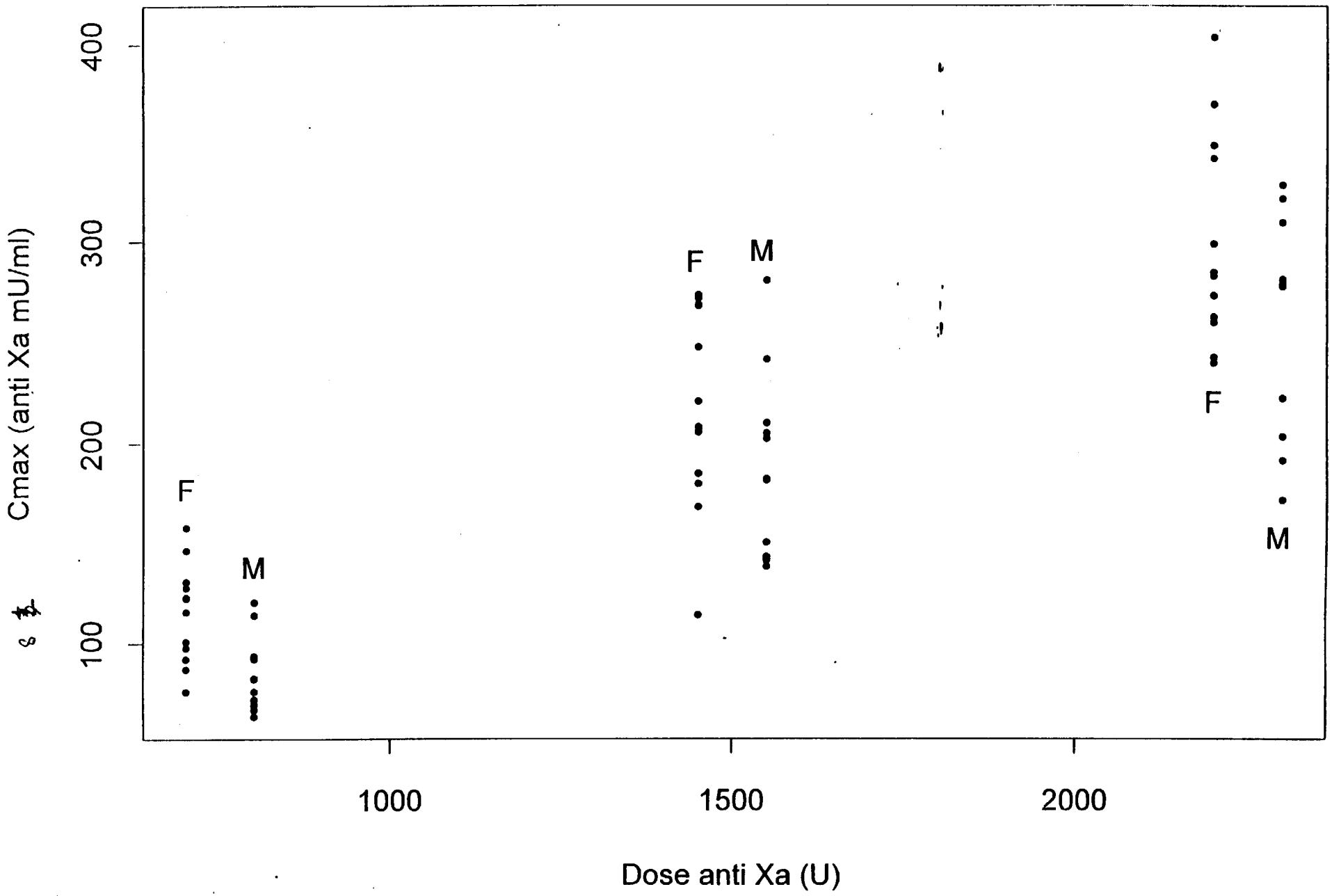
Fig 2


Dose Proportionality/AUCI
y = 2.219x - 189.12
R² = 0.7114
AUCI (antiXa mU.hr/ml)
7000
6000
5000
4000
3000
2000
1000
0
-1000
0
750
1500
2250
3000
Dose (anti Xa U)

Fig

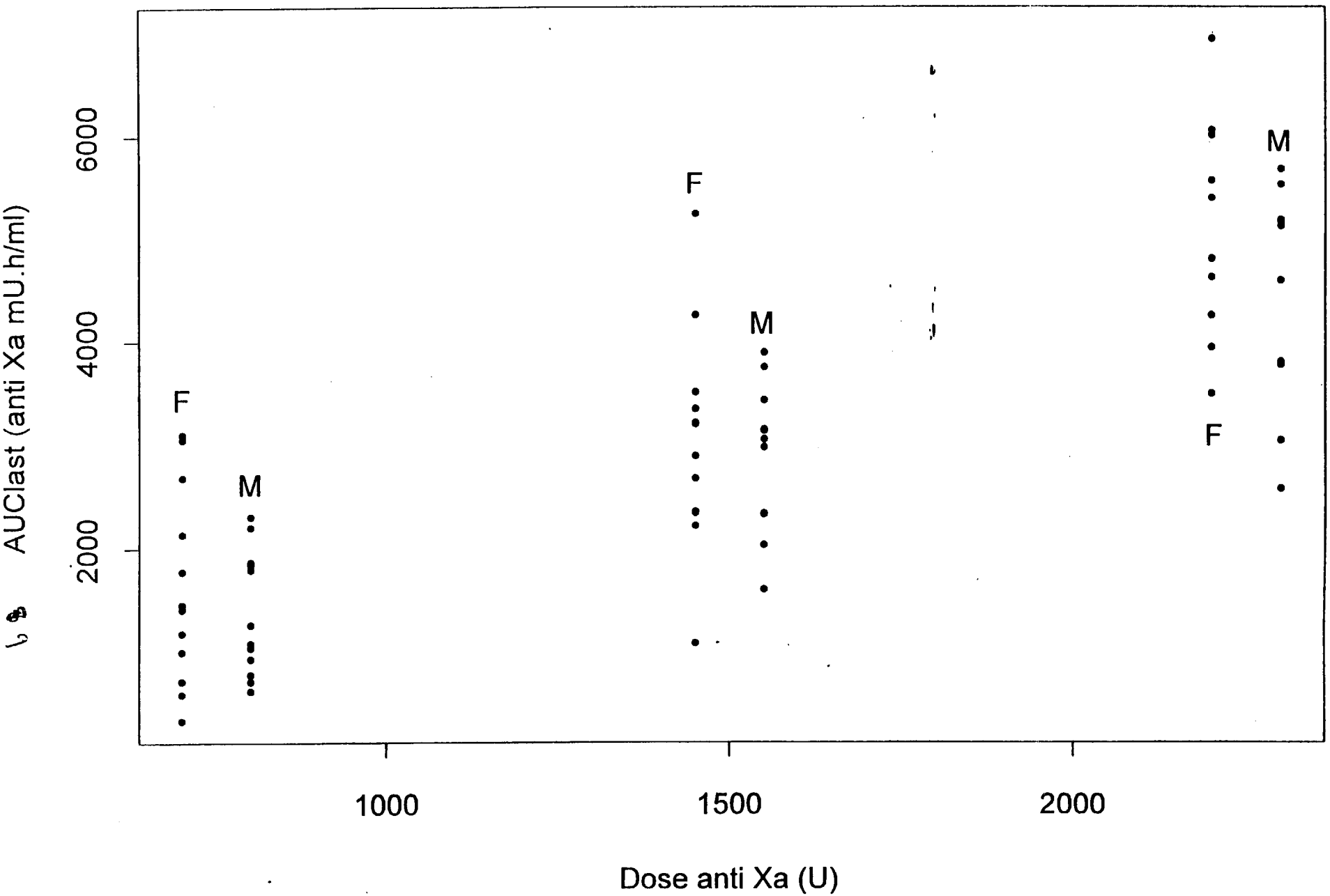
Dose Proportionality (Gender Effect)

Dose levels= 750, 1500 and 2250 anti Xa Units



Dose Proportionality (Gender Effect)

Dose levels= 750, 1500 and 2250 anti Xa Units



Tab
Study t .5
Route = IV

ID	Tmax	Cmax	Beta	t1/2	AUCl(obs)	AUCinf(ob)	AUCinf(pr)	AUMCl	AUMCin(ob)	AUMCin(pr)	MRTl	MRTinf(ob	MRT(pr)
57	0.25	170.32	0.038	18.1	998.9	1456.4	1448.2	7340.0	30238.8	29830.8	7.3	20.8	20.6
58	0.08	240.04	0.126	5.5	947.2	1184.4	1189.2	3891.2	8627.9	8722.4	4.1	7.3	7.3
59	0.25	167	0.013	52.7	1958.2	3431.9	3431.9	31717.6	214398.0	214398.0	16.2	62.5	62.5
60	0.25	170.16	0.097	7.2	759.7	1043.2	1031.7	3001.2	9332.3	9077.1	4.0	8.9	8.8
61	0.08	171.04	0.087	8.0	780.0	1122.1	1122.1	3310.4	11370.1	11370.1	4.2	10.1	10.1
62	0.08	173.96	0.050	13.9	1293.3	1810.5	1782.3	10653.5	33405.1	32163.3	8.2	18.5	18.0
63	0.08	188.48	0.104	6.7	814.0	1099.1	1099.1	3485.8	9652.3	9652.3	4.3	8.8	8.8
64	0.08	246.52	0.020	35.2	1043.4	3081.5	3081.5	4232.3	132333.2	132333.2	4.1	42.9	42.9
65	0.08	268.6	0.018	38.3	1682.2	2712.4	2712.4	17816.5	111845.2	111845.2	10.6	41.2	41.2
66	0.08	205.4	0.056	12.5	1295.3	1640.9	1633.1	9253.9	23771.2	23443.5	7.1	14.5	14.4
67	0.08	248.36	0.008	90.5	1794.4	4255.3	4255.3	27028.8	466604.2	466604.2	15.1	109.7	109.7
68	0.08	216.92	0.048	14.4	1299.5	1777.0	1777.0	10415.2	31784.8	31784.8	8.0	17.9	17.9
69	0.08	292.64	0.053	13.2	1536.7	1963.7	1963.7	10895.8	29257.4	29257.4	7.1	14.9	14.9
70	0.08	188.64	0.199	3.5	553.4	688.6	688.4	1531.6	3292.0	3289.9	2.8	4.8	4.8
71	0.08	253.36	0.006	119.8	1037.4	9007.3	9007.3	4369.5	1477257.0	1477257.0	4.2	164.0	164.0
72	0.08	263.96	0.114	6.1	1040.5	1336.0	1336.0	4239.2	10381.6	10381.6	4.1	7.8	7.8
73	0.08	265.32	0.011	61.2	1664.0	3219.3	3219.3	16796.6	210209.2	210209.2	10.1	65.3	65.3
74	0.08	221.6	0.044	15.6	1284.8	1762.8	1762.8	9698.0	31930.3	31930.3	7.5	18.1	18.1
75	0.08	228.68	0.134	5.2	831.5	1048.3	1039.0	3516.3	7741.8	7560.1	4.2	7.4	7.3
76	0.08	276.08	0.018	39.6	1817.3	2817.2	2817.2	18102.3	111294.5	111294.5	10.0	39.5	39.5
77	0.08	225.92	0.045	15.6	1364.4	1921.8	1921.8	10647.4	36538.3	36538.3	7.8	19.0	19.0
78	0.08	247.72	0.065	10.7	1431.8	1737.1	1734.8	10397.2	22439.5	22348.1	7.3	12.9	12.9
79	0.08	315.44	0.009	78.3	3154.6	5274.2	5274.2	68566.2	460637.1	460637.1	21.7	87.3	87.3
80	0.08	222.76	0.111	6.3	946.1	1253.9	1253.9	4076.6	10546.6	10546.6	4.3	8.4	8.4
	Mean	227.87	0.061	28.2	1305.4	2360.2	2357.6	12291.0	145620.3	145519.8	7.7	33.9	33.8
	SD	42.11	0.050	31.1	546.1	1807.1	1808.3	14247.5	312342.0	312381.8	4.6	39.1	39.1

Ta —

Study 00-

Route = SC

	ID	DOSE	Tmax	Cmax	Beta	t1/2	AUCl(obs)	AUCinf(ob)	AUCinf(pr)	AUMCl	AUMCinf(ob)	AUMCinf(pr)	MRTl	RTinf(ob)	RTinf(pr)
	57	750	3	68.88	0.051	13.64	810.4	1155.1	1142.1	7332.7	22386.5	21821.0	9.05	19.38	19.11
	58	750	3	116.28	0.004	157.61	2357.6	6969.1	6832.4	59680.6	1440298.9	1399372.1	25.31	206.67	204.82
	59	750	2	84.44	0.023	29.59	1836.0	2712.0	2652.7	32885.5	112338.0	106955.2	17.91	41.42	40.32
	60	750	3	84.36			747.2			4121.0			5.52		
	61	750	3	77.88			652.5			3462.6			5.31		
	62	750	1.5	73.92			1073.8			10765.0			10.02		
	63	750	6	71.32	0.042	16.40	1114.8	1753.5	1753.5	11150.4	41589.5	41589.5	10.00	23.72	23.72
	64	750	3	95.72	0.022	31.57	1909.2	2765.6	2676.0	31372.2	111489.0	103103.7	16.43	40.31	38.53
	65	750	3	94.4	0.060	11.66	1298.6	1754.7	1746.8	12442.7	31060.1	30737.2	9.58	17.70	17.60
	66	750	3	95.04			1878.2			25506.4			13.58		
	67	750	2	122.88	0.014	51.22	2254.1	3761.5	3487.7	58317.1	278255.5	238300.5	25.87	73.97	68.33
	68	750	4	65.72	0.059	11.86	965.1	1316.8	1310.1	9308.0	23762.9	23488.6	9.64	18.05	17.93
	69	750	3	133.28	0.064	10.87	1496.7	1869.9	1869.9	12676.8	27485.7	27485.7	8.47	14.70	14.70
	70	750	4	77.96			622.9			3404.4			5.47		
	71	750	2	125.4			748.5			2971.5			3.97		
	72	750	3	118.24	0.068	10.22	1300.2	1622.9	1603.3	10688.7	23192.7	22435.4	8.22	14.29	13.99
	73	750	3	149.16	0.024	29.22	2184.8	3253.7	3002.7	33679.3	130039.0	107410.8	15.42	39.97	35.77
	74	750	3	160.64	0.030	23.45	3157.1	3948.9	3903.6	48158.3	112959.2	109254.9	15.25	28.61	27.99
	75	750	1.5	94.28			372.3			1063.7			2.86		
	76	750	4	124.96	0.009	74.52	2743.2	4932.2	4905.8	69655.6	462613.0	457877.2	25.39	93.79	93.33
	77	750	4	89.28	0.038	18.19	1030.8	1543.0	1543.0	8808.1	34536.4	34536.4	8.54	22.38	22.38
	78	750	4	99.92	0.003	223.09	1816.2	7802.6	8263.7	29889.8	2243974.8	2414504.8	16.46	287.59	292.18
	79	750	4	130.24	0.008	87.32	3107.7	6327.7	6201.7	81120.4	718620.3	693671.3	26.10	113.57	111.85
	80	750	3	103.08	0.073	9.55	1217.9	1489.5	1483.3	10426.8	20685.9	20450.8	8.56	13.89	13.79
Mean			3.125	102.39	0.035	47.65	1529.0	3234.0	3198.7	24120.3	343252.2	344293.8	12.62	62.94	62.14
SD			0.980794	26.13	0.024	59.41	794.4	2110.9	2150.1	23352.2	611154.4	640930.0	7.21	76.52	77.22

Ta 5

Study 06

Route = SC

	ID	DOSE	Tmax	Cmax	Beta	t1/2	AUCl(obs)	AUCinf(ob)	AUCinf(pr)	AUMCl	AUMCinf(ob)	AUMCinf(pr)	MRTl	RTinf(ob)	RTinf(pr)
	57	1500	2	145.8	0.070	9.85	1637.1	1975.8	1975.8	13610.3	26550.8	26550.8	8.31	13.44	13.44
	58	1500	3	184.96	0.037	18.74	2390.1	2916.9	2916.9	26065.2	59276.1	59276.1	10.91	20.32	20.32
	59	1500	3	204.52	0.017	39.94	3936.4	5356.1	5185.7	90253.0	274269.0	252182.9	22.93	51.21	48.63
	60	1500	6	184.28	0.041	16.83	2373.9	2919.8	2881.8	26006.8	58913.4	56624.0	10.96	20.18	19.65
	61	1500	3	143.96	0.034	20.64	2068.6	2895.1	2813.4	25620.6	79980.4	74607.5	12.39	27.63	26.52
	62	1500	4	152.88	0.012	55.78	3477.5	4858.3	4890.0	76909.1	287435.1	292266.1	22.12	59.16	59.77
	64	1500	4	282.84	0.055	12.50	3197.9	3794.7	3726.1	38955.6	71203.9	67501.2	12.18	18.76	18.12
	65	1500	4	207.24	0.030	23.46	3793.5	4435.2	4270.0	78677.0	146606.4	129111.7	20.74	33.05	30.24
	66	1500	3	212.16	0.032	21.74	3186.1	3970.3	3945.2	47881.9	110114.3	108125.3	15.03	27.73	27.41
	67	1500	3	243.68	0.034	20.65	3027.8	3614.1	3573.7	41106.4	86724.3	83575.2	13.58	24.00	23.39
	68	1500	6	140.84	0.007	101.50	3104.0	5880.4	5818.2	68565.5	675043.0	661447.4	22.09	114.79	113.69
	69	1500	2	249.84	0.041	17.13	3554.4	4095.0	4026.2	47965.5	87274.1	82272.4	13.49	21.31	20.43
	70	1500	3	170.88	0.029	23.68	2262.2	2944.1	2944.1	25239.3	73084.6	73084.6	11.16	24.82	24.82
	71	1500	4	273.8	0.012	59.24	4287.4	6328.3	6189.0	86931.7	408290.4	386356.3	20.28	64.52	62.43
	72	1500	3	222.76	0.048	14.32	2946.4	3524.2	3476.1	33163.9	65902.0	63180.9	11.26	18.70	18.18
	73	1500	1.5	275.4	0.037	18.67	3264.8	3739.9	3715.7	41418.1	77017.9	75205.8	12.69	20.59	20.24
	74	1500	2	116.36	0.027	26.07	1110.8	1819.4	1819.4	8634.2	52296.3	52296.3	7.77	28.74	28.74
	75	1500	2	187.76	0.047	14.73	2732.9	3341.5	3297.6	32553.7	67899.7	64886.0	11.91	20.17	19.68
	76	1500	3	270.2	0.039	17.82	3393.9	3960.6	3840.7	43367.2	85142.5	76300.2	12.78	21.50	19.87
	77	1500	4	210.08	0.033	21.36	2391.5	3458.9	3181.3	27310.6	98628.8	80085.7	11.42	28.51	25.17
	78	1500	4	182.52	0.025	28.31	3248.8	4268.2	4195.8	48257.0	138828.8	132391.3	14.85	32.53	31.55
	79	1500	1.5	270.6	0.005	138.18	5264.3	11372.4	11372.4	112813.8	1770258.5	1770258.5	21.43	155.66	155.66
	80	1500	3	207.72	0.081	8.53	2405.1	2850.3	2833.8	20426.0	36589.9	35991.2	8.49	12.84	12.70
Mean			3.2	206.1	0.034	31.73	3002.4	4100.8	4038.6	46162.3	210297.0	204503.4	14.29	37.40	36.55
SD			1.2	49.0	0.019	31.03	894.4	1930.4	1926.9	27120.3	370826.3	370526.1	4.80	34.15	34.15

Tab

Study 00-

Route = SC

	ID	DOSE	Tmax	Cmax	Beta	t1/2	AUCl(obs)	AUCinf(ob)	AUCinf(pr)	AUMCl	AUMCinf(ob)	AUMCinf(pr)	MRTl	RTinf(ob)	RTinf(pr)
	57	2250	3	205.92	0.070	9.92	2627.8	2907.7	2870.3	27814.5	41894.4	40012.8	10.58	14.41	13.94
	58	2250	3	283.4	0.019	36.60	5703.4	6763.6	6789.5	111897.8	244220.8	247452.3	19.62	36.11	36.45
	59	2250	4	331.28	0.028	24.85	5137.9	6016.8	5840.0	106350.9	201133.4	182062.9	20.70	33.43	31.18
	60	2250	2	324.2	0.035	19.65	4610.9	5240.2	4997.8	87132.3	150279.2	125956.6	18.90	28.68	25.20
	61	2250	4	174.72	0.032	21.56	3088.6	3751.7	3682.8	45126.4	97583.5	92130.6	14.61	26.01	25.02
	62	2250	3	194.48	0.047	14.90	3844.3	4328.5	4325.5	61265.8	94914.7	94706.9	15.94	21.93	21.89
	64	2250	6	281.16	0.050	13.97	5148.8	5705.1	5668.3	76685.2	114598.9	112092.7	14.89	20.09	19.78
	65	2250	3	311.96	0.019	37.52	5184.2	6255.9	6260.0	101580.7	236746.8	237260.1	19.59	37.84	37.90
	66	2250	4	281.04	0.016	44.43	5199.3	6791.7	6646.5	100400.7	317121.9	297362.2	19.31	46.69	44.74
	67	2250	3	279.88	0.021	32.42	5552.1	6521.3	6506.9	108150.5	223275.6	221564.3	19.48	34.24	34.05
	68	2250	4	224.44	0.032	21.67	3815.1	4557.8	4509.0	54396.7	113268.0	109396.4	14.26	24.85	24.26
	69	2250	4	344.92	0.019	36.51	6047.4	7313.6	7249.0	111499.1	269366.4	261307.0	18.44	36.83	36.05
	70	2250	6	242.2	0.040	17.57	3537.6	4229.0	4229.0	39140.4	81553.3	81553.3	11.06	19.28	19.28
	71	2250	3	351.64	0.029	23.77	4821.4	5739.1	5738.7	63533.3	139052.1	139018.0	13.18	24.23	24.22
	72	2250	4	301.12	0.020	34.95	5590.1	6636.9	6583.7	103440.9	231605.3	225090.4	18.50	34.90	34.19
	73	2250	2	372.28	0.015	46.65	6093.6	7706.1	7707.2	115510.1	340122.1	340274.8	18.96	44.14	44.15
	74	2250	4	262.2	0.020	34.64	4823.5	5739.1	5671.0	88256.8	199942.9	191631.6	18.30	34.84	33.79
	75	2250	4	286.88	0.019	37.05	5414.6	6526.4	6531.8	101330.4	240811.1	241494.8	18.71	36.90	36.97
	76	2250	6	264.92	0.019	36.45	6039.4	7339.5	7305.6	120552.7	282530.9	278314.6	19.96	38.49	38.10
	77	2250	3	405.44	0.051	13.72	3980.7	4462.0	4355.2	49290.4	81916.9	74677.4	12.38	18.36	17.15
	78	2250	3	244.88	0.031	22.41	4639.6	5547.3	5564.0	67713.6	140620.6	141967.2	14.59	25.35	25.52
	79	2250	4	285.16	0.037	18.94	6970.2	7656.7	7506.3	140097.0	208289.4	193355.8	20.10	27.20	25.76
	80	2250	2	275.44	0.056	12.40	4284.9	4735.7	4630.6	58676.3	88378.3	81455.7	13.69	18.66	17.59
Mean			3.7	283.9	0.031	26.63	4876.3	5759.6	5703.0	84341.0	179966.4	174353.8	16.77	29.72	29.01
SD			1.2	56.2	0.015	10.92	1052.9	1308.6	1311.2	30175.3	83616.1	82963.6	3.16	8.82	8.83

Pharmacokinetics of Org (danaparoid) after IV and subcutaneous administration to elderly volunteers with particular reference to bioavailability

Study# 85014

Investigator and Site:

Study Period: April to June 1985

Objectives: To determine the bioavailability of Org when administered subcutaneously (SC) and to compare the kinetics of various effect parameters of Org after IV and sc administration to healthy elderly male and female subjects.

Study Design: This was a single center, open, randomized, balanced, crossover study. Twelve volunteers, 6 male and 6 female (aged 55-68 yr) participated in the study. Each volunteer received a single i.v. and a single s.c. dose of 3250 anti-Xa units of Org approximately two weeks apart.

Formulations: Injection contained 1250 anti-Xa units of Org per ml (CP 084126/7; batch 10172 BG).

Assay:

Results:

The total glycosaminoglycuronan pharmacokinetic analysis was carried out by the sponsor by fitting the data to a two-compartment open body model superimposed upon a supposedly constant baseline. This base line tended to differ between sexes: its mean ± standard deviation for the 6 male subjects was 910 ± 250 ng/ml, and for the 6 female subjects 590 ± 310 ng/ml. The SC glycosaminoglycuronan data had more variability than IV data. The IV data were used to estimate the disposition parameters and the combined IV and SC data were used to estimate the rate of absorption.

The following table illustrate mean and SD of kinetic parameters of plasma glycosaminoglycuronan.

Parameter	IV	SC
Absorption Half-Life (hr)	1.9 ± 0.7	2.1 ± 0.5
Absolute Bioavailability		86 ± 25.0
Elimination Half-Life (hr)	2.5 ± 0.7	4.5 ± 2.6
Central Distribution Volume (L)	5.5 ± 1.7	4.6 ± 1.5
Total Distribution Volume (L)	13.3 ± 3.9	15.9 ± 7.0
Clearance (L/hr)	5.2 ± 1.3	4.2 ± 1.3

A summary of the pharmacokinetic parameters estimated by route of administration and gender for anti-Xa are listed below:

Parameter	IV Males		IV Females		SC Males		SC Females	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cmax anti Xa mU/ml	764.3	154.8	824.0	94.2	339.5	96.9	431.9	34.1
AUCinf anti Xa mU.hr/ml	8186	4068	8296	1419	7565	2573	8459	1333
CL ml/hr	463.5	174.2	401.8	71.9	475.7	175.4	392.9	66.3
T1/2 hr	21.8	15.3	20.1	10.7	21.8	15.3	20.1	10.7

No significant difference in clearance was observed between the routes of administration. Women showed slightly lower clearance than men.

Anti IIa activity was only determined for subjects 1, 2, 3, 4, 5, 6 and 7. Most of the assayed values were found to be below 16 mU/ml. Due to high variation in data with most time point anti-IIa activity below assay quantitation, pharmacokinetic analysis of anti-IIa activity was not carried out.

Table 1 illustrates the mean hemostasis data. The prolongation of the bleeding time was greater in females than in males after IV and SC administration. The mean APTT values were slightly increased in both groups after IV and SC administration, until 5 hours after administration. Mean thrombin time values were increased until 5 hour after IV administration but not after SC administration.

Conclusion:
The study shows that SC administration of Org has close to 100% bioavailability as measured by plasma levels of anti-Xa activity. Elderly women showed slightly lower clearance than elderly men.

TABLE 9
MEAN HEMOSTASIS DATA

Parameter (Unit)	Route of Administration	Time of Assessment (h)	Male Volunteers (n=6)		Female Volunteers (n=6)	
			Mean	S.D.	Mean	S.D.
Bleeding Time (Sec)		At Screening	293	60	367	128
	i.v.	+0.25 h	336	97	531	172
	s.c.	+1.5 h	326	36	572	285
APTT (Sec)		At Screening	34.9	4.0	34.3	3.6
	i.v.	0	32.8	2.9	33.3	2.6
		0.25	38.3	3.7	36.7	3.4
		0.5	36.6	1.8	35.4	3.4
		0.75	37.9	3.9	37.3	3.6
		1	35.3	1.1	36.0	4.3
		5	35.2	3.2	36.1	2.7
		24	32.3	3.3	33.1	3.1
		48	32.2	2.7	31.8	3.5
	s.c.	0	31.7	1.7	32.4	2.2
		0.25	32.0	3.9	33.0	3.2
		0.5	34.3	5.8	33.0	2.5
		0.75	35.4	6.7	33.5	2.3
		1	36.6	6.2	34.3	4.0
		5	35.8	4.6	36.5	5.4
		24	32.9	1.9	32.7	3.8
		48	31.8	1.8	31.5	1.7
Thrombin time (Sec)		At Screening	19.2	1.1	19.0	0.7
	i.v.	0	20.4	1.0	20.6	0.9
		0.25	24.2	3.0	25.5	2.6
		0.5	23.2	2.1	23.6	1.7
		0.75	23.4	3.0	24.2	1.2
		1	23.2	2.3	23.3	1.2
		5	21.6	0.6	22.2	0.8
		24	20.2	0.3	20.4	1.9
		48	20.0	0.5	20.6	1.0
	s.c.	0	19.8	1.6	21.2	0.9
		0.25	19.9	0.7	21.1	1.7
		0.5	19.9	1.0	21.6	1.3
		0.75	20.1	0.9	21.1	1.2
		1	19.9	0.9	21.4	1.5
		5	20.6	0.8	23.4	3.5
		24	19.9	1.5	20.9	1.0
		48	20.1	1.3	20.8	1.0

An open, group-comparative, rising dose safety and pharmacokinetic study of Org administered intravenously once daily for 5 consecutive days to healthy male and female volunteers

Study: 81059

Investigator and Site:

Objectives: To assess the safety of iv administration of Org QD for 5 consecutive days. To assess the kinetic parameters of iv administered Org with respect to the effect on plasma anti-Xa activity.

Dosage Form: Injection fluid containing 800 anti-Xa U of Org (CP 081083) per ml., Thromboliquine® (Heparin sodium USP, 5000 IU per ml) and placebo (saline solution CP 081126).

Study Design: The following iv injections were administered daily at 08:00 am for 5 consecutive days:

Group	n	Treatment	Dose
1	6	Org	800 anti-Xa U
2	4	Org	1600 anti-Xa U
3	4	Org	2400 anti-Xa U
4	4	Org	3200 anti-Xa U
5	4	Heparin	5000 IU
6	3	Placebo	-

Specimen: Blood samples were collected for anti-Xa activity at the following time points
Day 1: -10 min, 5, 10, 20, 30 min and 1, 2, 4, 6, 8 and 10 hr
Day 2: -10 min and 10 hr
Day 3: -10 min and 10 hr
Day 4: -10 min and 10 hr
Day 5: -10 min, 5, 10, 20, 30 min and 1, 2, 4, 6, 8, 10 and 24 hr.

Assay: Information on assay of anti-Xa activity is not provided.

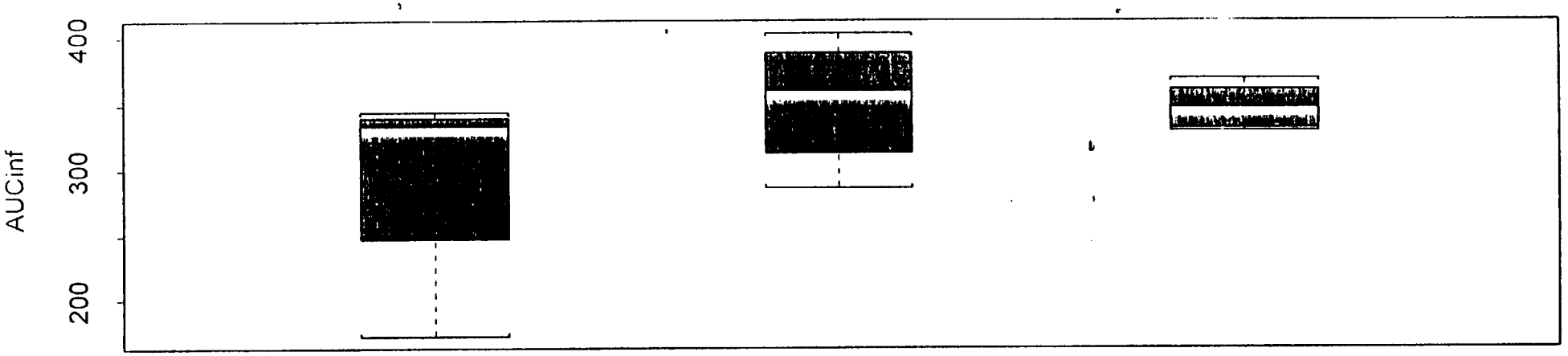
Results: Figure 1 illustrates the dose proportionality over dose range 1600 to 3200 anti-Xa U.

The plasma anti-Xa response in volunteers receiving 5 x 800 anti-Xa U of Org was very low and therefore the sponsor did not evaluate this dose. Figure 1 suggests that pharmacokinetics of Org to be non-linear. However, the comparison shown in Figure 1 is between different subjects. Figures 2, 3 and 4 show pharmacokinetic profiles of anti-Xa activity after QD administration of 1600, 2400 and 3200 anti-Xa U of Org respectively. The accumulation index for QD dosing is about 1.5. For individual subject pharmacokinetic profile, the AUC_{inf} after first dose could predict AUC_{SS}^{T} (area under curve at steady state over dosing period).

No clinically significant changes were seen in the hemostasis parameters investigated (APTT, Thrombin time and PT) for Org. Heparin consistently increased the APTT, Thrombin time and PT.

Conclusion:
Once daily iv administration of Org at doses 800 - 3200 anti-Xa units to healthy young male and female volunteers is well tolerated. No obvious changes are found in the hemostatic parameters apart from the expected dose-related increases in plasma anti-Xa activity. In this parallel group design study, Org at higher doses show a trend towards nonlinearity.

Dose Proportionality



Dose Proportionality

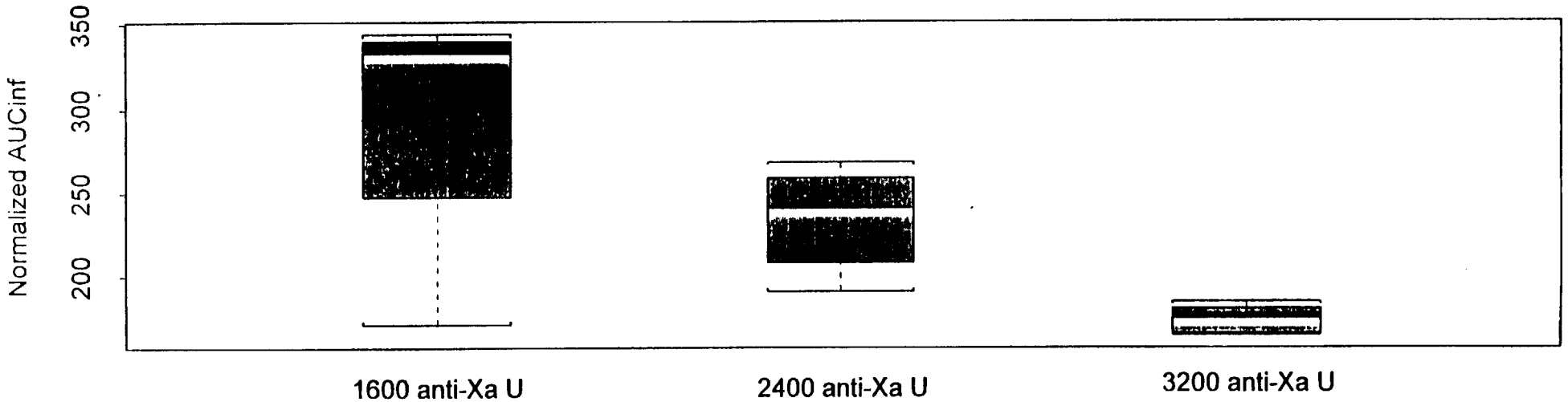


fig 2

Individual best-fitted curves of plasma anti-Xa effect.

Group 2 (1600 anti-Xa u Org 10172)

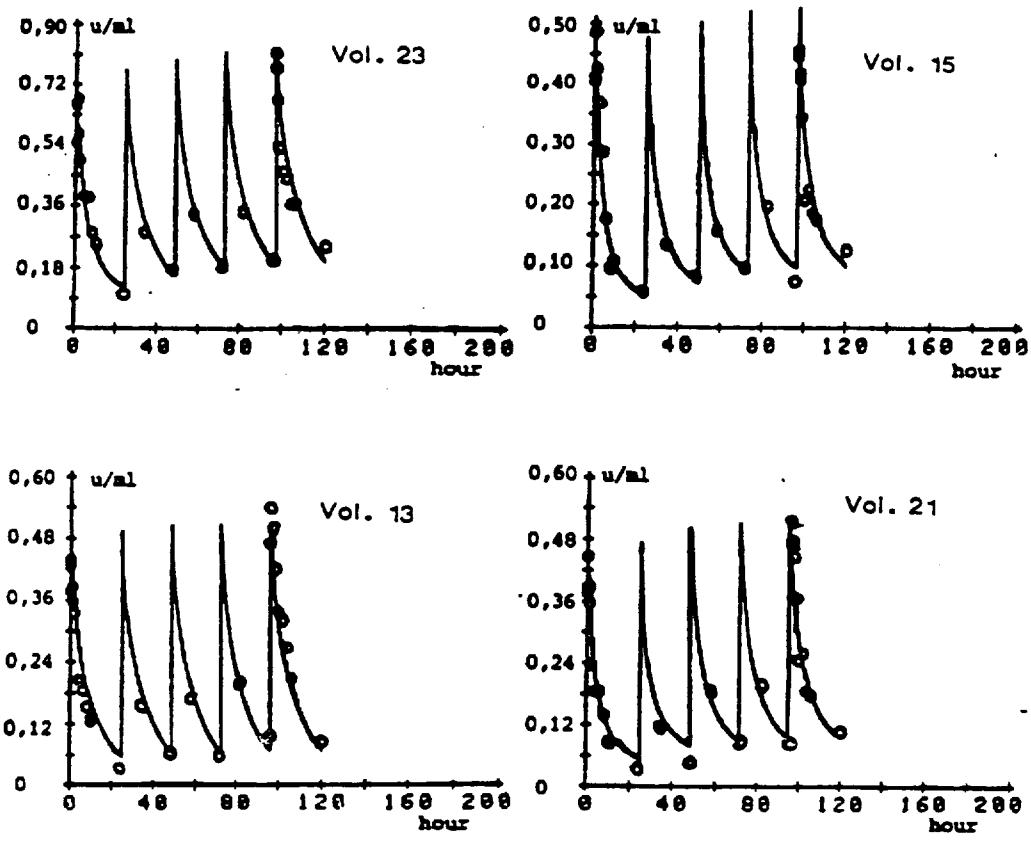


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Fig 3

Group 3 (2400 anti-Xa u Org 10172)

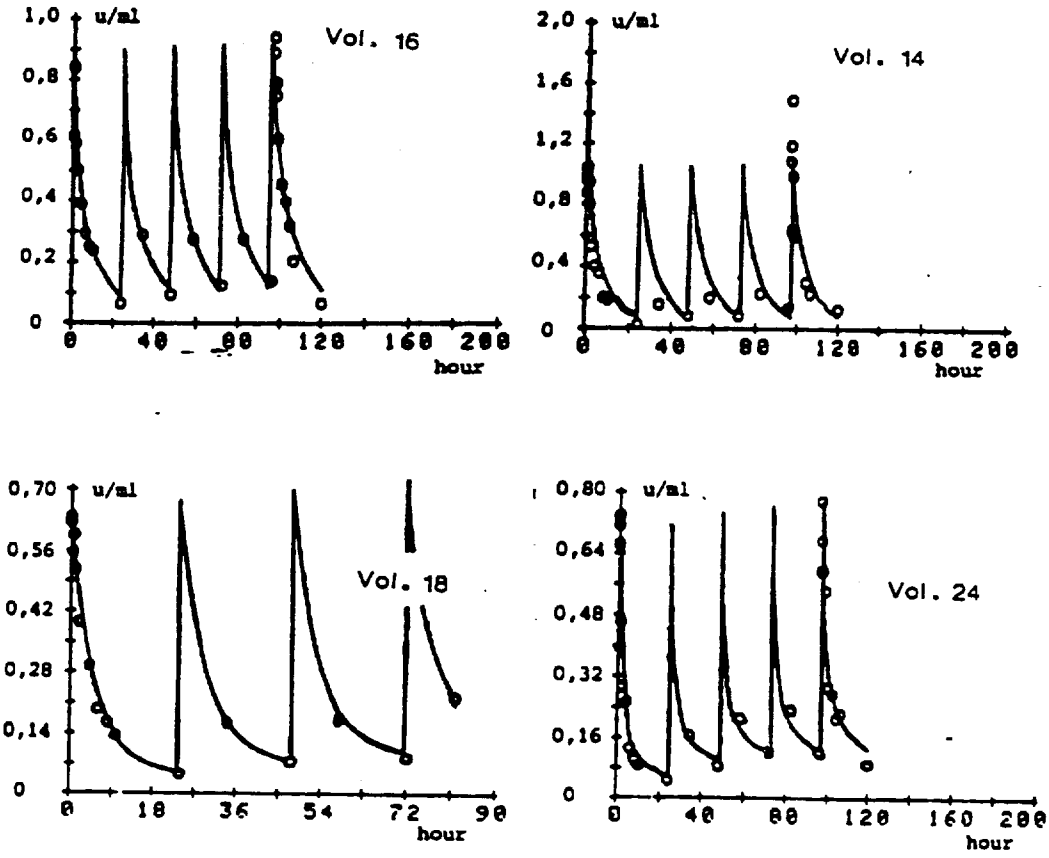
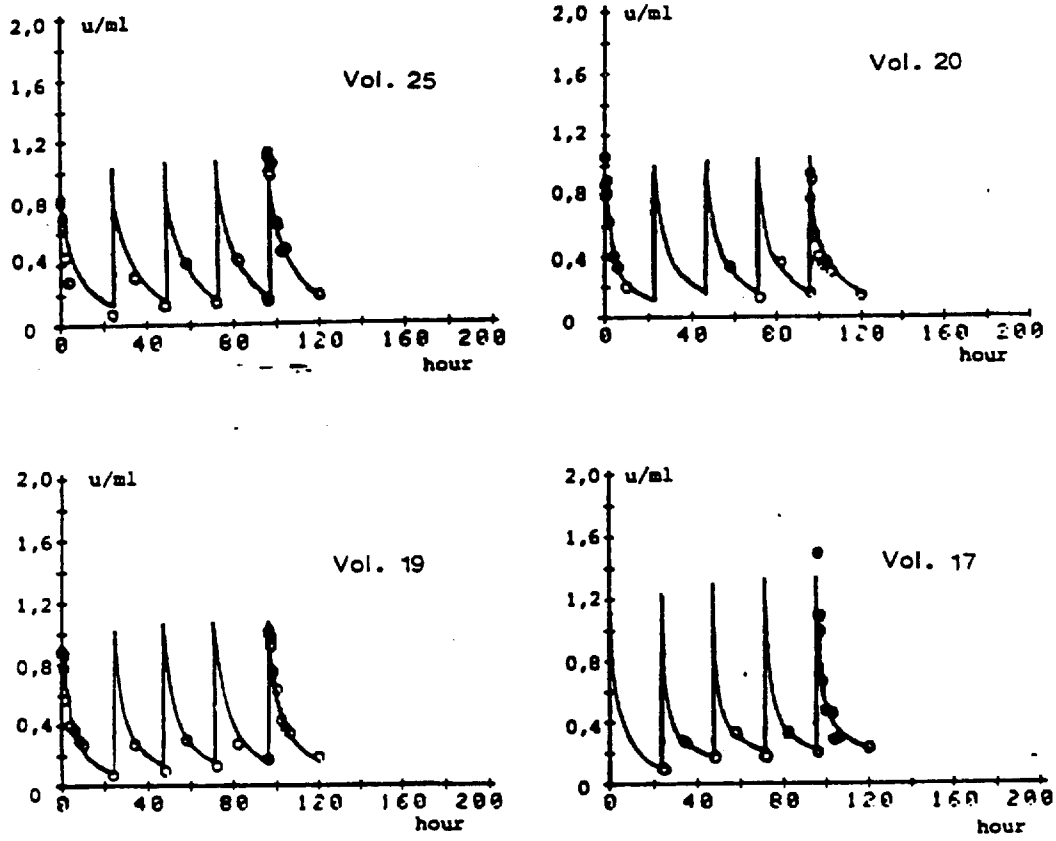


Fig 4

Group 4 (3200 anti-Xa u Org 10172)



An open, safety and pharmacokinetic study of two doses of Org administered subcutaneously twice daily for 5.5 days to healthy male volunteers

Study# 83018

Investigator and Site

Study Period: March to June 1983

Objectives: To ascertain the tolerance and safety. To assess the kinetic parameters of the plasma anti-Xa activity. To examine the relationship between the amidolytic and clotting assay of plasma anti-Xa activity.

Study Design: Two groups of 8 volunteers each received subcutaneous injections of Org twice a day for 5.5 days (11 doses in total) according to the following schedule:

Treatment Group	Dose of Org (anti-Xa U)				Expected plasma peak levels of anti-Xa activity[b]
	Day 1		Day 2-6		
	09:00 h	18:00 h	09:00 h	18:00 h[a]	
1	1200	800	480	800	0.19-0.22 u/ml
2	1800[c]	1200	720	1200	0.22-0.30 u/ml

a: does not given on Day 6
b: SS peak levels to be expected after 3 or 4 doses
c: administered as a divided dose into 2 separate anterior abdominal wall sites

Subjects: Sixteen healthy male subjects, aged 19-46 years, were enrolled into this study.

Formulations: Injection fluid containing 800 anti-Xa u of Org (CP 081083) per ml.

Assay:

Results: Figure 1 and 2 show the best fitted curves for the plasma anti-Xa levels, derived from clotting and the amidolytic assays, respectively. The clotting assay showed larger inter-subject variability in anti-Xa levels than did the amidolytic assay. Figures 3 and 4 show the individual data derived from the clotting assay versus the amidolytic assay. The mean PK parameters obtained from two different assays are shown in the following table. Figure 5 to 8 show simulated SS anti-Xa levels for different dose regimens.

Parameters	Clotting Assay			Amidolytic Assay		
	Mean	Range	SD	Mean	Range	SD
t1/2 (hr) eli	16.4	12.8 - 26.3	3.3	18.8	13.0 - 29.3	3.5
t1/2 (hr) abs	0.7	0.3 - 0.9	0.2	0.9	0.6 - 1.6	0.3
Vapp (l)	8.9	4.0 - 14.6	2.6	7.0	5.5 - 10.6	1.3
CL (ml/h)	383	168 - 599	116	262	218 - 349	37

Haemostasis: No obvious effects of Org on AT-III level, platelet reactivity towards ADP and fibrinogen were observed.

Comment: This study was reviewed only to get an idea about SS anti-Xa activity levels after bid dosing. This study does not have assay validation information. This was a serious deficiency and therefore making conclusions based on this data was difficult.

In Figure 1 to 4, the sponsor do not show how well the predicted values fit the observed concentrations. A residual plot would have been very useful.

Conclusion:
BID subcutaneous injection of Org at a daily dose of 1280 or 1920 anti-Xa u is safe. The clotting and amidolytic assay allow monitoring of plasma anti-Xa levels, but the individual data derived from the two assays show a poor correlation. Accumulation index for BID dosing ranged from 2.5 to 2.8.

7 pages
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Study of possible drug interaction between Org (danaparoid) and Acenocoumarol in six healthy male volunteers

Study # 85023

Investigator and Site:

Study Period: 05-05-1986 to 10-17-1986

Objectives: To investigate the effect of the combined treatment on the pharmacokinetics and pharmacodynamics of Org and acenocoumarol.

Study Design: This was multicenter, randomized, cross-over study in subjects between the age of 19 to 24 yr. Two groups of three volunteers were randomly assigned to treatment order B, C, A (group 1) or A, C, B (group 2). The between-treatment "wash-out" period were as follows:

For group 1; between treatment B and C = 24 days
between treatment C and A = 14 days
For group 2; between treatment A and C = 5 days
between treatment C and B = 12 days

Treatment A: On Day 1, 3250 anti Xa units of Org were administered intravenously over a period of 3 -5 min.
Treatment B: On Days -1, 0 and 1 oral treatment with acenocoumarol was given at doses of 6, 4, and 2 mg respectively. Starting on Day 2, there was daily acenocoumarol administration in doses that were individually adjusted to keep the thrombotest values between 10% and 15%. When steady-state thrombotest values were reached (after at least 14 days of acenocoumarol administration), one dose of 3250 anti Xa units of Org was administered intravenously. Acenocoumarol administration was continued at the individually adjusted dose for two more days.
Treatment C: On Days -1, 0 and 1 oral treatment with acenocoumarol was given at doses of 6, 4, and 2 mg respectively. On day 1, the volunteers were given 3250 anti-Xa units of Org intravenously.

Specimens: Blood samples were withdrawn at the times of Org administration 0 hr and at 0.25, 0.5, 1, 1.5, 2, 3.5, 5, 6.5, 8, 10, 13, 16, 24, 28, 32, 36, 40, 48, 56 and 72 hr.

Assay: Same as study 85014.

Hemostasis Parameters: The influence of liver enzyme induction on the pharmacodynamics of Org was sought on the basis of coagulation tests. The coagulation tests, thrombin time, APTT, PT and thrombotest were carried out. The tests were carried out on a KC 10

coagulometer after adding calcium chloride 0.025 M and the following reagents: thrombin reagent, automated APTT-reagen calcium thromboplastin and thrombotest reagen, respectively.

Results: Specific haemostasis variables are summarized in Table 1 to 2. The Thrombotest was affected for up to 5 hr after the iv administration of Org. The pharmacokinetic parameters estimated with and without acenocoumarol and during steady-state are shown below.

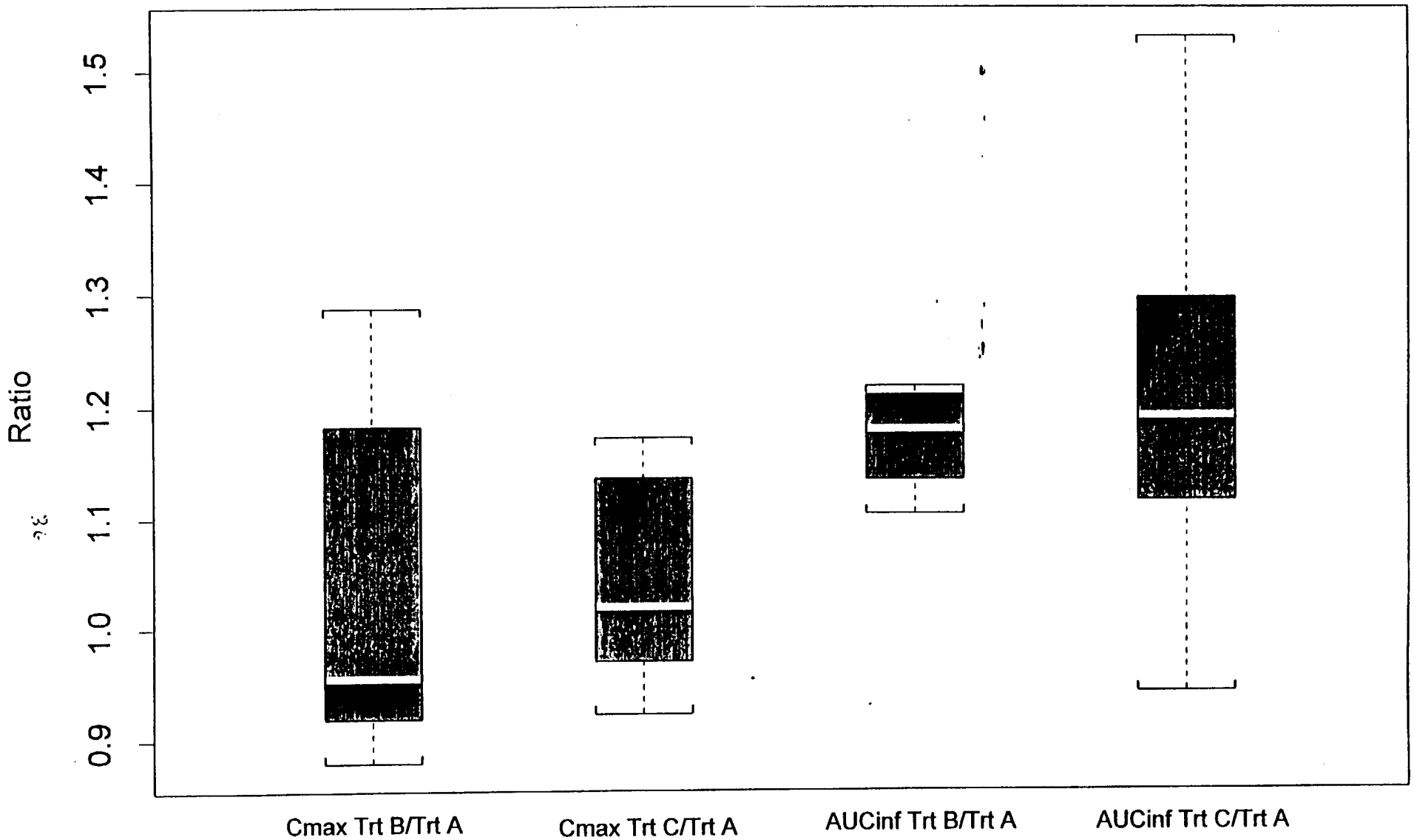
Parameter	Unit	Trt A	Trt B	Trt C
Cmax	mU/ml	738	772	763
Tmax	hr	0.25	0.25	0.25
AUCinf	mU*hr/ml	7629	9138	8945
Vss	ml	10201	8053	10132
$t_{1/2}$	hr	19.8	16.1	23.4

LS means

Figure 1 show the boxplots of ratios of Cmax and AUCinf for the three treatments. The Org anti-Xa activity was found to have approximately 17% lower AUCinf in the absence of acenocoumarol treatment.

Conclusion:
There appears to be a reduction of the plasma clearance of anti-Xa activity when Org at a dose of 3250 U was administered with acenocoumarol. This resulted in approximately 17% higher AUCinf for anti-Xa activity.

Drug Interaction of Danaparoid with Acenocoumarol



Prothrombin time (expressed as percentage of the control plasma)

Treatment scheme*	Time of assessment	Volunteers 1	2	3	4	5	6
A	0						
	15 min						
	30 min						
	60 min						
	5 h						
	24 h						
	48 h						
	72 h						
B_1	-48 h						
	-24 h						
	0						
	5 h						
	10 h						
	15 h						
	24 h						
B_2	0						
	15 min						
	30 min						
	60 min						
	5 h						
	24 h						
	48 h						
	72 h						
C	0						
	5 h						
	10 h						
	15 h						
	24 h						
	48 h						

* A : Covers a period of 72 hours after injection of Org 10172

B_1: Covers a period of 24 hours after 3 days of Acenocoumarol (6/4/2 mg) administration

B_2: Covers a period of 72 hours after injection of Org 10172 and continuation of daily administration of an optimal dose of acenocoumarol at time points 0, 24 and 48 hours

C : Covers a period of 48 hours after injection of Org 10172 and after 3 days of acenocoumarol (6/4/2 mg) administration

Thrombotest (expressed as coagulation time in s)

Treatment scheme*	Time of assessment	Volunteers 1	2	3	4	5	6
A	0						
	15 min						
	30 min						
	60 min						
	5 h						
	24 h						
	48 h						
	72 h						
B_1	-48 h						
	-24 h						
	0						
	5 h						
	10 h						
	15 h						
	24 h						
B_2	0						
	15 min						
	30 min						
	60 min						
	5 h						
	24 h						
	48 h						
	72 h						
C	0						
	5 h						
	10 h						
	15 h						
	24 h						
	48 h						

* A : Covers a period of 72 hours after injection of Org 10172

B_1: Covers a period of 24 hours after 3 days of Acenocoumarol (6/4/2 mg) administration

B_2: Covers a period of 72 hours after injection of Org 10172 and continuation of daily administration -0,24 h, 48 h- of optimal dosage of Acenocoumarol

C : Covers a period of 48 hours after injection of Org 10172 and after 3 days of Acenocoumarol (6/4/2 mg) administration

Study of possible drug interactions between Digoxin and Org in six healthy male volunteers

Study # 85024

Investigator and Site:

Objectives: To investigate the effect of digoxin on the pharmacodynamics and pharmacokinetics of Org.

Dosage Form: Digoxin 0.25 mg tablets (Lanoxin, Wellcome, Beckenham, Kent, U.K.)
Org was supplied by Organon, Oss, The Netherlands, as injection fluid containing 1250 anti-Xa units of Org per ml (CP 084126; 0.6 ml per ampule and CP 084127; 1.0 ml per ampule).

Study Design: Six subjects participated in a randomized, three period, three sequence, crossover-design trial.

Treatment A Org 3250 anti-Xa units was given IV over 3 to 5 minutes.
Treatment B Oral treatment with digoxin 0.25 mg once daily was given for eight days. With seventh dose of digoxin 3250 anti-Xa units of Org was administered intravenously.
Treatment C Oral treatment with digoxin 0.25 mg once daily was given for eight days. No Org was administered in this treatment.
Washout periods of at least one week were maintained between the three treatments. The subjects received normal diet and were abstained from alcohol and drugs.

Specimens: Blood samples were collected for anti-Xa assay at following times for treatment A and B.
At screening, 0, 1/4, 1, 1.5, 2, 3.5, 5, 6.5, 8, 10, 12, 18, 24, 28, 32, 36, 48, 56 and 72 hours.
Ten mililiter blood samples were collected for digoxin assay at following times for treatment B and C.
-24 hr, 0, 0.5, 1, 1.5, 2, 3. 5, 6.5, 8, 10, 24, 48, 72, 96 and 120 hours.
For treatment C blood sampling (10 ml) was done only for digoxin at following times.
0, 0.5, 1, 1.5, 2, 3, 5, 6.5. 8, 10, 24, 48, 72, 96 and 120 hours.

Assay: Anti-Xa activity was assayed in the same way as study 85014.
Digoxin concentrations in serum and urine were determined by means of a digoxin solid-phase, radioimmunoassay method The mean recovery of spiked samples was 92%. The intra-assay and inter-assay variation (%CV) of serum samples was 4.7% (n=60) and 5.2% (n=60) respectively. The detection limit of the method was 0.1 ng/ml. Org was shown not to interfere with this analysis.

Hemostasis Parameters: The influence of liver enzyme induction on the pharmacodynamics of Org was sought on the basis of coagulation tests. The coagulation tests, thrombin time, APTT, PT and thrombotest were carried out. The tests were carried out on a KC 10 coagulometer after adding calcium chloride 0.025 M and the following reagents: thrombin reagent , automated APTT-reagen calcium thromboplasti and thrombotest reagent , respectively.

Results:

Kinetics of anti-Xa activity of Org: Fig. 1 shows the plasma anti-Xa activity time curves after Org alone and during the combined treatment. The following table show the pharmacokinetic parameters (mean ± sd) for Org with and without digoxin.

Parameters	Units	Org alone	Org with Digoxin
AUC	mU*hr/ml	7715 ± 1090	7117 ± 917
Cmax	mU/ml	688 ± 92	638 ± 59
AUCinf	mU*hr/ml	9997 ± 1842	9214 ± 1544
Clinf	ml/hr	335 ± 65	361 ± 61
Vss	ml	14141 ± 2455	12792 ± 1901
$t_{1/2elim}$	hr	31.7 ± 11.2	26.1 ± 8.4

Digoxin produced a slight increase in the clearance of plasma anti-Xa activity.

Pharmacokinetics of digoxin: The average time course of serum digoxin following the seventh digoxin tablet after digoxin alone and on combined treatment is presented in Fig. 2. The following table demonstrates the pharmacokinetic interaction.

AUC (ng.ml/hr)		CLr (ml/min) (144-168 hr)		Cavg (ng/ml) (144-168 hr)	
-Org	+Org	-Org	+Org	-Org	+Org
20	17	120	126	0.83	0.72
5	4	18	9	0.18	0.18

In the presence of Org there was a reduction in the average serum concentration of digoxin. The renal clearance of digoxin did not show a significant change. The following table shows the statistical evaluation of coagulation tests. Digoxin did not influence the effects of Org on the clotting test.

Statistical Evaluation of Coagulation Tests:

	Time (min)	Contrast Org minus Org +digoxin (sec)	95% Confidence Interval		
Thrombin time	0	0.13	-0.74	to	1.01
	15	0.45	-0.38	to	1.28
	60	0.77	-0.12	to	1.66
	1440	0.82	-0.03	to	1.66
APTT	0	-0.30	-5.50	to	4.80
	15	2.20	-5.00	to	9.30
	60	2.00	-4.40	to	8.40
	1440	1.20	-4.00	to	6.30
PT	0	0.42	-0.61	to	1.44
	15	0.17	-1.18	to	1.52
	60	0.38	-0.92	to	1.69
	1440	0.05	-1.62	to	1.72
Thrombotest	0	-0.30	-3.10	to	2.50
	15	-0.20	-3.20	to	2.90
	60	-0.20	-2.70	to	2.40
	1440	0.30	-2.50	to	3.20

	Time (min)	Contrast Org + digoxin minus digoxin (sec)	95% Confidence Interval		
Thrombin time	0	-0.42	-1.29	to	0.46
	15	0.67	-0.17	to	1.50
	60	0.27	-0.62	to	1.16
	1440	-0.58	-1.43	to	0.26
APTT	0	-0.33	-5.49	to	4.82
	15	9.00	1.83	to	16.17
	60	5.33	-1.02	to	11.69
	1440	0.17	-4.98	to	5.31
PT	0	-0.27	-1.25	to	0.76
	15	0.27	-1.08	to	1.62
	60	0.22	-0.97	to	1.41
	1440	0.38	-1.28	to	2.05
Thrombotest	0	-1.20	-4.00	to	1.60
	15	6.00	2.90	to	9.10
	60	3.00	0.40	to	5.60
	1440	0.80	-2.00	to	3.70

Conclusion:
At the dose studied Org 's average clearance of anti-Xa activity was increased (7%). Therefore one can not rule out the possibility of higher reduction in the plasma anti-Xa activity in patients when Org and digoxin are administered simultaneously. However this study design does not permit an estimate of the magnitude of the change.

The simultaneous administration of Org with digoxin caused 14% reduction in the 24 hr AUC and average serum concentration of digoxin (renal clearance was unchanged). The plausible explanation is a change in the systemic clearance of digoxin.

1 page
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Study of Drug Interaction between Cloxacillin and Org in six healthy male subjects

Study#: 85025

Investigator and Site:

Objectives: To investigate the safety and tolerance and any possible clinical interaction of Cloxacillin and Org. To investigate the effects of cloxacillin on the pharmacodynamics of Org.

Dosage Form: Org; 1250 anti-Xa units (CP 084126; 0.6 ml per ampule and CP 084127; 1 ml per ampule).
Cloxacillin: Cloxacillin sodium (Orbenin® ; Beecham, Belgium) 500 mg capsules were supplied by the pharmacist of Leiden University Hospital.

Study Design: This was single center, cross-over, randomized study in six healthy male subjects (age 21 to 27 years and body weight 60 to 79 kg). On occasions 2 weeks apart, subjects received
Treatment A: On day 1, the volunteers were given 3250 anti-Xa units of Org administered intravenously for a period of 3 to 5 minutes.
Treatment B: Oral cloxacillin, 500 mg four times daily, was given for three days. Twenty four hour after the start of the cloxacillin treatment, 3250 anti-Xa units of Org was administered intravenously immediately after the fifth cloxacillin dose.

Specimens: Blood samples for anti-Xa activity were collected at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6.5, 8, 10, 13, 16, 24, 28, 32, 36, 48, 56 and 72 hr following administration of Org. Blood samples for the coagulation tests were collected at 0, 0.25, 1, 6.5 and 24 hr after administration of Org.

Assay: Anti-Xa activity was assayed in the same way as study 85014.

Hemostasis Parameters: The influence of liver enzyme induction on the pharmacodynamics of Org was sought on the basis of coagulation tests. The coagulation tests, thrombin time, APTT, PT and thrombotest were carried out. The tests were carried out on a KC 10 coagulometer after adding calcium chloride 0.025 M and the following reagents: thrombin reagent, automated APTT-reagent, calcium thromboplastin and thrombotest reagent, respectively.

Results: Large skin hematomas on the forearms, localized at venipuncture sites were developed in four volunteers (two receiving Org and two receiving the combined treatment). The following table summarizes the pharmacokinetic analysis of anti-Xa activity for two treatments.

Parameter	Org alone (N=6)		Org with Cloxacillin (N=5)*	
	Mean	S.D.	Mean	S.D.
Cmax (mU/ml)	801.48	153.59	773.21	208.33
AUCinf (mU.hr/ml)	12383	5166	11843	2806
CLinf (ml/hr)	300.97	118.09	284.71	54.75
t1/2 (hr)	27.4	14.3	33.5	13.2

* Subject #5 showed very high AUCinf in treatment B and was treated as an outlier

The half life of anti-Xa activity increased significantly when Org was combined with cloxacillin. The following table illustrate the comparison of coagulation tests for Org with and without administration of cloxacillin.

Statistical Evaluation of Coagulation Tests:

	Time (min)	Contrast Org minus Org + cloxacillin (sec)	95% Confidence Interval		
Thrombin time	0	1.63	0.94	to	2.33
	15	2.18	1.47	to	2.90
	60	2.70	1.64	to	3.76
	360	1.65	1.20	to	2.10
	1440	2.45	1.56	to	3.34
APTT	0	-3.21	-5.00	to	-1.30
	15	-4.80	-8.90	to	-0.80
	60	-2.20	-7.80	to	3.50
	360	-0.70	-2.80	to	1.50
	1440	-1.00	-3.10	to	1.10
PT	0	-0.49	-1.25	to	0.28
	15	0.45	-0.46	to	1.36
	60	0.50	0.00	to	1.00
	360	0.12	0.82	to	1.05
	1440	0.22	-0.75	to	1.19
Thrombotest	0	-1.00	-2.54	to	0.54
	15	-1.30	-3.10	to	0.50
	60	-0.50	-1.60	to	0.60
	360	1.00	-2.40	to	4.40
	1440	1.30	-0.50	to	3.10
Bleeding Time	0	24.5	-90.9	to	139.9
	15	89.0	7.50	to	170.5

Conclusion:

The coagulation tests revealed no important interactions at the pharmacodynamic level. The mean bleeding time after concomitant administration was less than 540 sec and therefore clinically unchanged. The half-life of anti-Xa activity increases when Org is administered with cloxacillin, however, clinical implication of this observation is not very clear. Such implication will depend upon extrapolation of anti-Xa activity to antithrombotic effect in humans.

Study of possible interaction between Org and Ticarcillin sodium in six healthy male volunteers.

Study 85026

Investigator and Site:

Objectives: To investigate the safety and tolerance of the combined medication of ticarcillin and Org as measured by the effects on clinical and laboratory parameters, to investigate the effect of ticarcillin on the pharmacokinetics and pharmacodynamics of Org and vice versa.

Dosage Form: Org; 1250 anti-Xa units (CP 084126; 0.6 ml per ampule and CP 084127; 1 ml per ampule).
Ticarcillin sodium (Ticar®; Beecham; Heppignies, Belgium) 1 gm vials were supplied by the pharmacist of

Study Design: This was a single centered, randomized, crossover study in six healthy subjects (age 23 to 26 years & body weight 62 to 89 kg).
Treatment A: On day one the subjects were given 3250 anti-Xa units of Org intravenously over a period of 3-5 minutes.
Treatment B: On day one intravenous treatment with 2 gm ticarcillin six times daily was started and continued for 2 days. Immediately after the morning injection of ticarcillin on Day 1, 3250 anti-Xa units of Org were administered intravenously.
Treatment C: All subjects received an iv bolus injection of 2 gm of ticarcillin alone.
Washout period of two weeks were scheduled between the three treatments.

Specimens: Blood samples were collected for ticarcillin concentration at 0, 5, 15, 30, 45, 60, 90, 120, 150, 180, 240 min, and 1 and 2 days. Blood samples were collected for anti-Xa activity at screening and at 0, .25, .5, 1, 1.5, 2, 3.5, 5, 6.5, 8, 10, 13, 16, 24, 28, 32, 36, 40, 48, 56 and 72 hours.

Assay: Anti-Xa and anti-IIa activity was assayed in the same way as study 85014.

Hemostasis Parameters: The coagulation tests, thrombin time, APTT, PT and thrombotest were carried out. The tests were carried out on a KC 10 coagulometer after adding calcium chloride 0.025 M and the following reagents: thrombin reagent , automated APTT-reagent calcium thromboplastin and thrombotest reagent respectively.

Results: The summary of pharmacokinetic data based on anti-Xa activity with and without the administration of ticarcillin are shown in the following table.

Parameters	Units	Treatment A	Treatment B
Cmax	mU/ml	771±171	669 ± 139
Tmax	hr	0.30 ± 0.11	0.33 ± 0.13
AUC inf	mU*hr/ml	7323 ± 1071	6770 ± 1075
CL	ml/hr	539 ± 98.9	621 ± 80
Vss	ml	9292 ± 1972	10732 ± 4987
t1/2	hr	17.0 ± 5.3	18.8 ± 10.8

There was 14.3% decrease in AUC, 7.5% decrease in AUCinf and 13.2% decrease in Cmax when ticarcillin was coadministered with Org.

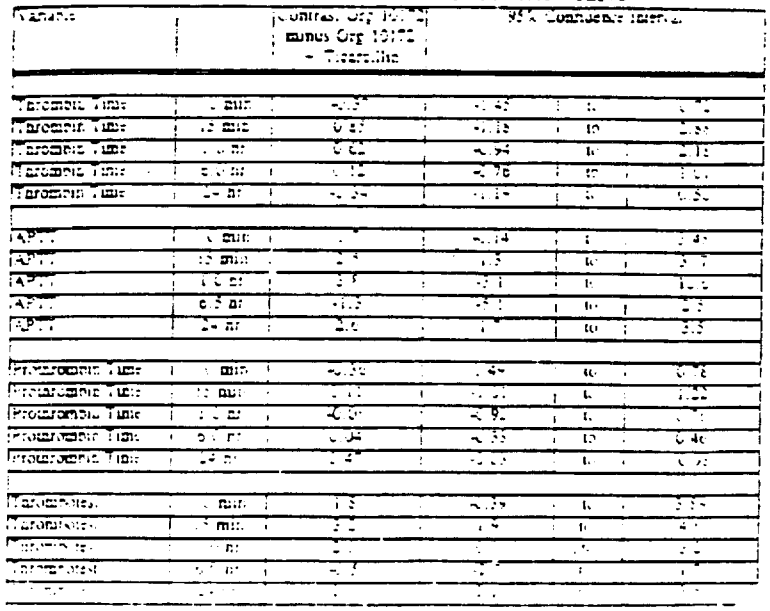
For ticarcillin although the time course of the individual plasma level profiles was quite in agreement with expectations, the concentrations were about 10 times lower than anticipated on the basis of literature data. The sponsor has used the validated assay. The sponsor has studied the stability of ticarcillin in whole blood, in plasma during freezing and thawing. The sponsor has also studied the absorption of ticarcillin on plastic components. However the sponsor could not explain the low ticarcillin concentrations seen in this study.

Following are the pharmacokinetic parameters estimated on the basis of plasma ticarcillin concentrations

Parameters	μg*min/ml (mean ± sd)
AUC (Ticarcillin only)	1409 ± 378
AUC (Ticarcillin + Org on day 1)	1209 ± 321

The following table shows the comparison on coagulation tests for treatment A and B.

STATISTICAL EVALUATION OF COAGULATION TESTS



The APTT and thrombotest showed slight but significant differences at 15 min and 24 hr time point. However these changes appears to be of minor importance.

The anti-IIa activity was highly variable with many sampling times below the level of quantitation no pharmacokinetic analysis was conducted.

Conclusion:

The study demonstrates that simultaneous administration of Org and Ticarcillin is safe and does not change the pharmacokinetics of Org measured as anti-Xa activity. Ticarcillin concentrations observed in this study were 10 times lower than seen in literature. Therefore additional study may be necessary to evaluate the effect of Org on pharmacokinetics of ticarcillin.

Study of possible drug interaction between Chlorthalidone and Org in six healthy male volunteers

Study 86007

Investigator and Site:

Objectives: To investigate the safety and tolerance of the combined administration of chlorthalidone and Org and effect of chlorthalidone on the pharmacodynamics and pharmacokinetics of Org.

Dosage Form: Chlorthalidone (Hygroton®, Ciba Geigy, Basel, Switzerland) 100 mg tablets were supplied by the pharmacist of
Org was supplied by NV Organon, Oss, The Netherlands, as injection fluid containing 1250 anti-Xa units of Org per ml (CP 084126; 0.6 ml per ampule and CP 084127; 1 ml per ampule)

Study Design: Six volunteers (ages 20 to 36 years, weight 69.5 to 80 kg) participated in a randomized, crossover-design trial.
Treatment A: On day 1, the volunteers were given 3250 anti- Xa units of Org administered intravenously over a period of 3-5 minutes.
Treatment B: Oral treatment with chlorthalidone 100 mg (tablet) at 11:00 pm. was given in the evening, before the day of Org administration. The following morning, 3250 anti-Xa units of Org were administered intravenously.
A washout period of two weeks was scheduled between treatment A and B.

Specimens: Blood samples were collected for anti-Xa and anti-IIa activity at the following time points after administration of Org: 0, 0.25, 1, 1.5, 2, 3.5, 5, 6.5, 8, 10, 12, 18, 24, 28, 32, 36, 48, 54, 60 and 72 hours.

Assay: Anti Xa and anti-IIa activities were assayed in the same way as study 85014.
Because of the relatively wide therapeutic margin of chlorthalidone, the effect of Org on the pharmacokinetics of chlorthalidone was not studied.

Hemostasis Parameters: The coagulation tests, thrombin time, APTT, PT and thrombotest were carried out. The tests were carried out on a KC 10 coagulometer after adding calcium chloride 0.025 M and the following reagents: thrombin reagent , automated APTT-reagent calcium thromboplastin , and thrombotest reagent respectively.

Results: The summary of pharmacokinetic data based on anti-Xa activity with and without

administration of chlorthalidone is shown in the following table.

Parameter*	Org	Org + Chlorthalidone	p-value
AUC (mU.hr/ml)	5215 ± 745	6034 ± 1052	0.0109
Cmax (mU/ml)	722.8 ± 69.1	799.6 ± 79.3	0.0563
AUCinf (mU.hr/ml)	6641 ± 395.2	7050 ± 1119	0.4388
CLinf (ml/hr)	490.8 ± 28.8	471.6 ± 80.8	0.6164
MRT (hr)	23.4 ± 6.2	20.9 ± 4.0	0.5105
Vss (ml)	11387 ± 2577	9713 ± 1626.7	0.1599
t1/2 (hr)	18.5 ± 5.8	16.2 ± 4.8	0.5492

* LS Means from ANOVA

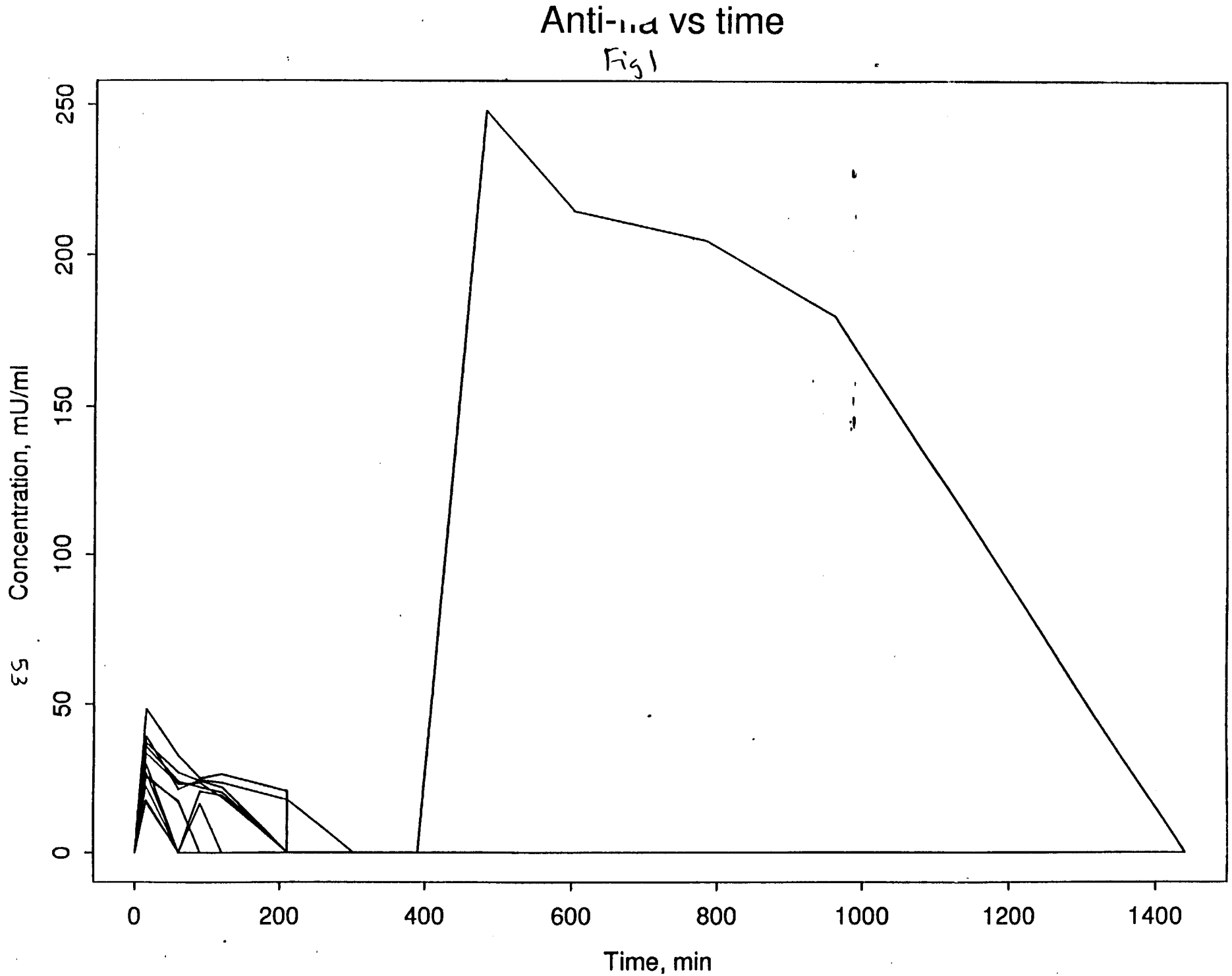
There was a increase in AUC (16%) of Org when it was administered concomitantly with chlorthalidone. The anti-IIa activity was highly variable with many sampling times below the level of quantitation, therefore no pharmacokinetic analysis was conducted. Figure 1 shows the anti-IIa activity versus time plot. The following table shows the comparison on coagulation tests for treatment A and B.

Parameters	Time	Contrast* (min)	95% Confidence Interval		
Thrombin Time	Pre-Injection	0.6	-0.4	to	1.6
	1 hr	0.7	-1.1	to	2.6
	24 hr	0	-0.5	to	0.5
APTT	Pre-Injection	-0.2	-2.8	to	2.5
	1 hr	1.8	-3.5	to	7.1
	24 hr	-1.2	-4.3	to	2.0
PT	Pre-Injection	-0.3	-0.9	to	0.3
	1 hr	0	-0.7	to	0.7
	24 hr	-0.5	-0.8	to	-0.2
Thrombotest	Pre-Injection	0.3	-3.7	to	4.4
	1 hr	-0.8	-3.9	to	2.2
	24 hr	-0.8	-2.3	to	0.6

* Org administered without Chlorthalidone and with Chlorthalidone

Conclusions:
There was a modest increase (< 20%) in anti-Xa activity (AUC, Cmax) when Org was administered concomitantly with chlorthalidone.

Fig 1


Anti-Ha vs time
Concentration, mU/ml
0
50
100
150
200
250
0
200
400
600
800
1000
1200
1400
Time, min

Study of the Effect of Liver Enzyme Induction by Pentobarbital on pharmacokinetic parameters of Org after IV administration in six healthy male volunteers

Study 86026

Investigator and Site:

Objectives: To investigate the effect of the enzyme inducing drug pentobarbital on the pharmacokinetic and pharmacodynamic parameters of Org.

Dosage Form: Org was supplied by NV Organon, Oss, The Netherlands, as injection fluid containing 1250 anti-Xa units of Org per ml (CP 084126; 0.6 ml per ampule and CP 084127; 1 ml per ampule). Pentobarbital 100 mg capsules and antipyrine were supplied by the Pharmacy

Study Design: Six healthy volunteers were randomly assigned to one of two treatment groups

Treatment A:	Day -2	Antipyrine 500 mg orally
	Day 1	Org 3250 anti-Xa units iv
	Days 5 - 16	Pentobarbital 100 mg/day orally
	Day 13	Antipyrine 500 mg orally
	Day 15	Org 3250 anti-Xa units iv

Treatment B:	Days -11 to Day 2	Pentobarbital 100 mg/day orally
	Day -2	Antipyrine 500 mg orally
	Day 1	Org 3250 anti-Xa units iv
	Day 4-26	Wash-out
	Day 26	Antipyrine 500 mg orally
	Day 28	Org 3250 anti-Xa units iv

Specimen: Blood samples were collected for anti-Xa and anti-IIa activity at the following time points

Treatment A: Blood samples for antipyrine concentration were collected at -2 day and 3, 6, 9, 12, 24, 28, 32, 48 hr after -2 day and at day 13 and 3, 6, 9, 12. 24, 28, 32, 48 hr after 13th day. Blood samples for anti-Xa, anti-IIa activity were collected at Day 1 and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6.5, 8, 10, 13, 16, 24, 28, 32, 36, 40, 48, 56, 72 hrs after day 1 and at day 15 and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6.5, 8, 10, 13, 16, 24, 28, 32, 36, 40, 48, 56, 72 hrs after day 15.

Treatment B: Blood samples for antipyrine concentration were collected at -2 day and 3, 6, 9, 12, 24, 28, 32, 48 hr after -2 day and at day 26 and 3, 6, 9, 12. 24, 28, 32, 48 hr after 26th day. Blood samples for anti-Xa, anti-IIa activity were collected at Day 1 and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6.5, 8, 10, 13, 16, 24, 28, 32, 36, 40, 48, 56, 72 hrs after day 1 and at day 28 and 0.25, 0.5, 1,

1.5, 2, 3, 4, 5, 6.5, 8, 10, 13, 16, 24, 28, 32, 36, 40, 48, 56. 72 hrs after day 28.

Assay: Anti Xa and anti-IIa activities were assayed in the same way as study 85014.

Hemostasis Parameters: The influence of liver enzyme induction on the pharmacodynamics of Org was sought on the basis of coagulation tests. The coagulation tests, thrombin time, APTT, PT and thrombotest were carried out. The tests were carried out on a KC 10 coagulometer after adding calcium chloride 0.025 M and the following reagents: thrombin reagent (automated APTT-reagent calcium thromboplastin and thrombotest reagent respectively.

Results: Because the pentobarbital induction may not washout during the study, three treatments were considered:

Org administered prior to administration of pentobarbital (N=3)
Org administered after washout of administrations of pentobarbital (N=3)
Org administration during the 11th day of administrations of pentobarbital (N=6)

The summary of pharmacokinetic data based on anti-Xa activity is shown in the following table.

Parameters mean ± sd	Org	Org after Pentobarbital washout	Org with Pentobarbital
Cmax (mU/ml)	698.1 ± 38.6	677.9 ± 113.4	675.2 ± 114.8
AUCinf (mU.hr/ml)	6263.2 ± 120.2	7538.9 ± 1661.7	7429.4 ± 1815.3
CLinf (ml/hr)	519.0 ± 10.0	446.3 ± 104.1	456.8 ± 99.7
t1/2 (hr)	16.4 ± 5.4	28.4 ± 10.1	22.4 ± 16.8

The summary of pharmacokinetic data based on anti-IIa activity is shown in the following table. The paucity of data and a large number of data points near the level of quantitation made the terminal slope flat. Therefore, only Cmax and AUC_{last} were used to make comparisons among the treatment groups.

Parameters mean ± sd	Org	Org after Pentobarbital washout	Org with Pentobarbital
Cmax (mU/ml)	24.1 ± 4.1	25.5 ± 9.7	23.8 ± 5.2
AUC_{last} (mU.hr/ml)	86.8 ± 73.4	181.4 ± 83.3	135.9 ± 83.7

The following table shows the comparison on coagulation tests for Org administered alone and Org administered with pentobarbital.

Parameters	Time	Contrast* (min)	95% Confidence Interval		
Thrombin Time	Pre Inj.	-0.63	-1.64	to	0.38
	15 min	0.9	-1.37	to	3.17
	1 hr	-1.47	-3.45	to	0.52
	6 hr	-1	-3.00	to	1.00
APTT	Pre Inj.	1.7	-4.3	to	7.6
	15 min	4.5	-7.1	to	16.1
	1 hr	3.3	-5.9	to	12.5
	6 hr	2.5	-3.7	to	8.7
PT	Pre Inj.	-0.15	-0.71	to	0.41
	15 min	-0.13	-1.32	to	1.05
	1 hr	0.17	-0.34	to	0.67
	6 hr	-0.32	-0.67	to	0.04
Thrombotest	Pre Inj.	-0.3	-2.2	to	1.5
	15 min	-0.3	-2.2	to	1.5
	1 hr	-0.8	-2.1	to	0.4
	24 hr	-0.7	-2.7	to	1.4

Conclusion:

The Org anti-Xa activity was not affected by pentobarbital induced metabolism in the liver as monitored by reduction in the half-life of antipyrine. The Org anti-IIa activity tend to show decrease in Org clearance after pentobarbital treatment. However, the variability in the Org's pharmacokinetic parameters (Cmax, AUCinf and CL) was higher when liver metabolism was induced by pentobarbital.

Study of possible interactions between aspirin and Org in eight healthy male volunteers

Study# 87020

Investigator and Site:

Study Dates: 5-18-1987 to 7-14-1987

Objectives: To investigate the safety and tolerance of the combined medication of aspirin and Org.
To investigate the acute effect of the combined medication of Org and aspirin on the bleeding time, platelet aggregation and haemostatic tests.
To investigate the effect of subcutaneous "chronic" Org treatment on the restoration of platelet function following the inhibition of cyclo-oxygenase activity by aspirin ingestion.

Dosage Form: Acetylsalicylic acid (Aspirin;) 500 mg tablets were used. Org was supplied by Organon International B.V., OSS, The Netherlands, as injection fluid containing 1250 anti-Xa units of Org per ml (CP 084126; 0.6 ml per ampoule and CP 084127; 1.0 ml per ampoule).

Study Design: Eight subjects participated in this randomized cross-over designed trial. The randomization scheme and treatment sequences were changed after administering the combination of two drugs to subject 5 and 6 as they showed greatly prolonged bleeding time. (The treatment order was not balanced because the combined administration of both drugs during the first treatment period was prevented). The Table 1 shows the final assignment.

Treatment A: The subjects received 3250 anti-Xa units of Org as an IV bolus injection at time 0 over a period of 3-5 min. The same day 7.5 hr later SC Org treatment was started and continued twice daily at 750 anti-Xa units for 8 days.

Treatment B: Two aspirin tablets of 500 mg with an interval of 12 hr

Treatment C: The subjects ingested 500 mg aspirin 14 and 2 hr before the administration of 3250 anti-Xa units of Org as an IV bolus injection at time 0. 7.5 hr later SC Org treatment BID, 750 anti-Xa units, was started and continued for 8 days.

These dose levels reflect the normal dose of aspirin and normal dose of Org recommended for clinical use for DVT (deep venous thrombosis) prophylaxis.

Specimen: Table 2 show the schedule for the collection times.

Assay:

Assay validation is not provided by the sponsor.

Haemostatic parameters and methods: The coagulation tests, thrombin time, APTT, PT and thrombotest were performed by standard methodology[1] within three days of collection. The tests were performed on a KC 10 coagulometer (after adding calcium chloride 0.025 M and the following reagents: thrombin reagent automated APTT- reagent calcium thromboplastin and thrombotest reagent respectively.

The bleeding time was determined by the method of Mielke et al[2] using the . The proximal tourniquet was inflated to 40 mmHg and two horizontal incisions (5 mm long and 1 mm deep) were made on the volar aspect of the forearm.

Platelet aggregation was measured in a at 37 °C. Aggregation in platelet rich plasma (PRP) (400000 platelets/ml) was induced by collagen. Aggregation was measured by measuring the optical density. First maximum aggregation was assessed by addition of an excess of collagen. Thereafter in other PRP samples the collagen concentration was decreased until no aggregation was measured. The ID50 value (the collagen concentration expressed in µg/ml by which 50% aggregation is reached) of each PRP sample was calculated from aggregation curves.

Results:

Hemostatic Parameters

Thrombin time: The statistical evaluation is presented in table 3 and 4. Figure 1 show the time courses of the means of the thrombin time. Compared to pre-injection values, increased thrombin time values were seen for Org combined with aspirin at 15 min.

Activated partial thromboplastin time: The statistical evaluation is presented in table 3 and 4. Figure 2 show the time courses of the means of APTT. Compared to intersubject values, increased APTT values were seen during Org alone and Org combined with aspirin at 15 min and to a lesser extent at 90 min after the administration of Org.

Prothrombin time: The statistical evaluation is presented in table 3 and 4. Figure 3 show the

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1 Biggs R. Human blood coagulation, haemostasis and thrombosis. 2nd ed. Oxford: Blackwell Scientific Publications (1972).

2 Mielke C. H. et al. The standardised normal Ivy bleeding and its prolongation by aspirin. Blood 34:204-210 (1969)

time courses of the means of the prothrombin time. Compared to intersubject values, increased PT values were seen during Org alone and Org combined with aspirin at 15 min and to a lesser extent at 90 min after the administration of Org.

Bleeding time: The individual data, means, medians and ranges on the bleeding time are presented in table 5. Figure 4, a bar chart, show the mean bleeding time values in 8 volunteers. Subjects 4, 5 and 8 showed significant increase in bleeding time for combined administration.

Platelet aggregation : The individual data, means and standard deviation (SD) on the platelet aggregation time are presented in table 6. Figure 5, a bar chart, shows the collagen ID50 values for all subjects. The figure show that there was no clear influence of Org on the inhibiting effect of aspirin on platelets. Org alone did not inhibit the collagen induced platelet aggregation.

Anti-Xa activity in plasma: Figure 6 show mean time courses (n=7) respectively of plasma anti-Xa activity during treatment A, B and C for 8 days. The results of volunteer 5 were not used for the calculations of the mean curves. Volunteer 5 did not receive his SC Org injections on days 2, 5, 6, 7 and 8 because of strongly prolonged bleeding times on days 1 and 5. Figure 5 show that after a peak plasma anti-Xa activity of about 0.85 U/ml at 15 min after the IV Org injection during treatment A and C, steady state levels of plasma anti-Xa activity (slightly above 0.1 U/ml) were reached 3 days after the start of the Org treatment.

Conclusion:

Although this study shows that there were no interaction between Org and aspirin, it may be necessary to take special precautions when aspirin and Org are used concomitantly because of the increased bleeding risk due to aspirin.

Table 1 Personal data at screening and treatment order.

Vol. No.	Age (Y)	Height (cm)	Weight (kg)	Smoking	Drinking per day	Order of treatment *
1	23	190	75	yes	≤ 2 glasses of beer	1
2	27	178	73	never	≤ 2 glasses of beer ≤ 2 glasses of wine	1
3	24	190	75	never	≤ 2 glasses of beer	3
4	20	180	70	yes	≤ 2 glasses of beer	3
5	20	186	72	never	≤ 2 glasses of beer	2
6	21	181	63	yes	≤ 2 glasses of beer ≤ 2 glasses of wine	2
7	20	170	63	never	≤ 2 glasses of beer	3
8	21	193	87	never	≤ 2 glasses of beer ≤ 2 glasses of wine	1

* 1 = Org 10172 (Treatment a) followed by Aspirin (Treatment b) followed by Org 10172 + Aspirin (Treatment c)

2 = Org 10172 (Treatment a) followed by Org 10172 + Aspirin (Treatment c) followed by Aspirin (Treatment b)

3 = Aspirin (Treatment b) followed by Org 10172 (Treatment a) followed by Org 10172 + Aspirin (Treatment c)

Table 2 Assessment schedule

For each treatment period a, b and c.

Days of the week	F	M	T	W	T	F	S	S	M	T	washout 2 weeks	M etc
Medical history	X^a	X^b	X^b	X^b	X^b	X^b	X^b	X^b	X^b	X^b		X^b
Physical exam.	X	X^c	X^c	X^c	X^c	X^c	X^c	X^c	X^c	X^c		X^c
Routine biochem.	X^a	X								X		X
Urinalysis	X^a											
Routine haematol.	X^a	X				X				X		X
AT-III, fibrinogen	X^a											
APTT, PT,TT	X					X				X		X^d
Plasma anti-Xa, [redacted]$_2$	X^e	X^d	X	X	X	X	X	X	X	X		X^d
Platelet aggreg.		X^d				X				X		X^d
Simplate BT		X^f								X		X^f
Plasma acetylsalicylate level	X	X										X

X = once only before the morning injection. X^a = only before the first treatment period.

X^b = a modified history concerning current illness and unscheduled drug intake.

X^c = a modified examination for signs of bleeding/bruising or for skin rash or other allergic manifestation.

X^d = 4 samples/tests i.e. i one just before the first injection
ii one 15 minutes after the first injection
iii one 90 minutes after the first injection
iv one 5 hours after the 1st injection, (but excluding a platelet aggregation test)

X^e = 3 baseline/before injection samples

X^f = 2 bleeding times (BT) one before and one 15 minutes after the first injection is given.

Table 3 Results statistical evaluation of coagulation tests by means of analysis of variance.

Variable		Comparison treatment	P (two sided)
Thrombin time	0 min	a, b and c	0,06
	15 min		0,05
	90 min		0,11
	5 h		0,04
	96 h		0,53
	192 h		0,81
APTT	0 min	a, b and c	0,59
	15 min		<0,0001
	90 min		0,0006
	5 h		0,002
	96 h		0,03
	192 h		0,07
Prothrombin time	0 min	a, b and c	0,96
	15 min		0,001
	90 min		0,03
	5 h		0,005
	96 h		0,79
	192 h		0,09

Treatment a: Org 10172 alone
Treatment b: Aspirin alone
Treatment c: Org 10172 + Aspirin.

Table 3a Results statistical evaluation of coagulation tests. Multiple comparisons among treatment groups by means of Scheffé's technique.

Variable	Time of assessment	Contrast treatment	Contrast value	95% confidence interval
Thrombin time	15 min	a minus b	0.63	-0.70 to 1.96
		a minus c	-1.41	-2.74 to -0.08
		b minus c	-2.05	-3.38 to -0.72
	5 h	a minus b	0.60	-0.34 to 1.54
		a minus c	-0.71	-1.65 to 0.22
		b minus c	-1.31	-2.25 to -0.37
APTT	15 min	a minus b	15.5	10.3 to 20.7
		a minus c	4.1	-1.0 to 9.3
		b minus c	-11.4	-16.5 to -6.2
	90 min	a minus b	6.3	2.2 to 10.3
		a minus c	1.3	-2.8 to 5.3
		b minus c	-5.0	-9.1 to -0.9
	5 h	a minus b	4.3	0.8 to 7.7
		a minus c	0	-3.4 to 3.4
		b minus c	-4.3	-7.7 to -0.8
	96 h	a minus b	1.6	-0.5 to 3.8
		a minus c	-0.5	-2.7 to 1.7
		b minus c	-2.1	-4.3 to 0
Prothrombin time	15 min	a minus b	1.58	0.73 to 2.42
		a minus c	0.46	-0.38 to 1.30
		b minus c	-1.11	-1.95 to -0.27
	90 min	a minus b	0.75	-0,27 to 1.77
		a minus c	0	-1.02 to 1.02
		b minus c	-0.75	-1.77 to 0.27
	5 h	a minus b	0.19	-0.23 to 0.60
		a minus c	-0.36	-0.78 to 0.05
		b minus c	-0.55	-0.97 to -0.13

Treatment a: Org 10172 alone
Treatment b: Aspirin alone
Treatment c: Org 10172 + Aspirin

Fig 1 MEAN (+/- S.D.) TIME COURSES
THROMBIN TIME (n=8)

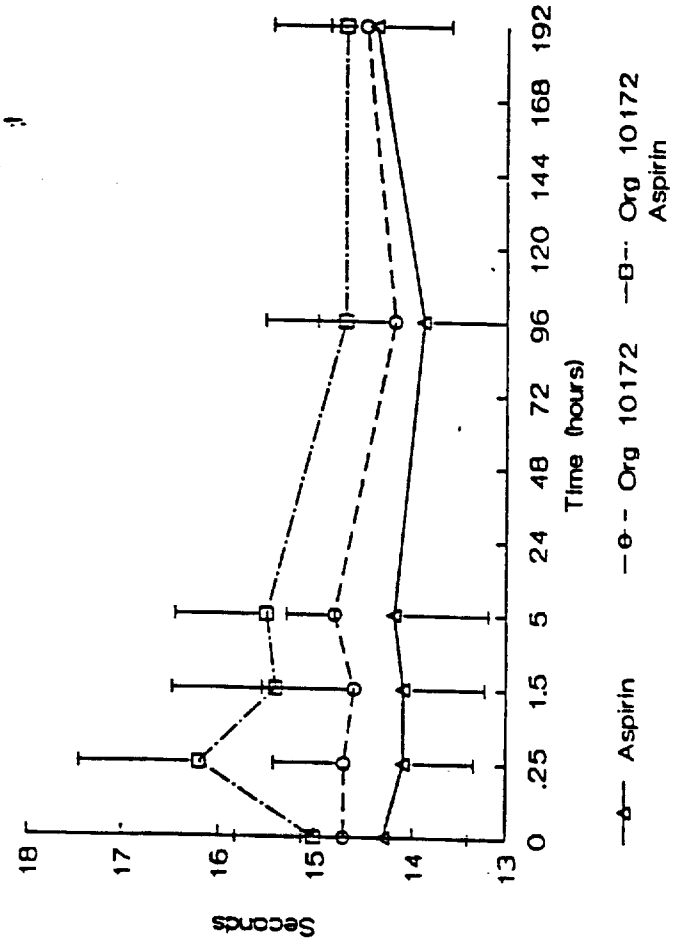


Fig 2 MEAN (+/- S.D.) TIME COURSES APTT (n=8)

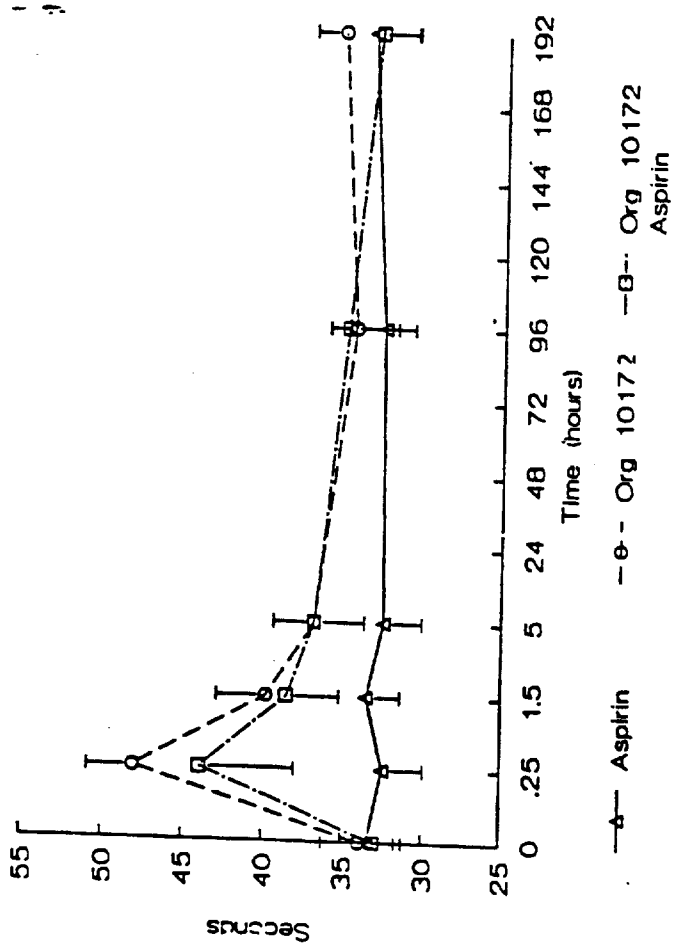


Fig 3 MEAN (+\- S.D.) TIME COURSES
PROTHROMBIN TIME (n=8)

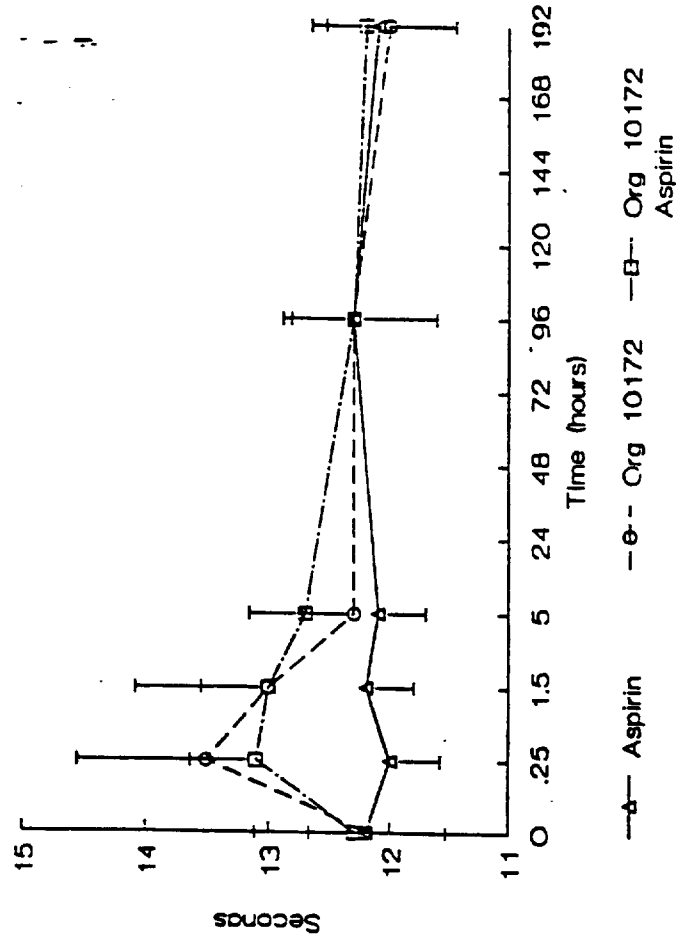


Table: 10 Individual data, mean, median and range of bleeding time.

Normal range < 9 min.

Org 10172

Subject	Day 1 Pre inj.	Day 1 15 min.	Day 2	Day 3	Day 5	Day 9
1						
2						
3						
4						
5						
6						
7						
8						
Mean	5.1	6.5				5.7
Median	4.5	6.0				5.5
Range						

Aspirin

Subject	Day 1 Pre inj.	Day 1 15 min.	Day 2	Day 3	Day 5	Day 9
1						
2						
3						
4						
5						
6						
7						
8						
Mean	>12.8	13.8				9.6
Median	9.0	10.3				6.5
Range						

Org 10172 + Aspirin

Subject	Day 1 Pre inj.	Day 1 15 min.	Day 2	Day 3	Day 5	Day 9
1						
2						
3						
4						
5						
6						
7						
8						
Mean	9.8	>20.1	11.4	9.1		5.4
Median	8.0	14.3	12.8	7.8		4.8
Range						

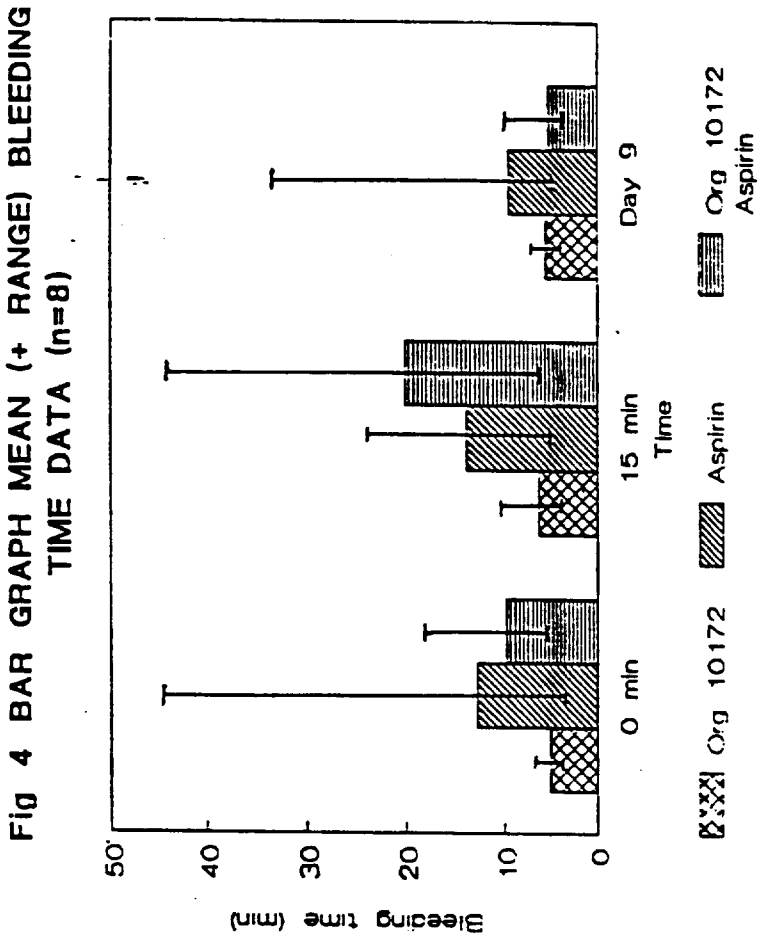


Fig 4 BAR GRAPH MEAN (+ RANGE) BLEEDING TIME DATA (n=8)
Bleeding time (min)
0
10
20
30
40
50
0 min
15 min
Day 9
Time
Org 10172
Aspirin
Org 10172
Aspirin

6

Table: ~~11~~ Collagen induced platelet aggregation, expressed in ID 50 values (µg/ml).

Subject No.	Day 1 T=0'	Day 1 T=15'	Day 1 T=90'	Day 5	Day 9
Org 10172 treated group					
1					
2					
3					
4					
5					
6					
7					
8					
Average	0.22	0.20	0.16	0.14	0.10
S.D.	0.19	0.21	0.16	0.15	0.09
SEM	0.07	0.08	0.05	0.05	0.03
Aspirin treated group					
1					
2					
3					
4					
5					
6					
7					
8					
Average	3.10	3.25	3.65	0.43	0.20
S.D.	1.92	1.88	2.09	0.27	0.14
SEM	0.68	0.66	0.74	0.10	0.05
Org 10172 + Aspirin treated group					
1					
2					
3					
4					
5					
6					
7					
8					
Average	3.38	3.27	3.41	0.41	0.14
S.D.	0.99	1.05	1.01	0.16	0.13
SEM	0.35	0.37	0.36	0.06	0.05

Fig 5 BAR GRAPH MEAN (+\- S.D.) COLLAGEN INDUCED PLATELET AGGREGATION (n=8)

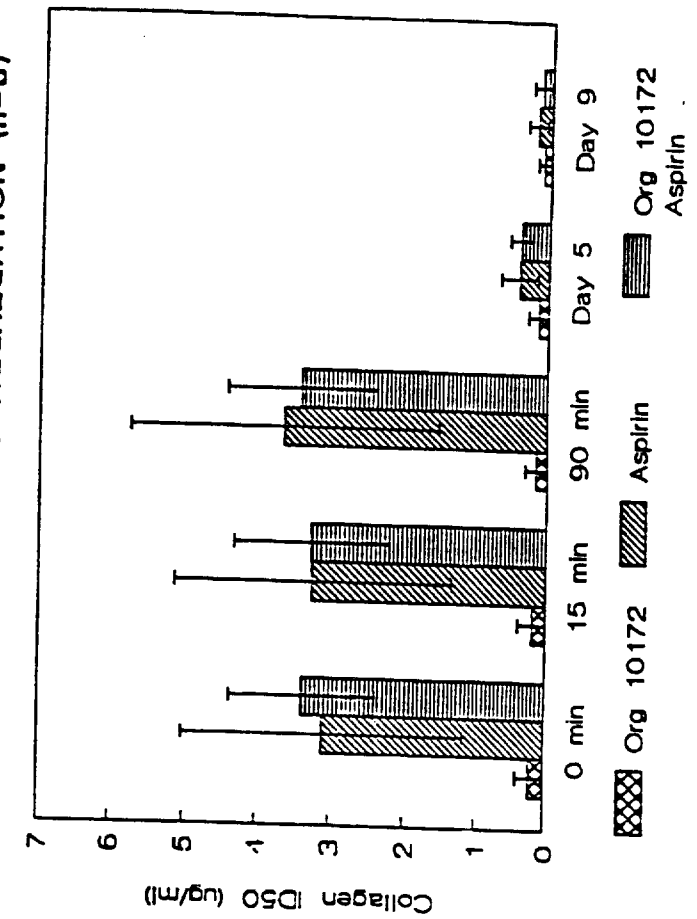
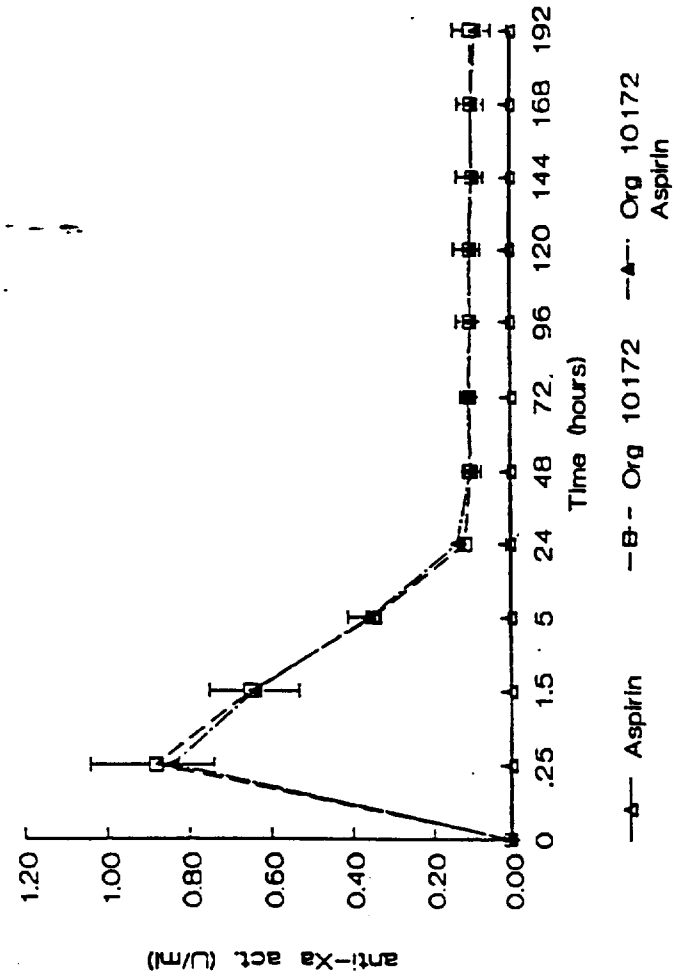


Fig 9 MEAN (+\- S.D.) TIME COURSES
PLASMA anti-Xa ACTIVITY (n=7)



Summary of anti-Xa activity Population Pharmacokinetics of Orgaran

Background: Orgaran (Org) was developed as an antithrombotic product to have a better benefit (antithrombotic properties)/risk (bleeding enhancement) ratio than other standard antithrombotic regimens currently available. The sponsor has proposed that Orgaran has been shown to enhance antithrombotic efficacy while minimizing the risk of hemorrhagic complications in both animal models and clinical studies.

The sponsor has submitted a population pharmacokinetic (PPK) analysis carried out on twelve pharmacokinetic studies in this NDA. This analysis consists of 110 subjects who were administered intravenous (iv) bolus doses of Org ranging from 750 to 6400 anti-Xa units. Forty four subjects out of 110 subjects also received Org via the subcutaneous (sc) route in doses ranging from 750 to 3250 anti-Xa units. Six of twelve studies consisted of a single iv dose of 3250 anti-Xa units of Org and were designed to determine the effects of the concomitant administration of acenocoumarol, digoxin, cloxacillin, ticarcillin, pentobarbital and chlorthalidone on the pharmacokinetics (pk) of Org. Three pk studies with 36 subjects were conducted to compare the pk of various batches of Org.

Subjects: Subjects were normal with height (mean ± sd) 176.3 ± 10.8, age (mean, range) 31.0, 19 - 68 and weight (mean ± sd) 74.4 ± 11.2.

Design: Either iv or sc administration of Org was done and extensive blood sampling was carried out.

Sponsor's Analysis: The sponsor used P Pharm software to carry out the PPK analysis. Based on a two stage approach a two compartment linear model was selected to fit the population data. The parameters estimated were viz., CL (K10*Vc), Vc, K12 and K21 for sc route additional parameter such as F and Ka were estimated. Following is a summary of PPK parameters for iv administration.

pk parameters	Unit	Mean	SD
K10*Vc	L/hr	0.3777	0.1272
K10	1/hr	0.0903	
Vc	L	4.1828	0.6325
K12	1/hr	0.1426	0.0804
K21	1/hr	0.1330	0.1483

The following is a summary of PPK parameter for sc administration.

pk parameters	Unit	Mean	SD
K10*Vc	L/hr	0.3733	0.1198
K10	1/hr	0.0944	
Vc	L	3.9555	0.4721
K12	1/hr	0.1322	0.0422
K21	1/hr	0.0863	0.0475
Ka	1/hr	0.4497	0.1122
F		0.96	0.1232

The sponsor found that the covariate height explained 28.4% of the variation in the volume of the central compartment. A variation of 34.8% is accounted by the weight covariate.

Div. of Pharmaceutical Evaluation II's Analysis:

Using the computer program NONMEM (version IV, level 1) estimates were obtained for

1. the population means of pk parameters with a two compartment open model
2. variance of the population means
3. variance of residual random error
4. std. error of estimates

Intersubject variability in clearance (CL), central and peripheral volume of distribution (V2 and V3), absorption rate constant (Ka) and intercompartmental clearance Q were modelled with exponential error model.

$CL_j = CL\ EXP(n_j^{CL})$
$V2_j = V2\ EXP(n_j^{V2})$
$Ka_j = Ka\ EXP(n_j^{Ka})$
$Q_j = Q\ EXP(n_j^{Q})$
$V3_j = V3\ EXP(n_j^{V3})$

The residual intrasubject error was modelled with additive and proportional model.

$$C_{ij} = C_{Mij} + C_{Mij}*\epsilon_{1ij} + \epsilon_{2ij}$$

Model Building:

Models	df	LLD	Variance
1. TVCL=θ_1 TVV2=θ_2 TVKA=θ_3 TVQ=θ_4 TVV3=θ_5			%CV on CL = 25.9 V2 = 16.4 KA = 26.0 Q = V3 = 29.3 %Error Prop = 10.8 Additive = 106
2. TVCL=θ_1 TVV2=θ_2*(HT/175)**θ_7 TVKA=θ_3 TVQ=θ_4 TVV3=θ_5	1	-97 (2 vs 1)	%CV on CL = 25.9 V2 = 13.8 KA = 27.2 Q = V3 = 26.4 %Error Prop = 10.6 Additive = 91.7
3. TVCL=θ_1*(HT/175)**θ_7 TVV2=θ_2*(HT/175)**θ_8 TVKA=θ_3 TVQ=θ_4 TVV3=θ_5	2	-109 (3 vs 1)	%CV on CL = 26.3 V2 = 13.7 KA = 27.2 Q = V3 = 26.1 %Error Prop = 10.6 Additive = 90.7
4. TVCL=θ_1*(HT/175)**θ_7 TVV2=θ_2*(HT/175)**θ_8 * (WT/74.4) ** θ_9 TVKA=θ_3 TVQ=θ_4 TVV3=θ_5	3	-128 (4 vs 1)	%CV on CL = 26.3 V2 = 12.7 KA = 26.5 Q = V3 = 26.0 %Error Prop = 10.6 Additive = 92.4

Fig. 1, 2, 3 and 4 show goodness of fit plots done on model 1 estimates. The following are the final population parameter estimates.

Parameter	Mean	CV on Mean = (SE/mean)*100	Interindividual Variation#	Variance	CV on Variance (SE/mean)*100
CL (L/hr)	0.363	3.5%	26.3%	0.069	19.9%
V2 (L)	4.15	1.6%	13.7%	0.016	20.2%
Ka (1/hr)	0.453	5.1%	27.2%	0.070	31.2%
Q (1/hr)	0.423	3.4%	26.7%	0.071	19.7%
V3 (L)	6.26	5.0%	26.1%	0.068	21.2%

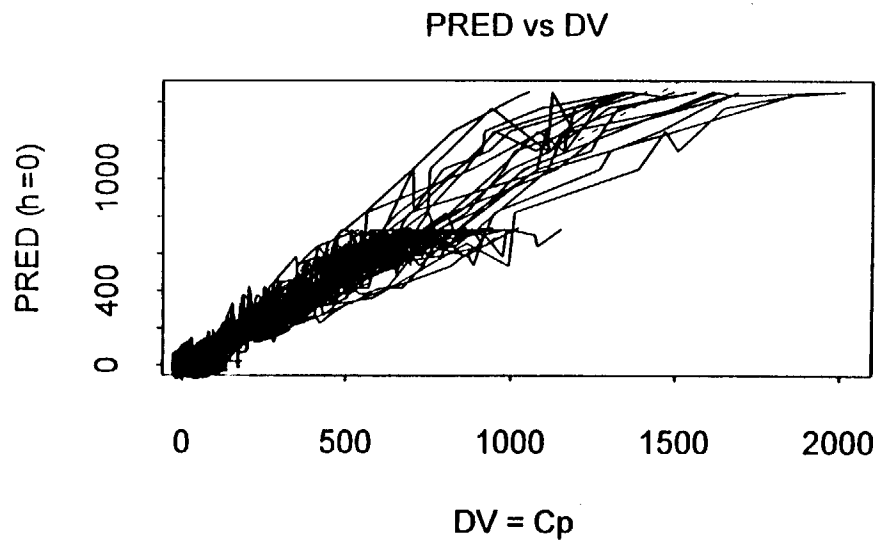
Interindividual Variance = Sqrt ot Variance * 100

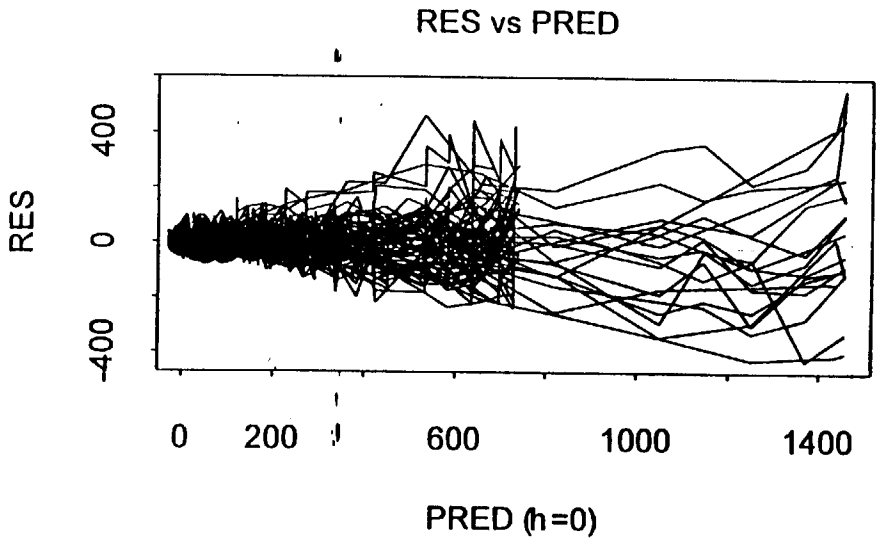
The beta (elimination rate constant) was calculated to be 0.0257 hr^{-1}. Thus the estimated half life of about 27 hr.

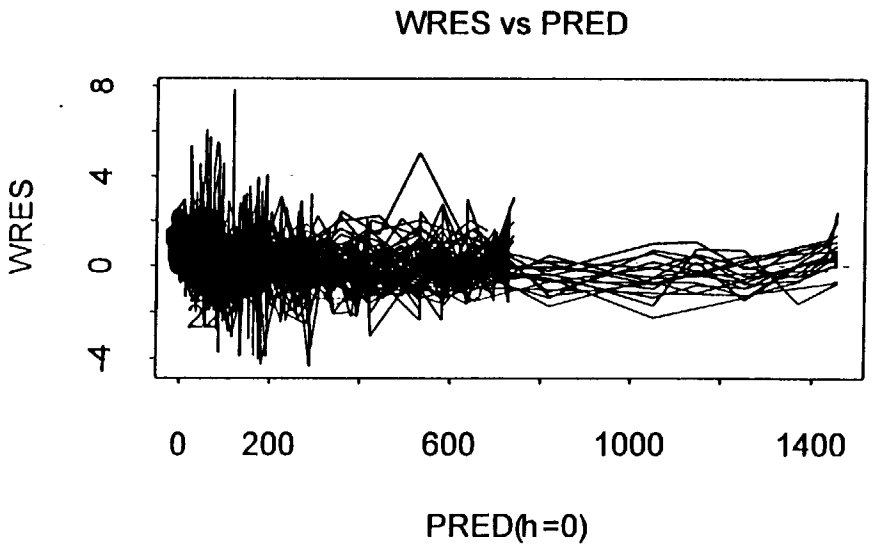
The estimated bioavailability of Org subcutaneous route was about 100%.

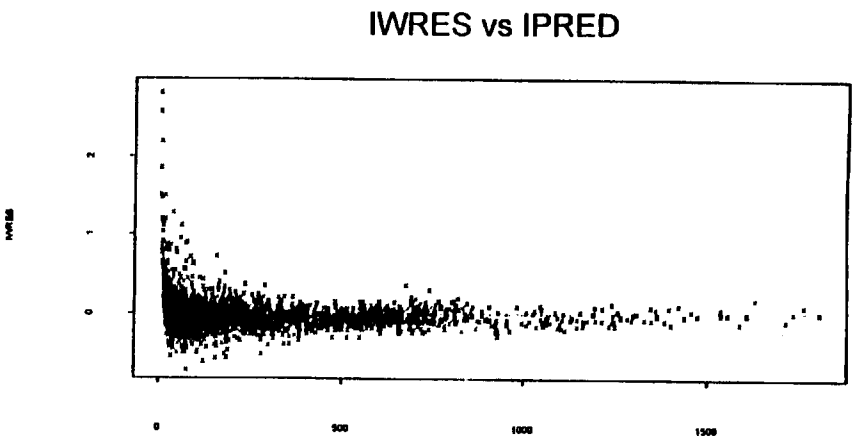
Danaparoid - Basic -Goodness of Fit

Solid:indiv data. Short dash: Unit, RES|WRES=0. Long dash: smooth. #:id)



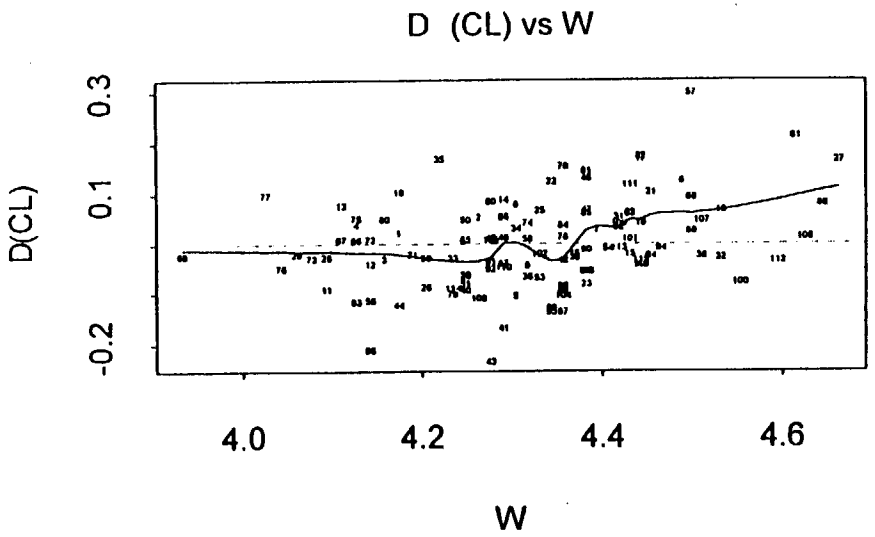


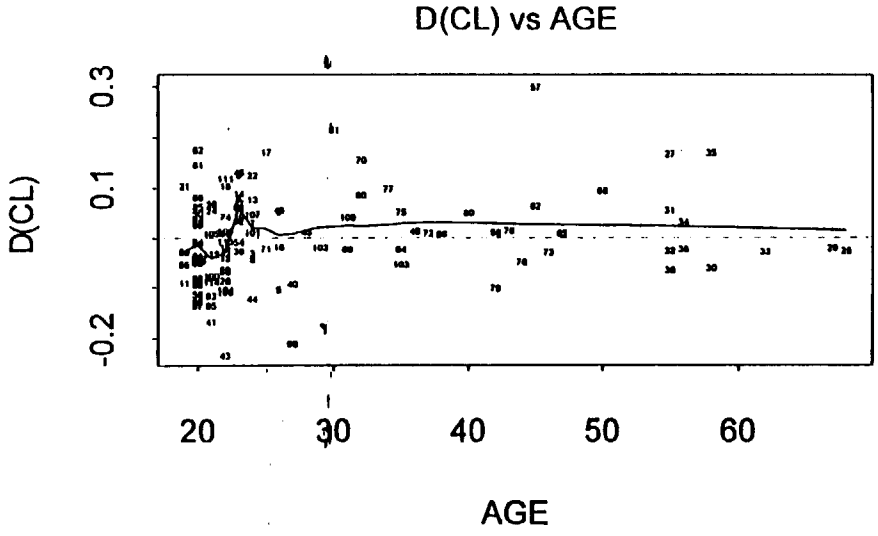


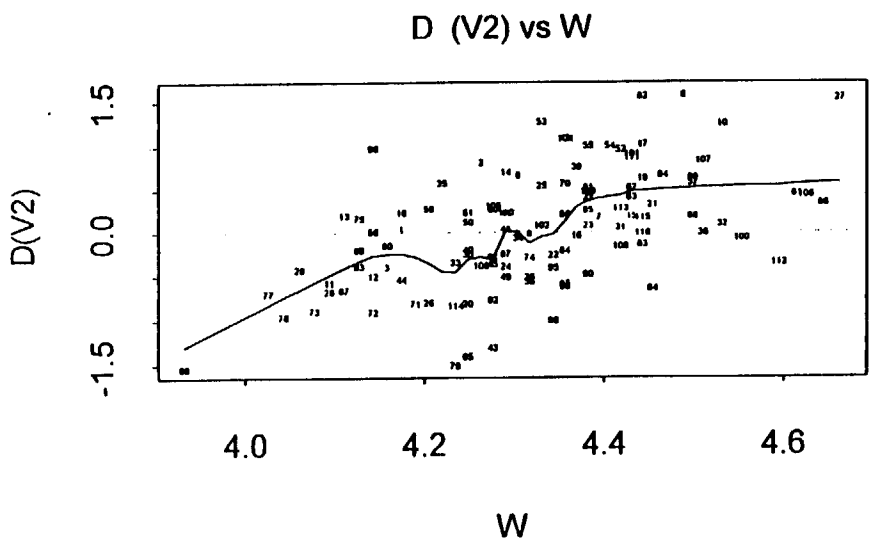


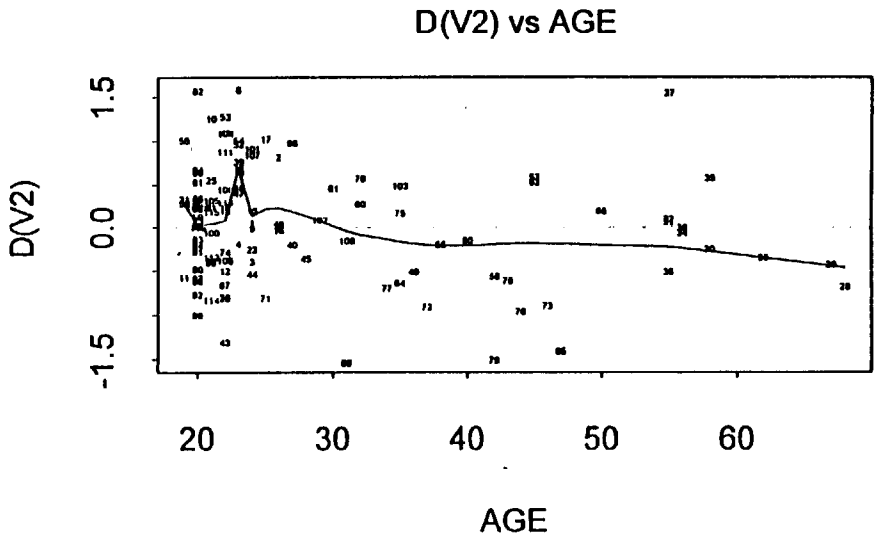
Danaparoid - Basic - Model Building

(Dash: D=0. Solid: smooth. #: id) D: Δ (difference) W: Ln(WT)





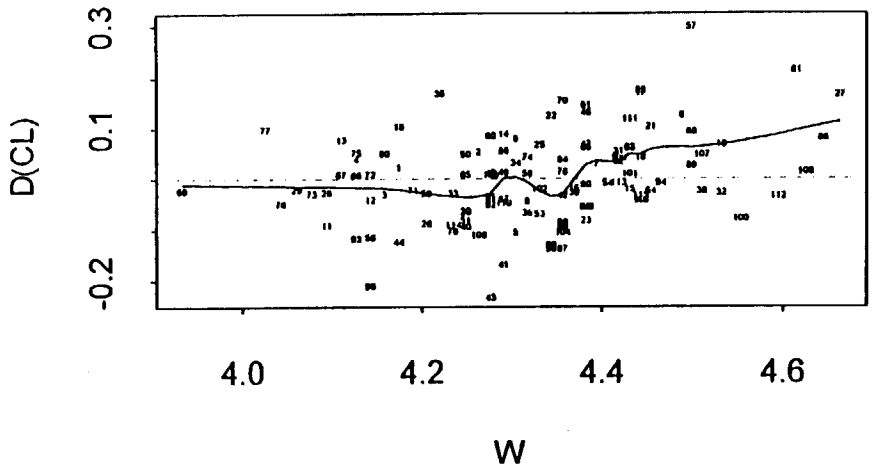




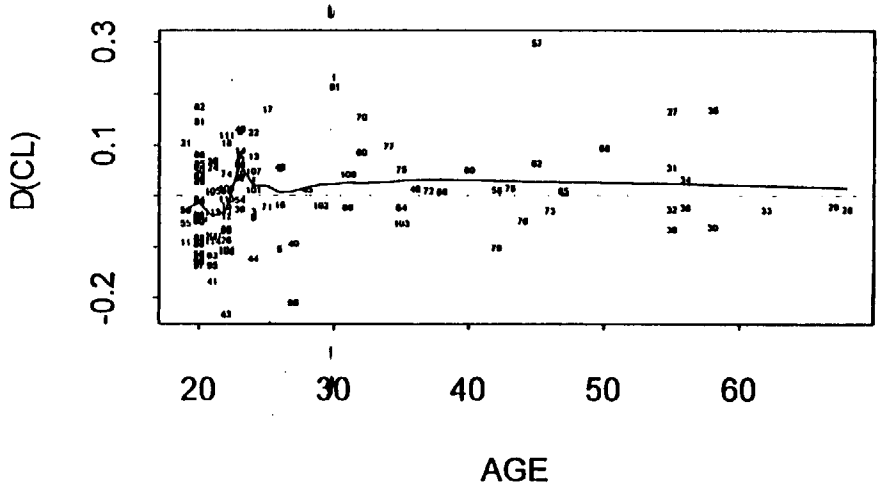
Danaparoid - Basic - Model Building

(Dash: D=0. Solid: smooth. #: id) D: Δ (difference) W: Ln(WT)

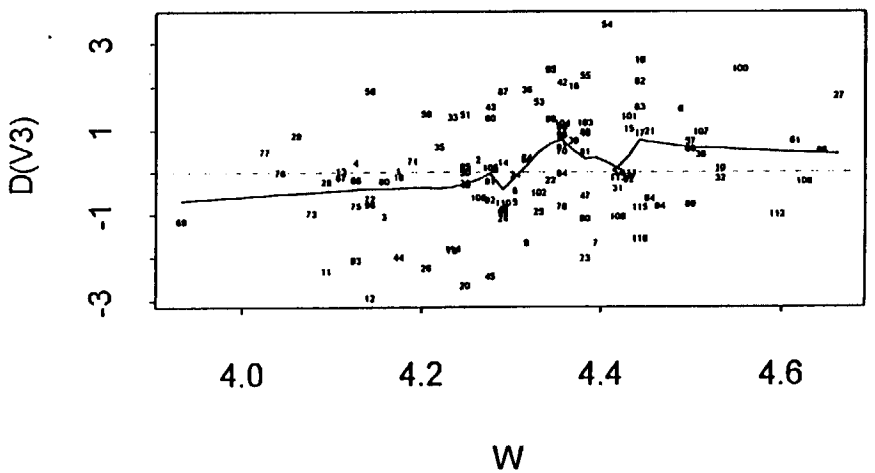
D (CL) vs W



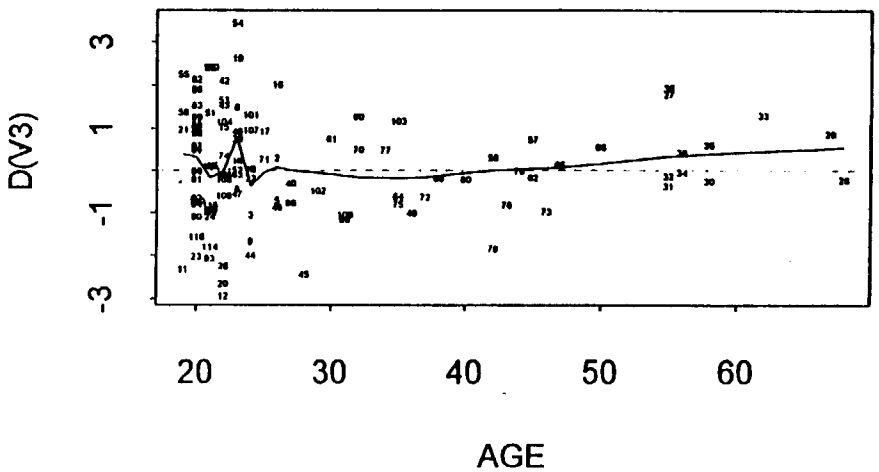
D(CL) vs AGE



D (V3) vs W

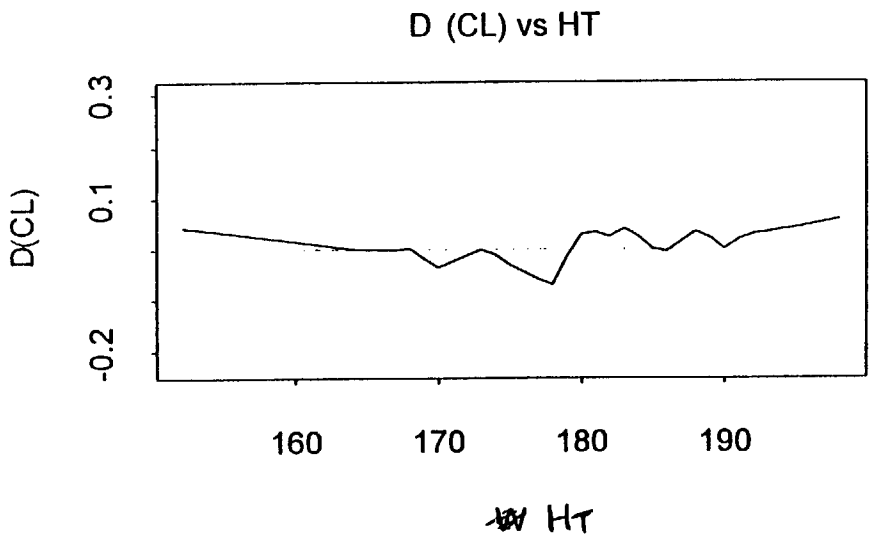


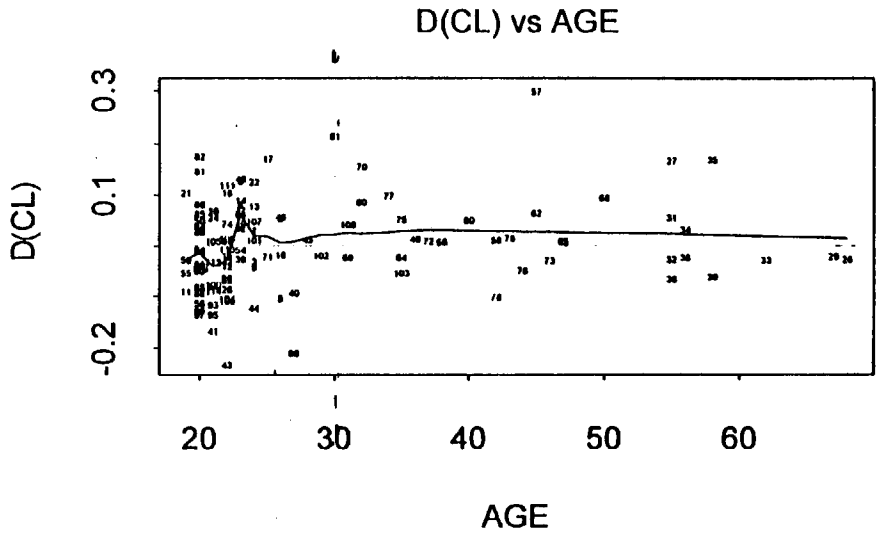
D(V3) vs AGE

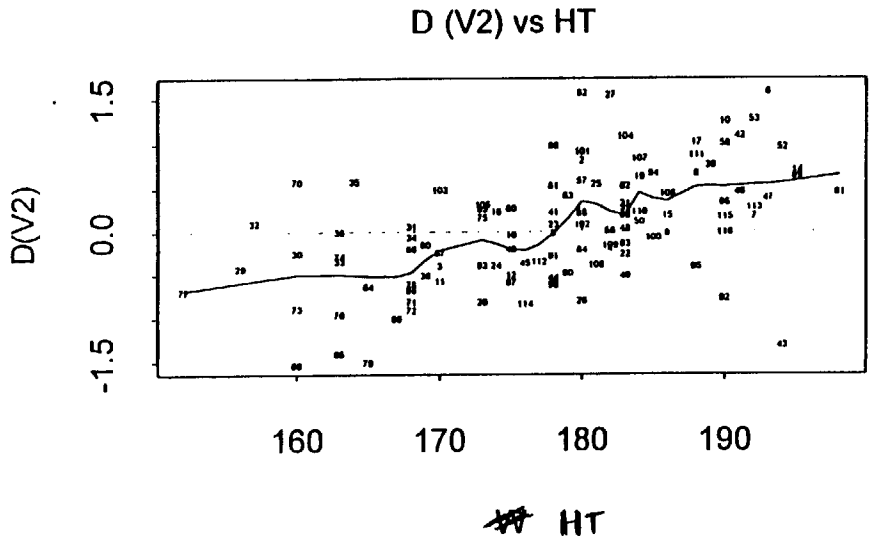


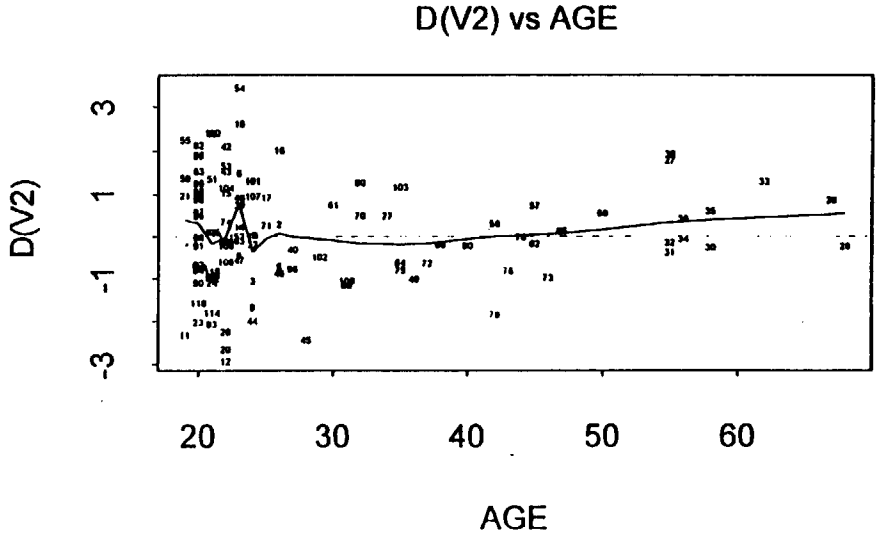
Danaparoid - Basic - Model Building

(Dash: D=0. Solid: smooth. #: id) D: Δ (difference) W: Ln (WT)









CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020430

CHEMISTRY REVIEW

Oliver

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

MAY - 2 1995

NDA:# 20-430 CHEM REVIEW # 1 REVIEW DATE: February 28, 1995

SUBMISSION TYPE			DATES			
DOCUMENT	CDER	ASSIGNED	REVIEW	NUM	LETTER	ST
ORIGINAL	29DEC94	4JAN95	28FEB95	1		

NAME & ADDRESS OF APPLICANT:
Organon Inc.
375 Mt. Pleasant Avenue
West Orange, NJ 07052

DRUG PRODUCT NAME:

Proprietary:	Orgaran
Nonproprietary/USAN:	Danaparoid Sodium
Code Name/#:	ORG 10172
Chem.Type/Ther.Class:	1S

PHARMACOLOGICAL CATEGORY: Anticoagulant

INDICATION: Prophylaxis of postoperative deep venous thrombosis and pulmonary embolism in patients undergoing orthopedic hip surgery

DOSAGE FORM: Injectable

STRENGTH: 1250anti-Xa U/mL

ROUTE OF ADMINISTRATION: Subcutaneous

HOW DISPENSED? __X__ RX _____ OTC

Chemical Name:
Sulfated glycosaminoglycans: a mixture of straight-chain anionic mucopolysaccharides.

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020430

ENVIRONMENTAL ASSESSMANT AND FONSI

Olivier

ENVIRONMENTAL ASSESSMENT

AND

FEB - 8 1996

FINDING OF NO SIGNIFICANT IMPACT

FOR

NDA 20-430

Orgaran™

(danaparoid sodium)

Injection

750 ANTI-X_a units

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS (HFD-180)

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-430

Orgaran™

(Danaparoid sodium

Injection

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

The Food and Drug Administration, Center for Drug Evaluation and Research has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their new drug application for **Orgaran™ Injection, Organon Inc.** has prepared an environmental assessment in accordance with 21 CFR 25.31a(b)(5) (attached) which evaluates the potential environmental impacts of the manufacture, use and disposal of the product.

Danaparoid sodium is a natural substance that is isolated from porcine mucosa, the same source of heparin products. The drug will be administered by injection in the treatment of deep vein thrombosis and pulmonary embolism. The drug substance will be manufacture by and the drug product will be manufactured at Organon Inc., West Orange, NJ. The finished drug product will be used in hospitals.

Danaparoid sodium may enter the environment from excretion by patients, from disposal of pharmaceutical waste or from emissions from manufacturing sites. Use of this product is expected to replace the use of other heparin-based products and is not

expected to significantly alter the distribution of the product in the environment.

Disposal may result from production waste such as out of specification lots, returned goods and user disposal of empty or partly used product and packaging. Pharmaceutical waste will be disposed of by the manufacturer at a licensed landfill. At U.S. hospitals, empty or partially empty packages will be disposed according to hospital procedures.

Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured, used and disposed of without any expected adverse environmental effects. Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.

2/2/96
DATE

PREPARED BY
Nancy B. Sager
Acting Supervisor
Environmental Assessment Team
Center for Drug Evaluation and Research

2/6/96
DATE

CONCURRED
Roger L. Williams, M.D.
Deputy Center Director for Pharmaceutical Science
Center for Drug Evaluation and Research

Attachment: Environmental Assessment

Project: Orgaran™
Document No.: PDR-102.07

ORGANON INC.
West Orange, New Jersey 07052

ENVIRONMENTAL IMPACT ASSESSMENT REPORT FOR ORGARAN™ INJECTION

REVISION 07

Pharmaceutical Development Department
Product Development and Government Affairs
December 4, 1995

ENVIRONMENTAL IMPACT ASSESSMENT REPORT FOR ORGARAN™ INJECTION - REVISION 07

Table of Contents

Table of Contents (continued)

1. **Date:** December 4, 1995

2. **Name of Applicant:** Organon Inc.

3. **Address:** 375 Mt. Pleasant Avenue
West Orange, New Jersey 07052

4. **Description of Proposed Action:**

A. Requested Approval

Organon Inc. has filed a new drug application, NDA No. 20-430, pursuant to Section 505 (b) of the Food, Drug, and Cosmetic Act requesting approval to manufacture, package, distribute and sell its parenteral dosage form of Orgaran™ Injection, an anti-thrombotic agent. Orgaran™ Injection is supplied as a sterile, isotonic, nonpyrogenic solution for injection containing 0.15% (w/v) sodium sulfite. Each 1 mL ampule or 1 mL pre-filled syringe contains 750 units of anti-X_a activity as 0.6 mL of a 1250 U/mL solution. It is intended to be administered subcutaneously or in appropriate indications may be given by the intravenous route. This Environmental Impact Assessment Report is being submitted in accordance with 21 CFR 25.31 (a) (b) in abbreviated format, for a substance that occurs naturally in the environment, as directed in item (5) of the previously cited code.

B. Need for Action

Approval of this application will offer patients in the United States an effective and reliable anti-thrombotic drug product.

I. Clinical Pharmacology

Orgaran™ Injection is characterized by coagulation factor X_a inhibitory activity and a low anti-thrombin activity and no significant effects on blood platelet function. Orgaran™ Injection has been studied clinically to demonstrate safety and efficacy for anticoagulant therapy in prophylaxis of deep venous thrombosis (DVT) and pulmonary embolism (PE).

Orgaran™ Injection has been shown both in animal models and in human studies to be an effective anti-thrombotic agent. Orgaran™ Injection has a negligible effect on hemostatic plug formation, platelet function and platelet aggregatibility, and exhibits thereby only a small, bleeding enhancing effect at therapeutic doses.

ii. Dosage Regimen

For routine prophylaxis of deep venous thrombosis (DVT) in elective hip surgery, Orgaran™ Injection is administered by deep subcutaneous injection. A preoperative dose not exceeding 750 units of anti-X_a activity should be given 1 to 4 hours before surgery. In general, Orgaran™ Injection doses of 750 units of anti-X_a activity are given twice daily up to 14 days.

iii. Drug Absorption/Metabolism

The active ingredient of Orgaran™ Injection, danaparoid sodium (ORG 10172), is rapidly absorbed after administration with an absolute bioavailability of 100%. The excretion rate of danaparoid sodium is approximately 50% (unchanged) in the urine.

C. Site of Drug Substance Production and Environmental Settings

The active ingredient, danaparoid sodium, will be manufactured by

The , a town of 50,000 inhabitants. The factory is located on a 160,000 square meter site together with other properties of the Organon group including research and development laboratories, technical services offices, control laboratories, etc. The site borders a meat canning company, a railway station, a housing development and administrative offices.

D. Site of Drug Product Production and Environmental Settings

Orgaran™ Injection will be manufactured by Organon Inc., 375 Mount Pleasant Avenue, West Orange, New Jersey 07052, which is situated on property bordering an electric utilities substation, a private golf course, an electric utilities switching station, an office building and condominiums.

Packaging and distribution of Orgaran™ Injection will be performed by Organon Inc. at our facility located at 6350 Hedgewood Drive in Allentown, Pennsylvania 18105. The Pennsylvania facility borders several other storage warehouses in an industrial park. The following materials will be used in the packaging of Orgaran™ Injection:

I. Ampules

a. Container:

Source:

Size:

Alternate Source:

b. Thermoformed Tray:

Style:

Material:

Suppliers:

c. Package Insert:

Folded Size:

Stock:

Printing:

Suppliers:

d. Carton:

Style:

Stock:

Suppliers:

ii. Syringes

a. Container:

Source:

Closure:

Source:

Shield:

Source:

b. Label:

Material:
Stock:

Adhesive:

Liner:

Size:

Suppliers:

c. Thermoformed PET Tray:

Material:

Suppliers:

d. Blister Pack Cover:

Material:

Style:

Suppliers:

e. Package Insert:

Folded Size:

Stock:

Printing:

Suppliers:

f. Carton:

Style:

Stock:

Suppliers:

Orgaran™ Injection is marketed for hospital use, by order from a licensed physician and under the direct supervision of a licensed physician.

E. Site of Drug Product Disposal

Organon Inc. considers Orgaran™ Injection to be non-hazardous. Material Safety Data Sheets for all inactive ingredients are provided in ATTACHMENT B. Since Orgaran™ Injection is considered non-hazardous, permits are not required for disposal.

Orgaran™ Injection will be used in hospitals for in-patient use throughout the United States. Disposal of empty ampules and syringes by the hospitals using the product, to the best of Organon Inc.'s knowledge, will be in compliance with federal, state and local statutes and regulations.

Expired and/or rejected drug product will be returned to Organon Inc., West Orange, New Jersey, where the materials are size reduced in a material shredder. Sawdust is added when necessary as an absorbent. The shredded materials are disposed of in an on-site dumpster which is collected by a contracted waste hauler. The non-product packaging material waste is transported to the Essex County Resource Recovery Facility where it is destroyed. Product waste is transferred to

to be disposed in a landfill designated by the State. Unused non-product packaging materials generated by the Allentown, PA facility are disposed of in the municipal trash except cardboard which is sent to a recycler. Product waste is returned to the West Orange facility where they are destroyed as mentioned above.

Listed below are the firms proposed for disposal activities.

I. The current waste hauler for the West Orange, New Jersey facility is:

ii. The waste is destroyed by either:

Essex County Resource Recovery Facility
183 Raymond Boulevard
Newark, New Jersey 07105
(NJDEP Facility Number 0714X)

This facility is operated by:

Or by:

iii. Cardboard and paper are shredded by:

iv. The current waste disposal firms for the Allentown, Pennsylvania facility are:

5. **Identification of Chemical Substances that are the Subject of the Proposed Action:**

The nomenclature, chemical and physical properties of the active ingredient in Orgaran™ Injection are as follows:

Chemical Nomenclature

United States Adopted Name (USAN):	Danaparoid Sodium
CAS Registry Number:	83513-48-8
Laboratory Code Name:	ORG 10172
Trade Name:	Orgaran™
Chemical Name:	Sulfated Glycosaminoglycuronan

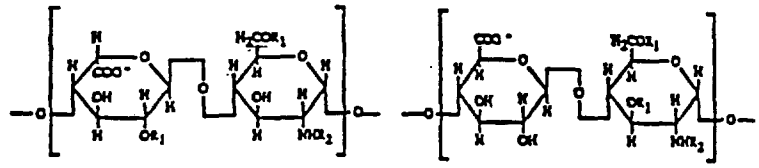
Physical and Chemical Characteristics

Appearance: White to creamy-white, amorphous powder

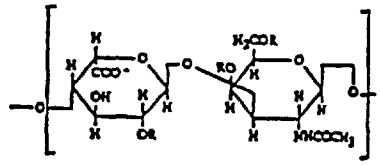
Molecular Mass: 4000 - 8000 Daltons

Structural Formula:

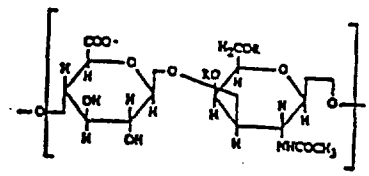
Main Repeating Disaccharide Units



Heparan Sulfate: R_1 = H or SO_3^-, R_2 = $COCH_3$ or SO_3^-



Dermatan Sulfate



Chondroitin Sulfate

Any product derived from natural sources may contain impurities originating from the starting material or chemicals used in the manufacturing process. In the case of the manufacture of danaparoid sodium, the starting material may include intrinsic components of the intestine (i.e. proteins, nucleic acids, micro-organisms, viruses, endotoxins and heavy metals). Chemicals used in the manufacturing process and detailed descriptions of the impurities and impurity specifications are provided in CONFIDENTIAL ATTACHMENT IA

6. **Introduction of Substances into the Environment:**

This section has been formatted according to the CFR format requirements for an abbreviated Environmental Impact Assessment for a naturally occurring product.

As stated in Section 4.C, the manufacture of the drug substance, danaparoid sodium, will be carried out by Therefore, no environmental release resultant of this operation is expected in the United States, either in West Orange, New Jersey or Allentown, Pennsylvania.

The air emission from the production area to the surrounding environment is regulated by the The clean-up water, which may contain pharmaceutical waste obtained from the cleaning process, is cooled and pretreated according to regional permissions, and in accordance with the and is discharged to the waste water treatment company. ATTACHMENT A contains letters certifying that is in compliance with applicable air and water emissions requirements and has been granted authority to produce and distribute danaparoid sodium by authorities.

During the production of Orgaran™ Injection in West Orange, New Jersey, the following chemical substances may be released into the occupational, atmospheric, and aquatic environments from the manufacturing and equipment cleaning processes.

Material Safety Data Sheets (MSDS) for each of the chemical substances are provided in ATTACHMENT B.

Active Ingredient
Danaparoid Sodium

Inactive Ingredients
Sodium Sulfite Anhydrous (Reagent grade)
Sodium Chloride, USP
Hydrochloric Acid, NF
Sodium Hydroxide, NF
Nitrogen, NF

Schematic Process Flow Diagrams for Orgaran™ Injection, presented in CONFIDENTIAL APPENDIX I, provide information on the processing capacity of chemical substances and the type of equipment used to manufacture the drug product.

In the manufacturing process, the weighing of dry chemicals and dispensing of liquid chemicals to prepare the batch, the processing of these chemicals into the bulk solution, and the equipment and room cleaning processes could allow for the release of pharmaceutical wastes from the sterile production manufacturing area into the environment. A detailed description of the environmental controls employed during the manufacture of Orgaran™ Injection is provided in CONFIDENTIAL APPENDIX II.

The air emission from the manufacturing area to the outside environment is regulated by the Department of Environmental Protection. The clean-up water, which may contain pharmaceutical waste obtained from the cleaning process, is diluted, neutralized and discharged into the public sewer for transport to the wastewater treatment company. The disposal of the wiping cloths used during clean-up is carried out by way of routine refuse removal.

A statement confirming Organon Inc.'s compliance with all federal, state and local environmental laws is provided in ATTACHMENT C. The approval of this application will have no effect upon Organon Inc.'s compliance with current emission requirements.

It is expected that sales of Orgaran™ Injection will replace sales of currently marketed heparin-based anticoagulants. Therefore, the net increase, if any, of heparin-based anticoagulants entering the environment should be negligible.

7. **Fate of Emitted Substances in the Environment:**

This section has been formatted according to the CFR format requirements for an abbreviated Environmental Impact Assessment for a naturally occurring product.

Danaparoid sodium, the active constituent of Orgaran™, is isolated from porcine mucosa, the same starting material as used for commercial heparin and low molecular weight heparin products. The manufacturing of the active material, danaparoid sodium, will take place at

Through the parenteral administration of Orgaran™ Injection to patients in the United States, it is expected that danaparoid sodium will enter the environment through the excrement of patients. It is expected that the quantity of danaparoid sodium and its metabolites released into the environment through human excrement will displace existing quantities of active materials and metabolites of currently marketed heparin-based anticoagulants. Even if this displacement is not taken into consideration, the expected introduced concentration (EIC) will be significantly less than one part per billion as established for Tier O evaluations (Guidelines for Industry for the Submission of an Environment Assessment in Human Drug Applications and Supplements, CDER, November 1995). Routinely, at concentration levels below one part per billion, drugs have been shown to have no significant effect on relevant standard test organisms and therefore unlikely to have a significant effect on the environment. The calculations for the expected introduced concentration are found in CONFIDENTIAL APPENDIX IB.

Based on the above, the introduction and use of Orgaran™ into the environment is not expected to significantly alter the concentration and distribution of the product in the natural environment.

Product waste is transferred to to be disposed in a landfill designated by the state.

8. **Environmental Effects of Released Substances:**

This section has been formatted according to the CFR format for an abbreviated Environmental Impact Assessment for a naturally occurring product.

Danaparoid sodium is a low molecular weight heparinoid comprised of a heterogeneous mixture of natural sulfated glycosaminoglycans isolated from the mast cells of porcine intestinal mucosa, where they are biosynthesized and stored. Therefore, it is a naturally occurring biological substance, analogous to heparin. At present, no adverse environmental effects on animals, plants, humans and other organisms have been recognized as a result of, or attributed to, the use of heparin or heparinoids.

It is anticipated that no adverse short or long-term side effects are predicted as a consequence of the release of Orgaran™ Injection into the environment at levels associated with its production, use and disposal.

A summary of available nonclinical toxicological data on the acute and chronic toxicity of danaparoid sodium is provided in CONFIDENTIAL APPENDIX III. See CONFIDENTIAL APPENDICES V through XXXVI for the nonclinical toxicology study reports.

9. **Use of Resources and Energy:**

The impact of Orgaran™ Injection, including the packaging components, on the use of resources and energy is nominal and is not excessive. The raw materials utilized to manufacture Orgaran™ Injection are common compounds, all of which are in ample commercial supply. Only very small increases in the utilization of energy is anticipated since production occurs at an existing facility. The expected product volume will not significantly increase the consumption of those resources beyond levels presently experienced.

No effects upon endangered or threatened species and upon property listed in or eligible for listing in the National Register of Historic Places are anticipated.

10. **Mitigation Measures:**

No known potential adverse environmental impact exists for current anticoagulant agents or for Orgaran™ Injection.

The manufacture, distribution, use and disposal of Organon™ Injection takes place under highly regulated and controlled conditions which further mitigate against negative environmental consequences.

11. **Alternatives to the Proposed Action:**

Not applicable since potential adverse environmental impacts brought on by the production, use, and disposal of Orgaran™ Injection have not yet been identified. An alternative action is no action.

12. **Preparers:**

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West Long Branch, New Jersey

13. **Certification:**

The undersigned official certifies that the information presented is true, accurate and complete to the best of the knowledge of the firm or agency responsible for preparation of the environmental assessment.

Patrick J. Osinski
Vice President,
Product Development and Government Affairs

Dec. 20, 1995
Date

14. **References:**

1. Anticoagulant activity of heparin separation of high activity and low activity heparin species by affinity chromatography on immobilized antithrombin, Febs Lett 66 90-93 (1976), HööK, M.; Björk, T.; Hopwood, J.; Lindahl, U.

2. The separation of active and inactive forms of heparin, Biochem Biophys Res Comm 69 570-577 (1976), Lam, L.H.; Silbert, J.E.; Rosenberg, R.D.

3. Purification of antithrombin iii by affinity chromatography, Thromb Res 5, 439-452 (1974), Miller-Andersson, M.; Borg, H.; Andersson, L.

See APPENDIX IV for the above three references.

STATEMENT OF COMPLIANCE

Organon Inc. states that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the production and packaging of Orgaran™ Injection, as well as emission requirements set forth in applicable federal, state and local statutes and regulations applicable to the production and packaging of Orgaran™ Injection at its facilities in West Orange, New Jersey and Allentown, Pennsylvania.

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020430

MICROBIOLOGY REVIEW

Oliver

OCT 4 1995

CONSULTATIVE REVIEW TO HFD-180
DIVISION OF MEDICAL IMAGING, SURGICAL, AND DENTAL DRUG PRODUCTS
MICROBIOLOGIST'S REVIEW NO. 1
October 4, 1995

MICROBIOLOGY REVIEWER: CAROL K. VINCENT

A. 1. NDA No.: 20-430
PRODUCT NAME: Orgaran (danaparoid sodium) Injection

APPLICANT: Organon Inc.
375 Mt. Pleasant Avenue
West Orange, NJ 07052

2. DOSAGE FORM AND ROUTE OF ADMINISTRATION: Two 0.6 mL (750 anti-Xa units) presentations: ampul, room temp storage [2-30°C], and pre-filled syringe, disposable with 25 ga needle, refrigerated storage [2-8°C]. For deep sub-cutaneous injection into fold of skin, twice daily, 6-14 days.

3. METHOD(s) OF STERILIZATION:

4. PHARMACOLOGICAL CATEGORY AND / OR PRINCIPAL INDICATION:
For prevention of post-operative deep vein thrombosis (DVT) in patients undergoing orthopedic hip surgery.

5. DRUG PRIORITY CLASSIFICATION: 1 S

B. 1. INITIAL APPLICATION DATE: 09-08-94
2. AMENDMENT: 11-22-94
3. RESUBMISSION DATE: 12-29-94
4. APPLICATION FILED: 02-28-95
5. RECEIVED FOR REVIEW: 09-28-94; 11-28-94; 01-06-95; 02-22-95
6. AMENDMENT: 02-06-95

C. REMARKS:

D. CONCLUSION: We recommend this application for approval for microbiological quality and sterility assurance based on the sterilization process validation information submitted on 12-29-94. A minimal number of items need clarification or additional information that the applicant can provide on a post-approval basis. The nature of these items does not change the recommendation for approval from Microbiology.

NB: *information provided for liquid-filled containers and rubber stoppers is not relevant to the Orgaran (danaparoid sodium) injection filled in syringes or glass ampuls, subject of this NDA, and is not reviewed at this time.*

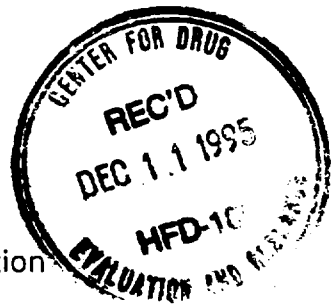
cc:
Orig. NDA 20-430
HFD-180/Gibbs/Al-Hakim/KOliver
HFD-160/Consult file/CKVincent
Drafted by: CKVincent/06-27-95/10-03-95
R/D Init by: PHCooney/10-04-95

Carol K. Vincent, HFD-160 10-4-95

PHC 10/4/95

DEC [illegible] 1995

REVIEW FOR DIVISION OF MEDICAL IMAGING AND RADIOPHARMACEUTICAL DRUG PRODUCTS
OFFICE OF NEW DRUG CHEMISTRY
MICROBIOLOGIST'S REVIEW NO. 2
December 5, 1995



MICROBIOLOGY REVIEWER: Carol K. Vincent, Microbiology Staff, HFD-805

A. 1. NDA No.: 20-430
PRODUCT NAME: Orgaran (danaparoid sodium) Injection

APPLICANT: Organon Inc.
375 Mt. Pleasant Avenue
West Orange, NJ 07052

2. DOSAGE FORM AND ROUTE OF ADMINISTRATION: Two 0.6 mL (750 anti-Xa units) presentations: ampul, room temp storage [2-30°C], and pre-filled syringe, disposable with 25 ga needle, refrigerated storage [2-8°C]. For deep sub-cutaneous injection into fold of skin, twice daily, 6-14 days.

3. METHOD(s) OF STERILIZATION:

4. PHARMACOLOGICAL CATEGORY AND / OR PRINCIPAL INDICATION: For prevention of post-operative deep vein thrombosis (DVT) in patients undergoing orthopedic hip surgery.

5. DRUG PRIORITY CLASSIFICATION: 1 S

B. 1. AMENDMENT: 11-28-95

2. RECEIVED FOR REVIEW: 12-04-95

C. REMARKS: The applicant's 11-28-95 amendment, subject of this review, responds to the Division of Gastrointestinal and Coagulation Drug Products' October 19, 1995 Information Request letter. This letter conveyed a minimal number of minor items needing clarification or additional information that were discussed in the previous review (SEE MICROBIOLOGIST'S REVIEW NO. 1 FOR NDA 20-430 DATED OCTOBER 4, 1995). The minor nature of these items does not change the recommendation for approval from the Microbiology perspective.

D. CONCLUSION: We recommend NDA 20-430 for approval for microbiological quality and sterility assurance based on the sterilization process validation information submitted on 12-29-94, 02-06-95, and 11-28-95.

NB: *information provided for liquid-filled containers and rubber stoppers is not relevant to the Orgaran (danaparoid sodium) injection filled in syringes or glass ampuls, subject of this NDA, and is not reviewed at this time.*

cc:
Orig. NDA 20-430
HFD-180/Gibbs/Al-Hakim/KOliver
HFD-160/Consult file/CKVincent [HFD-805]
Drafted by: CKVincent/12-04-95
R/D Init by: PHCooney/12-05-95

Carol K. Vincent, HFD-160

12-5-95

HFD-12/07/95

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020430

PPHARMACOLOGY REVIEW

Reviewer: Tanveer Ahmad, Ph.D.
Pharmacologist, HFD-180

APR - 4 1995

Sponsor & Address: Organon Inc.
West Orange, NJ 07052

Date of Review: March 17, 1995

Date of HFD-180 Receipt: September 9, 1994

Date of Submission: September 8, 1994

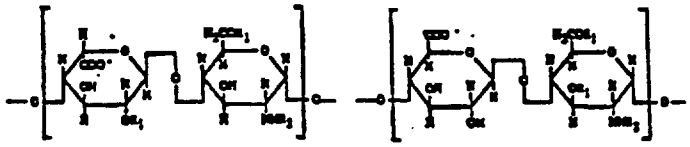
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA (Original Summary)

Drug: Orgaran™/Danaparoid Sodium/ORG 10172 (S.C. Injection)

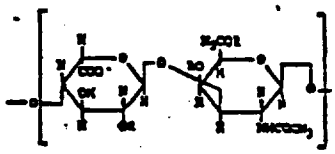
Chemical Name: Sulfated Glycosaminoglycuronan

Structural Formula:

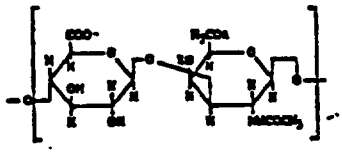
Main Repeating Disaccharide Units:



Heparan Sulfate: R_1 = H or SO_3^-, R_2 = $COCH_3$ or SO_3^-



Dermatan Sulfate



Chondroitin Sulfate

Average M.W. = 4000 - 8000 Daltons

Formulation: Each 1.0 ml of sterile isotonic aqueous solution contains 1200 anti-Xa units of ORG 10172 along with inactive ingredients such as sodium sulfite (1.5 mg), sodium chloride, HCl, NaOH and nitrogen.

Category: Antithrombotic agent.

Proposed Marketing Indication: Prophylactic therapy for post-operative deep venous thrombosis (DVT) and pulmonary embolism (PE) in patients undergoing orthopedic hip surgery.

Dose: 750 anti-Xa units b.i.d. via s.c. route for 7-10 days (15 anti-Xa u/kg, b.i.d., 50 kg body wt. assumed).

PRECLINICAL STUDIES AND TESTING LABORATORIES

Type of Study	Study #	Drug Lot #	Testing Laboratories
Pharmacology			
Absorption:			
Rat, Rabbit & Dogs			
Distribution:			
Not done			
Metabolism:			
Not done			
Excretion:			
Rat			
Acute Toxicity:			
Rat			
i.v.	SDG RR 2207	E	
s.c.	SDG RR 2208	E	
Dog			
i.v.	SDG RR 2308	BK	
s.c.	SDG RR 3222	BU	
Subacute/Subchronic/Chronic Toxicity:			
Rat			
6-Week (i.v.)	SDG RR 1268	K	ORDSL
6-Week (s.c.)	SDG RR 1269	K	ORDSL
6-Month (i.v.)	SDG RR 2448	IPA 87017/3	

Dog			
6-Week (i.v.)	SDG RR 1270	K	ORDSL
6-Week (s.c.)	SDG RR 1271	K	ORDSL
6-Month (i.v.)	SDG RR 2449	IPA 87018/3	
		IPA 87018/4	
		IPA 87018/5	
Reproductive Toxicity:			
Fertility & Reproductive Performance (Segment I)			
Rat (i.v.)	SDG RR 2258	IPA 82026	ORDSL
Teratology (Segment II)			
Rat (i.v.)	SDG RR 3128	CP 087143	
Rabbit (i.v.)	SDG RR 2209	IPA 82019	ORDSL
Perinatal/Postnatal (Segment III)			
Rat (i.v.)	SDG RR 3129	CP 087143	
Mutagenicity:			
Ames Test	1636/2900	382/0112, 390/0146	ORDSL
Chromosomal Aberration Test (in vitro)	LSR-051005-M-07086	084127	
UDS Assay in Hela Cell Cultures	LSR-051007-M-07286	084127	
CHL/HGPRT Forward Gene Mutation Assay	LSR-051006-M-07186	084127	
Mouse Micronucleus Test (in vivo)	2023	084127	
Special Toxicity Studies:			
Local Tolerance Study in rats (i.v.)	SDG RR 2460	BJ, K	
Local Tolerance Study in rats (s.c.)	SDG RR 2461	BJ, K	
2-Week i.v. Toxicity in Rats Using Batch K + 0.15% Sulfite	SDG RR 2305	K	ORDSL
2-Week i.v. Toxicity in Rats Using Batch BF + 0.15% Sulfite	SDG RR 2306	BF	ORDSL
Antigenicity Test in Mouse	SDG RR 2351		
Antigenicity Test in Guinea-Pigs	SDG RR 2275		

ORDSL = Organon Drug Safety R & D Labs, Schaijk, Netherlands

GOOD LABORATORY PRACTICE REGULATIONS:

A statement of compliance with GLP regulations and QAUI was not included for the following studies:

6-Week i.v. toxicity study in rats (SDG RR 1268)
6-Week s.c. toxicity study in rats (SDG RR 1269)
6-Week i.v. toxicity study in dogs (SDG RR 1270)
6-Week s.c. toxicity study in dogs (SDG RR 1271)

According to Dr. I. Zayed (Department Head of the Drug Safety R & D Labs., Organon Inc.), the above mentioned studies "were not inspected, final reports were not reviewed and no statement was prepared and signed by Quality Assurance Unit. However, the final reports were verified for accuracy of the raw data and the text". Even though the above mentioned studies were not done strictly under GLP regulations, there were sufficient detail information to assess the studies and these reports are acceptable.

ORG 10172 is sulfated glycosaminoglycuronans which is related chemically and pharmacologically to heparin. ORG 10172 contains hepran sulfate (~84%), dermatan sulfate (~12%) and small amount of chondroitin sulfate (~4%). On the basis of AT-III affinity ORG 10172 can be separated into two fractions: high affinity (HA) fraction (5%) and low affinity (LA) fraction (95%). HA-fraction consists of hepran sulfate and LA-fraction consists of hepran sulfate dermatan sulfate and minor amount of chondroitin sulfate.

In various experiments, ORG 10172 dose was expressed in mg/kg or anti-Xa unit/kg. The potency of ORG 10172 in terms of biological activities were as follows:

1 mg = 11-17 anti-Xa u = 0.5 anti IIa units.

PHARMACOLOGY:

Primary Activities

1. **Anti-thrombotic Activity:**

Anti-thrombotic activity of ORG 10172 was assessed in various animal models.

a. Venous Stasis Model in Rats and Rabbits:

Rats:

Venous stasis/thromboplastin induced thrombus was dose-dependently inhibited by ORG 10172, heparin or fragmin (10, 20 and 40 anti-Xa u/kg) when given 5 min. before the administration of thromboplastin suspension (0.1 ml/rat; 1:50 v/v diluted). All three drugs were equipotent with respect to anti-thrombotic activity (ED_{50} about 15 anti-Xa u/kg, i.v.).

Rabbits:

Venous stasis/thromboplastin induced thrombus was dose-dependently inhibited by ORG 10172 (20-30 anti-Xa unit/kg) or heparin (3-10 anti-Xa unit/kg) when given intravenously at 1.5 min. before the administration of thromboplastin (250 μg/kg). The ED_{50} values were 25 and 5 anti-Xa u/kg for ORG 10172 and heparin respectively. Hence, heparin is about 5 times more potent than ORG 10172 when administered intravenously as anti-thrombotic agent in this experiment. However, when ORG 10172 or heparin (100-400 anti-Xa u/kg) was given via s.c. route then their anti-thrombotic activities were comparable in this model.

b. AV-Shunt Model in Rats and Rabbits:

Rats:

Both heparin (200-1600 anti-Xa unit/kg) and ORG 10172 (200-1600 anti-Xa unit/kg) dose-dependently inhibited thrombus formation when given via s.c. route in A-V shunt model in rats. The ED_{50} values were 364 and 497 anti-Xa units/kg for ORG 10172 and heparin respectively. In another separate experiment in which drug (heparin [20-320 anti-Xa units/kg], ORG 10172 [20-320 anti-Xa units/kg] or fragmin [LWH: 20-320 anti-Xa units/kg]) was given via intravenous route, the thrombus formation was inhibited dose-dependently by all three drug. The ED_{50} values were 104, 81 and 64 anti-Xa units/kg for ORG 10172, heparin and fragmin respectively.

In a separate experiment, anti-thrombotic effect of ORG 10172 was assessed in A-V shunt model in rats by measuring platelet and fibrin-deposit along with thrombus weight, ORG 10172 (20-960 anti-Xa u/kg) and heparin (70-700 anti-Xa u/kg) both dose-dependently inhibited thrombus formation and fibrin deposit when given intravenously. In addition heparin also dose-dependently reduced the platelet deposit in thrombus, in contrast platelet deposit in the thrombus was not affected by ORG 10172 (up to 640 anti-Xa units/kg) treatment.

The above experiment was repeated and ED_{50} values for thrombus formation, fibrin deposit and platelet deposit were calculated when ORG 10172 (20-640 anti-Xa u/kg), heparin (40-640 anti-Xa u/kg) or fragmin (20-640 anti-Xa u/kg) was given intravenously. ORG 10172, heparin and fragmin dose-dependently inhibited thrombus formation (ED_{50} values: 36, 40 and 36 anti-Xa u/kg respectively) and fibrin deposit (ED_{50} values: 55, 54 and 46 anti-Xa u/kg respectively). A dose of 640 anti-xa u/kg (i.v.) of ORG 10172 inhibited thrombus weight and fibrin deposition by 86% and 88% respectively. In contrast to ORG 10172 (ED_{50} = >640 anti-Xa u/kg) both heparin and fragmin inhibited platelet deposition in thrombi (ED_{50} values: 180 and 270 anti-Xa u/kg respectively).

Effects of ORG 10172 Sub-fractions on AV-Shunt Model in Rats:

In this experiment effects of ORG 10172 (parent drug), ORG 10849 (high-affinity fraction), ORG 30561 (low-affinity fraction) and its subfractions ORG 30955 (hepran sulphate) and ORG 31287 (dermatan sulphate) on thrombus formation were assessed in AV-shunt model in rats. In addition, fibrin deposition (via ^{125}I-labelled fibrinogen) and platelet deposition (via $^{51}C_{\gamma}$-labelled platelet) into the thrombi were also assessed.

ORG 10172 and its subfractions dose dependently inhibited thrombus formation and fibrin deposition into thrombi. Platelet deposition into thrombi was not affected by ORG 10172 or its subfractions.

Compound	ED_{50} (Anti-Xa u/kg)	
	Thrombus Weight	Fibrin Deposit
ORG 10172 (parent drug)	72	111
	33	69
ORG 10849 (high-affinity fraction)	157	357
ORG 30561 (low-affinity fraction)	5.5	10
ORG 30955 (hepran sulphate)	5.6	19
ORG 31287 (dermatan sulphate)	<1.3	<2.5

The duration of anti-thrombotic activity of high-affinity fraction of ORG 10172 (ORG 10849) was similar to that seen with the parent drug (ORG 10172). According to the sponsor, the anti-thrombotic activity of low-affinity fraction (ORG 30561) lasted for a short time compared to ORG 10172 or ORG 10849 (different amount of anti-Xa unit were used in this model, therefore data could not be compared properly).

Duration of Anti-thrombotic Activity in AV-Shunt Model in Rats:

Irrespective of the dose (400, 800 or 1600 anti-Xa u/kg) of route of administration (i.v. or s.c.), the anti-thrombotic effect of ORG 10172 lasts at least twice as long as heparin in this model.

Rabbits:

In rabbit A-V shunt model, intravenous administration of ORG 10172, heparin or enoxaparin (200 and 400 anti-Xa u/kg) inhibited fibrin and platelet deposit in thrombi and the inhibition was comparable for all three drugs (data presented graphically).

c. **Fibrin Accretion Model in Rabbits**: ORG 10172, heparin, LMW-heparin (Cy 222) and enoxaparin (130-660 anti-Xa unit/kg, i.v. in 4 hours) dose dependently inhibited ^{125}I-fibrin deposit into the thrombus in this model. ORG 10172 was more potent anti-thrombotic agent in this model, the IC_{50} was approximately 130 anti-Xa unit/kg (total dose) while the IC_{50} values for other comparators were around 250 anti-Xa unit/kg (total dose) (data presented graphically).

d. **Injury Models in Rats**: In this experiment effects of a single s.c. administration of ORG 10172, heparin or 4 LMW-heparins on the occlusion of vessels injured by laser beams were assessed. All compounds showed anti-thrombotic effects which was dose dependent. The anti-thrombotic effect of ORG 10172 was "maximal at 24 hr after s.c. dose, while heparin after 48 hr, and LMW-heparin for up to 96 hr".

In arterial injury model, ORG 10172 (15 and 50 anti-Xa unit/kg/hr, i.v.), heparin (0.05-50 anti-Xa u/kg/hr, i.v.) and fragmin (15 and 50 anti-Xa unit/kg/hr) significantly inhibited intimal thickening after injury. In this experiment ORG 10172 and fragmin (LMH) has comparable potencies while heparin was most potent (curve shifted towards left of ORG 10172 and fragmin).

2. **Effects on Bleeding**: Effects of ORG 10172 on bleeding in various models (muscle bleeding in rats, subdermal bleeding in rats and rabbits, ear bleeding in rats and rabbits, hematoma formation in rats, bleeding time model in dogs and cardio-pulmonary bypass surgery in dogs) were assessed.

Muscle Bleeding Model in Rats: ORG 10172 (50-200 anti-Xa unit/kg, i.v.) did not increase blood loss in this model. Higher dose levels of ORG 10172 (400-1600 anti-Xa unit/kg, i.v.) resulted in significant increase in blood loss (126-175% of

control), but the effect was not dose related. In contrast, heparin (88-350 anti-Xa unit/kg, i.v.) dose-dependently increased the blood loss (178-425% of control) in this model.

Subdermal Bleeding Model in Rats and Rabbits:

Rat

In rats, ORG 10172 (1600-6400 anti-Xa u/kg, s.c.) and heparin (800-3200 anti-Xa u/kg, s.c.) increased blood loss dose-dependently. A 3-fold increase in blood loss compared to control was achieved by 2600 and 800 anti-Xa u/kg (s.c.) of ORG 10172 and heparin respectively. Additionally, bleeding enhancing activities of ORG 10172 after single (1600 anti-Xa u/kg) or subchronic (1600 anti-Xa u/kg b.i.d. for 4.5 days) s.c. administration were comparable (476% and 558% of control respectively).

In this model when drug was given via i.v. route, then ORG 10172 as well as heparin and fragmin (LMH) dose-dependently enhanced blood loss. A 3-fold increase in blood loss compared to control was achieved by 550, 180 and 375 anti-Xa u/kg of ORG 10172, heparin and fragmin respectively.

Effects of Subfractions of ORG 10172 on Blood Loss:

In subdermal bleeding model in rats, ORG 10172 (parent drug), ORG 10849 (high-affinity fraction), ORG 30561 (low-affinity fraction), and ORG 30955 (hepran sulphate) dose-dependently increased the blood loss. The effects of ORG 30955 (hepran sulphate) was comparable to ORG 30561 (low-affinity fraction) (data presented graphically).

Rabbits

Both ORG 10172 and heparin (100-400 anti-Xa u/kg, s.c. or 50-400 anti-Xa u/kg, i.v.) dose-dependently increased blood loss. The slope of dose-response curve of heparin is steeper than that of ORG 10172. Heparin was clearly more potent than ORG 10172 in this experiment (data presented graphically).

Ear Bleeding Model in Rats and Rabbits:

Rat

ORG 10172 (300 anti-Xa u/kg, i.v.) had no effect on rat ear bleeding time, while heparin (300 anti-Xa u/kg, i.v.) significantly increased the bleeding time (control = 98 sec, heparin = 251 sec).

Electron Microscopic Examination of Hemostatic Plug:

In rat ear bleeding model, rats were given a single i.v. dose of ORG 10172 or heparin (300 or 600 anti-Xa u/kg) at 1 or 5 min prior to bleeding induction. At 5, 15 or 30 min after bleeding induction, the wounds were excised and examined electron microscopically. Compared to heparin, ORG 10172 had less inhibiting effect on platelet degranulation and fibrin formation at the site of hemostatic plugs. In this experiment ORG 10172 had no effect on bleeding time while heparin significantly increased the bleeding time.

Rabbits

ORG 10172 (100-3200 anti-Xa u/kg, i.v.) had no effect on blood loss from rabbit ear in this model. Heparin (100-800 anti-Xa u/kg, i.v.) significantly dose dependently increased blood loss (data presented graphically) in this model.

Hematoma Formation in Rats:

In rats, both heparin (400-1600 anti-Xa u/kg, s.c.) and ORG 10172 (800-1600 anti-Xa u/kg, s.c.) induced hematoma formation, however, heparin was more potent than ORG 10172 (400 anti-Xa u/kg, [s.c.] of ORG 10172 had no effect on hematoma formation, while same dose of heparin produced hematoma in 100% of the animals).

Effects on Bleeding Time in Dogs:

Both ORG 10172 and heparin (50-400 anti-Xa u/kg, i.v.) dose-dependently increased bleeding time in dogs. In this model potencies of ORG 10172 and heparin were comparable (ORG 10172: 120-248% of control and heparin: 144-265% of control).

Effects on Cardiopulmonary Bypass (CPB) Surgery in Dogs:

In this experiment effect of ORG 10172 (260 anti-Xa u/kg, intracardiac followed by continuous infusion of 0.9% saline) and heparin (250 anti-Xa u/kg intracardiac followed by continuous infusion of 75 anti-Xa u/kg/hr) were assessed on postoperative blood loss after CPB surgery in dogs. According to the surgeon, the mean postoperative blood loss during the first 2.5 hr was 625 ml in ORG 10172 treated dogs and 806 ml in heparin treated dogs. Hence, ORG 10172 is slightly better than heparin with respect to minimizing the blood loss after CPB surgery.

3. **Effects on Coagulation Parameters**:

Effects on Intrinsic Coagulation Pathway [Activated Partial Thromboplastin Time (APTT)]:

In Vitro

In rat plasma ORG 10172 and heparin doubled the APTT at concentrations of 0.9 and 0.7 anti-Xa u/ml respectively, while in human plasma, concentrations of ORG 10172 and heparin needed for doubling the APTT were 0.9 and 0.4 anti-Xa u/ml respectively.

Ex Vivo

In rats, both ORG 10172 and heparin dose-dependently increased ex-vivo APTT after i.v. dose. ORG 10172 and heparin doubled APTT at doses of 200 and 18 anti-Xa unit/kg respectively. Hence, ORG 10172 is less potent than heparin.

Effects on Extrinsic Coagulation Pathway [Prothrombin Time (PT)]:

In Vitro

In rat plasma, ORG 10172 as high as 10 anti-Xa u/ml increased PT by 1.5 fold over the control values, while 6 anti-Xa u/ml of heparin doubled the PT. In human plasma, 4 anti-Xa u/ml of ORG 10172 increased the PT by 1.5 fold, while same concentration of heparin increased the PT by 7.5 fold. Therefore, ORG 10172 effects on PT was less pronounced than heparin.

Effects on Thrombin Time (TT):

The plasma thrombin time represents the rate of conversion of fibrinogen to fibrin by a standard amount of thrombin.

In Vitro

In rat plasma, ORG 10172 and heparin doubled the TCT at concentrations of 0.47 and 0.07 anti-Xa unit/ml respectively. In human plasma, the TCT was doubled at 0.33 and 0.038 anti-Xa unit/ml of ORG 10172 and heparin respectively. Hence, ORG 10172 effects on TCT was less potent than heparin.

Effects on Heptest (A Coagulation Assay Where Coagulation is Induced by Factor Xa):

In Vitro

In human plasma, ORG 10172 (0.02-0.5 anti-Xa u/ml) and heparin (0.02-0.5 anti-Xa u/ml) both prolonged dose-dependently factor Xa induced clotting time (heptest). In this experiment ORG 10172

was more potent then heparin (data presented graphically). This increased activity could be due to the inhibitory effects of ORG 10172 on thrombin generation and fibrin formation which also contributed to the effect in the heptest.

Effects on Amidolytic Anti-Xa Activity:

In vitro, ORG 10172 inhibited factor Xa amidolytic activity (using chromogenic substrate S2222) in human plasma as well as in an AT-III buffer system. The specific anti-Xa activity was 16.7 units/mg. According to sponsor, on weight basis, anti-Xa activity of heparin was about 10-15 times greater than ORG 10172. The data also indicated that anti-Xa activity of ORG 10172 resides mainly in high-affinity fraction (ORG 10849). Low-affinity fraction (ORG 30561), the subfraction hepran sulfate (ORG 30955) and the subfraction hepran sulphate plus dermatan sulphate (ORG 30995) had very little (negligible) anti-Xa activity.

In an ex-vivo experiment, rabbits were given 100 anti-Xa u/kg of ORG 10172 via s.c. route. The amidolytic anti-Xa activity reached to maximum at 4 hr after drug administration.

Effect on Amidolytic Anti-IIa Activity:

Thrombin (factor IIa) is neutralized by anti-thrombin III (AT-III) and heparin cofactor II (HC-II). Heparin and/or heparin like compound catalyzes the inhibiting activity of AT-III and/or HC-II. Anti-IIa activity was determined by using chromogenic substrate S2238 in the presence of AT-III and/or HC-II.

In vitro, ORG 10172 inhibits factor IIa amidolytic activity in human plasma. According to sponsor, on weight basis anti-IIa activity of ORG 10172 is approximately 150-200 times less than heparin. Similar results were seen when anti-IIa activity of ORG 10172 was measured in an AT-III or HC-II buffer system (ORG 10182: ID_{50} of anti-IIa activity via AT-III: 20 mcg/ml and via Hc-II: 24 mcg/ml). Anti-IIa activity of heparin was mostly mediated via AT-III (ID50: 0.03 mcg/ml). The anti-IIa activity of heparin mediated via HC-II was very small (ID_{50}: 1 mcg/ml). Data also indicated that antithrombin activity of ORG 10172 is mainly associated with high-affinity fraction, while HC-II mediated antithrombin activity is similar both in the high- and low-affinity fraction.

In an ex-vivo experiment, maximum anti-IIa activity (0.08 u/ml) was seen at 2 hr after s.c. administration of ORG 10172 (100 anti-Xa u/kg).

Effect on Thrombin Generation:

Effects of ORG 10172 on thrombin generation in vitro were measured by measuring IIa-generation inhibitory (IIaGI) activity in human plasma. Both ORG 10172 and heparin dose dependently inhibited thrombin generation in human plasma when induced by activated bovine factor Xa (ID_{50}: ORG 10172: 0.14 anti-Xa u/ml, heparin: 0.10 anti-Xa u/ml).

In another experiment IIaGI activity was assessed in buffer system containing AT-III or HC-II. ORG 10172 dose-dependently inhibited thrombin generation in buffer system containing AT-III or HC-II. Data also indicated that AT-III mediated inhibition of thrombin generation by ORG 10172 is mainly due to the high-affinity fraction, while HC-II mediated activity was similar of both (low and high) fractions of ORG 10172.

Effect on IIaGI Activity in Buffer System		
Compound	ID_{50} of IIaGI Activity (mcg/ml)	
	Via HC-II	Via AT-III
ORG 10172	52	10.6
ORG 10849 (high-affinity fraction)	80	0.9
ORG 30561 (low-affinity fraction)	75	98
Heparin	3	0.4

Inactivation of Thrombin (ex-vivo):

In rabbits, i.v. administration of heparin (800 anti-Xa u/kg), ORG 10172 (800 anti-Xa u/kg) or pentosan polysulfate (12 mg/kg) inactivated about 90% of thrombin. Heparin and enoxaparin inactivated thrombin via AT-III (percent complex with AT-III: 90% and percent complexed with HC-II: 10%) while ORG 10172 and pentosan sulfate inactivated thrombin via HC-II (percent complex with AT-III: 20% and percent complexed with HC-II: 80%). Thus AT-III is the main inhibitor of thrombin when heparin or enoxaparin is used, while ORG 10172 inhibits thrombin mainly through HC-II.

Duration of Anti-Xa, Anti-IIa and IIaGI Activities in Various Species After i.v. or s.c. Administration of ORG 10172, Heparin or Tedelparin:

Elimination half-lives (h)

Inhibitory activity	Compound	Route	Species		
			Rat	Rabbit	Dog
Anti-Xa	Org 10172	i.v.	4,0	6,7	12,4
		s.c.	3,7	6,2	11,6
	Heparin	i.v.	0,6	0,5	
		s.c.	0,7	1,6	
	Tedelparin	i.v.	0,8		
Anti-IIa	Org 10172	i.v.	2,6	6,7	
		s.c.	2,4	5,1	
	Heparin	i.v.	0,5	0,6	
		s.c.	0,7	3,1	
	Tedelparin	i.v.	0,4		
IIaGI	Org 10172	i.v.	10,0	7,0	
		s.c.	9,8	7,0	
	Heparin	i.v.	0,7	0,6	
		s.c.	1,0	3,0	
	Tedelparin	i.v.	0,4		

In rats and rabbits, the elimination half-lives of plasma anti-Xa, anti-IIa and IIaGI activities of ORG 10172 were significantly greater than that seen after heparin or tedelparin administration. The half-life of anti-Xa activity of ORG 10172 was significantly greater in dogs than in rats and rabbits. Furthermore, $t_{½}$ was not dependent on route of administration. In rats the s.c. bioavailability of ORG 10172 was 100% while only 41% for heparin based on anti-Xa activity (based on anti-IIa activity the respective values were 116% and 44%). In rabbit the s.c. bioavailability was approximately 100% for ORG 10172 and heparin based on anti-Xa activity, but s.c. bioavailability of heparin was only about 14% based on anti-IIa activity. The s.c. bioavailability of anti-Xa activity after ORG 10172 administration was about 100%.

Duration of Anti-Xa, Anti-IIa and IIaGI Activities in Rats After I.V. Administration of ORG 10849:

In this experiment rats were given a single i.v. dose of 400 anti-Xa u/kg of ORG 10849 (high-affinity fraction). At various time point 3 rats were sacrificed and blood samples were collected, then anti-Xa, anti-IIa and IIaGI activities were measured in plasma sample. The elimination half-lives of anti-Xa and IIaGI activities were 4 and 2.5 hr respectively (the elimination $t_{1/2}$ of anti-Xa and IIaGI activities after i.v. dose of ORG 10172 in rats were 4 and 10 hr respectively [report # 2404]). The $t_{1/2}$ of anti-IIa activity could not be calculated due to its short duration of action. The data indicated that duration of anti-Xa activity after ORG 10849 administration was similar to that seen after ORG 10172 administration, but duration of IIaGI activity after ORG 10172 was much greater than seen after ORG 10849 administration ($t_{1/2}$: 10 hr vs 2.5 hr). The duration of anti-Xa, anti-IIa and IIaGI activities in rats after the administration of low-affinity fraction could not be assessed due to its low magnitude of effects on these parameters.

4. **Effects on Fibrinolysis**:

Effect on fibrinolysis was assessed by measuring euglobulin clot lysis time.

In Vitro

ORG 10172 or heparin (0.32-5 anti-Xa u/ml) had no significant effect on euglobulin clot lysis time of rat plasma in vitro.

Ex-Vivo

Fibrinolysis activity of euglobulin fraction obtained from ORG 10172 (167-1336 anti-Xa u/kg, i.v.) treated rats was comparable to that seen in control rats. However, in heparin (175-700 anti-Xa u/kg, i.v.) treatment rats, the fibrinolytic activity of the euglobulin fraction was significantly and dose-dependently increased.

5. **Effects on Platelet**:

Effects on Platelet Aggregation:

In human platelet rich plasma, ORG 10172 (0.01-1 mg/ml = 0.8-8 anti-Xa u/ml) had no significant effect on ADP or Collagen induced platelet aggregation. ORG 10172 at high dose (10 mg/ml = 80 anti-Xa u/ml) inhibited platelet aggregation by 29% and 78%

when induced by ADP and Collagen respectively. Heparin (0.16-16 anti-Xa u/ml) potentiated platelet aggregation in human platelet rich plasma when induced by ADP (-11 to +131%) and Collagen (3 to 136%).

Both ORG 10172 and heparin dose-dependently inhibited platelet aggregation in human platelet rich plasma (PRP) when induced by thrombin (ID_{50}: ORG 10172 = 14 mcg/ml, heparin = 0.11 mcg/ml; 2 units/ml of thrombin used as aggregating agent). Similar results were seen when rabbit PRP was used.

In a separate experiment it was also shown that ADP and/or Collagen induced platelet aggregation in PRP obtained from ORG 10172 (1 anti-Xa u/ml) spiked human blood was similar to that seen in the control PRP. However, when the aggregation was induced by thrombin then compared to control 3-5 fold higher concentrations of thrombin were needed to get the same response.

Thrombin induced platelet aggregation in rabbit gel-filtered platelet in the presence of AT-III or HC-II was inhibited by ORG 10172 (ID_{50}: in the presence of AT-III = 159 mcg/ml, in the presence of HC-II = 198 mcg/ml) and heparin (ID_{50}: in the presence of AT-III = 0.16 mcg/ml, in the presence of HC-II = 8 mcg//ml). The data indicated that inhibition of thrombin induced platelet aggregation by ORG 10172 was mediated by AT-III and HC-II to the same extent, while heparin mainly works via AT-III (ID_{50} ration of HC-II/AT-III = 50/1).

Effects on Serotonin Release From Platelet (in vitro):

ORG 10172 (0.14-17.9 anti-Xa u/ml) had no effect on Collagen-induced serotonin release from rabbit platelet. In contrast heparin (0.35 - 39.2 anti-Xa u/ml) dose dependently inhibited Collagen-induced serotonin release from rabbit platelet.

ORG 10172 is sulfated glycosaminoglycuronans which is related chemically and pharmacologically to heparin. On the basis of AT-III affinity ORG 10172 can be separated into two fractions: high affinity (HA) fraction and low affinity (LA) fraction. HA fraction consists of hepran sulfate and the LA-fraction consists of hepran sulfate, dermatan sulfate and minor amount of chondroitin sulfate. ORG 10172 and heparin both had anti-thrombotic activity in various experimental models. In rats at equivalent doses (anti-Xa u/kg), the antithrombotic effect of ORG 10172 lasts at least twice as long as heparin. ORG 10172 also had less effect on bleeding (APTT and TCT) than heparin in various bleeding models at equivalent anti-thrombotic doses. Both ORG 10172 and heparin inhibited factor Xa and thrombin. On

weight basis, the anti-factor Xa activity of ORG 10172 is about 10-fold lower than heparin, and anti-thrombin activity of ORG 10172 is about 200-fold lower than heparin. This indicated that anti-Xa/anti-thrombin ratio of ORG 10172 is greater than heparin. The low-affinity fraction of ORG 10172 (ORG 3051), the subfraction hepran sulfate (ORG 30955) and the subfraction hepran sulphate plus dermatan sulphate (ORG 30995) has very little anti-Xa activity. Hence, anti-Xa activity of ORG 10172 resides mainly in high affinity fraction. Furthermore, anti-thrombin activity of heparin works mainly via AT-III while anti-thrombin activity of ORG 10172 works via AT-III as well as through HC-II. Data also indicated that anti-thrombin activity of ORG 10172 is mainly associated with high-affinity fraction, while HC-II mediated anti-thrombin activity is similar, both in the high- and low-affinity fractions. Unlike heparin, ORG 10172 had no significant effect on fibrinolytic activity in rats and very minimal or no effect on platelet function. ORG 10172 exert less and short-lasting bleeding enhancing activity than heparin or fragmin (LMW-heparin).

Secondary Activities

1. **Effects on Hemodynamics of Anesthetized Beagle Dogs**: In anesthetized dogs, ORG 10172 (5, 50 and 500 anti-Xa u/kg, i.v.; or 500 anti-Xa u/kg, s.c.) had no significant effect on heart rate, blood pressure, cardiac output, carotid and femoral blood flow, ECG recording and myocardial contractility.

2. **Effects on Gastrointestinal System in Rats**: ORG 10172 (400 anti-Xa u/kg, b.i.d. for 4.5 day, s.c.) had no significant effect on gastrointestinal motility, fecal production, body weight, body temperature and respiration rate in rats.

3. **Effects on Central Nervous System in Rats**: ORG 10172 (800 anti-Xa u/kg, s.c.) had no effect on motor co-ordination of rats in rotarod test.

4. **Effects on Autonomic Nervous System (in-vitro)**:

a. **In-Vitro Interaction With α_1-, β_1- and β_2-Adrenergic Receptors, Histamine H_1 and H_2 and Tryptamine D Receptors**: According to sponsor's summary, "ORG 10172 (up to 400 anti-Xa u/kg, i.v.) had no significant effect on blood pressure and heart rate of anesthetized cats or conscious rabbits" (report could not be located). ORG 10172 (2 mg/ml = 32 anti-Xa u/ml) as well as heparin (0.4 mg/ml = 64 anti-Xa u/ml) had no significant effect on isoprenaline (via β_1-receptors) or histamine (via histaminergic-H_2 receptor) induced increased beating frequency in guinea pig atrium. ORG 10172 at similar concentrations had no significant effect on isoprenaline (via β_2-receptors) induced

relaxation of guinea pig trachea, serotonin (via tryptamine D-receptors) induced contraction in rat fundus strip, noradrenaline (via 2_1 receptors) induced contraction in isolated rat vas deferens or histamine (via histamine-H_1 receptors) induced contraction in isolated guinea pig ileum. Thus ORG 10172 does not interact with α_1-, $ß_2$ adrenergic, H_1 and H_2 histaminergic and tryptamine-D receptors.

b. **In Vitro Interaction With Presynaptic α_2 Receptors**: In isolated rat vas deferens, ORG 10172 as well as heparin dose dependently inhibited electrically induced twitch response. The IC_{50} values for ORG 10172 and heparin were 1.1 mg/ml (18 anti-Xa u/ml) and 0.7 mg/ml (112 anti-Xa u/ml) respectively.

c. **In Vitro Interaction With Muscarinic Receptors**: According to sponsor summary, "ORG 10172 and heparin showed a weak concentration-independent interaction with muscarinic receptor at high concentrations (16 and 32 anti-Xa u/ml for ORG 10172 and 32 and 64 anti-Xa u/ml for heparin) (raw data could not be located) in isolated guinea pig ileum.

SPECIAL PHARMACOLOGY:

Drug Interaction Studies:

1. **Interaction With Carbenicillin (in-vitro)**: Carbenicillin is an antibiotic which is known to inhibit second phase of ADP-induced platelet aggregation in human platelet rich plasma (PRP). Carbenicillin (1 mg/ml), ORG 101722 (2 anti-Xa u/ml) and the combination of carbenicillin and ORG 10172 had no significant effect on ADP-induced first phase platelet aggregation in human PRP. Approximately 22-23% inhibition of second phase of ADP-induced aggregation was seen in system containing carbenicillin or ORG 10172, while inhibition of only 38% recorded when both compound were present which is slightly less than the additive effect of these compounds. Hence, carbenicillin does not interact with ORG 10172 in this platelet aggregation model.

2. **Interaction With X-Ray Contrast Agents (in-vitro)**: It has been reported that X-ray contrast agents (conray 30 and urografin 60) interfere with heparin in coagulation tests (Thromb. Hemost. 38: 160, 1977). In this experiment effect on X-ray contrast agents on anti-coagulant activity of ORG 10172 was assessed and compared with that seen with heparin. X-ray contrast agents prolonged prothrombin time (PT: control = 13.9 sec, conray (1:10) = 15.5 sec, urografin (1:10) = 19.6 sec), thrombin time (TT: Control = 18.8 sec, conray (1:10) = 38.1 sec, urografin (1:10) = 62.5 sec) and activated partial thromboplastin time (APTT:

control = 33.6 sec, conray (1:10) = 35.2 sec, urografin (1:10) = 39.6 sec) in human plasma in vitro. In the above in vitro experiment presence of X-ray contrast agents did not potentiate the activity of ORG 10172 or heparin in PT test, anti-Xa activity and anti-thrombin activity. However, presence of X-ray contrast agents potentiated anti-coagulant activity of heparin (1 anti-Xa u/ml) in APTT test (82-101%) and TT test (66-240%). Presence of X-ray contrasting agents did not potentiate the effect of ORG 10172 in APTT and TT test.

3. **In Vivo Interactions With Acetylsalicylic Acid, Various Cardiovascular Drugs and Antibiotics in Rat Muscle Bleeding Model**: In rat muscle bleeding model, combination of ORG 10172 or heparin (100 anti-Xa u/kg; 50 anti-Xa u/kg were used in experiment when acetylsalicylic acid was used) with one of the following drugs: acetylsalicylic acid (cyclo-oxygenase blocker: 10 or 100 mg/kg/day for 5 days), propanol (ß-blocker: 5 mg/kg i.v.), verapamil (calcium channel blocker: 2.5 mg/kg i.v.), nitroglycerin (vasodilator: 5 mg/kg i.v.) or antibiotics (carbenicillin: [70 mg/kg i.v.], amoxicillin: [15-30 mg/kg i.v. or 30-60 mg/kg s.c.], ampicillin: [30 mg/kg i.v. or 60 mg/kg s.c.], cloxacillin: [150-300 mg/kg i.v.] and penicillin G: [170,000 u/kg i.v.]) showed no synergistic effect with respect to blood loss.

4. **Ex-Vivo Interaction ORG 10172 and Warfarin in Monkeys**: In monkeys, administration of ORG 10172 (60 anti-Xa u/kg, i.v.) and warfarin (0.1-0.3 mg/kg, i.m.) did not produce synergistic effect with respect to prolongation of bleeding time, prothrombin time (extrinsic coagulation activity) or ellagic acid induced coagulation time (intrinsic coagulation activity), however, a synergistic effect was seen when stypven time (common final path or coagulation i.e. factor Xa induced coagulation) was measured (data presented graphically). Additionally, warfarin treatment had no significant effect on $t_{½}$ of anti-Xa activity of ORG 10172 ($t_{½}$: before warfarin = 4.9 ± 1 hr and after warfarin = 4.7 ± 1.5 hr).

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME):

ADME studies were conducted in rats, rabbits and dogs after i.v. administration of ^{3}H-labeled ORG 10172 (5% ^{3}H-ORG 10849 [fraction with high-affinity to AT-III] + 95% unlabeled ORG 30561 [fraction with low-affinity to AT-III]). ORG 10172 is a mixture of sulfated glycosaminoglycuronans (hepran sulfate, dermatan sulfate and chondroitin sulfate) therefore, pharmacokinetic parameters were assessed by measuring biological (anti-factor Xa, anti-factor IIa and thrombin generation inhibitory [IIaGI]) activities

as well as total radioactivity count. Anti-Xa, anti-IIa and IIaGI activities were measured by using amidolytic assay system (S2222 was used as chromogenic substrate for anti-Xa activity and S2238 was used as chromogenic substrate for anti-IIa and IIaGI activities). Radioactivity counts were monitored by liquid scintillation counting methods. Additionally, kinetics of the high- and low-affinity to AT-III fractions of the drug (ORG 10172) were also monitored (using competitive binding assay).

RAT:

Pharmacokinetics of ORG 10172, ORG 10849 and ORG 3056 After Single I.V. Dose
(Report # SDG RR 2340)

Methods: Male Wistar rats (n=4-6) were given a single i.v. injection of ^{3}H-ORG 10172 (300 anti-Xa u/kg = 13.6 mg/kg), ^{3}H-ORG 10849 (fraction with high-affinity for AT-III: 150 anti-Xa u/kg = 0.4 mg/kg) or ^{3}H-ORG 30561 (fraction with low-affinity for AT-III: 39 mg/kg). At various time points (approximately 5, 10, 30 min. and 1, 2, 3, 5 and 6 after drug administration) blood sample were collected and plasma samples were obtained. Total radioactivity and anti-Xa activity in plasma were monitored as mentioned above. Low-affinity fraction levels were measured by using competitive binding assay (thromb. Hemost, 54: 630-634, 1985). Various pharmacokinetic parameters (Cl, Vd and $t_{½}$) were also calculated.

Results: The half-life of anti-Xa activity after i.v. administration of ORG 10172 or ORG 10849 was about 3 hr. The pharmacokinetic parameters of ORG 10172 or ORG 10849 based on anti-Xa activity was comparable to that obtained using radioactivity count. The pharmacokinetic parameters of ORG 30561 (fraction with low-affinity for AT-III) obtained from its plasma levels were also comparable to that obtained using radioactivity count. The half-life of ORG 30561 was about 0.5 hr. Thus $t_{½}$ of parent drug (ORG 10172) and ORG 10849 (fraction with high-affinity for AT-III) were significantly higher than the $t_{½}$ of ORG 30561 (3 hr vs 0.5 hr). The data also indicated that $t_{½}$ of ORG 30561 did not influence the kinetic of ORG 10849 since $t_{½}$ of ORG 10849 was similar to that seen for ORG 10172, based on anti-Xa activity (or radioactivity). The Cl_p (plasma clearance) for ORG 30561 was faster than that of ORG 10849 (0.5 ml/hr/g vs 0.04 ml/hr/g).

Pharmacokinetic Parameters After Single I.V. Dose				
	^{3}H-ORG 10172	^{3}H-ORG 10849	^{3}H-ORG 30561	
Parameters	Anti-Xa Activity	Anti-Xa Activity	% of Dose	Radioactivity
Cl (ml/hr/g)	0.03 ± 0.003	0.04 ± 0.01	0.54 ± 0.12	0.53 ± 0.16
V_d Central (ml)	12.4 ± 3.7	14.4 ± 2.3	26 ± 15	27 ± 12
$t_{½}$ Alpha (hr)	0.4 ± 0.4	0.4 ± 0.2	0.06 ± 0.03	0.06 ± 0.02
$t_{½}$ Beta (hr)	3.2 ± 1.9	3.3 ± 1.7	0.4 ± 0.1	0.5 ± 0.2

Elimination of ORG 10172, ORG 10849 and ORG 30561 in Nephrectomized Rats (Report # SDG RR 2324)

Methods: In this study influence of renal failure (nephrectomized) on the elimination of ^{3}H-ORG 10172 (labeled in high-affinity fraction), ^{3}H-ORG 10849 (fraction with high-affinity for AT-III) and ^{3}H-ORG 30561 (fraction with low-affinity for AT-III) were assessed on rats. Nephrectomized and sham-operated rats (n=3-4/group) were given a single i.v. injection of ^{3}H-ORG 10172 (300 anti-Xa u/kg or 93 anti-Xa u/kg), ^{3}H-ORG 10849 (3100 anti-Xa u/kg) or ^{3}H-ORG 30561 (5.74 or 7-8 mg/kg). At various time points (approx. 5, 10, 15, 30, 45 min and 1.5, 2.5, 3.8 and 6.0 hours after drug administration) blood samples were collected and plasma samples were obtained. Total radioactivity in plasma was determined by LSC methods.

Results: Nephrectomy almost completely inhibited the elimination of ORG 10172, ORG 10849 and ORG 30561. This indicated clearly that elimination of these compounds are mainly through kidney.

	^{3}H-ORG 10172		^{3}H-ORG 10849		^{3}H-ORG 30561	
Parameters	Sham-op.	Nephr.	Sham-op.	Nephr.	Sham-op.	Nephr.
Clp (ml/hr/g)	0.06 ± 0.006	---	0.02 ± 0.007	---	0.47 ± 0.24	---
$t_{½}$ beta (hr)	1.8 ± 0.5	---	2.4 ± 0.1	---	1.2 ± 0.4	---

Sham-op = Sham operated rats
Nephr. = Nephrectomized rats
--- = Very little (almost zero) disappearance of radioactivity

Elimination of Anti-Xa, Anti-IIa and IIaGI Activities After a Single I.V. Dose of ORG 10172 (unlabeled) (Thromb. Res., 48: 549-558, 1987)

Methods: Male Wistar rats (n=6/dose group) were given a single i.v. dose of ORG 10172 (400, 800 or 1600 anti-Xa u/kg) or heparin (400, 800 or 1600 anti-Xa u/kg). Blood samples were collected from 3 rats/sampling time point (time intervals were not clearly identified) and plasma samples were obtained to measure anti-Xa, anti-IIa and IIaGI activities as a function of time.

Results: The elimination half-lives of anti-Xa, anti-IIa and IIaGI were increased dose dependently after heparin administration. The half-lives of these activities after ORG 10172 administration were significantly greater than that seen with heparin. Additionally, in contrast to heparin, increasing the dose of ORG 10172 did not increase the $t_{1/2}$ of anti-Xa, anti-IIa and IIaGI activities.

Compound	Dose	$t_{1/2}$ Elimination (min)		
	anti-Xa	anti-Xa	anti-IIa	IIaGI
ORG 10172	400	180	35	100
	800	240	65	80
	1600	240	65	100
Heparin Sodium USP	400	25	10	10
	800	25	15	15
	1600	70	35	35

Elimination of Anti-Xa, Anti-IIa and IIaGI Activities After a Single I.V. Dose of ORG 10849 (fraction with high-affinity to AT-III) (Report # SDG RR 2495)

Methods: Rats were given a single i.v. dose (400 anti-Xa u/kg) of ORG 10849. Blood samples were collected from 3 rats/time point (time intervals are not clearly identified), plasma samples were obtained for measuring anti-Xa, anti-IIa and IIaGI activities (for detail see above).

Results: In this study the elimination half-lives were calculated graphically (not a proper method to calculate $t_{½}$ beta). The $t_{½}$ beta values were 4 and 2.5 hour for anti-Xa and IIaGI activities respectively. The $t_{½}$ beta for anti-IIa activity could not be calculated due to low short lasting anti-IIa activity. Even in this crude experiment, the data indicated that $t_{½}$ beta for anti-Xa activity after ORG 10849 administration was similar to that seen after the administration of ORG 10172. This indicates that ORG 30561 (fraction with low-affinity for AT-III) does not alter the kinetics of ORG 10849 (fraction with high-affinity for AT-III).

Absorption, Elimination and Bioavailability of ORG 10172 After a Single I.V. or S.C. Dose
(Report # SDG RR 2404)

This study was also submitted under pharmacology, and has been reviewed as such (see pharmacology portion of the review). This study cannot be considered as regular pharmacokinetic study because of the following reasons: **(1)** blood sampling time points were not clearly identified, **(2)** elimination half-lives of anti-Xa, anti-IIa and IIaGI activities were estimated graphically. However, s.c. bioavailability (relative to i.v. route) was estimated from AUC's values (but AUC values were not reported). It is worthwhile to restate some of the basic findings:

The estimated $t_{½}$ of anti-Xa activities of ORG 10172 was approximately twice as long as anti-IIa activities (i.v.: 4.0 hr vs 2.6 hr, s.c.: 3.7 hr vs 2.4 hr). The $t_{½}$ of IIaGI activities of ORG 10172 was twice as long as anti-Xa activities (i.v.: 10.0 hr vs 4.0 hr, s.c.: 9.8 hr vs 3.7 hr). Based on anti-Xa activities, bioavailability of ORG 10172 after s.c. dose was complete (100%) and was about 2.5 times greater than that found after s.c. dose of heparin (41%). Similar results for s.c. bioavailability of the drugs (ORG 10172 and heparin) based on anti-IIa or IIaGI activities were reported.

RABBITS:

Absorption, Elimination and Bioavailability of ORG 10172 After a Single I.V. or S.C. Dose
(Report # SDG RR 2404)

This is not a pharmacokinetic study (for criticism of report see above). Briefly, rabbits were given a single injection of ORG 10172 (640 anti-Xa u/kg = 61 anti-IIa u/kg = 500 IIaGI u/kg)

or heparin (640 anti-Xa u/kg = 640 anti-IIa u/kg = 800 IIaGI u/kg) via i.v. or s.c. route. Sponsor calculated $t_{½}$ graphically and estimated s.c. bioavailability from AUC's values.

Inhibitory Activity	Compound	Route	$t_{½}$ (h)	Bioavailability (%)
Anti-Xa	ORG 10172	I.V.	6.7	100
		S.C.	6.2	130
	Heparin	I.V.	0.5	100
		S.C.	1.6	94
Anti-IIa	ORG 10172	I.V.	6.7	100
		S.C.	5.1	95
	Heparin	I.V.	0.6	100
		S.C.	3.1	14
IIaGI	ORG 10172	I.V.	7.0	100
		S.C.	7.0	63
	Heparin	I.V.	0.6	100
		S.C.	3.0	37

The $t_{½}$ of anti-Xa, anti-IIa and IIaGI are significantly longer for ORG 10172 (5.1-7.0 hr) than for heparin (1.6-3.1 hr). The s.c. bioavailability of anti-Xa, anti-IIa and IIaGI after ORG 10172 ranged 63-130%, while heparin resulted in variable bioavailability (14-94%).

In the above experiment sponsor also included dogs (n=2), which were given a single dose of ORG 10172 (800 anti-Xa u/kg) via i.v. or s.c. route. The $t_{½}$ of anti-Xa activities were 12.4 hr. and 11.6 hr. for i.v. and s.c. dose respectively, and the s.c. bioavailability of anti-Xa activities was reported to be 128% i.e. completely bioavailable.

Distribution:

Sponsor has not conducted any distribution study because volume of distribution of ORG 10172 in rat was 12.4 ± 3.7 ml (based on anti-Xa activity) which is close to the volume of blood compartment.

Protein Binding (in vitro) (Report # SDG RR 2302): Binding of ^{3}H-labeled ORG 10172 (5% ^{3}H-ORG 10849 [fraction with high-affinity for AT-III] + 95% unlabeled ORG 30561 [fraction with low-affinity for AT-III]), ^{3}H-ORG 10849 and ^{3}H-ORG 30561 to human serum albumin (4 mg/ml), At-III (0.01 mg/ml) and HC-II (0.01 mg/ml) were assessed by equilibrium dialysis method. Dialysis was performed at 37°C for 17 hour. It should be noted that concentrations of AT-III and HC-II were below the physiological levels (0.17-0.3 mg/ml) in this experiment. Additionally, 17 hr. of dialysis is not sufficient enough to equilibrate ("according to sponsor even after 140 hr. no equilibrium was reached"). The data indicated that binding of ORG 10172, ORG 10849 or ORG 30561 to human serum albumin, AT-III and HC-II were about 90-93%, 89-98% and 89-93% respectively. The data also indicated that ORG 30561 presence did not displace the binding of ORG 10849 to proteins since protein binding of ORG 10172 was comparable to that seen with ORG 10849.

Binding of ORG 10172, ORG 10849 and ORG 30561 to Cardiovascular Tissue in Isolated Rabbit Heart (Report # SDG 2379)

Binding of ^{3}H-labeled ORG 10172 (5% ^{3}H-ORG 10849 [fraction with high affinity for AT-III] + 95% unlabeled ORG 30561 [fraction with low affinity for AT-III]), ^{3}H-ORG 10849 and ^{3}H-ORG 30561 to vascular tissue were assessed during perfusion in the isolated rabbit heart. As a function of time an aliquot of perfusion samples were taken to measure radioactivity. During 31-60 min. of perfusion about 4.7% and 0.8% of the radioactivity from ^{3}H-ORG 10849 (or ^{3}H-ORG 10172) and ^{3}H-ORG 30561 were "bound" (most likely adsorbed) to vascular tissue. Upon washing the system with buffer all the bound radioactivity was recovered, hence binding to vascular tissue was reversible.

Placental Transfer of ^{3}H-ORG 10172 in Guinea Pigs:

In guinea pigs, about 4.1% of maternal plasma level of ORG 10172 (based on anti-Xa activity) was seen in fetal plasma. Therefore, ORG 10172 crosses placental barrier.

Metabolism:

ORG 10172 is a complex mixture of sulfated polysaccharides therefore, a conventional metabolism study could not be done. However, role of liver in the metabolism of ORG 10172 was investigated.

Clearance of ORG 10172, ORG 10849 and ORG 30561 in Perfused Rat Liver (in situ) (Report # SDG RR 2340)

Methods: Bile duct, portal vein and thoracic vena cava of male rats were cannulated. Additionally, hepatic artery was ligated. Liver was perfused with buffer containing ^{3}H-ORG 10172, ^{3}H-ORG 10849 or ^{3}H-ORG 30561. Perfusate (1 ml) was removed at 0, 2, 5, 10, 15, 30, 60, 75, 90, 105 and 120 minutes after the addition of drug. Bile samples were collected at every 15 min. for up to 2 hr. Perfusate and bile samples were analyzed for total radioactivity, anti-Xa activity and glycosaminoglycan (ORG 30561) levels.

Results: About 5-15% of the radioactivity from ORG 10172, ORG 10849 and ORG 30561 were distributed in various liver compartment during the first 15 min. of perfusion. No decrease in radioactivity was seen. Similar pattern was seen when anti-Xa activities or ORG 30561 levels were measured. Hence, liver does not metabolize ORG 10172 or its subfractions. According to sponsor, less then 1% of the administered radioactivity was excreted in the bile during a 24 hour period.

Hepatic Cytochrome P-450 Induction Study (Report # SDG RR 3200)

Methods: Male Wistar rats (n=4/dose group) were given ORG 10172 (200 or 3200 anti-Xa u/kg/day) via i.v. or s.c. route for 7 consecutive days. Control group rats received vehicle (0.15% Na_2SO_3 and 0.09% NaCl, ph 7.65) in similar fashion. An additional group or rats was included which received phenobarbital (80 mg/kg/day for 7 days) via i.p. route. Twenty-four hours after the last dose animals were sacrificed, livers were isolated and weighed. Hepatic content of cytochrome P-450 and 4-aniline hydroxylase activities were monitored.

Results: ORG 10172 had no significant effects on liver weight, hepatic cytochrome P-450 content and 4-aniline hydroxylase activities. Positive control (phenobarbital) produced expected results (liver wt., cytochrome P-450 content and 4-aniline hydroxylase activities were increased by 32%, 64% and 75% respectively). Hence, ORG 10172 is not a hepatic enzyme inducer.

Effect of Liver and Kidney Exclusion on the Plasma Anti-Xa and IIaGI Activities After I.V. Administration of ORG 10172 in Cats
(Report # SDG RR 2350)

Methods: In this experiment cats were given a single i.v. injection of 400 anti-Xa u/kg (or 400 anti-Xa u/kg i.v. bolus plus 37.5 anti-Xa u/kg/hr i.v. for 5 hours) of ORG 10172. Three hours after ORG 10172 administration liver was excluded (by clamping hepatic artery and portal vein, and the portal vein was connected with vena cava by a shunt; control cats were sham operated in which veins were not clamped and shunt was not opened), at various time intervals (not clearly identified) blood samples were collected, anti-Xa and IIaGI activities were measured.

Results: Data were presented graphically, and no pharmacokinetic parameters were calculated. Hence, no conclusion can be made. However, by just looking at the slopes of the time-response curves of anti-Xa and IIaGI activities, sponsor concluded that "liver plays no role in the plasma elimination of the anti-Xa and IIaGI activities after i.v. administration of ORG 10172 to cats". The above experiment was repeated in cats whose kidneys were excluded from circulation (both renal arteries and veins were clamped 3 hr. after ORG 10172 [400 anti-Xa u/kg] administration). Again data were presented graphically, and no pharmacokinetic parameters were calculated. Hence, no conclusion can be made.

Excretion:

Excretion of Radioactivity After a Single I.V. Dose of ^{3}H-ORG 10172, ^{3}H-ORG 10849 and ^{3}H-ORG 30561
(Report # SDG RR 2340)

Methods: Male and female Wistar rats were given a single injection of ^{3}H-ORG 10172 (222 anti-Xa u/kg), ^{3}H-ORG 10849 (241 anti-Xa u/kg) and ^{3}H-ORG 30561 (10.5 mg/kg). Twenty-four hours urine, feces and expired air samples were collected for up to 4 days after drug administration. Total radioactivity in each sample was measured by LSC methods.

Results: About 68-74% and 4-10% of the administered radioactivity of ^{3}H-ORG 10172 were eliminated in urine and feces respectively during the first 96 hours, and less than 2% of the administered radioactivity was detected in expired air during 0-72 hour. When ^{3}H-ORG 10849 was administered then 62-64%, 6-7% and 0.1-0.5% of the administered radioactivity were excreted in

urine, feces and expired air during the above mentioned time period. The corresponding amounts of excretion after ^{3}H-ORG 30561 were 47-65%, 13-15% and 1.4% respectively. The data clearly indicated that renal excretion is the main route of elimination of ORG 10172 and its subfractions.

Biliary Excretion in Rats
(Report # SDG RR 2340)

Methods: Bile duct cannulated rats were given a single i.v. injection of ^{3}H-ORG 10172 (165 anti-Xa u/kg), ^{3}H-ORG 10849 (170 anti-Xa u/kg) and ^{3}H-ORG 30561 (10 mg/kg). Bile samples were collected during 0-6 hr. and 6-24 hr. after drug administration. Total radioactivity in each sample was measured by LSC methods.

Results: During 0-24 hr., less than 1% of the administered radioactivity (ORG 10172, ORG 10849 or ORG 30561) was excreted in the bile and 98% of the biliary excreted radioactivity represented tritiated water. Hence, biliary excretion of ORG 10172 or its subfractions in rats was negligible.

Based on anti-Xa activity, bioavailability of ORG 10172 after s.c. dose in rats, rabbits and dogs were complete (100%), while s.c. bioavailability of anti-Xa activity after heparin dose were 41% and 94% in rats and rabbits respectively (s.c. bioavailability of anti-Xa activity after heparin in dogs were not measured). Therefore, most of the pharmacokinetic studies were conducted after i.v. dose. Furthermore, pharmacokinetic parameters of ORG 10172 (or its subfractions) obtained using biological activities (or levels) were comparable to that obtained using radioactivity counts.

In rats, the $t_{½}$ of anti-Xa activity of ORG 10172 (drug) or ORG 10849 (fraction with high-affinity for AT-III) was about 3 hr. while $t_{½}$ of ORG 30561 (fraction with low-affinity for AT-III: based on its levels) was about 0.5 hr. It should be noted here that ORG 10172 contains only 5% of ORG 10849 and the rest (95%) ORG 30561. From $t_{½}$ of anti-Xa activity, it is evident that elimination half-life of ORG 30561 did not influence the $t_{½}$ of ORG 10849 since $t_{½}$ of ORG 10849 was similar to the $t_{½}$ of ORG 10172. Plasma clearance of ORG 30561 was greater than that of ORG 10849 (0.5 ml/hr/g vs 0.04 ml/hr/g). In rat, volume of distribution after i.v. dose of ORG 10172 was 12-14 ml which is close to the volume of blood compartment. In a published report, it was shown that $t_{½}$ of anti-Xa, anti-IIa and IIaGI activities after i.v. dose of ORG 10172 ($t_{½}$: anti-Xa = 180-240 min., anti-IIa = 35-65 min. and IIaGI = 80-100 min.) were significantly

greater than that seen after i.v. dose of heparin ($t_{½}$: anti-Xa = 25-0 min., anti-IIa = 10-35 min. and IIaGI = 10-35 min.) (Tromb. Res., 48: 549-558, 1987). In a pharmacodynamic experiment, irrespective of route of administration, it was also shown that estimated $t_{½}$ (graphically) of anti-Xa activities of ORG 10172 was approximately twice as long as the $t_{½}$ of anti-IIa activities, and the $t_{½}$ of IIaGI activities of ORG 10172 was twice as long as anti-Xa activities ($t_{½}$ of anti-IIa and anti-Xa activities were similar after heparin dose). In rabbits, irrespective of route of administration, the $t_{½}$ (calculated graphically: not a proper method to calculate $t_{½}$ beta) of anti-Xa, anti-IIa and IIaGI activities were significantly greater for ORG 10172 (5.1-7.0 hr) than for heparin (1.6-3.1 hr). In contrast to rats, the $t_{½}$ of anti-IIa activity in rabbit was similar to $t_{½}$ of anti-Xa activity after ORG 10172 administration. In dogs, $t_{½}$ (graphically) of anti-Xa activity after i.v. or s.c. dose of ORG 10172 was about 12 hours.

Distribution of ORG 10172 in rat was not assessed because the drug is mainly confined to the blood compartment. However, ORG 10172 crosses placental barrier in pregnant guinea pigs. In vitro ORG 10172 and its subfractions binds to human serum albumin (90-93%).

Metabolism of ORG 10172 is not known, because no study was conducted using ^{35}S labeled ORG 10172. However, liver perfusion experiment indicated that drug or its subfractions were not metabolized by rat liver. In rats about 68-74%, 4-10% and <2% of the administered i.v. radioactivity of ^{3}H-ORG 10172 were excreted in urine, feces (in a separate experiment, it was shown that in rat less than 1% of the administered radioactivity was excreted in the bile during 0-24 hr. and most of the radioactivity represented tritiated water) and expired air respectively during 0-96 hours. Hence, renal is the main route of elimination of ORG 10172 and its subfractions.

TOXICOLOGY:

Acute Toxicity:

Methods: Acute i.v. and s.c. toxicity of ORG 10172 was studies in rats (SDG RR 2207 and SDG RR 2208) and dogs (SDG RR 2308 and SDG RR 3222). Control animals received the vehicle (0.9% saline) in similar fashion. All animals were observed for clinical signs and mortality for 7 days (14 days for dogs). At the end of observation period, animals were sacrificed and necropsied.

Results: In rats, the clinical signs were bradypnea, prostration and twitching after i.v. administration of ORG 10172. Subcutaneous administration of the drug into rats and dogs and i.v. administration of the drug into dogs resulted in subcutaneous swelling and hematoma at the injection sites. The highest non-lethal doses in female rats were 250 mg/kg (950 anti-Xa u/kg) and 2000 mg/kg (7600 anti-Xa u/kg) after i.v. and s.c. dose respectively (lowest tested dose produced mortality in male rats). The minimal lethal doses were 250 mg/kg (950 anti-Xa u/kg) and 1000 mg/kg (3800 anti-Xa u/kg) after i.v. dose in male and female rats respectively. After s.c. dose, minimal lethal doses were 1000 mg/kg (3800 anti-Xa u/kg) and 4000 mg/kg (15200 anti-Xa u/kg) in male and female rats respectively. In dogs, the only tested dose (i.v.: 2000 mg/kg = 28000 anti-Xa u/kg and s.c.: 2000 mg/kg = 24600 anti-Xa u/kg) dose did not produce any mortality.

Acute I.V. and S.C. Toxicity of ORG 10172

Species/Strain	No/Dose/ Sex	Route	Dose Levels mg/kg	LD_{50} mg/kg	Highest Non-Lethal Dose mg/kg	Minimum Lethal Dose mg/kg	
						Male	Female
Rat/Wistar	2-6	I.V.	0, 250*, 1000* & 2000*	1000	250*	250	1000
Rat/Wistar	6	S.C.	0, 1000*, 2000*, & 4000*	4000	2000*	1000	4000
Dog/Beagle	2	I.V.	0, 2000[b]	>2000	2000	ND	ND
Dog/Beagle	1	S.C.	0, 2000[c]	>2000	2000	ND	ND

* = only for females
a = 1 mg contains 3.8 anti-Xa units
b = 1 mg contains 14 anti-Xa units
c = 1 mg contains 12.3 anti-Xa units

Subacute/Subchronic/Chronic Toxicity:

Rat:

6-Week I.V. Toxicity Study in Rats
(SDG RR 1268)

Testing Laboratories: Organon Drug Safety R & D Labs
Schaijk, Netherlands

Study Started: August 26, 1980

Study Completed: June 19, 1982 (report date)

GLP Requirements: Non-GLP Study (see page 4)

Animals: Cpb: WU Spf-bred Wistar rats (males: 206-248 g and females: 144-186 g)

Drug Batch No.: K

Methods: In this study dose selection was based on 2-week i.v. dose range study (SDG RR 2362) in which doses of 0, 200 (760 anti-Xa u) and 400 (1520 anti-Xa u) mg/kg/day were used. One high dose treated female died on day 1 of the study. The cause of death could not be ascertained, however, sponsor suggested that death could be related to the high potassium content (6.7%) in the batch (E) of drug used in this dose-ranging study. Clinical signs (reduced activity, labored respiration, hunched posture and loss of righting reflex) were seen in some of the high dose treated rats (both sexes) and on one low dose treated female. Highest tested dose also produced increases in thrombocytes (males: 17% and females: 12%), serum levels of beta-lipoprotein (males: 12% and females: 20%) and triglycerides (males: 38%). Histopathological examination revealed small foci of centrolobular swollen and vacuolated hepatocytes in 2/6 males and slight hyperplasia in bone marrow in 2/6 males. Based on these findings, sponsor selected 0, 25 (200 anti-Xa u), 100 (800 anti-Xa u) and 400 (3200 anti-Xa u) mg/kg/day for 6-week i.v. toxicity study in rats.

In the present study, groups of rats (8/sex/group) were given i.v. injection of ORG 10172 at daily doses of 25 (200 anti-Xa u), 100 (800 anti-Xa u) and 400 (3200 anti-Xa u) mg/kg/day for 6 weeks. The control group rats were given vehicle (0.9% saline) in similar fashion. The volumes of administration were 4, 0.25, 1 and 4 ml/kg/day for control, low dose, mid dose and high dose respectively. The rate of i.v. administration was fixed at 1 ml/min. Additionally, two groups (6/sex/group) were included in this study, one received the vehicle and the other received high dose and used for 3-week recovery study. All animals were observed for clinical signs and mortality daily, body weights and food consumptions were recorded weekly. Blood samples were collected at 3 and 6 weeks of the study and at the end of recovery period for hematology and serum chemistry tests. Urine samples were collected during week 3 and 6 of the study and at the end of recovery period for urinalysis. All surviving rats were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. **Observed Effects**: In some of the high dose treated rats, site of injections (tail) were bluish.

2. **Mortality**: A total 5 rats (1 female from control group, 1 male from low dose group, 1 female from mid dose group and 2 females from high dose group) died or killed during study period. These deaths were not treatment related.

3. **Body Weight/Food Consumption/Water Consumption**: At high dose, body weight gains were reduced by 5% and 8% in males and females respectively when compared to corresponding control values. Food intakes were not affected by the treatment.

4. **Hematology/Coagulation/Bone Marrow**: No treatment related effects were seen.

5. **Blood Chemistry and Urinalysis**: No treatment related effects were seen.

6. **Vital Signs/Physical Examination/Ophthalmic Examination**: Not done.

7. **Organ Weights**: No treatment related effects were seen, except adrenal weights were increased by 15% compared to control values in high dose treated females, and this effect was not seen at the end of recovery period.

8. **Gross Pathology**: Increased incidence of bluish discoloration of the tail was seen in mid and high dose treated rats. Slightly enlarged adrenal glands were seen in some of the mid and high dose treated females (data were not provided in tabulated form).

9. **Histopathology**: Slight to excessive perivascular hemorrhages and/or edema were seen in high dose treated rats (male: 2/8 and females: 5/8). Slight hyperplasia of the lymphoreticular cells in the spleen were seen in 2/8 males and 1/8 females of high dose group. Additionally, small foci of extramedullary hematopoiesis was seen in 1/8 high dose treated females. No abnormalities were seen at the end of recovery period.

The data indicated that 100 mg/kg/day (800 anti-Xa u/kg/day) is the no effect dose in this study. The highest tested dose 400 mg/kg/day (3200 anti-Xa u/kg/day) can be considered as well tolerated dose, since it produced slight decrease in body weight gains (5-8%), perivascular hemorrhages and/or edema, slight hyperplasia of the lymphoreticular cells in the spleen and small foci of extramedullary hematopoiesis.

6-Week S.C. Toxicity Study in Rats
(SDG RR 1269)

Testing Laboratories: Organon Drug Safety R & D Labs
Schaijk, Netherlands

Study Started: September 2, 1980

Study Completed: June 19, 1982 (report date)

GLP Requirements: Non-GLP Study (see page 4)

Animals: Cpb: WU spf-bred Wistar rats (males: 181-238 g and females: 141-185 g)

Drug Batch No.: K

Methods: In this study dose selection was based on 2-week s.c. dose range study (SDG RR 2358) in which doses of 0, 200 (760 anti-Xa u) and 400 (1520 anti-Xa u) mg/kg/day were used. At high dose 3/6 males and 2/6 females died/killed due to exaggerated pharmacological effects (excessive hemorrhages at the injection sites). Low dose produced subcutaneous hemorrhages and/or hematoma, hyperplasia of bone marrow (11/12), hyperplasia of lympho-reticular tissue along with extramedullary hematopoiesis (1/12). Based on these findings sponsor selected 0, 20 (160 anti-Xa u), 80 (640 anti-Xa u) and 200 (1600 anti-Xa u) mg/kg/day for 6-week s.c. toxicity study in rats.

In the present study, groups of rats (8/sex/group) were given s.c. injection of ORG 10172 at daily doses of 20 (160 anti-Xa u/kg), 80 (640 anti-Xa u) and 200 (1600 anti-Xa u) mg/kg/day for 6 weeks. The control group rats were given vehicle (0.9% saline) in similar fashion. The volumes of administration were 2.0, 0.20, 0.80 and 2.0 ml/kg/day. Additionally, two groups (6/sex/group) were included in the study, one received the vehicle and the other received high dose and used for 3 weeks recovery study. All animals were observed for clinical signs and mortality daily, body weights and food intakes were recorded weekly. Blood samples were collected at 3 and 6 weeks of the study and at the end of recovery period for hematological and serum chemistry tests. Urine samples were also collected at the above mentioned time points for urinalysis. All surviving rats were subjected to complete necropsy and histopathological examinations.

Results:

1. **Observed Effects**: Subcutaneous swellings and hemorrhages at the injection sites were seen in all treated rats and the effect was dose related. These signs disappeared at the end of recovery period in low and mid dose treated rats.

2. **Mortality**: A total of 9 rats (1 females from mid dose group and 2 males and 6 females from high dose group) died during study period and the cause of deaths was excessive s.c. hemorrhages at the injection sites.

3. **Body Weight/Food Consumption/Water Consumption**: No significant treatment related effects were seen, except in high dose treated females, body weight gains were increased by 36% compared to the control values and this increase was due to large s.c. hematoma seen in all females.

4. **Hematology/Coagulation/Bone Marrow**: At the end of treatment period, significant decreases in hemoglobin (males: 22% and females: 40%), red blood cells (males: 25% and females: 38%) and MCHC (males: 5% and females 14%) and increases in MCV (males: 8% and females: 13%), WBC (males: 90% and females: 173%), neutrophils (males: 100% and females: 167%) and thrombocytes (males: 67% and females: 30%) were seen in high dose treated rats. Some of the above mentioned changes of lesser magnitude were also seen in mid dose treated rats. All these findings are related to the loss of blood at the injection sites. No such abnormality was evident at the end of recovery period.

5. **Blood Chemistry and Urinalysis**: At the end of treatment period, serum bilirubin levels were increased by 28% and 52% in mid and high dose treated males rats, and the corresponding increases in females were 19% and 84% respectively, when compared with their respective control values. Additionally, serum levels of beta-lipoprotein were increased by 10% and 23% in high dose treated males and female rats respectively. No abnormality was seen in urinalysis.

6. **Vital Signs/Physical Examination/Ophthalmic Examination**: Not done.

7. **Organ Weights**: Spleen weights were increased by 42% and 120% in high dose treated males and females respectively. Additionally, liver weights were increased by 12% and 22% in high dose treated males and females respectively.

8. **Gross Pathology**: Enlarged spleen were seen in high dose treated rats (males: 4/8 and females: 3/8) and dose related s.c. hematoma was seen in treated rats.

9. **Histopathology**: Hyperplasia of the lymphoreticular cells in the spleen in mid (males: 2/8 and females: 2/8) and high (males: 4/8 and females: 4/8) dose treated rats. Hyperplasia of bone marrow was also seen in mid (males: 4/8 and females: 1/8) and high dose (males: 5/8 and females: 2/8) treated rats. Extramedullary hematopoiesis were seen in the livers of 1/8 mid dose treated male and 1/8 high dose treated female. In addition, small foci of swollen and vacuolated hepatocytes were also seen in high dose treated rats (males: 2/8 and females: 2/8). Most of these findings are secondary to the excessive hemorrhages at the site of injection. At the site of injection hemorrhages/edema/ granulocytic cell infiltration and fibroblast proliferation were seen in 5/8 males and 5/8 females of mid dose group. Furthermore, above mentioned findings at the site of injection along with fibrinoid degeneration and necrosis of dermal collagen fibers were also seen in 2/8 males of mid dose group and 1/8 females of high dose group. No abnormalities were seen at the end of recovery period.

In this study the lowest tested dose (20 mg/kg/day = 160 anti-Xa u/kg/day) can be considered as well tolerated dose since it only produced slight s.c. hemorrhages at the injection sites. The mid and high dose produced lethality due to excessive hemorrhages at the injection sites. In 6-week subacute toxicity studies in rats, different dose levels were used when drug was given via i.v. or s.c. route. Irrespective of route of administration (i.v. or s.c.), ORG 10172 produced similar toxicities in rats, except when ORG 10172 (80 or 200 mg/kg/day) was given via s.c. route it produced lethality while no mortality was seen after 400 mg/kg/day i.v. dose. Excessive toxicity is not due to higher plasma level after s.c. administration since plasma anti-Xa levels in rat measured immediately after i.v. administration were twice as high as the highest plasma level after s.c. administration of the same dose (SDG report # 2404). Most likely, mechanical damage of subcutaneous tissue and high local concentrations of the drug caused excessive hemorrhage and deaths. To avoid deaths sponsor selected i.v. route of administration for the 6-month toxicity study in rats.

Six-Month I.V. Toxicity Study in Rats
(SDG RR 2448)

Testing Laboratories

Study Started: January 26, 1988

Study Completed: January 4, 1990 (report date)

GLP Requirements: A Statement of Compliance with GLP regulations was included.

Animals: 7-Weeks old Charles River Sprague-Dawley rats (male: 222-281 g and females: 153-200 g).

Drug Batch No.: IPA 87017/3, IPA 87017/4 and IPA 87017/5.

Methods: In this study, dose selection was based on 6-week i.v. toxicity study in Wistar rats in which doses of 0, 25, 100 and 400 mg/kg/day (correspond to 0, 200, 800 and 3200 anti-Xa u/kg/day) were used. The highest tested dose was well tolerated dose, it only produced slight decrease (5-8%) in body weight gains and exaggerated pharmacological effects.

In the present study, groups of Sprague-Dawley rats (20/sex/group) were given intravenous injection of ORG 10172 at daily doses of 100, 400 and 1600 anti-Xa u/kg (7.14, 28.57 and 114.28 mg/kg/day) for six months. The control group animals received the vehicle (sterile isotonic solution containing sodium sulphite and sodium chloride) in similar fashion. The volume of administration was 1.28 ml/kg. Additionally, two groups (10/sex/group) were included in this study, one received the vehicle and the other received high dose of the drug and used for 4-week recovery study. All animals were observed for clinical signs and mortality twice daily. Body weights and food intakes were monitored during pre-test, weekly up to 13 weeks, monthly thereafter and bi-weekly during the recovery period. Blood samples from orbital sinus were collected at 6, 13 weeks and 6 months of the study and at end of recovery period for hematological and serum chemistry tests. Overnight urine samples were also collected during week-5, week 13 and at the end of treatment/recovery period for urinalysis. All surviving rats were sacrificed at the end of treatment/recovery period and subjected to complete necropsy and histopathological examinations.

Results:

1. **Observed Effects**: Increased incidence of alopecia of the extremities and snout were seen in mid and high dose treated rats during 18-70 days of treatment. This finding was not evident at the end of recovery period.

2. **Mortality**: A total of 15 rats (3 in the control, 4 in the low dose group, 6 in the mid dose group and 2 in the high dose group) died or killed during the study period. These deaths are considered not to be treatment related.

3. **Body Weight/Food Consumption/Water Consumption**: Body weights and food intakes were not affected by the treatment.

4. **Hematology/Coagulation/Bone Marrow**: No treatment related effects were seen.

5. **Blood Chemistry and Urinalysis**: At the end of treatment period drug had no biologically significant effect on blood chemistry parameters. There were some statistically significant increases/decreases in the values BUN, SGOT, SGPT, alkaline phosphatase and LDH in treated rats but unfortunately data were not robust (standard deviations were very large) and effects were not dose related. Results of urinalysis were comparable in all groups.

6. **Vital Signs/Physical Examination/Ophthalmic Examination**: Myelination of retinal nerve fibers (right eye) was seen in one mid dose treated male rat (# 3002). Myelination of retinal nerve fibers was not seen in rats treated with high dose. One high dose treated male (# 4006) had complete cataract in right eye and another high dose treated male (# 4022) had posterior subcapsular cataract in both eyes.

7. **Organ Weights**: At the end of treatment period, in males, right adrenal weights were increased by 17%, 12% and 20% at low, mid and high dose respectively. In high dose treated males, thyroid/parathyroid weights were increased by 22% compared to control value.

8. **Gross Pathology**: Edema, sores/scabs, bumps and swelling were seen at the injection sites in most of the animals (including animals from the control group).

9. **Histopathology**: No treatment related effects were seen.

10. **Serum Anti-Xa Levels (SDG RR 2521)**: Blood samples were collected at 24 hr. after the last dose during week 6 and 13 of the study to monitor serum anti-Xa levels.

Mean Serum Anti-Xa Levels (u/ml)			
Weeks	**Low Dose**	**Mid Dose**	**High Dose**
6	0.05 ± 0.02	0.17 ± 0.04	0.48 ± 0.06
13	0.06 ± 0.01	0.22 ± 0.04	0.64 ± 0.12

The data indicated that levels of anti-Xa activity during weeks 6 and 13 of the study were comparable.

In this study, the lowest tested dose (100 anti-Xa u/kg/day) was the no effect dose and higher dose levels did not produce any marked toxicity.

Dogs:

6-Week I.V. Toxicity Study in Dogs
(SDG RR 1270)

Testing Laboratories: Organon Drug Safety R & D Labs.
Schaijk, Netherlands

Study Started: September 16, 1980

Study Completed: July 13, 1982 (report date)

GLP Requirements: Non-GLP (see page 4)

Animals: 17-23 Months Old Beagle Dogs (males: 10.5-13.2 kg and females: 8.6-12.2 kg)

Drug Batch No.: K

Methods: In this study dose selection was based on 2-week i.v. dose ranging study (SDG RR 2364) in which doses of 0, 200 (760 anti-Xa u) and 400 (1520 anti-Xa u) mg/kg/day were used. High dose produced lethality in 50% (1/2) of dogs and cause of death was cardiac arrest which was attributed to high potassium content (6.7%) in the batch (E) of the drug used in dose-ranging study. Among the survivors, s.c. hemorrhages, lack of motor coordination and/or mild ataxia, increased heart rate (returned to pre-treatment values within 10 min. after dosing) and increased liver weights (22-35%) were seen in treated dogs. Additionally, thrombocytopenia was seen in 2/2 high dose treated male dogs and in 1/2 mid dose treated female dog (all high dose females died) and 1/2 high dose treated dog had increased APTT (control: 22.5 sec and high dose: 31 sec). Histopathological examinations revealed hyperplasia of lymphoreticular cells in the spleen. Based on these findings, sponsor selected 0, 25 (200 anti-Xa u), 100 (800 anti-Xa u) and 400 (3200 anti-Xa u) mg/kg/day for 6-week i.v. toxicity study in dogs.

In the present study groups of dogs (3/sex/group) were given i.v. injection of ORG 10172 at daily doses of 25 (200 anti-Xa u), 100 (800 anti-Xa u) and 400 (3200 anti-Xa u) mg/kg/day for 6 weeks. The control group dogs were given vehicle (0.9% saline) in similar fashion. The volumes of administration were 4, 0.25, 1 and 4 ml/kg/day for control, low dose, mid dose and high dose respectively. Additionally, two groups (1/sex/group) were

included in this study, one received the vehicle and the other received high dose and used for recovery study. All dogs were observed for clinical signs and mortality daily, body weights and food consumptions were recorded weekly. ECG recordings were monitored once a week immediately after drug administration. Blood samples were collected at pre-test, week 3 and 5 of the study period and at the end of recovery period for hematological and serum chemistry tests. Urine samples were also collected at above mentioned time period for urinalysis. All surviving dogs were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. **Observed Effects**: At the injection sites, perivascular thickenings were seen in low dose (1/3 males and 3/3 females), mid dose (3/3 males and 3/3 females) and high dose (4/4 males and 4/4 females) treated dogs. Additionally, 1/4 high dose treated dogs had large hematoma and ulcer in one of the limbs which was not used for injection. At the end of recovery period, no such signs were evident.

2. **Mortality**: One female dog each from mid and high dose groups were killed in extremis due to severe post-hemorrhagic anemia.

3. **Body Weight/Food Consumption/Water Consumption**: No treatment related effects were seen.

4. **Hematology/Coagulation/Bone Marrow**: At the end of 5-weeks of treatment, hemoglobin and red blood cells were decreased by 31% and 27% respectively in high dose treated males when compared to control values. Most of the data were not legible, according to sponsor's summary, increased number of thrombocytes were seen in high dose treated dogs (2/4 males and 2/4 females). At the end of recovery period these changes were not seen.

5. **Blood Chemistry/Urinalysis**: In high dose treated males, serum alkaline phosphate and beta-lipoprotein levels were increased by 43% and 41% respectively, when compared to control values.

6. **Vital Signs/Physical Examination/Ophthalmic Examination/ECG**: Just after dosing, increased heart rates (up to 70%: 170 beats/min) were seen in high dose treated dogs. According to sponsor, no conduction disturbances were seen.

7. **Organ Weights**: No treatment related effects were seen.

8. **Gross Pathology**: Moderate s.c. hemorrhages and hematomas were seen at the injection sites in mid and high dose treated dogs.

9. **Histopathology**: Extramedullary hematopoiesis in the spleen along with slight hyperplasia of the lymphoreticular cells and/or increased number of megakaryocytes were seen in 2/4 high dose treated dogs. Bone marrow hyperplasia was seen in 2/4 males and 1/4 females of high dose group.

The data indicated that lowest tested dose (25 mg/kg/day = 200 anti-Xa u/kg/day) can be considered as no effect dose since it only produced slight s.c. hemorrhages. Mid dose and high dose levels produced moderate s.c. hemorrhages and hematomas at the injection sites in dogs of both sexes and mortalities among female dogs.

6-Week S.C. Toxicity Study in Dogs (SDG RR 1271)

Testing Laboratories: Scientific Development Group
Organon, OSS
The Netherlands

Study Started: Not given

Study Completed: July 7, 1982 (report date)

GLP Requirements: Non-GLP (see page 4)

Animals: 14-23 months old Beagle dogs (males: 9.2-12.8 kg and females: 8.1-11.2 kg)

Drug Batch No.: K

Methods: In this study dose selection was based on 2-week s.c. dose-ranging study (SDG RR 2363) in which dose of 0, 200 (760 anti-Xa u) and 400 (1520 anti-Xa u) mg/kg were used. One out of 2 male dogs from low dose group was killed during study period, and the cause of morbidity was excessive hemorrhages and anemia. Among the survivors, s.c. hemorrhages, anemia, hematomas (abdominal region and whole back), hyperplasia of lympho-reticular cells in the spleen, hyperplasia in bone marrow, extra-medullary hematopoiesis in the liver were seen in treated dogs. Based on these findings, sponsor selected 0, 20 (160 anti-Xa u), 60 (480 anti-Xa u) and 200 (1600 anti-Xa u) mg/kg/day for 6-week s.c. toxicity study in dogs.

In the present study groups of dogs (low dose: 3/sex, mid dose: 5/sex and high dose: 4/sex) were given s.c. injection of ORG 10172 at daily doses of 20, 60 and 200 mg/kg/day for 6-weeks. The control group dogs (4/sex) were given vehicle (0.9% saline) in similar fashion. The volumes of administration were 2, 0.2, 0.6 and 2 ml/kg/day for control, low dose, mid dose and high dose respectively. One dog each/sex from control and mid dose groups were used for 4-week recovery study. All dogs were observed for clinical signs and mortality daily, body weights and food intakes were recorded weekly. Ophthalmic examinations were performed at pre-test and at the end of treatment/recovery period. Blood samples were obtained at pre-test, 3 and 6 weeks of treatment and 3 weeks after the last dose for hematological and serum chemistry tests. Urine samples were also collected at above mentioned time period for urinalysis. All surviving dogs were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. **Observed Effects**: Dose related hematoma and s.c. thickenings were seen at the injection site. Additionally, subcutaneous abdominal and inguinal hemorrhages were also seen in most of the treated dogs. These effects are related to the anticoagulant properties of the drug. Most of signs reduced/disappeared at the end of recovery period.

2. **Mortality**: A total of 8 dogs (one male and one female from mid dose group, and 3 males and 3 females from high dose group) died or killed during the study period. In all cases, the cause of death was post-hemorrhagic anemia.

3. **Body Weight/Food Consumption/Water Consumption**: In high dose group only one dog left at the termination of the study, therefore no meaningful statistical comparison is possible. However, treatment had no significant effect of body weights of low and mid dose treated dogs (variation seen in body weight during the course of study was due to the presence of s.c. hematoma). Food intakes were comparable in all groups.

4. **Hematology/Coagulation/Bone Marrow**: At the end of treatment period, decreases in hemoglobin (24-29%), red blood cells (27-36%) were seen in mid and high dose treated dogs of both sexes. Additionally, white blood cells count were increased by 87% and 64% in mid and high dose treated male dogs. All these changes are related to s.c. hemorrhages. At the end of recovery period these changes were not seen in mid dose treated group (high dose treated dogs were not used for recovery study).

5. **Blood Chemistry/Urinalysis**: No treatment related effects were seen.

6. **Vital Signs/Physical Examination/Ophthalmic Examination**: (See observed effect.) No drug related changes were seen in ophthalmic examinations.

7. **Organ Weights**: Spleen weights were increased by 67% and 87% in mid and high dose treated male dogs, when compared to the control values. There was also an indication of increased liver weight (18%) in high dose treated male dogs. These results should be viewed with caution because there was only one male dog in high dose group at termination. Furthermore, these changes could be related to compensatory extramedullary hematopoiesis following extensive hemorrhages.

8. **Gross Pathology**: Moderate s.c. hemorrhages, hematoma were seen in treated dogs. Enlarged spleen and enlarged liver were seen in 1/1 male dog of high dose group.

9. **Histopathology**: No summary table was provided. According to sponsor, extramedullary hematopoiesis in the spleen and hyperplasia of bone marrow were seen in some dogs of low dose group and in most of the dogs from mid and high dose groups. Foci of extramedullary hematopoiesis in the liver were also seen in most of the mid and high dose treated dogs. Subcutaneous hemorrhages were also seen in most of low dose treated dogs and in all of mid and high dose treated dogs.

In this study, no effect dose was not established. Mid and high dose levels produced lethality. Low dose did not produce any mortality but produced moderate s.c. hemorrhages and hematomas at the injection sites. In 6-week subacute toxicity studies in dogs, different dose levels were used when drug was given via i.v. or s.c. route. Irrespective of route of administration (i.v. or s.c.), ORG 10172 produced similar toxicities in dogs. There was an increased incidence in mortality and hematoma formation in dogs after s.c. as compared to i.v. administration. Excessive toxicity is not due to higher plasma level after s.c. administration since plasma anti-Xa levels in dog measured immediately after i.v. administration were twice as high as the highest plasma level after s.c. administration of the same dose (SDG report # 2404). Most likely, mechanical damage of subcutaneous tissue and high local concentrations of the drug caused excessive hemorrhage and deaths. To avoid deaths sponsor selected i.v. route of administration for the 6-month toxicity study in dogs.

Six Month I.V. Toxicity Study in Dogs
(SDG RR 2449)

Testing Laboratories:

Study Started: April 22, 1988

Study Completed: January 4, 1990 (report date)

GLP Requirements: A Statement of Compliance with GLP regulations was included.

Animals: About 6 month old Beagle dogs (males: 7.4-10.1 kg and females: 6.2-8.6 kg).

Drug Batch No.: IPA 87018/3, IPA 87018/4 and IPA 87018/5

Methods: Groups of dogs (4/sex/group) were given i.v. injection of ORG 10172 at daily doses of 100 (6.62 mg/kg/day), 400 (26.49 mg/kg/day) and 1600 (105.96 mg/kg/day) anti-Xa u/kg/day for six months. The control group was given vehicle (sterile, isotonic solution containing sodium sulphite and sodium chloride) in similar fashion. The volume of administration was 1.28 ml/kg. Additionally, two groups (2/sex/group) were included in this study, one received the vehicle and the other received high dose and used for 12-week recovery period. All dogs were observed for clinical signs and mortality twice daily. Body weights and food intakes were recorded at pretest and weekly thereafter. Blood samples were collected at weeks 6, 11, 13 and 26 of the study and at 6 and 12 weeks after the stoppage of the drug in high dose group for hematological and serum chemistry tests. Overnight urine samples were also collected at the above mentioned time period for urinalysis. All surviving dogs were sacrificed at the end of treatment recovery period and subjected to complete necropsy and histopathological examinations.

Results:

1. **Observed Effects**: At the injection sites, scar tissue hematomas were seen in some of the mid dose and all of the high dose treated dogs. Additionally, open abscesses on the legs and severe swellings were seen in high dose treated dogs.

2. **Mortality**: One mid dose treated male died on day 164 due to hemothorax. One high dose treated male and 2 high dose treated females were killed in extremis on days 12, 53 and 56 of the study respectively. These dogs had excessive hemorrhages. Due to exaggerated pharmacological effects of the drug in high dose

treated dogs, sponsor stopped the treatment in high dose group after 10 weeks of treatment and sacrificed all high dose treated dogs except 2/sex which were used for 12-week recovery study.

3. **Body Weight/Food Consumption/Water Consumption**: Low and mid dose had no significant effect on body weight gains and food consumptions. In dogs which were treated with high dose for only 10 weeks, the body weight gains were reduced by 35% and 69% in males and females respectively. At the end of recovery period, body weights of females from high dose group were comparable to that seen in control dogs, but body weights of males from high dose group were still 11% lower than the corresponding control values. In high dose group, during the treatment period (10 weeks), food consumptions were reduced by up to 36%. However, during recovery period food intakes were comparable to that seen in control dogs.

4. **Hematology/Coagulation/Bone Marrow**: No treatment related effects were seen in low and mid dose treated dogs. Decreased hemoglobin (males: 15% and females: 16%), hematocrit (males: 11% and females: 11%) and red blood cells count (males: 17% and females: 19%) and increased platelet count (males: 49% and females: 56%) and APTT (males: 18% and females: 14%) were seen in dogs treated (10-weeks) with high dose. These effects were not seen at the end of recovery period.

5. **Blood Chemistry/Urinalysis**: No treatment related effects were seen.

6. **Vital Signs/Physical Examination/Ophthalmic Examination**: Ophthalmic examinations revealed no drug related effects.

7. **Organ Weights**: At the end of treatment period, increased weights of liver (up to 29%), spleen (up to 72%), kidney (up to 13%), thymus (up to 23%) and thyroid/parathyroid (up to 33%) were seen in low and mid dose treated males (high dose dogs were sacrificed at the end of 10-weeks of treatment). In low and mid dose treated females, submaxillary glands weight were reduced by 18-20% when compared to control values.

8. **Gross Pathology**: No tabulated summary was provided. According to sponsor, hematomas, s.c. hemorrhages and scar tissue were seen at the injection sites in high dose treated dogs.

9. **Histopathology**: Hyperplasia of the bone marrow and extramedullary hematopoiesis were seen in 3/3 males and 4/4 females of high dose group. Extramedullary hematopoiesis was also seen in spleen of 1/4 mid dose treated female dogs. S.C. hemorrhages/inflammation/necrosis were seen at the injection sites in most mid dose and all of the high dose treated dogs.

10. **Serum Anti-Xa Levels (SDG RR 2522)**: Blood samples were collected at 18 hr after the last dose during week 6 and 13 of the study to monitor serum anti-Xa levels.

Mean Serum Anti-Xa Levels (u/ml)			
Weeks	**Low Dose**	**Mid Dose**	**High Dose**
6	0.55 ± 0.08	1.47 ± 0.31	3.33 ± 0.47
13	0.54 ± 0.07	1.68 ± 0.12	ND

ND = not done

The data indicated that levels of anti-Xa activity during weeks 6 and 13 of the study were comparable.

In this study no effect dose was not established. Mid and high dose levels produced lethality. Low dose did not produce any mortality but produced slight s.c. hemorrhages at the injection sites and increased liver, spleen, kidney, thymus and thyroid weights without any accompanying histological abnormalities. Therefore, low dose can be considered as a well tolerated dose.

REPRODUCTIVE TOXICITY:

I.V. Segment I. Fertility and General Reproductive Performance Study in Rats (SDG RR 2258)

Testing Laboratories: Drug Safety RDL
Organon Oss, Schaijk

Study Started: Not given.

Study Completed: September 9, 1988

GLP Requirement: A Statement of Compliance with GLP regulations and quality assurance unit was included.

Animals: Male (75-120 g) and female (200-238 g) Charles River CD Sprague Dawley rats.

Drug Batch No.: I.P.A. No. 82026

Methods: The dose selection was based on preliminary i.v. fertility study in rats (SDG RR 2037), in which doses of 0 and 800 anti-Xa u/kg/day (100 mg/kg/day) were used. The male rats were treated from 14 days prior to mating with vehicle treated females and throughout the mating phase (maximum of 7 days). Females were treated for 7 days prior to mating with vehicle treated males and throughout mating, and up to 6 days gestation. On day 14 of gestation dams were sacrificed to examine number of implants sites, live embryos and signs of gross abnormalities. No effects were seen on fertility and general reproductive performance of rat. In view of these findings the highest i.v. dose selected for the present study was 800 anti-Xa u/kg/day (100 mg/kg/day).

In the main study, groups of 24 male and 24 female rats were given i.v. injection of ORG 10172 at daily doses of 0 (vehicle: isotonic solution containing 0.15% sodium sulphite and sodium chloride), 36 (390 anti-Xa u), 60 (650 anti-Xa u) and 100 (1090 anti-Xa u) mg/kg/day. The volume of administration was fixed at 1 ml/kg. The male rats were treated for 9 weeks prior to mating and throughout the mating phase and until they were sacrificed. Females were treated for 14 days prior to mating and throughout mating, gestation and 4 weeks of lactation. Additionally, 2 groups (24/sex) were also included in the study, one received high dose in similar fashion while the other was kept untreated, and treated males and females were mated with untreated females and males respectively. Parents were observed daily for mortality and toxic signs. Body weights and food/water consumptions were recorded weekly. The mating performance and fertility of both sexes were evaluated. About one-half of pregnant rats were sacrificed on day 20 of gestation, and was 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. Fetuses were eviscerated and one-half of fetuses were examined for skeletal major/minor abnormalities, the remaining fetuses were examined for visceral abnormalities and variations. The remaining dams were allowed to deliver spontaneously. The number of live/dead pups were recorded, and the live pups were weighed and sexed. The offspring were reared by the dams until weaning. On week 4 of post partum all dams were sacrificed and necropsied and examined as mentioned above. Postnatal body weight changes of the pups were recorded until the age of 28 days. At day 28 of post partum, 2 males and 2 females from each litter were randomly chosen for F1 generation study. At 10 weeks of age they were continuously mated, and females (including males) were sacrificed on day 14 of gestation and examined for gross visceral changes.

Results: Subcutaneous hemorrhages were seen in one dam treated with mid dose and in 3 dams treated with high dose. Additionally, blood in the vagina was seen in 2 dams of mid dose group and in 3 dams of high dose group. One male each from low dose and mid dose group and 2 females from high dose group died during course of study. The cause of death of one male from mid dose group was hemothorax and the cause of death of one female from high dose was pulmonary edema. The cause of remaining deaths were not known. Body weight gains and food intakes were not affected by treatment in males or females. The estrous cycle of the female rats revealed no differences between the control and treated groups. Precoital intervals, mating rates and pregnancy rates were comparable in all groups.

Parameters	Control	Low Dose	Mid Dose	High Dose
# of Female Pairs	24	24	24	22
# of Females Mated	24	21	23	21
Mating Rate (%)	100	87.5	95.8	95.4
# of Pregnant	22	20	22	20
Pregnancy Rate (%)	91.7	95.7	95.2	91.7

Dams Sacrificed at Day 21:

No treatment related gross lesions were seen in female rats of F_0 generation. There were no significant changes in pregnancy parameters (pre- and post implantation loss, litter size, sex ratios, and mean fetal weights). No treatment related major malformation was seen in fetuses. No treatment related minor skeletal/visceral anomalies/variations were seen.

Dams Sacrificed on Day 21 of Pregnancy				
Parameters	**Control**	**Low Dose**	**Mid Dose**	**High Dose**
# of Pregnant Dams	11	10	11	10
# of Corpora Lutea	150	150	157	142
Mean # of Corpora Lutea/Dam	13.6	15.0	14.3	14.2
# of Implants	141	128	156	131
Mean # of Implants/Dam	12.8	12.8	14.2	13.1
Total # of Fetuses	141	128	156	131
# of Live Fetuses	123	124	143	120
Mean # of Live Fetuses/Dam	11.2	12.4	13.0	12.0
# of Dead Fetuses	18	4	13	11
Mean # of Dead Fetuses/Dam	1.6	0.4	0.12	1.1
Pre-implantation Loss (%)	5.5	14.2	0.6	6.5
Post-implantation Loss (%)	14.3	2.8	8.5	8.6
Mean Fetal Weight (g)	5.13 ± 0.41	5.18 ± 0.51	5.32 ± 0.34	5.39 ± 0.54
Sex Ratio (% females)	53.9	47.9	56.1	48.5

Dams Allowed to Deliver: No significant differences in the gestation period between the groups were noted. Litter size, sex ratios, viability 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. No treatment related gross lesions were seen in rats of F_1 generation.

Dams Allowed to Deliver				
Parameters	**Control**	**Low Dose**	**Mid Dose**	**High Dose**
# of Pregnant Dams	11	10	11	10
Gestation Length (days)	22.5	22.3	22.5	22.3
# of Live Pups	148	124	138	126
# of Dead Pups	0	0	0	0
Litter Size at Delivery	13.4	12.4	12.6	12.6
Litter Size at Weaning	13.3	12.3	11.6	11.2
Sex Ratio (% females)	44.7	40.1	48.2	39.2

In conclusion, there were no abnormal effects on the fertility and mating performance of the treated male and female rats at i.v. doses up to and including 100 mg/kg/day (1090 anti-Xa u/kg/day; about 36 times the proposed clinical dose [30 anti-Xa u/kg/day]) of ORG 10172.

Segment II. Teratology Study in Rats (SDG RR 3128)

This report was submitted under IND as an Amendment which was reviewed on July 29, 1992. The review is reproduced below:

Testing Laboratories:

Study Started: September 3, 1990

Study Completed: February 21, 1991

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

Test Species: Pregnant SPF Sprague Dawley rats (Crl: CD SD BR VAF/plus strain).

No. of Animals: 40 pregnant rats/group

Drug Batch No.: CP087143

Placebo Batch No.: CP088070

Methods: In the main study, pregnant rats were given i.v. doses of 0, 100, 400 and 1600 anti-Xa units/kg/day from day 6 to 17 day of gestation. Control animals received the vehicle throughout the same period. The volume of administration was fixed at 1.28 ml/kg. The selection of the doses were based on the 6-month chronic toxicity study (SDGRR No. 2448) and a fertility study (SDGRR No. 2258). Pregnant dams were observed daily for mortality and clinical signs. Body weights were recorded on days 1 (day 1 of the pregnancy), 2, 3, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation. Twenty dams rats were sacrificed on day 20 of gestation, and was 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. Approximately one-half of the fetuses eviscerated and examined for skeletal major/minor abnormalities, the remaining fetuses were examined for visceral abnormalities and variations. The remaining of the dams (about 20 /group) were allowed to deliver spontaneously. The number of live/dead pups were recorded, and the live pups were weighed and sexed. Culling was carried out to make 8 offspring (4 male and 4 female) per dam. Pups were also weighed on days 4, 8, 12, 16 and 21 of post partum. The offspring were reared by the dams until day 21 of post partum. On day 21 of post partum all dams were sacrificed and necropsied, and examined as mentioned above. During the nursing period the growth and differential of the pups were observed, and development parameters were assessed (righting reflex, pupil reflex, pinnae detachment, upper incisor eruption, eye opening, learning ability test, open field test). At week 12, 10 pairs of the animals per group were continuously mated for 20 days. F_1 dams were weighed on day 0, 3, 7, 10, 14, 17 and 20 of gestation. Dams were allowed to deliver normally and rear their offspring (F_2) to weaning. At day 21 of postpartum all F_1 dams and F_2 pups were sacrificed and examined as mentioned above.

Results:

Dams Sacrificed at Day 20: One dam from 100 anti-Xa units/kg/day group was found dead on day 16 of the study and the cause of death could not be established. No significant effect on body weight or food consumptions were seen in treated rats, however during lactation period the body weight gains of the treated mothers were reduced by 35-43% compared to the control group. Food consumptions during lactation period were not affected. The number of corpora lutea, the number of implants, pre- and post-implant losses, numbers of live/dead embryos, weights of fetuses and sex ratio did not show any significant difference between the treated groups and the control group. External examination

revealed forelimb flexures/malrotated hind limbs in 2 fetuses (from 1 litter) of mid dose group and 3 fetuses (from 1 litter) of high dose group. The incidence rates of this malformation were 0% (0 out of 20 litters), 0% (0 out of 20 litters), 0.8% (1 out of 20 litters) and 1.2% (1 out of 20 litters) which is dose related and statistically significant ($p = 0.0198$; Exact trend test). There were no other treatment related anomalies.

Effect of ORG 10172 on Maternal and Fetal Parameters in Rats

Parameters Measured	Control	Low Dose	Mid Dose	High Dose
Total Mated	40	40	40	40
# of Pregnant	36	33	39	35
% Pregnant	90	82.5	97.5	87.5
# Dam examined	20	20	20	20
# Corpora lutea/Dam	14.3	14.9	14.3	14.8
# Implants/Dam	13.0	13.9	13.2	13.3
# Pre implant loss/dam (%)	8.2	6.4	6.8	9.6
# Post implant loss/dam (1%)	4.6	5.6	5.8	5.6
# Early Embryonic Deaths/dam	0.6	0.7	0.7	0.6
# Late Embryonic Deaths/dam	0.1	0.1	0.0	0.2
# Total Embryonic Deaths/dam	0.7	0.8	0.7	0.8
# Live fetuses/dam	12.4	13.1	12.5	12.6
Mean Fetal wt (g)	3.82	3.81	3.88	3.91
Sex Ratio (M/F)	0.91	1.05	0.97	1.00

Morphological Findings of Fetuses

Gross Malformations:

# Examined	247	262	250	251
Forelimb flexures/ malrotated hind limb	0	0	2	3

Dams Allowed to Deliver: No significant differences in the gestation period between the groups were noted. One dam of mid dose group gave birth to only 2 dead pups. There were no significant effects on postnatal development and differentiation.

There was no significant effect on fertility test and mating performance test of F_1-generation rats. No drug related abnormalities were seen in F_2 pups at necropsy.

In this study, external examinations of the fetuses revealed forelimb flexures/malrotated hind limbs in 2 fetuses (from 1 litter) of mid dose group and 3 fetuses (from 1 litter) of high dose group. The incidence rates of this major malformation were 0% (0 out of 20 litters), 0% (0 out of 20 litters), 0.8% (1 out of 20 litters) and 1.2% (1 out of 20 litters) in control, low, mid and high dose groups respectively. The historical incidence rate from 14 embryotoxicity studies (conducted by Huntingdon Research Center, England) for bilateral fore limb flexure with distorted rib cage is 0.95% (range: 0-12.5%) on the basis of litters affected and 0.18% (0-2.4%) on the basis of fetuses affected. The incidences of the above mentioned malformation in segment II teratology study in rats is higher than the mean incidence rate observed in the historical controls but it remains within the historical incidence range. Furthermore, in Segment I study in rats, no treatment related major malformation was seen in fetuses sacrificed on day 21 of pregnancy. Thus no teratogenic effect at dosage up to 1600 anti-Xa u/kg/day was seen in rats. The postnatal development and the fertility of the offspring were comparable in all groups.

I.V. Segment II. Teratology Study in Rabbits (SDG RR 2209)

Testing Laboratories: Drug safety R & D Labs.
Organon, OSS, Schaijk, Netherlands.

Study Started: September 19, 1982

Study Completed: May 17, 1988

GLP Requirement: A Statement of Compliance with GLP regulations and quality assurance unit was included.

Test Species: Adult Dutch Rabbits (2.02-2.99 kg)

No. of Animals: 25 pregnant females/group

Route of Administration: I.V.

Dose Levels: 36, 60 and 100 mg/kg/day (280, 470 and 780 anti-Xa u/kg/day respectively).

Drug Batch No.: I.P.A No. 82019

Methods: The selection of the doses were based on the preliminary Segment II teratology study (SDG RR 2038) in rabbits in which i.v. doses of 0 and 800 anti-Xa u/kg/day (100 mg/kg/day) were used. Pregnant rabbits were treated from day 6-18 of gestation. In this preliminary study, drug did not produce any maternal toxicity, embryotoxicity or teratogenic effects. Based on these results sponsor selected 100 mg/kg/day as the highest dose for the main study. In the main study, pregnant rabbits were given i.v. doses of 36, 60 and 100 mg/kg/day (280, 470 and 780 anti-Xa u/kg/day respectively) from day 6 to 18 day of gestation. Control animals received the vehicle (isotonic solution containing 0.15% sodium sulphite and sodium chloride) throughout the same period. The volumes of administration were 0.5 ml/kg for control and low dose groups and 1 ml/kg for mid and high dose groups. Pregnant dams were observed daily (each time the animals were handled) for mortality and clinical signs. Body weights and food intake were recorded during days 0, 6, 12, 19, 24 and 29 of the pregnancy. 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 and identification of any malformed fetuses or uterine abnormalities. Live fetuses were weighted, sexed and examined for external abnormalities. All fetuses were eviscerated and were examined for skeletal malformations and variations, and visceral abnormalities.

Results: One dam from mid dose group died on day 7 of pregnancy and the cause of death was post-hemorrhagic anemia. Additionally, one dam from mid dose group was killed on day 27 of pregnancy because it had a broken leg. At necropsy, one dam each from low, mid and high dose groups had discolored amniotic fluid, white spots on placenta and uterine horn filled with blood respectively. The pregnancy rates ranged from 68-84%. Treatment had no effect on ovarian weight, post-implantation loss, fetal/placental weight and sex ratio. No treatment related abnormalities were observed on external, skeletal and visceral examinations in any group.

Effect of ORG 10172 on Maternal and Fetal Parameters in Rabbits				
Parameters	**Control**	**Low Dose**	**Mid Dose**	**High Dose**
Total Mated	25	25	25	25
# of Pregnant	21	18	17	19
% Pregnant	84	72	68	76
# of Dams with Live Fetuses	18	17	15	18
# of Corpora Lutea	173	149	143	160
# of Implants	163	138	140	134
Post-implantation Loss/Dam (%)	29.3	18.5	21.0	22.5
# of Live Fetuses	119	113	112	102
Mean Fetal Wt. (g)	37.0 ± 5.0	37.9 ± 6.1	35.4 ± 6.6	38.8 ± 4.7
Sex Ratio (% female)	44.0	50.5	49.8	58.2
Placental Wt. (g)	3.13 ± 0.73	3.30 ± 0.76	2.86 ± 0.70	3.27 ± 0.69
Fetal Malformations				
# of Fetuses Examined	119	113	112	102
External	0	0	0	0
Skeleton	0	0	0	0
Visceral	0	0	0	0

Thus no teratogenic effect at dosage up to 100 mg/kg/day (780 anti-Xa u/kg/day) was seen in rabbits.

Segment III. Perinatal and Postnatal Study in Rats (SDG RR 3129)

This report was submitted under IND as an Amendment which was reviewed on July 29, 1992. The review is reproduced below:

Testing Laboratories:

Study Started: August 6, 1990

Study Completed: January 24, 1991

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

Test Species: Pregnant SPF Sprague Dawley rats (Crl: CD SD BR VAF/plus strain).

No. of Animals: 25 pregnant rats/group

Drug Batch No.: CP087143

Placebo Batch No.: CP088070

Route of Administration: I.V.

Dose Levels: 0, 100, 400 and 1600 anti-Xa units/kg/day (1.28 ml/kg body weight)

Methods: Pregnant rats were given i.v. doses of 0 (vehicle), 100, 400 and 1600 anti-Xa units/kg/day from day 15 of gestation to day 21 after parturition. All dams were observed for clinical signs daily, body weights were recorded on 0, 7, 13, 15, 17 and 20 day of gestation, and on days 0, 7, 14 and 21 of post partum. Food consumptions were recorded between day 13-20 of gestation. The number of live/dead pups were recorded, and the live pups were weighed and sexed. Culling was carried out to make 8 offspring (4 male and 4 female) per dam. Pups were also weighed on days 4, 8, 12, 16 and 21 of post partum. The offspring were reared by the dams until day 21 of post partum. On day 21 of post partum all dams were sacrificed and necropsied, and examined externally and internally for abnormalities. During the nursing period the growth and differential of the pups were observed, and development parameters were assessed (righting reflex, pupil reflex, pinnae detachment, upper incisor eruption, eye opening, learning ability test, open field test). At week 12, 20 pairs of the animals per group were continuously mated for 20 days. F_1 dams were weighed on day 0, 7, 10, 14, 17 and 20 of gestation. Dams were allowed to deliver normally and rear their offspring (F_2) to weaning. At day 21 of postpartum all F_1 dams and F_2 pups were sacrificed and examined as mentioned above.

Results: One dam of high dose group was found dead on day 27 of the study. Autopsy finding indicated that this animal was not pregnant and cause of death could not be established. Two dams from high dose group had pale extremities from day 18-27 of the study. Throughout gestation and lactation period, no other abnormalities were seen in clinical signs, body weight gains, food and water consumptions of F_0 dams. Pregnancy rates and length of gestation were comparable in all groups. No abnormalities were observed at autopsy of F_0 dams which would be

attributed to treatment. No drug related effects were seen in the F_1 pups during postnatal period except mean pup weights at 1600 anti-Xa unit/kg dose group were significantly less than the control values. Development of F_1 pups were comparable in all groups except vaginal opening (control = 32.9 days, low dose = 33 days, mid dose = 33 days and high dose = 33.6 days) and balano-preputial cleavage (control = 42.4 days, low dose = 42.7 days, mid dose = 43.3 days and high dose = 43.6 days) were delayed in treated groups. Reproductive performance of F_1 generation was not affected, and no external abnormalities were observed in the F_2 fetuses. Thus no adverse effect were seen in rats following i.v. administration of up to 1600 Anti-Xa units/kg/day of ORG 10172 during perinatal and postnatal period.

Segment III Perinatal and Postnatal Study in Rats

Parameters Measured	Control	Low Dose	Mid Dose	High Dose
Total Mated	25	25	25	25
# of Pregnant	25	25	22	24
% Pregnant	100	100	92	96
Length of Gestation (days)	21.5	21.5	21.3	21.4
# of Implants/Dam	13.1	13.5	14.2	13.1
# of Implant Loss/Dam	4.9	5.4	6.3	3.4
# of Live Fetuses/Dam	12.4	12.8	13.2	12.5
Mean Fetal Wt (g)				
Day 0	6.4	6.3	6.2	6.2
Day 4	10.3	10.1	9.8	9.7
Day 21	58.3	58.3	55.1	54.0

GENETIC TOXICOLOGY:

Ames Test
(SDG RR 1636 And SDG RR 2900)

Testing Laboratories: Drug safety R & D Labs.
Organon, OSS, Schaijk, Netherlands.

Dates Studies Started and Completed: October 26, 1982 and June 24, 1984 (report date).

Drug Batch No.: 382/0112 and 390/0146

Methods: Ames test was conducted to assess the mutagenic potential of the drug by measuring its ability to induce reverse mutations at selected loci of several strains of Salmonella typhimurium [TA 98, TA 1537 and TA 1538 (frame shift); TA 100 and TA 1535 (base pair substitution)] in the presence and absence of S-9 activation (Arocol 1254 induced rat liver microsomal enzyme mixture). The method used is plate incorporation method. Vehicle (distilled water), ORG 10172 (8-10000 mcg/plate) and positive controls [2-aminoanthracene (5 mcg/plate), 2-acetylaminofluorene (25 mcg/plate) and benzo[a]pyrene (7.5 mcg/plate)] were plated in triplicate with tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 in the presence and absence of S-9 mix and incubated for 48 hour. Revertant colonies were counted. Two fold increase in the number of revertant colonies above the solvent control value are consider positive.

Results: Drug was not mutagenic in any of the tester strains, irrespective of the treatment with metabolic activation system (S-9 Mix). Increase in mutant colonies was noted in all microbial strains employed in the presence of positive control (with or without S-9 Mix).

The above test was repeated in tester strain of E. coli WP2 UVrA PKM101 and the result was negative.

In Vitro Chromosome Aberration Test in CHO Cells
(LSR-051005-M-07086)

Testing Laboratories:

Dates Studies Started and Completed: July 30, 1986 and October 30, 1986.

Cells Employed: Chinese hamster ovary (CHO) cultured cells.

Concentration Employed: 46.4-10000 mcg/ml

Solvent Control: Phosphate buffered saline containing 0.15% sodium sulphite.

Positive Control: Mitomycin C (0.1 mcg/ml) and cyclophosphamide (6.6 mcg/ml).

Source of Metabolic Activation: Rat liver microsomal enzymes (S-9 mix).

Drug Batch No.: 084127

Methods: CHO cultured cells were treated with ORG 10172 in the presence and absence of metabolic activator (S-9 mix). Cells were harvested at 24 hours after the start of treatment (cells in the presence of S-9 mix were treated only for 3 hours then washed and incubated for additional 12 or 24 hours). The chromosomal aberrations were analyzed in cell sampled at 24 hr at three dose levels (2150, 1000 and 465 mcg/ml in the absence of S-9 mix and 10000, 4640 and 2150 mcg/ml in the presence of S-9 mix). The highest concentration selected for analysis at this time produced about 61% and 62% mitotic inhibition in the absence and presence of S-9 mix respectively. At 12 hr, in the presence of S-9 mix, the effect of three concentrations (10000, 4640 and 2150 mcg/ml) were assessed and at this time highest concentration produced about 32% mitotic inhibition. At the end of experiment 100 metaphases were examined per treatment group. If a statistically significant increases in proportion of structurally aberrant cells (without gaps) occurred at two consecutive dose levels or at the highest tested concentration compared to control and the incidence of aberration bearing cells in treated cultures exceed 5% (result must be reproducible in both replicate cell cultures) then the compound is genotoxic.

Results: Irrespective of the presence or absence of metabolic activation, treatment with ORG 10172 did not produce any significant reproducible increase in chromosomal aberration over the value obtained for the control group. The positive control gave expected results. Thus ORG 10172 had no clastogenic activity in this in vitro cytogenetic test.

Unscheduled DNA Synthesis in Hela Cell Cultures (in vitro)
(LSR-051007-M-07286)

Testing Laboratories:

Dates Studies Started and Completed: July 23, 1986 and September 4, 1986.

Cells Used: Cultured Hela Cells (HeLa S3)

Concentration Employed: 625-10000 mcg/ml

Solvent Control: Phosphate buffered saline containing 0.15% sodium sulphite.

Positive Control: 4-Nitroquinoline-n-oxide (NQO: 5 mcg/ml) and Benzo[a]pyrene (BP: 2.5 mcg/ml).

Source of Metabolic Activation: Rat liver microsomal enzymes.

Drug Batch No.: 084127

Methods: Hela cells were first incubated at 37^0C with hydroxyurea for 24 hr to limit replicative DNA synthesis. Then ORG 10172, negative control or positive control was incubated in Hela cell cultures in the presence and absence of S-9 mix for 3 hours. Tritiated thymidine was included in the incubation and the amount of radioactivity incorporated into DNA in the presence of hydroxyurea is the measure of DNA repair. If the mean cpm of the test substance in the presence of hydroxyurea is 50% greater than the mean dpm of the negative control in the presence of hydroxyurea at two consecutive dose levels or at a single dose level if that is the highest dose-level which can be tested, furthermore results must be reproducible, then the test substance is genotoxic in this test.

Results: Irrespective of the presence and absence of metabolic activator (S-9 mix) the unscheduled DNA synthesis were similar in control and treated samples. The unscheduled DNA synthesis were significantly higher in the presence of positive controls. Thus the drug at concentration as high as 10000 mcg/ml was not genotoxic in UDS test.

CHL/HGPRT Mammalian Cell-Forward Gene Mutation Assay (LSR-051006-M-07186)

Testing Laboratories:

Dates Studies Started and Completed: July 26, 1986 and August 25, 1986.

Strain Employed: V79 Chinese hamster lung cells

Concentration Employed: 625-10000 mcg/ml

Solvent Control: Phosphate buffered saline containing 0.15% sodium sulphite.

Positive Control: Ethyl methane sulfonate (10 mM) and N-dimethylnitrosamine (10 mM).

Source of Metabolic Activation: Rat liver microsomal enzymes.

Drug Batch No.: 084127

Criteria of Genotoxic Effect: Mutation frequency in the exposed culture must be at least 5-fold (usually 3-fold is acceptable criteria) greater than the mean mutation frequency of the negative control cultures at two consecutive dose levels or at the last non-toxic tested dose, furthermore results must be reproducible, then the test substance is genotoxic in this test.

Results: Sponsor has conducted 2 separate experiments. Mutation frequencies in ORG 10172 treated cultures (with and without S-9 Mix) were all within normal limits. Significant increases in the mutation frequencies of the positive test control cultures were observed. Thus ORG 10172 had no mutagenic potential in this test up to 10000 mcg/ml.

In Vivo Genotoxicity Study of ORG 10172 by the I.V. Route in the Mouse Macronucleus Test (Report # 2023)

Testing Laboratories:

Dates Studies Started and Completed: December 2, 1986 and January 29, 1987.

Test Species: Swiss CD-1 mice

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

Route of Administration: I.V.

Dose Levels: 374 and 749 mg/kg (10 ml/kg).

Drug Batch No.: 084127

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

Negative Control: 0.9% saline (10 ml/kg)

Positive Control: Mitomycin C (5 mg/10 ml/kg)

Methods: Animals were given a single dose of vehicle, ORG 10172 or positive control at 24, 48 and 72 hours prior to sacrifice and preparation of the bone marrow. On the Giemsa-stained slides, 1000 polychromatic erythrocytes per animal were examined for the presence of micronuclei.

Results: ORG 10172 did not induce an increase of micronucleated polychromatic erythrocytes in mice bone marrow. In contrast, the % of micronucleated polychromatic erythrocytes in mitomycin treated group was markedly higher than the negative control. These findings suggest that ORG 10172 is not mutagenic in this test system.

SPECIAL TOXICITY STUDIES:

Local Tolerance of ORG 10172 After 2-Week I.V. or S.C. Injection in Rats
(SDG RR 2460 & SDG RR 2461)

Testing Laboratories:

Methods: In this study local reaction at the injection site of two different batches (BJ and K; 3200 anti-Xa-units = 400 mg of batch K = 200 mg of batch BJ) of ORG 10172 was assessed when given via i.v. or s.c. route. Groups of rats (12/sex/group) were given daily doses of ORG 10172 (batch # BJ or # K) via i.v. (3200 anti-Xa u/kg/day) or s.c. (1600 anti-Xa u/kg/day) route for 2 weeks. Control animals were given vehicle (isotonic saline without and with 0.15% sodium sulfite) in similar fashion. The

volume of administration ranged from 2.56-4.0 ml/ kg/day for i.v. route and 1.28-2.0 ml/kg for s.c. route. At the end of treatment period animals were killed and injection sites were examined microscopically.

Results:

Drug Administered Via I.V. Route:

Two males and two females from batch K treatment group died/killed during the course of the study. All these deaths were related to internal hemorrhages and poor conditions (anemia etc.). Those animals which survived treatment with batch K had hematoma at the injection sites. No mortality was observed in group which was treated with batch BJ. However, in group treated with batch BJ, hematomas and/or hemorrhages were seen at the injection sites. Histopathological examinations indicated that both batches (BJ and K) of ORG 10172 produced perivascular hemorrhages at the injection sites and at equipotent anti-Xa doses (3200 anti-Xa u/kg/day) batch K was pharmacologically more active than batch BJ.

Drug Administered Via S.C. Route:

Four males and 4 females from batch BJ treatment group, and 5 males and 3 females from batch K treatment group died/killed during the study period. Deaths were related to poor condition (anemia etc.) and blood loss in hematomas. Hematomas and edema at the injection sites were seen in all surviving animals treated with ORG 10172 (batch BJ or K). Histopathological examinations of injection sites revealed s.c. hemorrhages, edema, inflammatory cell aggregation and hematomas in most of the ORG 10172 (batch BJ or K) treated animals. The data indicated that no batch related differences were seen when ORG 10172 was given via s.c. route.

2-Week I.V. Toxicity Study in Rats Using Two Different Batches of ORG 10172 (SDG RR 2305 and SDG RR 2306)

Testing Laboratories:

Study Started: June 19, 1984

Study Completed: December 7, 1988 (report date)

GLP Requirements: A Statement of Compliance with Netherlands GLP regulations was included.

Animals: Cpb: WU (Wistar) rats (age not given; males: 145-162 g and females: 122-143 g).

Methods: This study was conducted to assess the toxicity of two different batches of ORG 10172 (K and BF). Groups of rats (6/sex/group) were given i.v. injection of ORG 10172 (batch K or batch BF) at daily doses of 200, 800 and 3200 anti-Xa u/kg for 2 weeks. The control group animals received the vehicle (8 mg sodium chloride and 1.5 mg sodium sulphite in 1 ml water) in similar fashion. Various toxicological parameters (clinical signs, mortality, body weight, food intake, hematology and serum chemistry tests, urinalysis and histopathological examinations) were monitored.

Results: No clinical signs or mortality was seen in rats treated with batch K, while batch BF produced one death in high dose treated females. Cause of death could not be established. The data were not legible, sponsor summary report indicated that no overt signs of toxicities were seen in the study when two different batches of ORG 10172 (K and BF) were used.

Passive Cutaneous Anaphylactic (PCA) Reaction in Rats (SDG RR 2351)

Testing Laboratories:

Study Started: January 26, 1988

Study Completed: February 6, 1989 (report date)

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

Drug Batch No.: IPA 87020/2

Animals: Female TO mice (22-28 g) and male Wistar rats (190-237 g).

Methods: Antigenicity of ORG 10172 was examined with passive cutaneous anaphylactic (PCA) reaction in rats. Ten mice per group were sensitized by s.c. administration of vehicle (0.9% saline containing sodium sulphite) mixed with $Al(OH)_3$ or ORG 10172 (1, 10 or 100 mcg/mouse) mixed with $Al(OH)_3$ once a weeks, for 3 weeks. The positive control group received 10 mg/animal of ovalbumin (OVA) mixed with $Al(OH)_3$ subcutaneously on week 1 and 3 of the study. Additionally, mice were also

sensitized with ORG 10172 + human serum albumin + $Al(OH)_3$ subcutaneously on week 1 and 3 of the study. The PCA reaction was examined with serum collected from animals at 1-2 weeks after the last sensitization dose. Serial dilutions of the serum was injected intradermally into the dorsal skin of rats (recipients, n = 2/group). Forty-eight hours later these animals were challenged with the respective antigens (saline, OVA or ORG 10172) mixed with Evan's blue by injecting intravenously. After 30 min animals were killed and examined for dyed extravasation in the back. "Signs of blueing in the inner aspect of the skin were used to quantify production of antibody titers (IgE)."

Results: No PCA reaction was seen in rats. All positive control group animals had PCA reaction (PCA titer range: 1/32 - 1/128). Thus ORG 10172 did not elicit sensitization activity in passive cutaneous anaphylaxis test in rats.

Antigenicity Study of ORG 10172 in Guinea Pigs After Subcutaneous Administration (SDG RR # 2275)

Testing Laboratories:

Study Started: January 28, 1988

Study Completed: September 6, 1988 (report date)

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

Drug Batch No.: IPA 87020/2

Animals: Female Dunkin-Hartley guinea pigs (285-358 g).

1. Passive Cutaneous Anaphylactic (PCA) Reaction in Guinea Pigs

Methods: Antigenicity of ORG 10172 was examined with passive cutaneous anaphylactic (PCA) reaction in guinea pigs. Ten guinea pigs per group were sensitized by s.c. administration of vehicle (0.9% saline containing sodium sulphite) mixed with FCA (Freund's complete adjuvant) or ORG 10172 (7.14, 28.56 or 114.24 mg/kg) mixed with FCA once a weeks, for 4 weeks. The positive control group received 10 mg/animal of ovalbumin (OVA) subcutaneously on week 1 and 3 of the study or just one dose on week 1 of the study. The PCA reaction was examined with serum collected from

animals at 7/14/35 days after the last sensitization dose. Serial dilutions of the serum was injected intracutaneously on the back of guinea pigs (recipients, n = 2/group). Four or 48 hours later these animals were challenged with the respective antigens (saline, OVA or ORG 10172) mixed with Evan's blue by intracardiac injection. After 30 min animals were killed and examined for dyed extravasation in the back. "Signs of blueing in the inner aspect of the skin were used to quantify production of antibody titers (IgE)."

Results: No PCA reaction was seen in guinea pigs. All positive control group animals had PCA reaction (mean PCA titer: 1 in 774 for IgGIa and 1 in 448 for IgG1b/IgGE). Thus, ORG 10172 did not elicit sensitization activity in passive cutaneous anaphylaxis test in guinea pigs.

2. **Active Anaphylactic Reaction in Guinea Pigs**

Methods: Five guinea pigs per group were sensitized by s.c. administration of vehicle (0.9% saline containing sodium sulphite) mixed with FCA (Freund's complete adjuvant) or ORG 10172 (7.14, 28.56 or 114.24 mg/kg) mixed with FCA once a weeks, for 4 weeks. The positive control group (n = 2) received 10 mg/animal of ovalbumin (OVA) subcutaneously on week 1 and 3 of the study or just one dose on week 1 of the study. Two weeks after the last sensitization dose, active anaphylactic was examined following intracardial administration of respective antigens (saline, OVA or ORG 10172).

Results: No anaphylactic symptoms were noted in ORG 10172 treated animals when challenged with ORG 10172. Positive control group animals all had anaphylactic symptoms which resulted into deaths of all animals (4/4). Thus, ORG 10172 did not induce a specific IgE or IgG antibodies in guinea pigs.

3. **Cutaneous Hypersensitivity Reaction in Guinea Pigs**

Methods: Five guinea pigs per group were sensitized by s.c. administration of vehicle (0.9% saline containing sodium sulphite) mixed with FCA (Freund's complete adjuvant) or ORG 10172 (7.14, 28.56 or 114.24 mg/kg) mixed with FCA once a weeks, for 4 weeks. The positive control group (n = 2) received 10 mg/animal of ovalbumin (OVA) subcutaneously on week 1 and 3 of the study or just one dose on week 1 of the study. Two weeks after the last sensitization dose, cutaneous hypersensitivity was examined at 24, 48 and 72 hours following intradermal administration of respective antigens (vehicle, OVA or ORG 10172).

Results: No cutaneous hypersensitivity reaction were noted in ORG 10172 treated animals when challenged with ORG 10172. Positive control group animals had marked erythema. Thus, ORG 10172 did not induced cutaneous hypersensitivity reactions in guinea pigs.

4. Passive Hemagglutination Test in Guinea Pigs

Serum samples from all sensitized animals were incubated with tanned or untanned ORG 10172- or OVA-coated sheep red blood cells in the presence and in the absence of tannic acid (tannic acid increases the binding of proteins to erythrocytes). "The hemagglutination titer was taken as the highest serum dilution at which hemagglutination was present."

No serum agglutinating antibodies were seen in serum sample taken from ORG 10172 treated guinea pigs using passive hemagglutination test. Guinea pigs treated with OVA had passive hemagglutination antibody titers (mean titer = or > than 1888).

Proposed Text of the Labeling for Orgaran

The label (see Appendix I) is according to 21 CFR, 201.50, Subpart B (April 1, 1994). However, the following changes should be incorporated:

1. Pregnancy:

Sponsor's Version:

Evaluation:

In the Segment II teratology study in rats, i.v. doses of 0, 100, 400 and 1600 anti-Xa units/kg/day were used. In this study, external examinations of the fetuses revealed forelimb flexures/ malrotated hind limbs in 2 fetuses (from 1 litter) of mid dose group and 3 fetuses (from 1 litter) of high dose group. The incidence rates of this major malformation were 0% (0 out of 20 litters), 0% (0 out of 20 litters), 0.8% (1 out of 20 litters) and 1.2% (1 out of 20 litters) in control, low, mid and high dose groups respectively. The historical incidence rate from 14 embryotoxicity studies (conducted by Huntingdon Research Center, England) for bilateral fore limb flexure with distorted rib cage is 0.95% (range: 0-12.5%) on the basis of litters affected and 0.18% (0-2.4%) on the basis of fetuses affected. The incidences of the above mentioned malformation in segment II teratology study in rats is higher than the mean incidence rate observed in the historical controls but it remains within the historical incidence range. Furthermore, in Segment I study in rats, no treatment related major malformation was seen in fetuses sacrificed on day 21 of pregnancy. Thus, no teratogenic effect at dosage up to 1600 anti-Xa u/kg/day was seen in rats. The postnatal development and the fertility of the offspring were comparable in all groups.

In i.v. Segment II teratology study in rabbits, doses of 0, 280, 470 and 780 anti-Xa units/kg/day were used. No teratogenic effect at dosage up to 780 anti-Xa u/kg/day) was seen in rabbits.

Proposed Version:

Pregnancy: Teratogenic effects. Pregnancy category B. Reproduction studies have been performed in rats (up to 1600 anti-Xa units/kg/day) and rabbits (up to 780 anti-Xa units/ kg/day) at i.v. doses up to 53 and 26 times the human dose (750 anti-Xa units b.i.d. = 30 anti-Xa units/kg/day = 1110 anti-Xa units/sq. m.; 50 kg body weight assumed) on the basis of anti-Xa units/kg/day and up to 8.5 and 6.0 times the human dose on the basis of anti-Xa units/sq. m. respectively which revealed no evidence of impaired fertility or harm to the fetus due to Orgaran. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

OVERDOSAGE:

Sponsor's Version:

OVERDOSAGE

Accidental overdosage following administration of ORGARAN™ (danaparoid sodium) injection may lead to bleeding complications. The effects of ORGARAN™ on anti-Xa activity cannot be antagonized with any known agent at this time. Although protamine chloride partially neutralizes the anti-Xa activity of ORGARAN™ and can be safely co-administered, there is no evidence that protamine chloride is capable of reducing severe non-surgical bleeding during treatment with ORGARAN™. In the event of serious bleeding, ORGARAN™ should be stopped and the administration of fresh frozen plasma should be considered. Withdrawal of ORGARAN™ may be expected to restore the coagulation balance without rebound phenomenon.

Evaluation:

Sponsor did not provide any clinical or preclinical overdose data. The minimal lethal s.c. doses were 3800 anti-Xa u/kg and 15200 anti-Xa u/kg in male and female rats respectively. The s.c. LD50 value in rat was 15200 anti-Xa units/kg and the cause of death was post-hemorrhagic anemia.

Proposed Version:

The following sentences should be added to the sponsor's version:

The minimal lethal s.c. doses were 3800 anti-Xa u/kg and 15200 anti-Xa u/kg in male and female rats respectively. The s.c. LD50 value in rat was 15200 anti-Xa units/kg and the cause of death was post-hemorrhagic anemia.

SUMMARY AND EVALUATION:

ORG 10172 is sulfated glycosaminoglycuronans which is related chemically and pharmacologically to heparin. ORG 10172 contains hepran sulfate (~84%), dermatan sulfate (~12%) and small amount of chondroitin sulfate (~4%). On the basis of AT-III affinity ORG 10172 can be separated into two fractions: high affinity (HA) fraction (5%) and low affinity (LA) fraction (95%). HA-fraction consists of hepran sulfate and LA-fraction consists of hepran sulfate dermatan sulfate and minor amount of chondroitin sulfate. ORG 10172 and heparin both had anti-thrombotic activity in various experimental models. In rats at equivalent doses (anti-Xa u/kg), the antithrombotic effect of ORG 10172 lasts at least twice as long as heparin. ORG 10172 also had less effect on bleeding (APTT and TCT) than heparin in various bleeding models at equivalent anti-thrombotic doses. Both ORG 10172 and heparin inhibited factor Xa and thrombin. On weight basis, the anti-factor Xa activity of ORG 10172 is about 10-fold lower than heparin, and anti-thrombin activity of ORG 10172 is about 200-fold lower than heparin. This indicated that anti-Xa/anti-thrombin ratio of ORG 10172 is greater than heparin. The low-affinity fraction of ORG 10172 (ORG 3051), the subfraction hepran sulfate (ORG 30955) and the subfraction hepran sulphate plus dermatan sulphate (ORG 30995) has very little anti-Xa activity. Hence, anti-Xa activity of ORG 10172 resides mainly in high affinity fraction. Furthermore, anti-thrombin activity of heparin works mainly via AT-III while anti-thrombin activity of ORG 10172 works via AT-III as well as through HC-II. Data also indicated that anti-thrombin activity of ORG 10172 is mainly associated with high-affinity fraction, while HC-II mediated anti-thrombin activity is similar, both in the high- and low-affinity fractions. Unlike heparin, ORG 10172 had no significant effect on fibrinolytic activity in rats and very minimal or no effect on platelet function. ORG 10172 exert less and short-lasting bleeding enhancing activity than heparin or fragmin (LMW-heparin).

Sponsor submitted a new Drug Application for Orgaran (ORG 10172) for marketing it for prevention of deep vein thrombosis which may lead to pulmonary embolism in patients undergoing hip replacement surgery. The drug comes in prefilled syringes containing 750 anti-Xa unit of ORG 10172 in 0.6 ml of sterile isotonic solution. The recommended dose of ORG 10172 is 750 anti-Xa unit b.i.d. subcutaneously for 7-10 days (15 anti-Xa unit/kg, b.i.d., 50 kg body weight assumed).

In support of the new drug application for Orgaran, sponsor has submitted preclinical pharmacology studies; absorption, distribution, metabolism and excretion (ADME) studies in rats,

rabbits and dogs; acute toxicity studies in rats and dogs; 6-week (i.v. and s.c.) and 6-month i.v. toxicity studies in rats; 6-week (i.v. and s.c.) and 6-month i.v. toxicity studies in dogs; Segment I. i.v. fertility and general reproductive performance study in rats; Segment II. i.v. teratology studies in rats and rabbits; Segment III. i.v. perinatal and postnatal study in rats; Genotoxicity studies: Ames Test, in vitro chromosomal Aberration Test in CHO cells, UDS Assay in Hela Cell Cultures, CHL/HGPRT Forward Gene Mutation Assay and in vivo mouse micronucleus Test; and Special toxicity studies: local i.v. and s.c. tolerance study in rats, and antigenicity test in mice and guinea pigs.

Absorption, distribution, metabolism, and excretion studies were conducted in rats (i.v.), rabbits (i.v. and s.c.) and dogs (i.v. and s.c.). Based on anti-Xa activity, bioavailability of ORG 10172 after s.c. dose in rats, rabbits and dogs were complete (100%), while s.c. bioavailability of anti-Xa activity after heparin dose were 41% and 94% in rats and rabbits respectively (s.c. bioavailability of anti-Xa activity after heparin in dogs were not measured).

In i.v. pharmacokinetic studies in rats, the $t_{½}$ of anti-Xa activity of ORG 10172 (drug) or ORG 10849 (fraction with high-affinity for AT-III) was about 3 hr. while $t_{½}$ of ORG 30561 (fraction with low-affinity for AT-III) was about 0.5 hr. From $t_{½}$ of anti-Xa activity, it is evident that elimination half-life of ORG 30561 did not influence the $t_{½}$ of ORG 10849 since $t_{½}$ of ORG 10849 was similar to the $t_{½}$ of ORG 10172. Plasma clearance of ORG 30561 was greater than that of ORG 10849 (0.5 ml/hr/g vs 0.04 ml/hr/g). In rat, volume of distribution after i.v. dose of ORG 10172 was 12-14 ml which is close to the volume of blood compartment. In a pharmacodynamic experiment, irrespective of route of administration, it was also shown that estimated $t_{½}$ of anti-Xa activities of ORG 10172 was approximately twice as long as the $t_{½}$ of anti-IIa activities, and the $t_{½}$ of IIaGI activities of ORG 10172 was twice as long as anti-Xa activities ($t_{½}$ of anti-IIa and anti-Xa activities were similar after heparin dose). In rabbits, irrespective of route of administration, the estimated $t_{½}$ of anti-Xa, anti-IIa and IIaGI activities were significantly greater for ORG 10172 (5.1-7.0 hr) than for heparin (1.6-3.1 hr). In contrast to rats, the $t_{½}$ of anti-IIa activity in rabbit was similar to $t_{½}$ of anti-Xa activity after ORG 10172 administration. In dogs, estimated $t_{½}$ of anti-Xa activity after i.v. or s.c. dose of ORG 10172 was about 12 hours.

Distribution of ORG 10172 in rat was not assessed because the drug is mainly confined to the blood compartment. However, ORG 10172 crosses placental barrier in pregnant guinea pigs. In vitro ORG 10172 and its subfractions binds to human serum albumin (90-93%).

Metabolism of ORG 10172 is not known, because no study was conducted using ^{35}S labeled ORG 10172.

In rats about 68-74%, 4-10% and <2% of the administered i.v. radioactivity of ^{3}H-ORG 10172 were excreted in urine, feces and expired air respectively during 0-96 hours. Hence, renal is the main route of elimination of ORG 10172 and its subfractions.

In acute toxicity studies in rats, the minimal lethal doses were 950 anti-Xa u/kg and 3800 anti-Xa u/kg after i.v. dose in male and female rats respectively. After s.c. dose, minimal lethal doses were 3800 anti-Xa u/kg and 15200 anti-Xa u/kg in male and female rats respectively. In dogs, the only tested dose (i.v.: 28000 anti-Xa u/kg and s.c.: 24600 anti-Xa u/kg) dose did not produce any mortality. In rats, the clinical signs were bradypnea, prostration and twitching after i.v. administration of ORG 10172. Subcutaneous administration of the drug into rats and dogs and i.v. administration of the drug into dogs resulted in subcutaneous swelling and hematoma at the injection sites.

In the 6-week i.v. toxicity study in rats, doses of 25, 100 and 400 mg/kg/day (correspond to 200, 800 and 3200 anti-Xa u/kg/day) were used. The data indicated that 100 mg/kg/day (800 anti-Xa u/kg/day) is the no effect dose in this study. The highest tested dose 400 mg/kg/day (3200 anti-Xa u/kg/day) can be considered as well tolerated dose, since it produced slight decrease in body weight gains (5-8%), perivascular hemorrhages and/or edema, slight hyperplasia of the lymphoreticular cells in the spleen and small foci of extramedullary hematopoiesis.

In the 6-week s.c. toxicity study in rats, doses of 20, 80 and 200 mg/kg/day (correspond to 160, 640 and 1600 anti-Xa u/kg/day were used. In this study the lowest tested dose (20 mg/kg/day = 160 anti-Xa u/kg/day) can be considered as well tolerated dose since it only produced slight s.c. hemorrhages at the injection sites. The mid and high dose produced lethality due to excessive hemorrhages at the injection sites. In 6-week subacute toxicity studies in rats, different dose levels were used when drug was given via i.v. or s.c. route. Irrespective of route of administration (i.v. or s.c.), ORG 10172 produced similar toxicities in rats, except when ORG 10172 (80 or 200 mg/kg/day) was given via s.c. route it produced lethality while no mortality was seen after 400 mg/kg/day i.v. dose. Excessive toxicity is

not due to higher plasma level after s.c. administration since plasma anti-Xa levels in rat measured immediately after i.v. administration were twice as high as the highest plasma level after s.c. administration of the same dose (SDG report # 2404). Most likely, mechanical damage of subcutaneous tissue and high local concentrations of the drug caused excessive hemorrhage and deaths. To avoid deaths sponsor selected i.v. route of administration for the 6-month toxicity study in rats.

In the 6-month i.v. toxicity study in rats, doses of 7.14, 28.57 and 114.28 mg/kg/day (correspond to 100, 400 and 1600 anti-Xa u/kg/day) were used. In this study, the lowest tested dose (100 anti-Xa u/kg/day) was the no effect dose and higher dose levels did not produce any marked toxicity.

In the 6-week i.v. toxicity study in dogs, doses of 25, 100 and 400 mg/kg/day (correspond to 200, 800 and 3200 anti-Xa u/kg/day were used. The data indicated that lowest tested dose (25 mg/kg/day = 200 anti-Xa u/kg/day) can be considered as no effect dose since it only produced slight s.c. hemorrhages. Mid dose and high dose levels produced moderate s.c. hemorrhages and hematomas at the injection sites in dogs of both sexes and mortalities among female dogs.

In the 6-week s.c. toxicity study in dogs, doses of 20, 60 and 200 mg/kg/day (correspond to 160, 480 and 1600 anti-Xa u/kg/day were used. In this study, no effect dose was not established. Mid and high dose levels produced lethality. Low dose did not produce any mortality but produced moderate s.c. hemorrhages and hematomas at the injection sites. In 6-week subacute toxicity studies in dogs, different dose levels were used when drug was given via i.v. or s.c. route. Irrespective of route of administration (i.v. or s.c.), ORG 10172 produced similar toxicities in dogs. There was an increased incidence in mortality and hematoma formation in dogs after s.c. as compared to i.v. administration. Excessive toxicity is not due to higher plasma level after s.c. administration since plasma anti-Xa levels in dog measured immediately after i.v. administration were twice as high as the highest plasma level after s.c. administration of the same dose (SDG report # 2404). Most likely, mechanical damage of subcutaneous tissue and high local concentrations of the drug caused excessive hemorrhage and deaths. To avoid deaths sponsor selected i.v. route of administration for the 6-month toxicity study in dogs.

In the 6-month i.v. toxicity study in dogs, doses of 6.62, 26.49 and 105.96 mg/kg/day (correspond to 100, 400 and 1600 anti-Xa u/kg/day) were used. In this study no effect dose was not established. Mid and high dose levels produced lethality. Low dose did not produce any mortality but produced slight s.c.

hemorrhages at the injection sites and increased liver, spleen, kidney, thymus and thyroid weights without any accompanying histological abnormalities. Therefore, low dose can be considered as a well tolerated dose.

In Segment I fertility and general reproductive performance study in rats, i.v. doses of 0, 390, 650 and 1090 anti-Xa u/kg/day were used. There were no abnormal effects on the fertility and mating performance of the treated male and female rats at doses up to and including 1090 anti-Xa u/kg/day (about 36 times the proposed clinical dose [30 anti-Xa u/kg/day]) of orgaran.

In the Segment II teratology study in rats, i.v. doses of 0, 100, 400 and 1600 anti-Xa units/kg/day were used. No teratogenic effects at dosage up to 1600 anti-Xa u/kg/day (about 53 times the proposed clinical dose [30 anti-Xa u/kg/day]) were seen. The postnatal development and the fertility of the offspring were comparable in all groups.

In the Segment II teratology study in rabbits, i.v. doses of 0, 280, 470 and 780 anti-Xa u/kg/day were used. No teratogenic effects at dosage up to 780 anti-Xa u/kg/day (about 26 times the proposed clinical dose [30 anti-Xa u/kg/day]) were seen.

In Segment III prenatal and postnatal study in rats, i.v. doses of 0, 100, 400 and 1600 anti-Xa units/kg/day were used. No adverse effect were seen in rats following i.v. administration of up to 1600 anti-Xa u/kg/day of orgaran during perinatal and postnatal period.

No mutagenic potential was demonstrated when orgaran was tested in 5 different tests: Ames Test, in vitro chromosomal Aberration Test in CHO cells, UDS Assay in Hela Cell Cultures, CHL/HGPRT Forward Gene Mutation Assay and in vivo mouse micronucleus Test.

In the special 2-week (i.v. and s.c.) toxicity studies, administration of orgaran via i.v. or s.c. route produced perivascular hemorrhages and hematomas/edema at the injection sites. Orgaran (ORG 10172) did not elicit sensitization activity in passive cutaneous anaphylaxis test in rats and guinea pigs. ORG 10172 did not induced active anaphylactic reaction or cutaneous hypersensitivity reaction in guinea pigs. No serum agglutinating antibodies were seen in serum sample taken from ORG 10172 treated guinea pigs using passive hemagglutination test.

In humans the proposed route of administration is subcutaneous. Sponsor has adequately characterized Orgaran and conducted sufficient preclinical toxicity studies in different species. Irrespective of route of administration (i.v. or s.c.), ORG 10172

produced similar toxicities in rats and dogs. In both rats and dogs, no target organ of toxicity was identified, higher dose levels produced excessive hemorrhages at the injection sites. There was an increased incidence in mortality (rats and dogs), hemorrhage (rats and dogs) and hematoma formation (dogs) after s.c. as compared to i.v. administration. Excessive toxicity is not due to higher plasma level after s.c. administration since plasma anti-Xa levels in rat and dog measured immediately after i.v. administration were twice as high as the highest plasma level after s.c. administration of the same dose (SDG report # 2404). Most likely, mechanical damage of subcutaneous tissue and high local concentrations of the drug caused excessive hemorrhage and deaths. From a preclinical standpoint the application is approvable.

The label is according to 21 CFR, 201.50 Subpart B (April 1, 1991), however, it needs minor changes in the text as outlined in the review portion.

RECOMMENDATIONS:

From a preclinical standpoint the application is approvable. Sponsor should be asked to change the labeling as outlined in the review portion.

Tanveer Ahmad 3/17/95
Tanveer Ahmad, Ph.D.
Pharmacologist, HFD-180

cc:
Orig. NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Ahmad
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-345/Dr. James
HFD-102/Assistant Director-Pharmacology

TA/hw/3/17/95
C:\WPFILES\PHARM\N\20430411.0TA

1. Concur
2. The reviewer will address the preclinical safety assessment of sodium sulfite (an ingredient in danaparoid sodium injection) in a separate addendum.

4/4/95

NDA 20-430 (Orgaran)

ADDENDUM TO PHARMACOLOGY REVIEW DATED APRIL 4, 1995

Sodium sulfite is present in the Orgaran formulation. Vehicle containing sodium sulfite as well as drug containing sodium sulfite have been tested in various preclinical studies. In the following studies control animals were given 1.92 mg/kg/day of sodium sulfite along with the vehicle:

1. 6-Month i.v. toxicity studies in rats and dogs.

2. I.V. Segment I fertility and reproductive performance study in rats.

3. I.V. Segment II teratology studies in rats and rabbits.

4. I.V. Segment III perinatal/postnatal study in rats.

In none of the above mentioned studies allergic reactions were seen. Furthermore, s.c. administration (clinical route of administration) of vehicle containing sodium sulfite (0.6 mg/kg for rats and 0.3 mg/kg for guinea pigs) did not elicit sensitization activity in passive cutaneous anaphylaxis test in rats and guinea pigs nor it induced active anaphylactic reaction or cutaneous hypersensitivity reaction in guinea pigs.

Tanveer Ahmad 4/6/95

Tanveer Ahmad, Ph.D.
Pharmacologist, HFD-180

cc:
Orig. NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Ahmad
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
N\20430504.0TA

4/6/95

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020430

CORRESPONDENCE

NDA 20-430

SEP 1 4 1994

Organon, Inc.
Attention: Mr. Patrick Osinski
375 Mount Pleasant Avenue
West Orange, New Jersey 07052

Dear Mr. Osinski:

We have received your new drug application submitted under section 505(b)(1) of the Federal Food, Drug and Cosmetic Act for the following:

Name of Drug Product: Orgaran™ (danaparoid sodium) Injection

Date of Application: September 8, 1994

Date of Receipt: September 9, 1994

Our Reference Number: NDA 20-430

Unless we notify you within 60 days of our receipt date that the application is not sufficiently complete to permit a substantive review, this application will be filed under section 505(b)(1) of the Act on November 8, 1994 in accordance with 21 CFR 314.101(a).

If the application is filed, the regulatory due date is March 8, 1995 and the due date under the Prescription Drug User Fee Act of 1992 is September 8, 1995.

Under 21 CFR 314.102(c) of the new drug regulations and in accordance with the policy described in the Center for Drug Evaluation and Research Staff Manual Guide CDB 4820.6, you may request an informal conference with this division (to be held approximately 90 days from the above receipt date) for a brief report on the status of the review but not on the application's ultimate approvability. Please request the meeting at least 15 days in advance. Alternatively, you may choose to receive such a report by telephone. Should you wish a conference, a telephone report, or if you have any questions concerning this NDA, please contact me at (301) 443-0487.

Please cite the NDA number listed above at the top of the first page of any communications concerning this application.

Sincerely yours,

Karen Oliver
Consumer Safety Officer
Division of Gastrointestinal
and Coagulation Drug Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

cc:
Orig. NDA 20-430
HFD-180
HFD-181/CSO/K.Oliver
DISTRICT OFFICE
R/D init: K.Johnson 9/12/94
KO/September 13, 1994 Karen Oliver 9/13/94
KO/9/13/94/c:\wpwin\karenfil\20430409.0ko

ACKNOWLEDGEMENT